Using calcein-filled osmotic pumps to study the calcification response of benthic foraminifera to induced hypoxia under *in situ* conditions: An experimental approach

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Dissertations in Geology at Lund University,
Master's thesis, no 431
(45 hp/ECTS credits)

Department of Geology
Lund University
2015

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Cover Picture: Collecting foraminifera from the Gullmar Fjord, August 2014. Photo Anders Filipsson.

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Landgren, S., 2015: Using calcein-filled osmotic pumps to study the calcification response of benthic foraminifera to induced hypoxia under *in situ* conditions: An experimental approach. *Dissertations in Geology at Lund University*, No. 431, 32 pp. 45 hp (45 ECTS credits).

Abstract: Benthic foraminifera are extensively used for environmental monitoring, for example in the context of oxygen depletion in coastal waters, which is the fastest growing and most serious threat to marine ecosystems. Several studies have demonstrated that many foraminiferal species are able to survive, reproduce and calcify under hypoxic (low dissolved oxygen) and even anoxic (no oxygen) conditions. The responses to these conditions are species specific, for example lowering of the metabolism, storage of nitrate to support anaerobic metabolism, and vertical migration in the sediments. Here we show for the first time that osmotic pumps can be used to dispense the fluorescent calcite marker calcein in undisturbed marine sediments in order to analyse the calcification response of calcareous foraminifera to different oxygen conditions. In the present study we used osmotic pumps to deliver a 100 mg l^{-1} solution of calcein to marine sediment cores, which were collected from the Gullmar Fjord, wherein benthic foraminifera were cultured under normoxic and hypoxic *in situ* conditions during ~3.5 months. The average temperature in the seawater overlying the sediments was 8°C and the average salinity was 32.6. The osmotic pumps, which had a calculated flow rate of ~1 µl h^{-1}, were filled with 2 ml of the calcein solution and then placed, one per sediment core, 1 cm below the sediment-water interface. Our analysis of calcein-labelled benthic foraminifera in the 100–150 µm-fraction in the uppermost 1.5 cm of the sediments shows that the relative abundances of calcifying *Nonionoides turgida* and *Nonionellina labradorica* compared to *Bulimina marginata* are positively correlated with the oxygen levels. The results indicate that the calcein concentration decreases in a direction away from the osmotic pump, although calcein-labelled foraminifera were recovered at all distances within a radius of 4.5 cm from the point source. By using osmotic pumps culturing and calcein-labelling can be performed *in situ* in undisturbed sediment cores, which means that the advantages of both the field study and the laboratory experiment can be utilized – that is a better consistency with the natural conditions in combination with a rigorous control of environmental variables. Thus, osmotic pumps have a great potential to become an important tool in future studies of benthic foraminiferal ecology.

Keywords: benthic foraminifera, calcein, osmotic pump, calcification, hypoxia, Gullmar Fjord.

Supervisor(s): Helena L. Filipsson & Laurie Charrieau

Subject: Quaternary Geology

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Användning av calcein-fyllda osmotiska pumpar för att studera hur biomineraliseringen hos bentiska foraminiferer påverkas av inducerad syrebrist under *in situ* förhållanden: En experimentell ansats.

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Sammanfattning: Bentiska foraminiferer används i stor utsträckning för miljöövervakning, t ex i samband med syrebrist i kustnära vatten, vilket är det snabbast växande och mest allvarliga hotet mot marina ekosystem. Ett flertal studier har visat att många foraminiferarter har förmåga till överlevnad, förökning och biomineralisering under förhållanden med syrebrist (hypoxi) och till och med under helt syrefria förhållanden (anoxi). Reaktionen på dessa förhållanden är artspecifika, till exempel sänkt ämnesomsättning, lagring av nitrat för att understödja anaerob metabolism och vertikal förflyttning i sedimenten. Här visar vi för första gången att osmotiska pumpar kan användas för att dispensera den fluorescerande kalcitmarkören kalcein i orörda marina sediment med syfte att analysera hur biomineraliseringen hos kalkskalsbildande foraminiferer påverkas av olika syreförhållanden. I denna studie använde vi osmotiska pumpar för att tillföra en 100 mg l^{-1} kalceinlösning till marina sedimentkärnor, som togs från Gullmarn, vari bentiska foraminiferer odlades under normalt syresatta och hypoxi *in situ* förhållanden under ~3,5 månader. Medeltemperaturen i havsvattnet överliggande sedimenten var 8°C och den genomsnittliga salthalten var 32,6. De osmotiska pumparna, som hade en beräknad flödeshastighet av ~1 µl h^{-1}, fylldes med 2 ml av kalceinlösningen och placerades sedan, en per sedimentkärna, 1 cm under gränsytan mellan sediment och vatten. Vår analys av kalceinmärkta bentiska foraminiferer i 100-150 µm-fraktionen i de översta 1,5 cm av sedimenten visar att det relativa antalet biomineraliserande *Nonionoides turgida* och *Nonionellina labradorica* jämfört med *Bulimina marginata* är positivt korrelerade till syrenivåerna. Resultaten indikerar att kalceinkoncentrationen avtar i riktning bort från den osmotiska pumpen, även om kalceinmärkta foraminiferer återfanns på alla avstånd inom en radie på 4,5 cm från punktkällan. Genom att använda osmotiska pumpar kan odling och kalceinmärkning ske *in situ* i ostörda sedimentkärnor, vilket medför att både fältstudiens och laboratorieexperimentets fördelar kan utnyttjas – det vill säga en bättre överensstämmelse med naturliga förhållanden i kombination med en noggrann kontroll över miljövariabler. Detta innebär att osmotiska pumpar har en stor potential att bli ett viktigt verktyg i framtida studier av bentisk foraminiferekologi.

Nyckelord: bentiska foraminiferer, kalcein, osmotisk pump, syrebrist, Gullmarn.

Handledare: Helena L. Filipsson & Laurie Charrieau

Ämne: Kvartärgeologi

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1 Introduction

Many coastal marine ecosystems are exposed to multiple stressors as a result of human impacts such as harmful algal blooms (Anderson 2002), overfishing (Jackson et al. 2001), habitat loss (Airoldi & Beck 2007) and pollution (Shahidul Islam & Tanaka 2004). However, oxygen depletion has in relatively short time become one of the most severe and widespread threats to coastal ecosystems worldwide (Díaz & Rosenberg 2008). Oxygen depletion or hypoxia ($[O_2] < 1.4$ ml l^{-1}) occurs when the supply of oxygen is less than the consumption of oxygen by aerobic degradation of organic matter. This implies that increased primary production; an increase of the turnover time of the bottom water, for example by a strong stratification of the water column or changes in ocean circulation; and/or an increase in temperature which decreases the solubility of oxygen in water (e.g. Weiss 1970), increase the risk of oxygen depletion. Records of hypoxia in deep basins and fjords occur throughout the geological history. However, it is only in the last decades, due to human-induced eutrophication and climate change, that hypoxia has dramatically increased in shallow marine and estuarine areas (Díaz & Rosenberg 2008; Rabalais et al. 2010). Furthermore, hypoxia is predicted to increase as a consequence of global warming, circulation changes and continued eutrophication (Keeling et al. 2010; Rabalais et al. 2010; Bijma et al. 2013; Friedrich et al. 2014).

Changes in oxygen levels and food supply from primary productivity are reflected in the assemblages of benthic foraminifera, which have extensively been used for environmental monitoring both to evaluate changes due to human impact and to estimate the pre-impacted conditions, that is in those cases where such information have not been available (e.g. Alve 1991, 1995; Scott et al. 2001; Murray 2006; Nigam et al. 2006; Dolven et al. 2013). A number of studies have investigated the response of benthic foraminifera to different oxygen concentrations, in the field (in situ) (e.g. Kaiho 1994; Sen Gupta et al. 1996; Bernhard et al. 1997; Gustafsson & Nordberg 1999; Gooday et al. 2000; Sen Gupta & Platon 2006; Langlet et al. 2013) and under laboratory conditions (e.g. Bernhard & Alve 1996; Risgaard-Petersen et al. 2006; Pucci et al. 2009; Nardelli et al. 2014).

The complex relationships between biotic and abiotic components of an ecosystem cannot be recreated in a laboratory experiment (Murray 2006). On the other hand, the main problem in field studies is to separate the effect of the variable of interest from other variables that naturally interact. Here, we combine these methods by culturing benthic foraminifera in *in situ* undisturbed sediment cores to examine the response by calcareous benthic foraminifera to experimentally induced hypoxia applying osmotic pump technology in a promising new approach to the study of benthic foraminiferal ecology.

Detailed knowledge of the benthic foraminiferal microhabitat preferences under which calcification takes place is required in order to use stable isotopic signatures of foraminiferal test to interpret palaeoenvironmental conditions. One example is the $\delta^{13}C$ signal, a widely used proxy for productivity and circulation, which reflects the isotopic composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) of the ambient bottom water or interstitial water at the time of calcification. (McCorkle et al. 1990, 1997; Mackensen et al. 2000; Fontanier et al. 2006; Hoogakker et al. 2015). It is variations in the oxygen content that primarily determine the benthic foraminiferal migration to favourable microhabitats (Alve & Bernhard 1995; Geslin et al. 2004), which can be found at depths down to 10-15 cm (Corliss 1985).

One of the methods used in this study is calcein-labelling of foraminiferal tests in order to identify foraminifera that have calcified during the experiment. Calcein, (bis[*N*,*N*−bis(carboxymethyl) aminomethyl] fluorescein), is a compound that can be incorporated into the calcite during biomineralization, provided that the foraminifera are incubated in a solution of calcein and seawater (Bernhard et al. 2004). Calcite with integrated calcein will fluoresce with a green-yellow light when examined under an epifluorescence microscope. Thus, calcein can be used to fluorescently label calcite to identify which part of a foraminiferal test has been biomineralized during, before (e.g. Filipsson et al. 2010) or after (e.g. Dissard et al. 2010) the incubation. Bentov et al. (2009) show that some benthic foraminiferal species store seawater in internal vacuoles since calcite was labelled by calcein even after the incubation had ended. Dissard et al. (2009) investigated the effect of calcein on element incorporation into foraminiferal calcite and observed no significant impact of calcein on Mg/Ca and Sr/Ca. Bernhard et al. (2004) demonstrate that both the survival rate of foraminifera that were incubated in a concentration of 10 mg l^{-1} calcein during a period of 12 days (7°C) and the rate of reproduction and growth of foraminifera that were cultured for 10-24 days were similar to those of the control specimens. However, when foraminifera were exposed to calcein for more than 5-6 weeks (> 25°C) at concentrations even as low as 5 mg l^{-1}, this resulted in an increase of the proportion of abnormal specimen, mortality and stunted reproduction (Kurtarkar et al. 2015).

The experiment described in my Master's thesis, is the first experimental study in which osmotic pumps have been used in order to deliver calcein to fluorescently label calcite that were biomineralized by benthic foraminifera cultured under hypoxic and normoxic conditions.

The aim of the study is to

- test if a calcein solution could be delivered using osmotic pumps at a rate sufficient to fluorescently label the calcite;
- analyse the changing density of the calcein-labelled foraminifera with distance from the point source, that is to better understand if and how the calcein solution spreads in the sediment;
  and
- analyse the response of calcareous foraminifera to different oxygen conditions.

The benthic foraminifera used in this study were collected from the Gullmar Fjord. This silled fjord on the Swedish west coast is usually hypoxic at the end of the normal annual cycle between two deep-water exchanges. This means that it can be assumed that the benthic fauna is adapted to these conditions.

1.1 Foraminifera

Foraminifera constitute one of the most important and diverse components of the marine microorganism community (Sen Gupta 1999), and they provide a major toolbox of different palaeooceanographic proxies (reviewed by e.g. Katz et al. 2010). Their average size is small (<0.5 mm) (Pawlowski 2012), and they are found in abundance in all marine settings, from marsh and marginal marine areas, through shelf seas to deep sea (Goldstein 1999; Murray 2006). Most foraminiferal species have a test (or shell), which enables both preservation and identification. The test wall may consist of organic matter; it can be composed of foreign grains gathered by the foraminifera from the seafloor and agglutinated together by organic or biomineralized cements; or the test wall can be biomineralized in which case it consist of either calcite, aragonite or silica of which the latter (silica) is extremely rare (Sen Gupta 1999). Foraminifera have an excellent fossil record, which extends back to the Early Cambrian (Culver 1994; Vachard et al. 2010). These earliest forms are all agglutinated benthic foraminifera. The planktonic foraminifera are considered to have originated during the Jurassic (Caron & Homewood 1983; Hart et al. 2003).

1.2 Osmotic pumps

The osmotic pump, which works according to the principles described by Theeuwes & Yum (1976), has an outer cover with a semipermeable membrane and is filled with a solution of a predetermined concentration = osmotic agent. Inside the osmotic pump there is a delimited, non-permeable area called the solution compartment, which contains the solution to be dispensed. The wall of the solution compartment is mobile and is shaped by the pressure of the surrounding solution (Fig. 1). As the osmotic pump is placed in a solution with a concentration less than that of the osmotic agent, the water will, via osmosis, enter the container through the semipermeable membrane. Due to the increasing amount of water inside the osmotic pump, the solution compartment will be compressed and hence the solution will be delivered through the orifice at the same rate as the water flows into the pump. The rate J is calculated by the equation

$$J = KA(\sigma\Delta\pi - \Delta P) \quad (1)$$

where J is the volume of water transported per unit of time, K is the permeability of the membrane to water, A is the effective surface area, σ is the osmotic reflection coefficient of the membrane, and $\Delta\pi$ and ΔP is the difference in osmotic and hydrostatic pressure, respectively, between the inside of the osmotic pump containing the osmotic agent and the environment outside the pump. The rate is independent of the composition of the solution to be dispensed.

1.3 Hydrography

The Gullmar Fjord opens into the Skagerrak across a sill at 42 m (Fig. 2). It is 28 km long, 2 km wide, NW/SE oriented and has a maximum depth of approximately 120 m. Outside the fjord, west of the sill, the North Sea high-salinity water flows into the Skagerrak at depth, while the Baltic Sea brackish water flows in the opposite direction in the surface layer. These two water masses determine the stratification and turnover times (Arneborg 2004) for the upper part of the fjord water column, which is composed of a low-salinity (S = 24–27) surface layer down to 15 m and an intermediate layer (S = 32–33) down to 50 m. The third and deepest layer has small seasonal variation in salinity (S = 34.4 on average) and is usually only renewed once a year during late winter or spring. Short periods of hypoxia occur almost every year prior to the coming deep-water exchange. Between 1979 and 1998 several extended periods (>3 months) of severe hypoxia (oxygen concentration <1 ml l^{-1}) were measured (Filipsson & Nordberg 2004). In 2007 a new severe hypoxic event that lasted more than 3 months occurred according to hydrographical data obtained from the Swedish Meteorological and Hydrological Institute's (SMHI) publically available database SHARK (Svenskt HavsARKiv, www.smhi.se). In 2014 the deep-water exchange failed to occur and a new period of severe oxygen depletion began in July and was still ongoing as of December 2014 (Fig. 3).

2 Materials and Methods

2.1 Calcein delivery rate

In this experiment ALZET® Osmotic Pumps were used for a continuous and controlled delivery of the calcein solution in the sediment cores, following Bernhard et al. (2015). ALZET Osmotic Pumps are available in a variety of models with different sizes of the

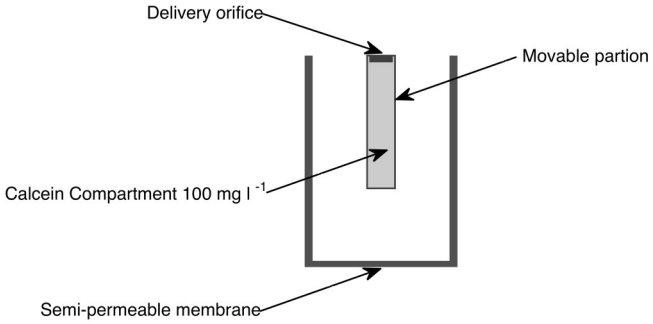

Fig. 1. Description of the osmotic pump, modified from Theeuwes & Yum (1976)

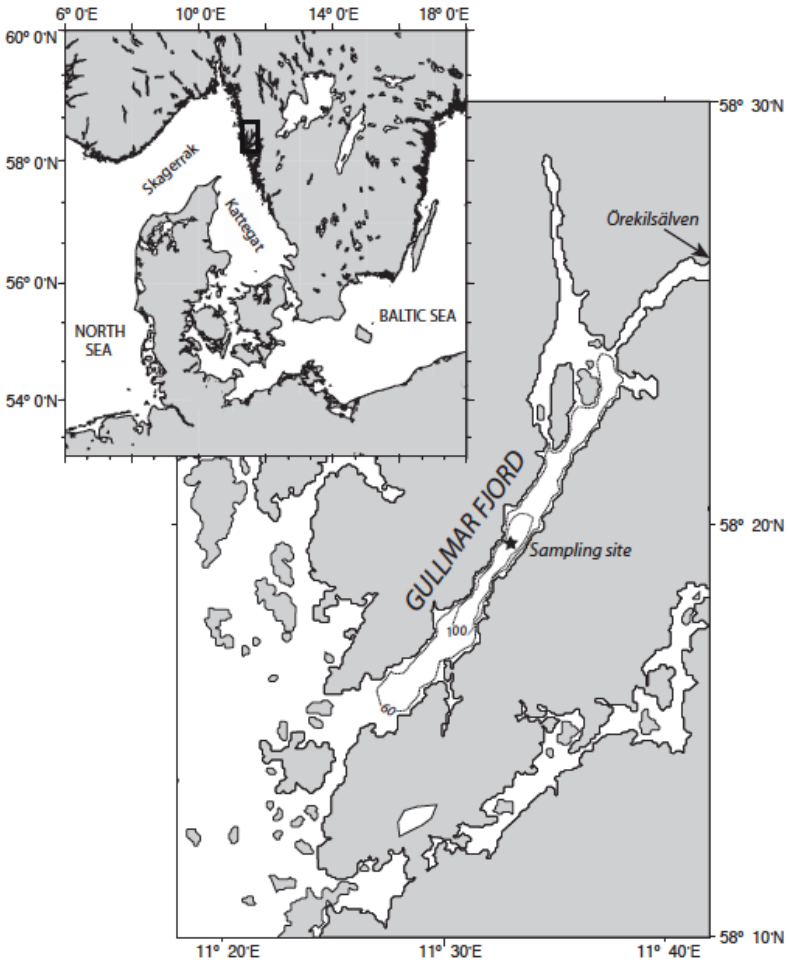

Fig. 2. Location map of the Gullmar Fjord with the coring site (58°19'35N 11°32'65E) indicated by a star.

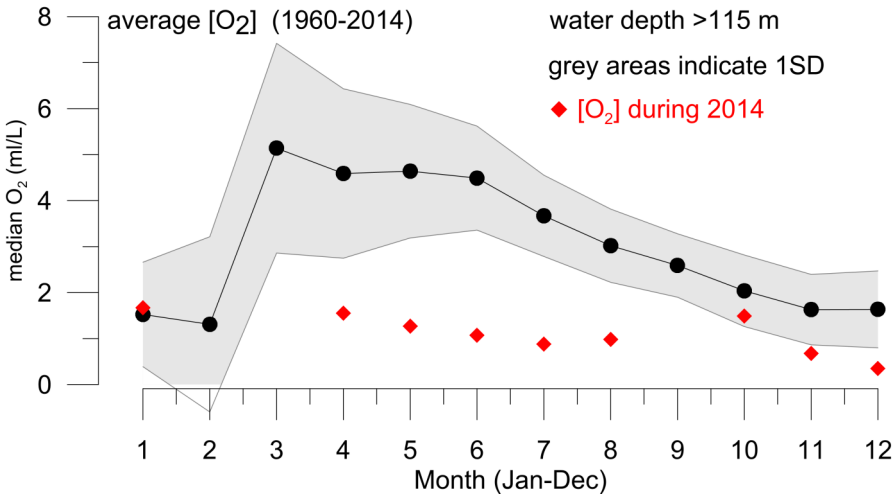

Fig. 3. The monthly average [O_2] 1960–2014 and [O_2] measured during 2014. The deep-water exchange failed to occur 2014 and thus also the subsequent increase in [O_2] which led to the development of a severe hypoxic condition.

solution compartment (reservoir volume), delivery rates and durations. 2ML1, 2ML2 and 2ML4 have equal reservoir volume (2 ml), which is the largest available, but they have different pump rates. The delivery rate Q of these pump models is calculated by the equation (ALZET 2015)

$$Q = Q_0\,(0.141\,e^{(0.051t)} - (0.007\pi) + 0.12) \qquad (2)$$

Q = the delivery rate (µl/h)
Q_0 = the specified pump rate per model at 37°C (µl/hr)
t = temperature (°C), $4 \le t \le 42$
π = the osmotic pressure of the solution outside the pump (atm), $0 \le \pi \le 25$
The accuracy of the calculated delivery rate is +/-10%.

In order to calculate the delivery rate Q in Eq. (2) the osmotic pressure π of the seawater in the sediment cores must be determined. This is accomplished using the van't Hoff equation supplemented with the osmotic coefficient Φ that compensates for the deviation of a solvent from its ideal behaviour. The osmotic pressure π is then given by the equation

$$\pi = \phi cTR, \qquad (3)$$

where c is the molarity of all solutes, R is the gas constant (R = 0.08206), and T is absolute temperature of the solution (seawater).

The osmotic coefficient Φ and the molarity of seawater c, in Eq. (3), were calculated using the Gibbs Seawater Oceanographic Toolbox (GSW) version 3.0, which contains functions for calculations of thermodynamic properties of seawater based on the International Thermodynamic Equation of Seawater – 2010 (TEOS-10) (IOC et al. 2010; McDougall & Barker 2011).

Absolute Salinity S_A, defined as the mass fraction of dissolved material in seawater (g kg^{-1}), cannot be measured directly (Millero et al. 2008), and usually salinity is reported as Practical Salinity S, which is a dimensionless quantity. However, to calculate Φ and c in Eq. (3), the mass of the sea salt in the seawater is required and thus S_A. GSW includes a function to calculate S_A from S, sea pressure p, and location. Sea pressure p is defined to be 0 (dbar) at the surface. Location (longitude and latitude) is required since the composition of the sea salt depends on the provenance of the seawater. The osmotic coefficient, Φ in Eq. (3) is calculated from S_A and temperature t of the seawater. The molarity of seawater c in Eq. (3) is calculated from

$$c = nV_{sw}^{-1} = mM^{-1}V_{sw}^{-1} = mM^{-1}\rho_{sw}m_{sw}^{-1}, \qquad (4)$$

where n is the moles of sea salt, V_{sw} is the volume of the seawater, m is the mass of the sea salt, M is the molar mass of sea salt, ρ_{sw} is the density of the seawater, and m_{sw} is the mass of the seawater. The molar mass, M, is set to the average atomic weight of sea salt ≈ 31.4038218 g mol^{-1} (Millero et al. 2008). GSW also includes a function to calculate the density of seawater ρ_{sw} from S_A and sea pressure p. The mass of the seawater m_{sw} is assumed to be 1000 g.

During the experiment total dissolved solids TDS was measured, not salinity S, which is required to calculate S_A as described above. The electrical conductivity EC can be calculated from TDS by the equation

$$TDS = k_e EC, \qquad (5)$$

where k_e is the correction factor used by the conductivity meter during the measurements and EC is electrical conductivity at 25°C (e.g. Walton 1989). Next salinity S can be calculated using a function in GSW, gsw_SP_from_C, which calculate S from electrical conductivity EC, temperature t, and sea pressure p.

2.2 Collection and maintenance

Six undisturbed sediment cores with living foraminiferal specimens were collected on 15 August 2014 from 118 m water depth in the Gullmar Fjord (58°19'35N 11°32'65E) using a GEMAX gravity corer from the R/V *Oscar von Sydow*. The internal diameter of the plastic core liner is 9 cm and the tube length is 100 cm. The lengths of the sediment cores GFOA–GFOD were ~62 cm each, while the cores GFOE and GFOF were ~70 cm each. 60 l of seawater (32 m water depth, S = 32.8) was collected in thoroughly rinsed plastic containers from the deep-water intake at the Sven Lovén Centre for Marine Sciences in Kristineberg. The sediment cores were wired with blue ice in order to maintain a constant temperature during transportation to the laboratory at the Department of Geology, Lund University. Immediately upon arrival in Lund the sediment cores were placed in a cold room for storage until the start of the experiment.

The experiment was initiated on the 26 August 2014 and was terminated 15–18 December 2014. All six sediment cores were placed in a climate chamber at 8°C, which is ~1.5°C above the average temperature at the site of collection (SHARK). The temperature in the climate chamber was monitored using two DS1921G Thermochron iButtons (± 1°C). Most of the equipment required for microprofiling was set up inside the climatic chamber in order to keep the doors closed during measurements and thus avoid variations in temperatures. GFOC, GFOD, GFOE were decided to be maintained as hypoxic during the experiment and GFOA, GFOB, GFOF as normoxic. All hypoxic cores were sealed with Parafilm M®, duct tape, and plastic plugs.

The ALZET osmotic pump model 2ML2 was used for this experiment (diameter 1.4 cm and length 5.1 cm). After replacement of the so-called Flow Moderator, in original of stainless steel, with Flow Moderators made of PEEK ™ (polyetheretherketone), the 2 ml compartments of the osmotic pumps were filled with 100 mg l^{-1} solution of calcein and seawater. Then the osmotic pumps were placed, one in each sediment core, 1 cm below the sediment-water interface.

Each week oxygen concentrations, pH, temperature and total dissolved solids (TDS) were measured. Vertical oxygen and pH profiles were measured in the uppermost sediments of the cores GFOB and GFOD using microsensors (Ox-100, pH-100/pH-200, Unisense, Denmark), except in November when no pH profiles were measured. Lovibond SensoDirect 150 was used for measurements of water chemistry above the sediment surface: oxygen concentrations (± 0.4 mg l^{-1}) and

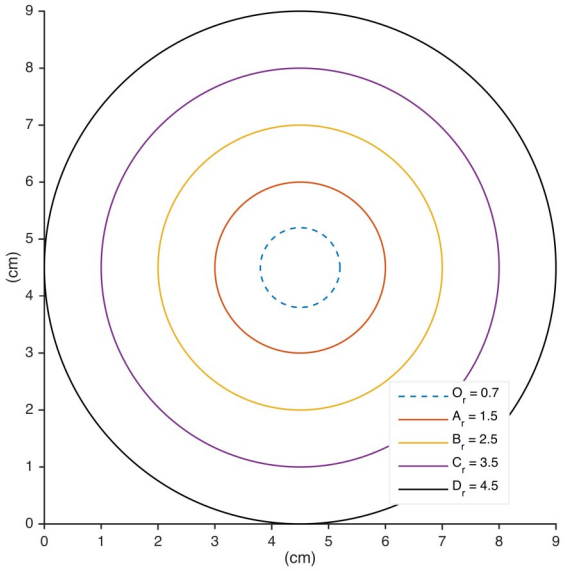

Fig. 4. The cores were sliced into 0.5 cm intervals until 2 cm depth and into 1 cm intervals until 6 cm depth. These samples were at the same time divided into 4 subsamples, A-D, with the radii r = 1.5, 2.5, 3.5 and 4.0 cm.

pH (± 0.02 pH) were measured in the cores GFOE and GFOF, and TDS (± 2.640 mg l^{-1}) was measured in all cores. In addition, electrical conductivity EC (± 4 mS cm^{-1}) was measured in all cores on two occasions. After completion of the measurements the seawater in the cores was carefully replaced with seawater from the fjord. The hypoxic cores received seawater that was nitrogen bubbled to lower the oxygen concentration.

The foraminifera were fed weekly by a mixture of the marine microalgal strains *Dunaliella tertiolecta* Butcher and *Isochrysis galbana* Parke following Wilson-Finelli et al. (1998), 2 x 2 ml each per core. The microalgae starter cultures were obtained from the University of Gothenburg GU Marine Algal Culture Collection (GUMACC). The cultures were grown to 400 ml by using growth media, then concentrated by centrifuging at 3000 rmp for 8 min, which reduced 400 ml to about 24 ml, and finally frozen as 2 x 12 ml portions. As a consequence of the supply rate of organic material in combination with low oxygen concentrations, the redox front was seen to rise in the sediment cores and approaching the sediment-water interface in the hypoxic cores about 9 weeks after the start of the experiment. In the hypoxic cores the food supply was reduced by half the following four weeks in order to avoid the development of anoxic conditions in the uppermost centimetres.

After experiment termination all cores were sliced into 0.5 cm intervals until 2 cm depth and into 1 cm intervals until 6 cm depth. The sample volume was calculated on the basis of the inner diameter of the core (9 cm) from which the diameter of the osmotic pump was subtracted (0.7 cm); a 0.5 cm slice therefore has a volume of 31 cm^3. In order to analyse if the density of the calcein-labelled foraminifera changes with distance from the calcein point source (that is the orifice of the osmotic pump), each sample was further divided into concentric circles with radii: 1.5, 2.5, 3.5 and 4.5 cm (Fig. 4). These subsamples were transferred to plastic containers, weighed, labelled A–D and stored in a cool storage until the microscope examination. Samples that were not analysed were freeze-dried; all samples are archived at the Department of Geology, Lund University.

2.3 Foraminiferal analyses

In this study only calcareous calcein-labelled specimens in the 100–250 µm-fraction of the uppermost 1.5 cm of the sediments cores GFOB, GFOD, GFOE and GFOF were analysed. The fraction 100–250 µm was chosen in order to compare the results with other analyses of foraminifera from the Gullmar Fjord, e.g. Nordberg et al. (2000), Filipsson et al. (2004), and Polovodova et al. (2011). A total of 48 subsamples (A–D) were prepared. The subsamples were placed in a solution of sodiumdiphosphate ($Na_2P_2O_7$) for 1 h, in order to disintegrate sediment aggregates, and thereafter washed over a nested set of 63-, 100- and 250 µm sieves. Ultimately the 100–250 µm-fractions were wet picked using a Nikon SMZ1500 stereomicroscope equipped with an epi-fluorescence attachment (excitation 485 nm, emission 520 nm). All calcein-labelled specimens in a subsample were picked, sorted and counted.

The species identification was based on the following studies of the fauna in Gullmar fjord: Höglund (1947) and Polovodova Asteman & Nordberg (2013). The original description and synonyms of the species were extracted from the World Modern Foraminifera Database (Hayward et al. 2015).

Total absolute abundances (individuals/10 cm^3 of the uppermost 1.5 cm of the sediments cores), vertical absolute abundances (as the sum of the specimens counted in the subsamples A–D per interval 0–0.5, 0.5 –1, 1–1.5 cm and normalized to 10 cm^3), and horizontal absolute abundances (individuals/10cm^3 per subsample A-D), as well as relative abundances and diversity as species richness (count of number of taxa), were calculated.

The average living depth (ALD_x) is a way to describe the vertical distribution of the total foraminifera fauna or of individual taxa, and to obtain a general idea about the microhabitat patterns. Jorissen et al. (1995) defined the ALD_x as

$$ALD_x = \sum_{i=0,x} (n_i \times D_i)/N$$

where x is the lower limit of deepest sample, n_i is the number of specimen in interval i, D_i is the midpoint of sample interval i, and N is the total number of individuals for all sample levels. In this study, the $ALD_{1.5}$ (Average Living Depth calculated for the upper 1.5 cm, in 0.5 cm intervals) was calculated for the cores GFOB, GFOD, GFOE and GFOF for the total calcein-labelled fauna.

Statistical analyses were processed using MATLAB (2014). Principal component analysis (PCA) was performed to demonstrate possible differences in the calcification response of the calcareous foraminiferal species to the different oxygen condi-

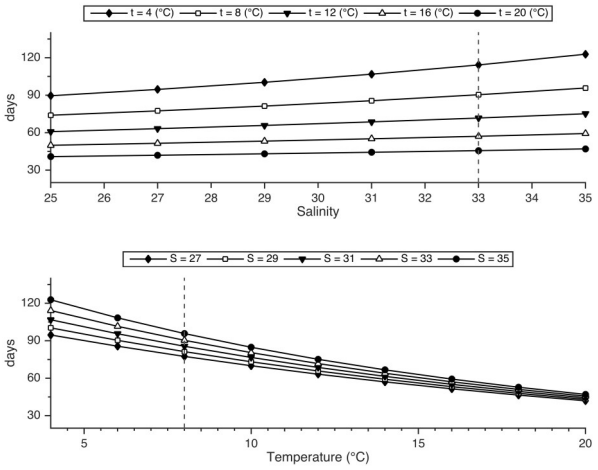

Fig. 5. The duration of the osmotic pump was calculated for temperatures t = 4, 8, 12, 16 and 20°C, picture above, and salinities S = 27, 29, 31, 33 and 35, picture below. The dashed vertical lines mark the conditions used in this experiment: t = 8°C and S = 33.

tions in this experiment. The PCA was based on the relative abundances of the dominant species (>5%) of the uppermost 1.5 cm of the sediments cores. Pairwise comparisons of absolute abundances of calcein-labelled foraminifera from subsamples A–D were performed using the 'Wilcoxon signed-ranks test' to examine whether there were significant differences of the abundances due to the spreading of the calcein from a point source (p values < 0.05 were considered significant).

In order to quantify a possible overestimation of the number of calcein-labelled foraminifera due to cytoplasmic fluorescence, which was noted in some specimens by Bernhard et al. (2004), a number of calcein-labelled foraminifera from one of the hypoxic cores (0.5–1.0 cm depth) were divided into two groups and exposed to 5% sodium hypochlorite for 15 minutes and 1 hour, respectively. The specimens were selected from a hypoxic subsample since the risk of overestimation, due to undecayed cytoplasm, is probably higher under low-oxygen conditions.

3 Results

3.1 A MATLAB function 'alzet_flowrate'

A function, alzet_flowrate, was developed and implemented in MATLAB (2014) to calculate the delivery rate Q in Eq. (2). The alzet_flowrate.m code is given in Appendix A. Input arguments to alzet_flowrate are observed values of salinity S, temperature t, together with longitude and latitude and pump model: 2ML1, 2ML2 or 2ML4. The function implements Eq. (2–4) and utilizes functions in the Gibbs Seawater Oceanographic Toolbox (GSW) as previously detailed. The delivery rate Q (µl hrs^{-1}) and the estimated duration of the osmotic pump in days are returned. The salinity S values were calculated from the measured TDS values. First the correction factor k_e in Eq. 5 was calculated using measurements of TDS and EC at two occasions (Table 1). Then k_e (= 0.67) was used to calculate EC

Table 1. Total dissolved solids TDS and electrical conductivity EC measured at two occasions together with calculated values of the correction factor ke (average 0.67).

Date	**TDS (g l^{-1})**	**EC (mS cm^{-1})**	**k_e**
04/09/2014	32.9	50	0.66
04/09/2014	34.1	51	0.67
04/09/2014	33.8	51	0.66
04/09/2014	33.3	50	0.67
04/09/2014	33.5	50	0.67
04/09/2014	33.5	50	0.67
12/09/2014	33.3	50	0.67
12/09/2014	33.3	50	0.67
12/09/2014	33.1	50	0.66
12/09/2014	31.9	48	0.66
12/09/2014	33.2	50	0.66
12/09/2014	33.2	50	0.66

from the measured TDS, and finally the function gsw_SP_from_C was called and the S values were returned. The function alzet_flowrate was called with the following input arguments: S = 33, t = 8, long = 11.54, lat = 58.32, and model = '2ML2'

```
[days, flowrate]=alzet_flowrate(33, 8, 11.54, 58.32, '2ML2')
```

and returned: days = 90.4, flowrate = 0.88 µl h^{-1} ± 10%. This implies that the duration of the experiment, 112–115 days, slightly exceeded the estimated duration of the osmotic pumps. The duration of the osmotic pump was calculated for different temperatures (range 4–20°C) and salinities (range 27–35) (Fig. 5).

3.2 Water chemistry

Water chemistry of the overlying waters was consistent for all sediment cores with respect to salinity S, which varied between 31.7 and 33.6 (average 32.6, SD 0.5) (Fig. 6A). The temperature for all cores varied between 7.3°C and 8.6°C (average 8.0°C, SD 0.3) and pH varied between 7.0 and 8.3 (average 7.8, SD 0.3). The temperature in the cores GFOB and GFOD was consistently lower than in the cores GFOE and GFOF; initially the average temperatures were 0.3–0.6°C lower and from mid-October 0.1–0.2°C lower (Fig. 6B). The pH values in the nitrogen-bubbled, hypoxic cores GFOD and GFOE were 0.2–0.3 pH units higher than the normoxic cores (Fig. 6C). The oxygen concentration in the hypoxic core (GFOE) varied between 0.3 and 5 ml l^{-1} (average 2 ml l^{-1}, SD 1) and usually rose above the hypoxic (>1.4 ml l^{-1}) level before the water was exchanged the coming week (Fig. 6D). The oxygen concentration in the normoxic core GFOF varied between 0.7 and 5 ml l^{-1} (average 4, SD 1) (Fig. 6D). The minimum value was measured at one occasion in November otherwise the oxygen concentration kept above the hypoxic level.

The average air temperatures in the climate cham-

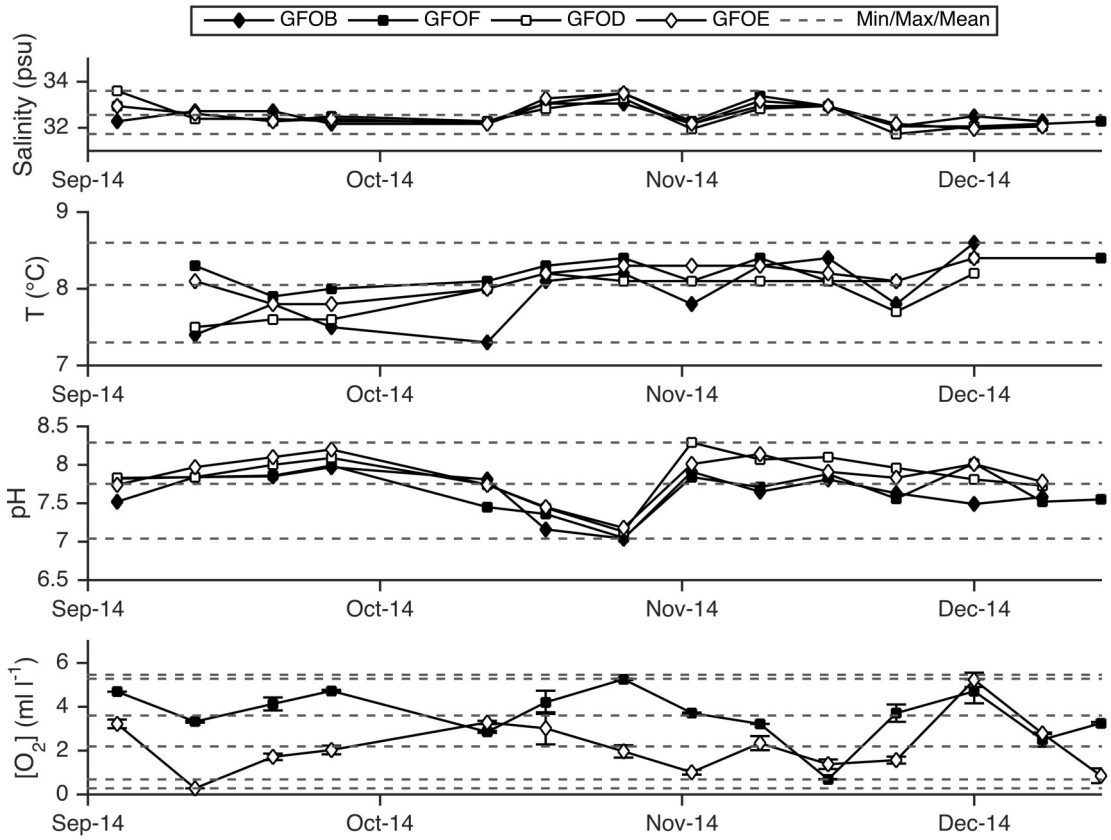

Fig. 6. Water chemistry was measured weekly in the normoxic cores GFOB and GFOF (full symbols) and the hypoxic cores GFOD and GFOE (empty symbols) during the experiment. **A**. Salinity. **B**. Temperature. **C**. pH. **D**. $[O_2]$ was measured weekly in the water above the sediment in the cores GFOE and GFOF.

ber measured with the two Thermochron iButtons differed by about 1°C: (average 6.9°C, SD 0.3 and average 7.8°C, SD 0.3) (Fig. 7). Minimum and maximum values are associated with the weekly measurements during which a constant temperature in the climate chamber was not possible to maintain.

Some examples of oxygen profiles measured in the uppermost sediments of cores GFOB (normoxic) and GFOD (hypoxic) are shown in Fig. 8A–D. The oxygen concentration at the sediment-water interface in GFOB remained well above the hypoxic level throughout the duration of the experiment (average 4.4 ml l^{-1}, SD 0.5) (Table 2). In the core GFOD the oxygen concentration at the sediment-water interface was initially at or below hypoxic level (average 2 ml l^{-1}, SD 1), but as of 26 October 2014 the oxygen concentration increased gradually and a maximum value of 2.8 ml l^{-1} was measured on 23 November 2014. In both the normoxic and hypoxic core the oxygen concentration diminishes very quickly within the sediment, reaching zero at about 0.2–0.3 mm depth (Table 2).

Some examples of pH profiles in the cores GFOB and GFOD are shown in Fig. 9A–B. The pH measurements in the nitrogen-bubbled (hypoxic) core, GFOD, were significantly higher than those in the normoxic core, GFOB. The exception with lower values on 26 October 2014 was most likely due to a broken pH microsensor.

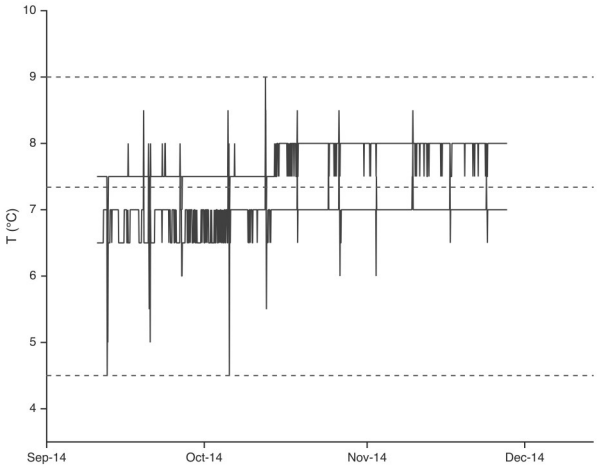

Fig. 7. Air temperatures in the climate chamber were measured continuously by two Thermochron iButtons (temperature loggers). The average temperatures measured differed by ~1°C between the two loggers. Minimum and maximum values are associated with the weekly measurements during which a constant temperature in the climate chamber was not possible to maintain.

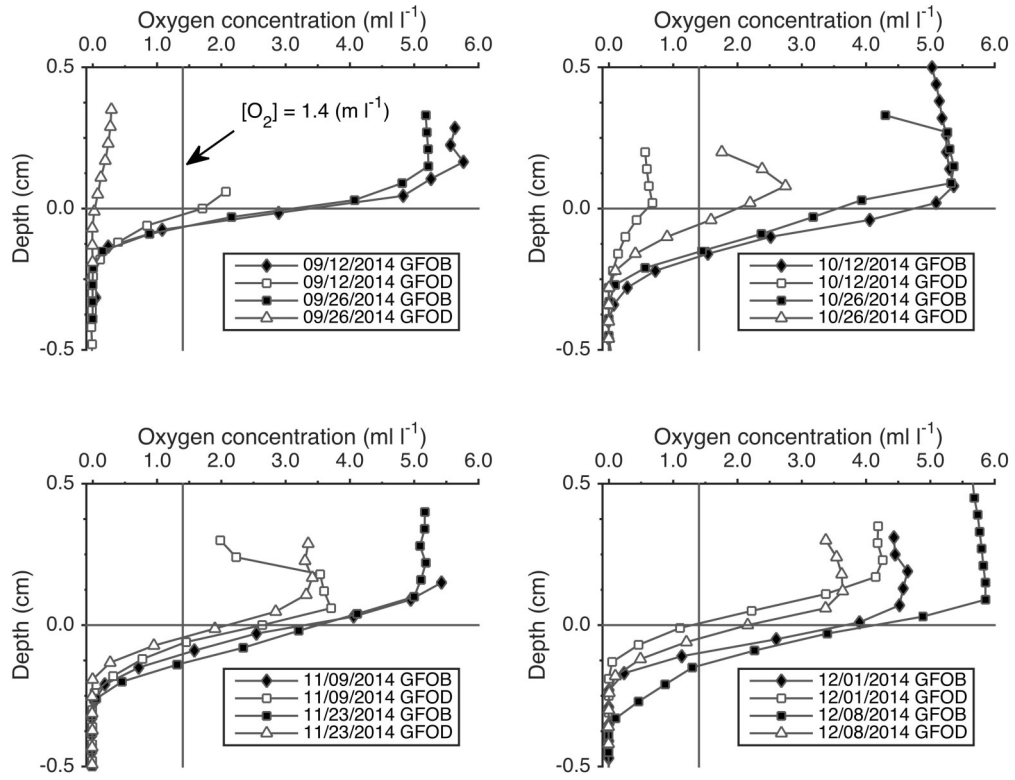

Fig. 8. **A–D**. Examples of oxygen profiles in the nitrogen-bubbled core GFOD (empty symbols) and normoxic core GFOB (full symbols).

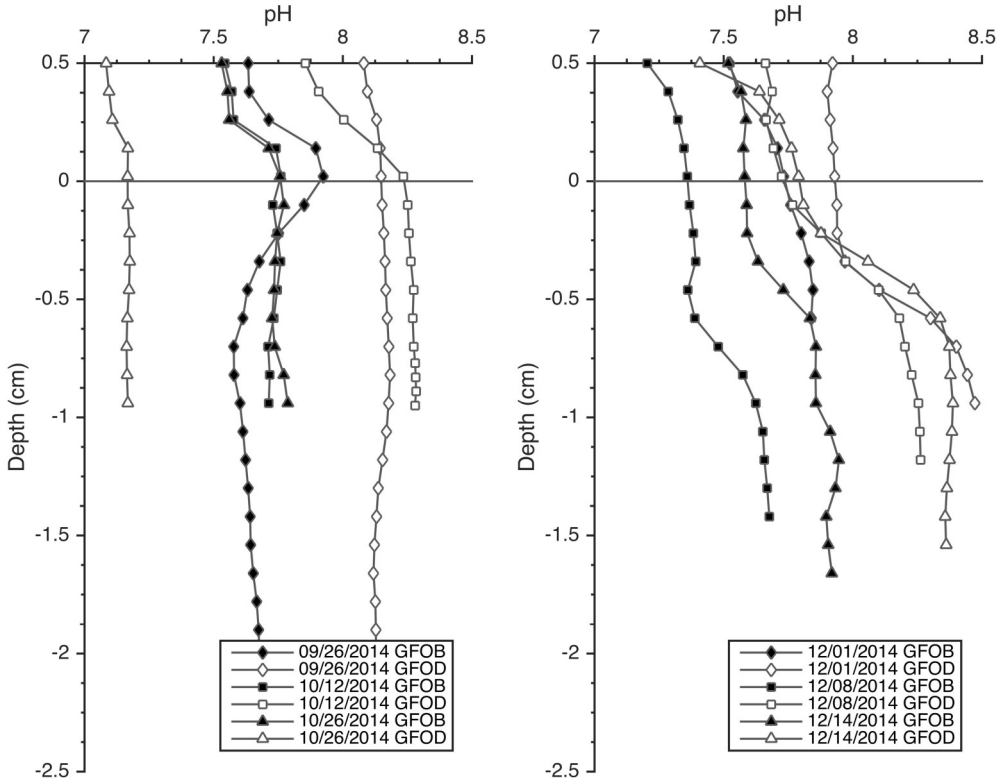

Fig. 9. **A–B**. Examples of pH profiles in the nitrogen-bubbled core GFOD (empty symbols) and the normoxic core GFOB (full symbols). The levels of pH were in general higher in GFOD compared to GFOB. The measured values from the 26th of October 2014 are most likely incorrect because of a broken pH-microsensor; the following 4 weeks no pH-profiles were performed due to this.

3.3 Foraminifera

3.3.1 Composition

Total abundances of the calcein-labelled specimens in the 100–250 µm-fraction of the uppermost 1.5 cm of the sediments cores were: 106 and 300 in the normoxic cores GFOB and GFOF, which corresponds to approximately 11 and 32 individuals/10 cm^3; and 228 and 275 in the hypoxic cores GFOD and GFOE, which corresponds to approximately 24 and 30 individuals/10 cm^3. The highest value of species richness (range: 13–21, average 17) was found in GFOD (hypoxic) and the lowest was found in GFOB (normoxic).

The foraminiferal fauna in all cores are dominated by *Bulimina marginata*, *Cassidulina laevigata* and *Nonionoides turgida* (Table 3). *Nonionellina labradorica* is a dominant species in both the normoxic cores, whereas *Quinqueloculina* spp. are among the dominant species in the hypoxic core GFOD and common in the other hypoxic core (GFOE). *Eilohedra vitrea* (original name *Epistominella vitrea*) is common in one normoxic (GFOB) and one hypoxic (GFOE) core. *Bolivina skagerrakensis* is frequent in GFOD. The total counted individuals (*N*) and species richness (*S*) of six dominant species are shown in Fig. 10.

3.3.2 Vertical distribution

The vertical distribution of the dominant taxa (>5%) and the calculated values of the average living depth in the upper 1.5 cm ($ALD_{1.5}$) are shown in Fig. 10. In GFOB (normoxic) the highest value of absolute abundance per depth interval is 26 individuals/10 cm^3 observed in the interval 1.0–1.5 cm, $ALD_{1.5}$ is 1.1 cm, and *B. marginata*, *C. laevigata* and *N. turgida* have the highest abundance in the lowest interval analysed. In the other normoxic core GFOF the highest absolute abundance value of 51 individuals/10 cm^3 is found in the interval 0.5–1 cm and $ALD_{1.5}$ is 0.8 cm. Again, *B. marginata* and *C. laevigata* have the highest abundance in the lowest interval, but in this core *N. turgida* together with *N. labradorica* have the highest abundance in the middle interval. The highest value of absolute abundance in the hypoxic core GFOD is 35 individuals/10 cm^3 at depth interval (0.5–1.0 cm) and $ALD_{1.5}$ is 0.9 cm. In this core *B. marginata*, *N. turgida* and *Quinqueloculina* spp. all show the highest absolute abundance in the middle interval, while *C. laevigata* is more uniformly distributed. In GFOE, the other hypoxic core, a highest absolute abundance value of 41 individuals /10 cm^3 is found in the interval 1.0–1.5 cm and $ALD_{1.5}$ is 0.9 cm. Both *B. marginata* and *C. laevigata* have the highest abundance in the lowest interval, whereas *N. turgida* and *E. vitrea* have the highest abundance in the uppermost interval.

3.3.3 PCA analysis

The principal component analysis (PCA) was based on relative abundance of the six dominant taxa (>5%): *B. marginata*, *C. laevigata*, *E. vitrea*, *N. labradorica*, *N. turgida* and *Quinqueloculina* spp. The PCA resulted in two significant axes, explaining 97.8% of the total variance (Fig. 11). The first axis, which accounts for 77.3% of the total variance, is dominated by *N. turgida*, that together with *N. labradorica* loads on the positive side, while the negative side is loaded by *B. marginata*, *C. laevigata* and *Quinqueloculina* spp., together with *E. vitrea.* The second axis, which accounts for 20.5% of the total variance, is dominated by *B. marginata*, that together with *E. vitrea*, *N. labradorica* and *N. turgida* loads on the positive side, while the negative side is loaded by *C. laevigata* and *Quinqueloculina* spp.. The position of the four cores on the plane formed by the first two axes is shown in Fig. 11. Both the normoxic cores, which are relatively rich in *N. turgida* and *N. labradorica,* plot on the positive side on the first axis. The hypoxic core GFOE is dominated by *B. marginata* and plots on the positive side on the second axis, while the hypoxic core GFOD, which is relatively rich in *C. laevigata*, plots on the negative side of both axes.

Table 2. The oxygen concentration in the normoxic core GFOB (N) and hypoxic core GFOD (H) measured at the sediment-water interface (depth = 0 cm) are displayed in the first two columns to the left. The depths where the oxygen concentration reached zero ($[O_2]$ = 0 ml l^{-1}) during oxygen profiling are displayed in the third and fourth columns. The values within parenthesis are average oxygen concentrations and depths.

	GFOB (N)	**GFOD (H)**	**GFOB (N)**	**GFOD (H)**
	$[O_2]$ (ml l^{-1})	**$[O_2]$ (ml l^{-1})**	**Depth (cm)**	**Depth (cm)**
Date	**(4.4 ml l^{-1}, SD 0.5)**	**(2 ml l^{-1}, SD 1)**	**(-0.3 cm, SD 0.1)**	**(-0.2 cm, SD 0.1)**
09/12/2014	4.8	1.7	-0.2	-0.2
09/26/2014	4.1	0.1	-0.2	-0.01
10/12/2014	5.1	0.7	-0.4	-0.3
10/26/2014	3.9	2.2	-0.3	-0.3
11/09/2014	4.1	2.6	-0.3	-0.2
11/23/2014	4.1	2.8	-0.3	-0.2
12/01/2014	3.9	2.2	-0.2	-0.2
12/08/2014	4.9	2.2	-0.4	-0.2

Table 3. Relative abundances (%) for the foraminiferal taxa in the uppermost 1.5 cm of the cores GFOB, GFOD, GFOE and GFOF. (N) = normoxic and (H) = hypoxic. The grey boxes represent dominant taxa (>5%) in at least one of the cores.

Species	**GFOB (N)**	**GFOD (H)**	**GFOE (H)**	**GFOF (N)**
B. skagerrakensis	1	5	2	2
B. pseudopunctata	1	1	2	4
B. marginata	23	24	42	18
C. laevigata	8	27	16	13
Cribroelphidium albiumbilicatum/C.magellanicum	0	0	0	0
C. incertum	0	0	0	0
Cribroelphidium spp.	3	1	1	1
Elphidium excavatum clavatum	2	2	2	4
E. vitrea	7	2	8	1
Globobulimina auriculata	0	0	0	0
G. turgida	0	2	0	0
Hyalinea balthica	0	1	0	1
Lobatula lobatula	1	1	0	0
Miliolinella subrotunda	0	0	0	1
N. iridea	1	4	1	2
N. labradorica	13	2	4	7
N. turgida	37	11	12	41
Nonioninae spp.	3	0	0	2
Pyrgo williamsoni	0	0	2	0
Quinqueloculina spp.	1	13	5	0
Stainforthia fusiformis	0	1	0	0
Varia	0	3	1	2

3.3.4 Horizontal distribution

A majority of the calcein-labelled foraminifera were found in the subsamples labelled D, which were the outermost areas of the sediment slices when they were horizontally divided. However, a pairwise comparisons of the absolute abundances (individuals/10 cm^3) of calcein-labelled individuals from subsamples A–D showed that the absolute abundances are significantly higher in subsamples A, that is closest to the calcein point source (the osmotic pump), than in B, C and D (Wilcoxon signed-ranks test, $p < 0.005$)(Fig. 12). However, this was not the case neither when the absolute abundances of subsamples B were compared to the densities of the subsamples C and D, nor when the absolute abundances of subsample C was compared to the densities of subsamples D .

3.3.5 Cytoplasmic fluorescence

Cytoplasmic fluorescence may be misinterpreted as calcein-labelled calcite and hence there is a risk that the numbers of experimental calcifying foraminifera have been overestimated. We tested this potential source of error by exposing two samples, each containing a number of calcareous foraminifera that previously had been counted as calcein-labelled, to 5% sodium hypochlorite (NaClO) during 15 minutes respectively 1 hour (Table 4). In the group treated for 15 min 94% of the specimens were still fluorescent after treatment. Corresponding result was 77% in the group treated for 1 hour. Of the species examined only *C. laevigata* and *Quinqueloculina* spp. were counted different before and after treatment. The largest difference was noted for *C. laevigata* after 1 hour of treatment as only 40% of the total amount still was counted as calcein-labelled.

4 Discussion

Calcein-labelling as a method to distinguish experimental biomineralized calcite has been used in several studies since the introduction by Bernhard (2004). This, however, is the first time that osmotic pumps have been used to deliver calcein to analyse the calcification response of foraminifera to different oxygen conditions. The usage of osmotic pumps has an advantage compared to previous laboratory experiment utilizing calcein since the solution can be introduced directly in undisturbed sediment and thus provides an

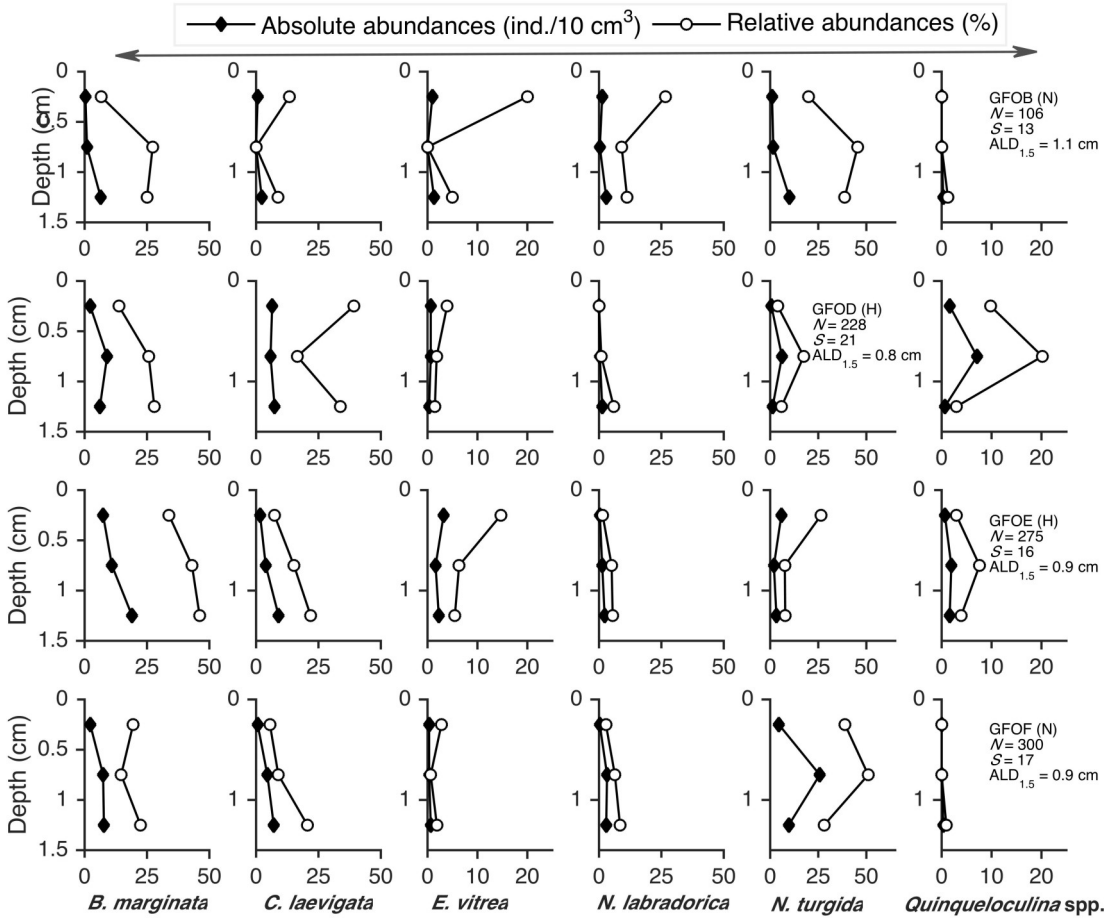

Fig. 10. Vertical distribution of the six foraminiferal taxa that had a relative abundance (>5%) in at least one of the cores plotted per depth intervals and core. (N) is a normoxic core; (H) is a hypoxic core. Note different scales on x-axes. The total number of specimens N in the upper 1.5 cm of each core is given, together with the species richness S and average living depth $ALD_{1.5}$.

opportunity to better mimic the natural complex environment. The calcein is continuously delivered at a low constant rate to the sediments. The delivery rate is a function of temperature and salinity or more precisely the difference in the osmotic pressure between the solution inside the osmotic pump and the surrounding seawater. We developed a MATLAB routine, 'alzet_flowrate', to calculate the delivery rate, which in our experiment is 0.88 µl h^{-1}. The function can be used in future experimental design to determine the duration of an experiment, select a pump model, and adjust the calcein concentration.

Table 4. Result of treatment of two groups with 5% sodium hypochlorite (NaClO) during 15 respectively 1 hour.

	Species	Before	After	%
15 min NaClO	*B. skagerrakensis*	4	4	100
	B. marginata	12	12	100
	C. laevigata	6	6	100
	N. turgida	5	5	100
	Quinqueloculina spp.	5	3	60
	All	32	30	94
1 h NaClO	*B. skagerrakensis*	2	2	100
	B. marginata	10	10	100
	C. laevigata	10	4	40
	N. turgida	4	4	100
	Quinqueloculina spp.	9	7	78
	All	35	27	77

The dispersion of the calcein in the sediment results from a combination of mechanical mixing and concentration gradients that drive the molecular diffusion, and the transport is influenced for example by temperature, porosity and bioturbation (Berner 1980). As part of the method development we wanted to understand how the calcein spreads in the sediment. For this reason we divided the sediment slices into four concentric circles, A–D, and counted the number of calcein-labelled foraminifera per circle (subsample). The majority of foraminifera, as the total sum, were found in the outer circles with the largest volume, while the highest absolute abundances (individuals/10 cm^3) were found in the innermost circle. Assuming an initially uniform distribution of foraminifera in the sediments and that the calcein spreads radially from the point source, then these results suggest that the lower abundances in the outermost areas represent a combination of a decrease both in calcein concentra-

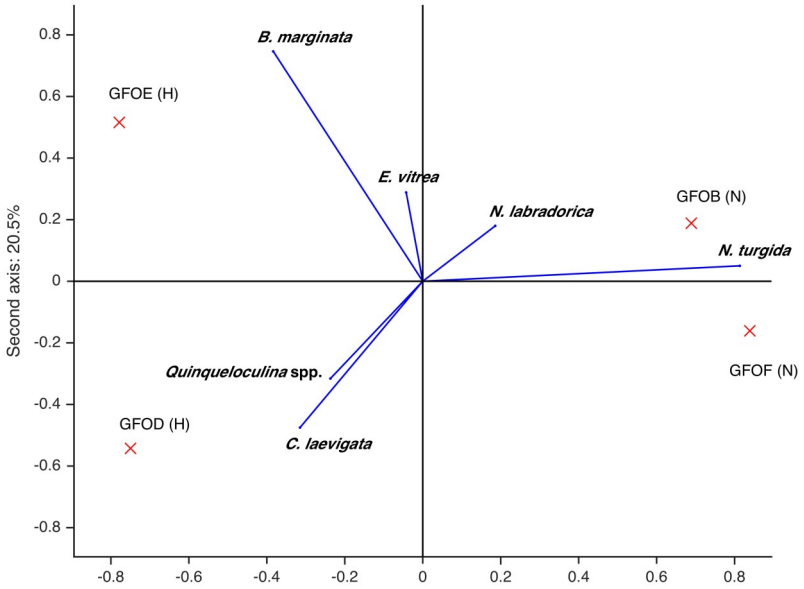

Fig. 11. Plot of the scores of the four cores (N = normoxic, H = hypoxic) and the species loadings along the first and second axes. The principal component analysis (PCA) was based on relative abundance of the six dominant taxa (>5%). The position of the four cores on the plane formed by the first two axes is shown. Both the normoxic cores, which are relatively rich in *N. turgida* and *N. labradorica,* plot on the positive side on the first axis. The hypoxic core GFOE is dominated by *B. marginata* and plots on the positive side on the second axis, while the hypoxic core GFOD, which is relatively rich in *C. laevigata*, plot on the negative side of both axes.

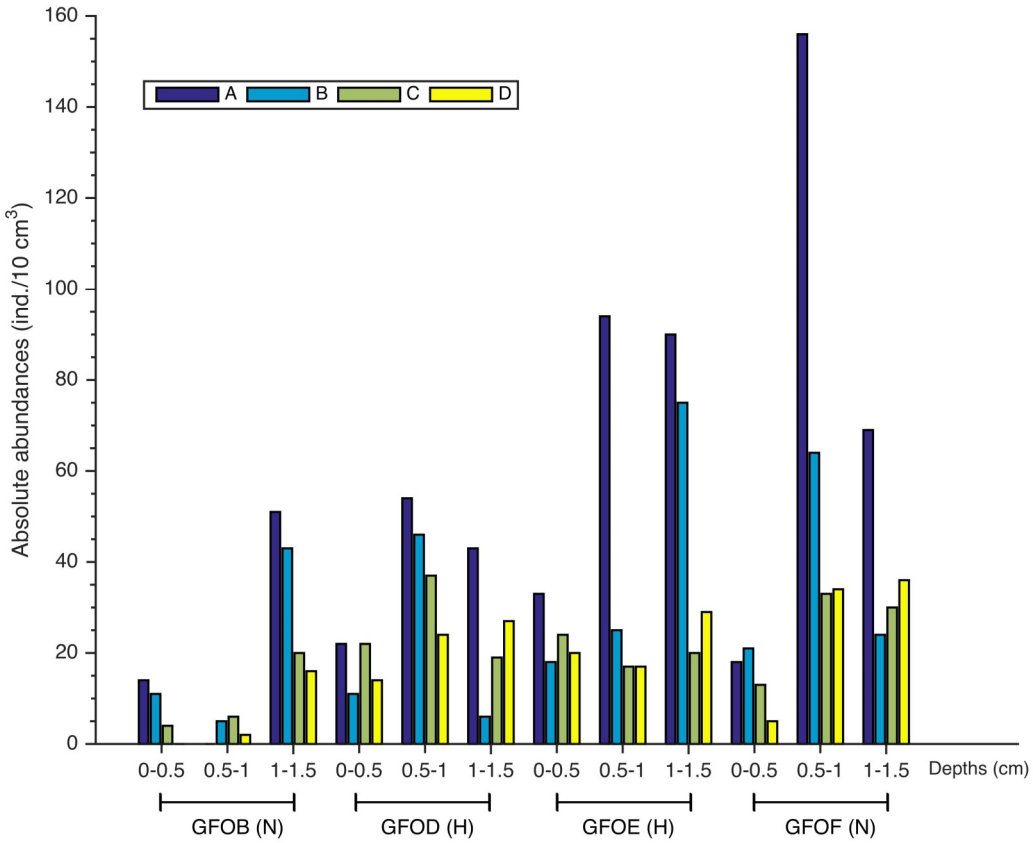

Fig. 12. Each bar is the number of calcein-labelled foraminifera found per subsample. Thus, the picture illustrates the distribution of calcein-labelled specimens both as a distance from the osmotic pump in the middle of the core and per depth and per core. (N) is a normoxic core. (H) is a hypoxic core. Absolute abundances of calcein-labelled specimens are significantly higher in subsamples A closest to the calcein point source (the osmotic pump), than in B, C and D (Wilcoxon signed-ranks test, $p < 0.005$).

tions in the direction away from the source and in survival rates as time elapses. That is when the calcein had spread to the outer areas fewer individuals with calcifying potential were alive.

In the sediment cores the foraminifera were exposed to multiple stressors: competition with macrofauna and predation (e.g. Buzas & Carle 1979; Gustafsson & Nordberg 2001; Murray 2006), eutrophication due to an excessive input of algae, and pulses of oxygen depleted water. In all cores polychaete worms co-existed with the foraminifera throughout the experiment. Even though our oxygen profiles indicated a maximum oxygen penetration depth <0.5 cm, polychaetes dwelled deeper in the cores down to about 5–10 cm. These observations are consistent with the results by Revsbech et al. (1980) where the oxygen penetration depth was 0.3–0.5 cm while the sediment was oxidized down to 3–10 cm by the activity of burrowing macrofauna. In the hypoxic cores, following the replacement of the water with nitrogen-bubbled water, the polychaetes were observed to migrate a couple of centimetres above the sediment-water interface attached to the plastic core liner. The polychaetes returned to their position within the sediments as the oxygen levels gradually rose during the week until next occasion of a hypoxic pulse, and as a consequence of this response to the varied oxygen conditions the bioturbation was enhanced.

In our experiment *Bulimina marginata, Cassidulina laevigata* and *Nonionoides turgida* account for between 60 and 70% of the total number of calcein-labelled foraminifera in all the cores. A relatively higher proportion of *N. turgida* 37–40% and the accessory species *Nonionellina labradorica* 7–13% distinguish the normoxic cores from the hypoxic cores in which the corresponding proportions are 11–12% and 2–4% respectively. Conversely, *C. laevigata* and the accessory species *Quinqueloculina* spp. constitute a higher proportion 16–27% and 5–13% in the hypoxic cores in comparison to 8–13% and <1% in the normoxic cores. In a study by Nardelli et al. (2014) the survival rates after two months of *B. marginata* and *C. laevigata* were 30–45% and 20–45%, respectively, without significant differences between normoxic and hypoxic conditions. For *B. marginata* this comparison was valid for anoxic conditions as well. Moreover, both species were able to calcify irrespective of oxygen conditions.

In previous studies of the foraminiferal fauna in the Gullmar Fjord (Nordberg et al. 2000; Filipsson & Nordberg 2004; Polovodova Asteman & Nordberg 2013) it has been shown that the recent foraminiferal fauna is dominated by *Stainforthia fusiformis* accompanied by high or increasing abundances of *Bolivinellina pseudopunctata*, *B. marginata*, *N. turgida*, *Textularia earlandi*, and *Quinqueloculina stalkeri*. All species, except *T. earlandi*, are known to occur in oxygen-depleted areas (e.g. Bernhard & Sen Gupta 1999; Pucci et al. 2009; Nardelli et al. 2014 and references therein). *Cassidulina laevigata*, *N. labradorica* and *Hyalinea balthica* are present in lower abundances, but they were common in the fjord until a nearly twenty-year-long phase with recurrent episodes of severe hypoxia was initiated in the late 1970s (Filipsson & Nordberg 2004; Polovodova et al. 2011).

The absence of *S. fusiformis* and *B. pseudopunctata* in our experimental set of dominant species could be explained by a difficulty to detect the fluorescence due the small sizes of the two species (Alve 1994; Alve & Goldstein 2010). However, since we only analysed the 100–250 µm-fraction this could potentially have introduced a bias. In a study by Moodley et al. (1997) it was found that genera with elongate morphology, e.g. *Stainforthia* spp. and *Bolivina* spp., were less abundant, ~5–30% and ~5–20%, respectively, in the 63 µm-fraction compared to the 38 µm-fraction and that the relative abundance of *Nonionella* spp. was overestimated under both normoxic and anoxic conditions. *Textularia earlandi* is not included at all, since this is an agglutinated species and, we only included calcein-labelled calcareous foraminiferal species.

The higher abundances of *C. laevigata* and *Quinqueloculina* spp. in the hypoxic cores compared to the normoxic are enigmatic, since at least the first species is considered to be less tolerant to low oxygen conditions (Filipsson & Nordberg 2004; Nardelli et al. 2014). The experiment performed, where a number of calcein-labelled calcareous foraminifera from a hypoxic core were exposed to 5% sodium hypochlorite (NaClO) during 15 minutes and 1 hour, respectively, could potentially provide us with an answer; all specimens counted as non-fluorescent after the treatment belonged to one of these two species. Interestingly, these non-fluorescent tests of *C. laevigata* and *Quinqueloculina* spp. had been altered in the same way; whereas the tests of the other species had been bleached and become more whitish, these tests instead had a rusty colouring, which might be explained by oxidation by the sodium hypochlorite of diagenetic pyrite previously formed. Sodium hypochlorite has been used to oxidize and remove pyrite from microfossils (Merrill 1980; Jeppsson & Anehus 1999). Decomposition of organic material by bacteria under anoxic conditions occurs through sulphate reduction, which can be written as (Berner et al. 1985)

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2CO_3^{-}.$$

Pyrite (FeS_2) then forms via the reaction of detrital iron minerals with hydrogen sulphide (H_2S). A search for previous studies about cytoplasmic fluorescence yielded few results. It is possible that the morphology of these tests, in contrast to tests of other species in our experiment, could provide an enclosed microenvironment in which aerobic degradation of organic matter faster would lead to a state of oxygen deficiency and thus sulphate reduction, which increases alkalinity, while the enclosure would protect pyrite from oxidation. Bacteria are known to induce carbonate precipitation by changing the system chemistry (e.g. Douglas & Beveridge 1998; Warren et al. 2001; Ercole et al. 2007), since alkalinity promotes carbonate precipitation. Furthermore, the average pH was 0.2–0.3 pH units higher in the hypoxic cores, which could be a consequence of the nitrogen bubbling (Gobler et al. 2014). Calcein could be incorporated in abiotic calcite as well as in biotic calcite (Dissard et al. 2009). An explanation of the different results with respect to fluorescence might be that the sodium hypochlorite-treatment dissolves the remaining residues of organic

material acting as a matrix for the bacterial induced calcein-labelled calcite and thus this disappears as the tests are rinsed following the treatment. Further Bernhard (2004) noted that tests of dead *Quinqueloculina* spp. were fluorescently labelled by calcein. In summary this indicates that the *Quinqueloculina* spp. should be omitted from the analysis and that the abundances of these two species are likely to have been overestimated. However, to test and quantify this hypothesis would require a separate analysis.

An unexpected result is that the lowest value of counted calcein-labelled foraminifera in the top 1.5 cm per core is found in a normoxic core. This result may partially be due to the lower temperatures measured for this core, on average 0.1–0.6°C lower, which presumably was a consequence of the placement of the core in the climate chamber, since the average temperatures in opposing corners of the chamber, as recorded by temperature loggers, differed by ~1°C. Worth noting is also that the $ALD_{1.5}$ for the dominant species *B. marginata* and *N. turgida* is >1.0 cm in this core (i.e. the abundance is concentrated to the lowest interval). In a study by Pucci et al. (2009) the ALD_5 of *N. turgida* varied between 1.6 and 2.3 in normoxic conditions. Nardelli et al. (2014) report no significant differences between calcification of *B. marginata* in normoxic (0–0.3 cm), hypoxic (0.3–1.3 cm) and anoxic layers (2.3–3.3 cm). Neither for *C. laevigata* no significant differences were observed between the ability to calcify under these conditions. However, while the survival rate of *B. marginata* were without significant differences, none of the *C. laevigata* had survived two months of incubation in the anoxic layer (Nardelli et al. 2014). This indicates that at least layers down to 4 cm should be included in the analysis to better understand the calcifying ability of the different species in our experiment, but this was not possibledue to time constraints.

On the other hand, the PCA analysis based on the relative abundances of the dominant species (>5%) visualizes a distinct negative correlation between the hypoxic cores and the relative abundances of calcifying *N. turgida* and *N. labradorica* compared to *B. marginata* and thereby suggesting that this could be an applicable indicator in the context of environmental monitoring in areas with recurrent hypoxic conditions. Thus, it is proposed that an analysis should be performed to examine whether time series analyses could be based exclusively on the relative abundance of these three species in the fraction 100–150 µm in the uppermost centimetre of the sediments.

Several studies have demonstrated that many foraminiferal species are able to survive, reproduce and calcify under hypoxic and even anoxic conditions (e.g. Alve & Bernhard 1995, Bernhard & Alve 1996, Duijnstee et al. 2003, Risgaard-Petersen et al. 2006, Pucci et al. 2009, Piña-Ochoa et al. 2010, Langlet et al. 2013, Nardelli et al. 2014). The responses to these conditions are species specific, for example lowering of the metabolism, storage of nitrate to support anaerobic metabolism, and vertical migration in the sediments. In order to use assemblages of foraminifera for environmental monitoring in the context of oxygen depletion, which is the fastest growing and most serious threat to marine ecosystems, it is necessary to continue the work to increase knowledge of these different strategies. We will hopefully contribute to these efforts with this presentation of a new method that has the potential of utilizing the advantages of both the field study and the laboratory experiment.

5 Conclusions

Our experimental approach shows that osmotic pumps can be used to introduce calcein directly to undisturbed marine sediments to fluorescently label calcite biomineralized by benthic foraminifera. In the present study we used osmotic pumps to deliver a 100 mg l^{-1} solution of calcein to sediment cores, which were collected from the Gullmar Fjord, wherein benthic foraminifera were cultured under normoxic and hypoxic *in situ* conditions during ~3.5 months. The osmotic pumps, which had a calculated flow rate of ~1 µl h^{-1}, were filled with 2 ml of the calcein solution and then they were placed, one per sediment core, 1 cm below the sediment-water interface.

Our analysis of calcein-labelled foraminifera in the 100–150 µm-fraction in the uppermost 1.5 cm of the sediments shows that the relative abundances of calcifying *Nonionoides turgida* and *Nonionellina labradorica* compared to *Bulimina marginata* are positively correlated with the oxygen levels. The results indicate that the calcein concentration decreases in a direction away from the osmotic pump, although calcein-labelled foraminifera were recovered at all distances within a radius of 4.5 cm from the point source.

By using osmotic pumps culturing and calcein-labelling can be performed *in situ* in undisturbed sediment cores, which means that the advantages of both the field study respective the laboratory experiment can be utilized – that is a better consistency with the natural conditions in combination with a rigorous control of environmental variables. This implies that the osmotic pumps have a great potential to become an important tool in future studies of benthic foraminiferal ecology.

6 Acknowledgements

First, I want to thank my supervisor Helena L. Filipsson for giving me the opportunity to work on this interesting and challenging project, for helpful and constructive reviews and, stimulating discussions related to this work, which has contributed to improving this manuscript.

I thank my co-supervisor Laurie Charrieau for having introduced me to the microsensor technology and epifluorescense microscopy. I also would like to give a special thank to Joan M. Bernhard, who proposed this new approach of using osmotic pumps, which has been the focus of the project.

I would also like to thank Per Carlsson at the Department of Biology, Lund University (LU), for valuable help with culturing of the microalgae.

I am very grateful to the Department of Physical Geography and Ecosystem Science, LU, for allowing us to use the climate chamber during the experiment and to Marcin Jackowicz-Korczynski for all the technical assistance.

I thank the captain and the crew of the R/V Oscar von Sydow for their assistance during the collection cruise.

None of this would have been possible without the granted study leave from IKEA IT. I thank my team-managers, Fredrik Åkerberg and Håkan Andersson, for their support during this time.

Finally, my most heartfelt thanks go to Kerstin Sällström and Catarina Sandström for supporting me during this inspiring journey.

7 References

Airoldi, L. & Beck, M.W., 2007: Loss, status and trends for coastal habitats of Europe. *Oceanography and Marine Biology: An Annual Review 45*, 345–405.

Alve, E., 1991: Foraminifera, climatic change and pollution: a study of Late Holocene sediments in Drammensfjord, SE Norway. *Holocene 1*, 243–261.

Alve, E., 1994: Opportunistic features of the foraminifer Stainforthia fusiformis (Williamson): evidence from Frierfjord, Norway. *Journal of Micropalaeontology 13*, 24.

Alve, E., 1995: Benthic foraminiferal responses to estuarine pollution: a review. *Journal of Foraminiferal Research 25*, 190–203.

Alve, E. & Bernhard, J.M., 1995: Vertical migratory response of benthic foraminifera to controlled oxygen concentrations in an experimental mesocosm. *Marine Ecology Progress Series 116*, 137–151.

Alve, E. & Goldstein, S.T., 2010: Dispersal, survival and delayed growth of benthic foraminiferal propagules. *Journal of Sea Research 63*, 36–51.

ALZET, 2015: *Pump Selection*, DURECT Corporation, Cupertino, CA, accessed from http://www.alzet.com/products/guide_to_use/pump_selection.html#duration on 15 September 2014.

Anderson, D.M., Glibert, P.M. & Burkholder, J.M., 2002: Harmful algal blooms and eutrophication nutrient sources, composition, and consequences. *Estuaries 25*, 704–726.

Arneborg, L., 2004: Turnover times for the water above sill level in Gullmar Fjord. *Continental Shelf Research 24*, 443–460.

Bentov, S., Brownlee, C. & Erez, J., 2009: The role of seawater endocytosis in the biomineralization process in calcareous foraminifera. *Proceedings of the National Academy of Sciences of the United States of America 106*, 21500–21504.

Berner, R.A., 1980: *Early Diagenesis: A Theoretical Approach*, Princeton University Press. 241 pp.

Berner, R.A., 1985: Sulphate reduction, organic matter decomposition and pyrite formation. *Philosophical Transactions of the Royal Society of London A 315*, 25–38.

Bernhard, J.M. & Alve, E., 1996: Survival, ATP pool, and ultrastructural characterization of benthic foraminifera from Drammensfjord (Norway): response to anoxia. *Marine Micropaleontology 28*, 5–17.

Bernhard, J. M. & Sen Gupta, B. K., 1999: Foraminifera of oxygen-depleted environments. In B. K. Sen Gupta (ed.): *Modern Foraminifera, 201–216*. Kluwer Academic Press, Dordrecht.

Bernhard, J.M., Sen Gupta, B.K. & Borne, P.F., 1997: Benthic foraminiferal proxy to estimate dysoxic bottom-water oxygen concentrations: Santa Barbara Basin, U.S. Pacific continental margin. *Journal of Foraminiferal Research 21*, 301¬–310.

Bernhard, J. M., J. K. Blanks, C. J. Hintz, & Chandler, G. T., 2004: Use of the fluorescent calcite marker calcein to label foraminiferal tests. *Journal of Foraminiferal Research 34*, 96–101.

Bernhard, J.M., Phalen, W.G., McIntyre-Wressnig, A., Mezzo, F., Wit, J.C., Jeglinski, M. & Filipsson, H.L., 2015: *Technical Note: Towards resolving in situ, centimeter-scale location and timing of biomineralization in calcareous meiobenthos—the Calcein-Osmotic pump method.* Manuscript submitted for publication.

Bijma, J., Pörtner, H.-O., Yesson, C. & Rogers, A.D., 2013: Climate change and the oceans – What does the future hold. *Marine Pollution Bulletin 74*, 495–505.

Buzas, M. A. & Carle, K., 1979: Predators of foraminifera in the Indian River, Florida. *Journal of Foraminiferal Research 9*, 336–40.

Caron, M. & Homewood, P., 1983: Evolution of early planktic foraminifers. *Marine Micropaleontology 7*, 453–462.

Corliss, B.H., 1985: Microhabitats of benthic foraminifera within deep-sea sediments. *Nature 314*, 435–438.

Culver, S., 1994: Early Cambrian foraminifera from the southwestern Taoudeni Basin, West Africa. *Journal of Foraminiferal Research 24*, 191–202.

Díaz, R. J. & Rosenberg, R., 2008: Spreading dead zones and consequences for marine ecosystems. *Science 321*, 926–929.

Dissard, D., Nehrke, G., Reichart, G. J., Nouet, J. & Bijma, J., 2009: Effect of the fluorescent indicator calcein on Mg and Sr incorporation into foraminiferal calcite. *Geochemistry, Geophysics, Geosystems 10*, 1–13.

Dissard, D., Nehrke, G., Reichart, G. J. & Bijma, J., 2010: Impact of seawater pCO2 on calcification and Mg/Ca and Sr/Ca ratios in benthic foraminifera calcite: results from culturing experiments with Ammonia tepida. *Biogeosciences 7*, 81–93.

Dolven, J.K., Alve, E., Rygg, B. & Magnusson, J., 2013: Defining past ecological status and in situ reference conditions using benthic foraminifera: a case study from the Oslofjord, Norway. *Ecological Indicators 29*, 219–233.

Douglas, S. & Beveridge, T.J., 1998: Mineral formation by bacteria in natural microbial communities. *Microbiology Ecology 26*, 79–88.

Duijnstee, A. P., Ernst, S. R. & van der Zwaan, G. J., 2003: Effect of anoxia on the vertical migration of benthic foraminifera. *Marine ecology progress series 246*, 85–94.

Ercole, C., Cacchio, P., Botta, A.L., Centi, V. & Lepidi, A., 2007: Bacterially induced minerali-

zation of calcium carbonate: The role of exopolysaccharides and capsular polysaccharides. *Microscopy and Microanalysis 13*, 42–50.

Filipsson, H.L. & Nordberg, K., 2004: Climate variations, an overlooked factor influencing the recent marine environment. An example from Gullmar Fjord, Sweden, illustrated by benthic foraminifera and hydrographic data. *Estuaries 27*, 867–881.

Filipsson, H. L., Nordberg, K., & Gustafsson, M., 2004: Seasonal study of δ18O and δ13C in living (stained) benthic foraminifera from two Swedish fjords. *Marine Micropaleontology 53*, 159–172.

Filipsson, H. L., Bernhard, J. M., Lincoln, S. A. & McCorkle, D. C., 2010: A culture-based calibration of benthic foraminiferal paleotemperature proxies: δ18O and Mg/Ca results. *Biogeosciences 7,* 1335–1347.

Fontanier, C., Mackensen, A., Jorissen, F.J., Anschutz, P., Licari, L. & Griveaud, C., 2006: Stable oxygen and carbon isotopes of live benthic foraminifera from the Bay of Biscay: Microhabitat impact and seasonal variability. *Marine Micropaleontology 58,* 159–183.

Friedrich, J., Janssen, F., Aleynik, D., Bange, H.W., Boltacheva, N., Cagatay, M.N., Dale, A.W., Etiope, G., Erdem, Z., Geraga, M., Gilli, A., Gomoiu, M.T., Hall, P.O.J., Hansson, D., He, Y., Holtappels, M., Kirf, M.K., Kononets, M., Konovalov, S., Lichtschlag, A., Livingstone, D.M., Marinaro, G., Mazlumyan, S., Naeher, S., North, R.P., Papatheodorou, G., Pfannkuche, O., Prien, R., Rehder, G., Schubert, C.J., Soltwede, T., Sommer, S., Stahl, H., Stanev, E.V., Teaca, A., Tengberg, A., Waldmann, C., Wehrli, B. & Wenzhöfer, F., 2014: Investigating hypoxia in aquatic environments: diverse approaches to addressing a complex phenomenon. *Biogeosciences 11*, 1215–1259.

Geslin, E., Heinz, P., Jorissen, F., & Hemleben, C., 2004: Migratory responses of deep-sea benthic foraminifera to variable oxygen conditions: laboratory investigations. *Marine Micropaleontology 53*, 227–243.

Gobler, C.J., DePasquale, E.L., Griffith, A.W. & Baumann, H., 2014: Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. *PLoS ONE 9*, e83648.

Goldstein, S.T., 1999: Foraminifera: A biological overview. In B.K. Sen Gupta (ed.): *Modern Foraminifera, 37–55*. Kluwer Academic Press, Dordrecht.

Gooday, A.J., Bernhard, J.M., Levin, L.A. & Suhr, S.B., 2000: Foraminifera in the Arabian Sea oxygen minimum zone and other oxygen-deficient settings: taxonomic composition, diversity, and relation to metazoan faunas. *Deep-Sea Research II 47*, 25–54.

Gustafsson, M. & Nordberg, K., 1999: Benthic foraminifera and their response to hydrography, periodic hypoxic conditions and primary production in the Koljö fjord on the Swedish west coast. *Journal of Sea Research 41*, 163–178.

Gustafsson, M. & Nordberg, K., 2001: Living (stained) benthic foraminiferal response to primary production and hydrography in the deepest part of the Gullmar Fjord, Swedish west coast, with comparisons to Höglund's 1927 material. *Journal of Foraminiferal Research 31*, 2–11.

Hart, M.B., Hylton, M.D., Oxford, M.J., Price, G.D., Hudson, W. & Smart, C.W., 2003: The search for the origin of the planktic Foraminifera. *Journal of the Geological Society 160*, 341–343.

Hayward, B.W., Cedhagen, T., Kaminski, M. & Gross, O., 2015: *World Foraminifera Database*, accessed from http://www.marinespecies.org/foraminifera on 21 March 2015.

Hoogakker, B.A.A., Elderfield, H., Schmiedl, G., McCave, I.N. & Rickaby, R.E.M., 2015: Glacial–interglacial changes in bottom-water oxygen content on the Portuguese margin. *Nature Geoscience 8*, 40–43.

Höglund, H., 1947: *Foraminifera in the Gullmar Fjord and the Skagerak*, Zoologiska Bidrag från Uppsala, Band 26, Uppsala University. 328 pp.

IOC, SCOR & IAPSO, 2010: *The international thermodynamic equation of seawater – 2010: Calculation and use of thermodynamic properties.* Intergovernmental Oceanographic Commission, Manuals and Guides No. 56, UNESCO (English). 196 pp.

Jackson, J.B, Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J. & Warner, R.R., 2001: Historical overfishing and the recent collapse of coastal ecosystems. *Science 293*, 629–637.

Jeppsson, L. & Anehus, R., 1999: A new technique to separate conodont elements from heavier minerals. *Alcheringa 23*, 57–62.

Jorissen, F.J., de Stigter, H.C. & Widmark, J.G.V., 1995: A conceptual model explaining benthic foraminiferal microhabitats. *Marine Micropaleontology 22*, 3–15.

Kaiho, K., 1994: Benthic foraminiferal dissolved-oxygen index and dissolved-oxygen levels in the modern ocean. *Geology 22*, 719–722.

Katz M., Cramer B., Franzese A., Hönisch B., Miller K., Rosenthal Y. & Wright J., 2010: Traditional and emerging geochemical proxies in foraminifera. *Journal of Foraminiferal Research 40*, 165–192.

Keeling, R., Körtzinger, A. & Gruber, N., 2010: Ocean deoxygenation in a warming world. *Annual Review of Marine Science 2*, 199–229.

Kurtarkar, S. R., Saraswat, R., Nigam, R., Banerjee, B., Mallick, R., Naik, D. K. & Singh, D. P., 2015: Assessing the effect of calcein incorporation on physiological processes of benthic foraminifera. *Marine Micropaleontology 114*, 36–45.

Langlet, D., Geslin, E., Baal, C., Metzger, E.,

Lejzerowicz, F., Riedel, B., Zuschin, M., Pawlowski, J., Stachowitsch, M. & Jorissen, F. J., 2013: Foraminiferal survival after long-term in situ experimentally induced anoxia. *Biogeosciences 10*, 7463–7480.

Mackensen, A., Schumacher, S., Radke, J. & Schmidt, D.N., 2000: Microhabitat preferences and stable carbon isotopes of endobenthic foraminifera: clue to quantitative reconstruction of oceanic new production. *Marine Micropaleontology 40*, 233–258.

MATLAB R2014b version 8.4.0.150421, 2014: *Computer software*. The MathWorks Inc., Natick, Massachusetts.

McCorkle, D.C., Keigwin, L.D., Corliss, B.H. & Emerson, S.R., 1990: The influence of microhabitats on the carbon isotopic composition of deep-sea benthic foraminifera. *Paleoceanography 5,* 161–185.

McCorkle, D.C., Corliss, B.H. & Farnham, C.A., 1997: Vertical distributions and stable isotopic compositions of live (stained) benthic foraminifera from the North Carolina and California continental margin. *Deep-Sea Research I 44*, 983–1024.

McDougall, T.J. & Barker, P.M., 2011: *Getting started with TEOS-10 and the Gibbs Seawater (GSW) Oceanographic Toolbox*. SCOR/IAPSO WG127. 28 pp.

Merrill, G.K., 1980: Removal of pyrite from microfossil samples by means of sodium hypochlorite. *Journal of Paleontology 54*, 633–634.

Millero, F.J., Feistel, R., Wright, D.G. & McDougall, T.J., 2008: The composition of Standard Seawater and the definition of the Reference-Composition Salinity Scale. *Deep-Sea Research I 55*, 50–72.

Moodley, L., van der Zwaan, G.J, Herman, P.M.J, Kempers, L. & van Breugel, P., 1997: Differential response of benthic meiofauna to anoxia with special reference to Foraminifera (Protista: Sarcodina). *Marine Ecology Progress Series 158*, 151–163.

Murray, J.W., 2006: *Ecology and Applications of Benthic Foraminifera.* Cambridge University Press, New York. 426 pp.

Nardelli, M.P., Barras, C., Metzger, E., Mouret, A., Filipsson, H.L., Jorissen, F. & Geslin, E., 2014: Experimental evidence for foraminiferal calcification under anoxia. *Biogeosciences 11,* 4029–4038.

Nigam, R., Saraswat, R. & Panchang, R., 2006: Application of foraminifers in ecotoxicology: Retrospect, perspect and prospect. *Environment International 32*, 273–283.

Nordberg, K., Gustafsson, M. & Krantz, A. L., 2000: Decreasing oxygen concentrations in the Gullmar Fjord, Sweden, as confirmed by benthic foraminifera, and the possible association with NAO. *Journal of Marine Systems 23*, 303–316.

Pawlowski, J., 2012: Foraminifera. In M. Schaechter (ed.): *Eukaryotic Microbes, 291–309*. Elsevier, San Diego, USA.

Piña-Ochoa, E., Høgslund, S., Geslin, E., Cedhagen, T., Revsbech, N. P., Nielsen, L. P., Schweizer, M., Jorissen, F., Rysgaard, S. & Risgaard-Petersen, N., 2010: Widespread occurrence of nitrate storage and denitrification among Foraminifera and Gromiida. *Proceedings of the National Academy of Sciences of the United States of America 107*, 1148–1153.

Polovodova Asteman, I. & Nordberg, K., 2013: Foraminiferal fauna from a deep basin in Gullmar Fjord: the influence of seasonal hypoxia and North Atlantic Oscillation. *Journal of Sea Research 79*, 40–49.

Polovodova, I., Nordberg, K. & Filipsson, H.L., 2011: The benthic foraminiferal record of the Medieval Warm Period and the recent warming in the Gullmar Fjord, Swedish west coast. *Marine Micropaleontology 81*, 95–106.

Pucci, F., Geslin, E., Barras, C., Morigi, C., Sabbatini, A., Negri, A. & Jorissen, F.J., 2009: Survival of benthic foraminifera under hypoxic conditions: Results of an experimental study using the CellTracker Green method. *Marine Pollution Bulletin 59,* 336–351.

Rabalais, N. N., Díaz, R. J., Levin, L. A., Turner, R. E., Gilbert, D. & Zhang, J., 2010: Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences 7*, 585–619.

Revsbech, N.P., Sørensen, J. & Blackburn, T.H., 1980: Distribution of oxygen in marine sediments measured with microelectrodes. *Limnology and Oceanography 25*, 403–411.

Risgaard-Petersen, N., Langezaal, A.M., Ingvardsen, S., Schmid, M.C., Jetten, M.S.M., Op den Camp, H.J.M., Derksen, J.W.M., Pina-Ochoa, E., Eriksson, S.P., Nielsen, L.P., Scott, D.B., Schafer, C.T. & Medioli, S., 2001: *Monitoring in coastal environments using foraminifera and thecamoebian indicators,* Cambridge University Press, Cambridge. 177 pp.

Sen Gupta, B.K., 1999: Systematics of modern Foraminifera. In B.K. Sen Gupta (ed.): *Modern Foraminifera, 7–36.* Kluwer Academic Press, Dordrecht.

Sen Gupta, B.K. & Platon, E., 2006: Tracking past sedimentary records of oxygen depletion in coastal waters: Use of the Ammonia-Elphidium Foraminiferal Index. *Journal of Coastal Research SI 39*, 1351-1355.

Sen Gupta, B.K, Turner, R.E. & Rabalais, N.N., 1996: Seasonal oxygen depletion in continental-shelf waters of Louisiana: Historical record of benthic foraminifers. *Geology 24*, 227–230.

Shahidul Islam, M., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Marine Pollution Bullentin 48*, 624–649.

Theeuwes, F. & Yum, S. I., 1976: Principles of the design and operation of generic osmotic pumps for the delivery of semisolid or liquid drug formulations. *Annals of Biomedical Engineering 4*, 343–353.

Vachard, D., Pille, L. & Gaillot, J., 2010: Palaeozoic Foraminifera: Systematics, palaeoecology and responses to global changes. *Revue de micro-*

paléontologie 53, 209–254.

Walton, N.R.G., 1989: Electrical conductivity and total dissolved solids – What is their precise relationship. *Desalination 72*, 275–292.

Warren, L.A., Maurice, P.A., Parmar, N. & Ferris, F.G., 2001: Microbially mediated calcium carbonate precipitation: Implications for interpreting calcite precipitation and for solid-phase capture of inorganic contaminants. *Geomicrobiology Journal 18*, 93–115.

Weiss, R. F., 1970: The solubility of nitrogen, oxygen and argon in water and seawater. *Deep-Sea Research 17*, 721–735.

Wilson-Finelli, A. E., Chandler, G. T. & Spero, H. J., 1998: Stable isotope behavior in paleoceanographically important benthic foraminifera: results from microcosm culture experiments. *Journal of Foraminiferal Research 28*, 312–320.

APPENDIX A. A MATLAB function alzet_flowrate

```
function [ days, flowrate ] = alzet_flowrate(SP, t, long, lat, pump)
%   alzet_flowrate              ALZET osmotic pump flow rate and duration
%==========================================================================
%   USAGE:
%      [ days, flowrate ] = alzet_flowrate(SP, t, long, lat, pump)
%
%   DESCRIPTION:
%   Calculates the ALZET osmotic pump flow rate from Practical Salinity, SP,
%   temperature t (C), location (long, lat) and ALZET pump (2ML1, 2ML2 or
%   2ML4). The function calls subroutines in GSW Oceanographic Toolbox that
%   need to be downloaded and added to the path of your MATLABinstallation.
%   Please see McDougall & Barker (2011) for additional information.
%
%   INPUT:
%   SP = Practical Salinity (PSS-78)                  (unitless)
%   p = sea pressure (not implemented, p = 0)          (dbar)
%   t = temperature                                  (°C)
%   long =  longitude in decimal degrees  [ 0 ... +360 ] or  [ -180 ... +180 ]
%   lat  =  latitude in decimal degrees north          [ -90 ... +90 ]
%
%   SA & t need to have the same dimensions [1 x N].
%   long, lat, pump need to have dimensions 1x1.
%
%   OUTPUT:
%   flowrate = flow rate                            (µl hrs^-1)
%   days = the duration in days                        (days)
%
%   AUTHOR:
%   Susanne Landgren (susanne.landgren@gmail.com)
%
%   VERSION NUMBER: 1.0 (March 2015)
%
%   REFERENCES:
%  ALZET Osmotic Pumps, 2015: Pump Selection, DURECT Corporation, Cupertino, CA, viewed 15 March 2015
%   <http://www.alzet.com/products/guide_to_use/pump_selection.html#duration>
%
%   McDougall, T.J. & P.M. Barker, 2011:
%   Getting started with TEOS-10 and the Gibbs Seawater (GSW) Oceanographic Toolbox,
%   SCOR/IAPSO WG127, ISBN 978-0-646-55621-5, 28 pp.%
%
%   The osmotic pressure, (pi), is calculated from equation
%              pi = phi c T R, osmotic pressure
%   phi = the osmotic coefficient, unitless
%   c = molarity of all solutes, mol l^-1, M
%   R = universal gas constant, 0.08206 l atm mol^-1 K^-1
%   T = absolute temperature, K (273.15 + ∞C)
%
%   The ALZET pumping rate is predicted by equation
%
%      Qt = Q0 *(0.141 * e^0.051t - (0.007*pi) + 0.12)
%
%   Qt = the pumping rate at temperature t ( µl/hrs )
%   Q0 = the specified pumping rate at 37∞C in µl/hrs
%   t = temperature in degrees Celsius, 4 < t < 42
%   pi = osmotic pressure of the solution outside the pump (atm), 0 < pi < 25
%   accuracy ± 10 %
%==========================================================================
%--------------------------------------------------------------------------
% Check variables and resize if necessary
%--------------------------------------------------------------------------
if ~(nargin==5)
   error('alzet_flowrate:  Requires five inputs')
end %if
```

```
[ms,ns] = size(SP):
[mt,nt] = size(t):

if (mt ~= ms | nt ~= ns)
   error('alzet_flowrate: SP and t must have same dimensions [1 x 1] or [1 x N]')
end

switch upper(pump)
   case '2ML1'
      Q0 = 10: % (∞C)
   case '2ML2'
      Q0 = 5: % (∞C):
   case '2ML4'
      Q0 = 2.5: % (∞C)
   otherwise
      error('alzet_flowrate: not a recognized ALZET osmotic pump, please use "2ML1", "2ML2", or "2ML4"'):
end
if any(long < 0 | long > 360)
   error('alzet_flowrate: longitude is out of range')
end
if any(abs(lat) > 90)
   error('alzet_flowrate: latitude is out of range')
end
if any(t < 4 | t > 42)
   error('alzet_flowrate: temperature is out of range, 4 < t < 42')
end
%--------------------------------------------------------------------------
% Start of the calculation
%--------------------------------------------------------------------------
% Constants
R = 0.08206: % universal gas constant (l atm mol^-1 K^-1)
p = 0: % sea pressure (dbar)
T = t + 273.15: % absolute temperature
% Variables
SA = 0: % absolute salinity (g/kg)
phi = 0: % osmotic coefficent
A = 0: % average atomic weight of sea salt (g mol ^-1)
sw_rho = 0: % density seawater (g l^-1)
c = 0: % molarity (mol l^-1)
pi = 0: % osmotic pressure of seawater (atm)

SA = gsw_SA_from_SP(SP, p, long, lat):
phi = gsw_osmotic_coefficient_t_exact(SA,t,0):
A = gsw_atomic_weight():
sw_rho = gsw_rho(SA, t, p):
solute_mol = SA./A: %  amount of solute (mol)
m_sw(1:length(SP)) = 1000: % (g) (assume 1000 g seawater)
volume_sw = m_sw./sw_rho: % volume (l)
c = solute_mol./volume_sw:
pi = phi.*c.*T.*R:

if any(pi < 0 | pi > 25)
   error(strcat('alzet_flow_rate: calculated osmotic pressure (pi) = ', num2str(pi), ' is out of range, 0 < pi < 25, t = ',
num2str(t), ' SP = ', num2str(SP))):
end
flowrate = Q0 * (0.141 * exp(t.*0.051) - pi.*0.007 + 0.12): % (µl/hrs)

%   Divide 95% of the average reservoir fill volume (µl) by
%   the average pumping rate (µl/hr) to allow for a 5 % residual
%   which cannot be displaced from the pump
PUMP_VOL(1:length(SP)) = 2E+3 * 0.95: % (µl)
hrs = PUMP_VOL./flowrate: % (hrs)
days(1:length(SP)) = hrs./24: % (days)
end % alzet_flowrate
```

APPENDIX B. Total abundances of calcein-labelled foraminifera

Sediment core	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**	**GFOB**
Section	A	B	C	D	A	B	C	D	A	B	C	D
Core depth (cm)	0-0.5	0-0.5	0-0.5	0-0.5	0.5-1	0.5-1	0.5-1	0.5-1	1.0-1.5	1.0-1.5	1.0-1.5	1.0-1.5
Sample weight (g)	4.52	5.52	3.82	10.02	4.92	10.02	11.02	11.92	5.22	8.32	10.12	14.82
Bolivina skagerrakensis		1										
Bolivinellina pseudopunctata										1		
Bulimina marginata	1					1	2		5	8	5	2
Cassidulina laevigata		2							1	3	2	1
Cribroelphidium albiumbilicatum C.magellanicum												
Elphidium excavatum clavatum		1					1					
Cribroelphidium incertum												
Eilohedra vitrea	1	1	1						2	1	1	
Cribroelphidium spp. Cushman &										1		2
Globobulimina auriculata												
Globobulimina turgida												
Hyalinea balthica												
Lobatula lobatula								1				
Miliolinella subrotunda												
Nonionella iridea									1			
Nonionellina labradorica	1	2	1				1			2	4	3
Nonionoides turgida	1		2			2	2	1	4	10	6	11
Nonioninae spp.									1	1		1
Pyrgo williamsoni												
Quinqueloculina spp.											1	
Stainforthia fusiformis												
Varia.												

APPENDIX B. Total abundances of calcein-labelled foraminifera

Sediment core	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**	**GFOD**
Section	A	B	C	D	A	B	C	D	A	B	C	D
Core depth (cm)	0-0.5	0-0.5	0-0.5	0-0.5	0.5-1	0.5-1	0.5-1	0.5-1	1.0-1.5	1.0-1.5	1.0-1.5	1.0-1.5
Sample weight (g)	3.42	5.12	5.52	5.42	5.32	7.52	12.02	16.62	4.32	5.72	8.22	13.42
Bolivina skagerrakensis			1	1	1	3	1	1		1	1	1
Bolivinellina pseudopunctata			1									1
Bulimina marginata			4	3	5	7	9	7	4		8	7
Cassidulina laevigata	3	3	10	4	1	5	6	6	2	1	4	16
Cribroelphidium albiumbilicatum C.magellanicum												
Elphidium excavatum clavatum							2		1			1
Cribroelphidium incertum		1										
Eilohedra vitrea				2				2	1			
Cribroelphidium spp. Cushman &			1				1		1			
Globobulimina auriculata	1											
Globobulimina turgida	1		2	1								
Hyalinea balthica												2
Lobatula lobatula		1				1						
Miliolinella subrotunda											1	
Nonionella iridea				2	2	1		1	2			
Nonionellina labradorica								1			1	3
Nonionoides turgida	1			1	5	5	6	3		1	1	2
Nonioninae spp.										1		
Pyrgo williamsoni				1								
Quinqueloculina spp.		1	2	2	1	5	7	9	1			1
Stainforthia fusiformis							1				1	
Varia.		1				2	2				1	

APPENDIX B. Total abundances of calcein-labelled foraminifera

Sediment core	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**	**GFOE**
Section	A	B	C	D	A	B	C	D	A	B	C	D
Core depth (cm)	0-0.5	0-0.5	0-0.5	0-0.5	0.5-1	0.5-1	0.5-1	0.5-1	1.0-1.5	1.0-1.5	1.0-1.5	1.0-1.5
Sample weight (g)	3.22	8.52	13.32	9.22	4.52	7.62	13.52	16.22	8.62	18.22	12.72	18.42
Bolivina skagerrakensis	1				2			1		1		1
Bolivinellina pseudopunctata				3	1		1					
Bulimina marginata	5	6	7	5	11	11	5	7	13	23	9	14
Cassidulina laevigata			4	1	1	1	4	6	3	14	4	7
Cribroelphidium albiumbilicatum C.magellanicum												
Elphidium excavatum clavatum					2				1	1		2
Cribroelphidium incertum												
Eilohedra vitrea		3	6	1		1	2	2	3	2	2	
Cribroelphidium spp. Cushman &										1		1
Globobulimina auriculata												
Globobulimina turgida												
Hyalinea balthica						1						
Lobatula lobatula												
Miliolinella subrotunda											1	
Nonionella iridea					2							
Nonionellina labradorica		1			2			2	1	1	1	4
Nonionoides turgida	2		4	12	2		1	3	4	3		3
Nonioninae spp.												
Pyrgo williamsoni			1		2							2
Quinqueloculina spp.			1	1	1	2	3			1	1	3
Stainforthia fusiformis	1											
Varia.		1		2							1	

APPENDIX B. Total abundances of calcein-labelled foraminifera

Sediment core	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**	**GFOF**
Section	A	B	C	D	A	B	C	D	A	B	C	D
Core depth (cm)	0-0.5	0-0.5	0-0.5	0-0.5	0.5-1	0.5-1	0.5-1	0.5-1	1.0-1.5	1.0-1.5	1.0-1.5	1.0-1.5
Sample weight (g)	2.92	4.22	4.52	6.32	7.22	8.32	15.02	17.52	6.82	11.02	16.12	18.32
Bolivina skagerrakensis							1	2		2		1
Bolivinellina pseudopunctata	2	1		1	5	1	1	2				
Bulimina marginata		2	4	1	7	6	3	7	2	3	7	12
Cassidulina laevigata			2		3	2	6	3	6	2	6	8
Cribroelphidium albiumbilicatum C.magellanicum	1											
Elphidium excavatum clavatum		1	2		1	1					3	3
Cribroelphidium incertum												
Eilohedra vitrea		1						1		2		
Cribroelphidium spp. Cushman &			1		1							2
Globobulimina auriculata												
Globobulimina turgida												
Hyalinea balthica								1		1		1
Lobatula lobatula												
Miliolinella subrotunda					2							
Nonionella iridea	1				2			4				
Nonionellina labradorica				1	5	1	4		1	1	3	4
Nonionoides turgida	1	8	3	2	17	24	16	23	8	3	7	12
Nonioninae spp.						3					2	1
Pyrgo williamsoni												
Quinqueloculina spp.										1		
Stainforthia fusiformis				1								
Varia.						2			2			1

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