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Titel der Masterarbeit / Title of the Master's Thesis

„The influence of hydrostatic pressure on prokaryotic activity in the deep sea"

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Abstract

Hydrostatic pressure as a physical parameter is considered to have a significant effect on prokaryotes, although in most studies on deep-sea microbes their metabolic rates have been largely determined under atmospheric pressure conditions. To investigate the role of hydrostatic pressure and its effects on biomass production and activity of different prokaryotic groups, an *in situ* microbial incubator (ISMI) was deployed in the Northern Atlantic during a cruise on board the research vessel *Meteor*. The ISMI is a device that collects seawater from defined water depths and allows incubating microbes under *in situ* conditions. In this study we compared the influence of hydrostatic pressure on metabolic activity and rates on samples collected at 450, 3000, and 4000 m depths. The fraction of active cells and their metabolic rate measured under *in situ* pressure conditions were compared to those obtained under atmospheric pressure conditions. Catalyzed reporter deposition fluorescence *in situ* hybridization combined with microautoradiography was applied for determining abundances of specific prokaryotic groups in combination with visualizing their rate of activity. Our results showed a generally lower activity under *in situ* pressure conditions than under atmospheric pressure. Our results indicate that a major fraction of the deep-sea prokaryotic community is piezosensitive, being more active under atmospheric pressure conditions. However, we also found that specific prokaryotic groups, like *Alteromonas*, have the tendency to be piezophilic, or at least piezotolerant. Overall, our data indicate that measuring deep-sea prokaryotic activity needs to be done under *in situ* hydrostatic pressure conditions to obtain realistic estimates on the deep-sea prokaryotic metabolism.

Introduction

The largest fraction of the ocean's volume is below 200 m depth, called the dark ocean or deep sea, and is considered as the least explored habitat of the biosphere on Earth. It is characterized by complete darkness, high hydrostatic pressure and low temperatures of 0–4°C, as well as high inorganic nutrients and low organic carbon concentrations (Lauro and Bartlett, 2008). The oceans average depth is about 3800 m with a maximal depth of 11,000 m at the Mariana Trench in the northwest Pacific (Bartlett, 2002). The dark ocean starts where the euphotic or epipelagic zone ends and is subdivided into the mesopelagic zone (usually between 200–1000 m depth), the bathypelagic (1000–4000 m depth), and the least explored abyssal zones (> 4000 m depth) (Arístegui et al., 2009). Lauro and Bartlett (2008) describe the deep sea as a physically uniform environment, only occasionally interrupted by outbursts of activity at the locations of hydrothermal vents (Prieur et al. 1995), whale falls (Smith and Baco 2003), and cold seeps (Elvert et al. 2000). The dark ocean contains 75% of the ocean's microbial cells and 50% of the prokaryotic biomass and production (Aristegui et al., 2009). Recent studies suggest that besides heterotrophic activity, chemoautotrophic metabolism is important in the dark ocean (Herndl et al., 2005; Arístegui et al., 2009; Reinthaler et al., 2010).

Prokaryotic activity and community composition is highly depth-stratified in the oceanic water column, reflecting the increasing recalcitrance of dissolved organic matter and decreasing temperature with depth (Delong et al., 2006). Biogeochemical processes and environmental parameters in relation to the phylogenetic composition of prokaryotic communities represent a highly studied area of research in microbial oceanography of the deep sea. Almost all studies on microbes in the deep sea measured their metabolic rates under atmospheric pressure conditions (Herndl et al., 2005; Arístegui et al., 2009; Lekunberri et al., 2013). Thus, the effect of hydrostatic pressure has been largely neglected, mainly due to the technical difficulty to measure metabolic rates at *in situ* pressure conditions.

Hydrostatic pressure as a physical parameter is considered to have a significant effect on prokaryotes. For instance, an essential cellular process inhibited by hydrostatic pressure is protein synthesis (Lauro and Bartlett, 2008). It also has been shown that pressure influences the physiology of microorganisms living in the bathypelagic realm (Tamburini et al., 2013). Pressure-adapted microorganisms are coined 'piezotolerant' (similar growth rate at atmospheric pressure and high pressure), 'piezophilic' (higher growth rate at high pressure

than under atmospheric pressure), or 'obligate piezophilic' (growth only at high pressure). Organisms growing best at atmospheric pressure are coined 'piezosensitive' (Yayanos (1995), reviewed by Fang et al. (2010) and Kato (2011)).

The role of elevated hydrostatic pressure in controlling deep ocean microbial activity is still not resolved and results from various studies are contradictory. First measurements on microbial activity in the deep sea without changing the pressure conditions were performed by Jannasch and Wirsen (1973). They found that elevated pressure decreases rates of growth and metabolic activity of deep-sea and surface waters microbial populations. The opposite conclusion was reached by Tamburini et al. (2013), who stated that deep-sea microorganisms are adapted to high pressure conditions and consequently, deep sea microbes incubated under atmospheric pressure exhibit lower activity than under *in situ* hydrostatic pressure. These two contrary conclusions indicate that investigating the role of hydrostatic pressure on deep-sea microbes remains to be resolved using state-of-the-art technology.

Prokaryotic populations are depth-stratified in terms of relative abundance, with *Alphaproteobacteria, Gammaproteobacteria, Actinobacteria* and *Thaumarchaeota* being abundant in the deep ocean (Arístegui et al., 2009; Salazar et al., 2016). *Thaumarchaeota*, as a major group of marine *Archaea*, are known to increase in relative abundances towards deeper oceanic layers (Schattenhöfer et al., 2009). The same pattern holds true for the *Chloroflexi*-type SAR202 cluster, which is more abundant in meso- and bathypelagic than in epipelagic waters (Varela et al., 2008; Schattenhöfer et al., 2009). The role of the SAR202 clade in the deep sea is not fully understood yet, but a study by Landry et al. (2017) concluded that they might play an important role in the degradation of recalcitrant organic matter. Other *Bacteria* abundant in deep waters are the genera *Colwellia, Alteromonas* and *Pseudoalteromonas* belonging to the *Gammaproteobacteria*. Although not much is known yet about their status in activity under the hydrostatic pressure conditions inherent to the deep ocean, a deep-sea ecotype of *Alteromonas macleodii* has been intensely investigated (López-López et al., 2005; Zaballos et al., 2006). Another abundant group in the dark ocean is SAR406 or Marine Group A, now referred to as *Marinimicrobia*, which are involved in the cycling of sulfur and nitrogen (Wright et al., 2014; Hawley et al., 2017).

To determine marine prokaryotic activity at *in situ* pressure conditions, an *in situ* microbial incubator (ISMI) was deployed in the North Atlantic Ocean. The ISMI is able to collect and incubate prokaryotes *in situ* after adding a labeled substrate. Prior to deployment

it can be programmed to fix a certain volume of the incubated samples at depth and specific time intervals. This allows obtaining insight into the prokaryotic activity at depth and prevents potential biases that might occur when bringing samples up to the surface, hence to atmospheric pressure conditions.

The main focus of this study is to understand how hydrostatic pressure influences the metabolic activity and biomass production of heterotrophic or mixotrophic prokaryotes in the meso- and bathypelagic realm of the ocean. The ISMI is a device targeting to solve this enigma. Hence, the goals of this study were 1) to determine whether there are differences in activity between *in situ* and atmospheric pressure conditions in heterotrophic microbial activity; 2) to investigate which prokaryotic groups are affected by changes in the hydrostatic pressure; 3) to classify selected prokaryotic groups in their response to changes in hydrostatic pressure and thus, determine whether they are piezosensitive, piezotolerant, piezophilic, or obligate piezophiles.

Materials and Methods

Description of the components of the ISMI and the incubation procedure

The ISMI is a device that collects seawater from defined depths in the water column and therefore, allows incubating microbes under *in situ* conditions. As depicted in Fig. 1, the ISMI can be roughly partitioned into two parts. The upper part consists of a tray, holding the incubation bottles and a peristaltic pump. The bottom part carries the essential electronic devices, such as the battery and the controller system, as well as a second peristaltic pump. The frame also holds the clamp unit and the bottles containing the fixative to stop the prokaryotes' metabolic activity. The clamp unit distributes the water into the different bottles and also regulates the rinsing steps in between filling the bottles. These cylindrical polycarbonate bottles can be removed for cleaning and later for collecting samples. There are two types of the cylindrical bottles, the incubation bottles for life samples and bottles for killed samples as abiotic controls.

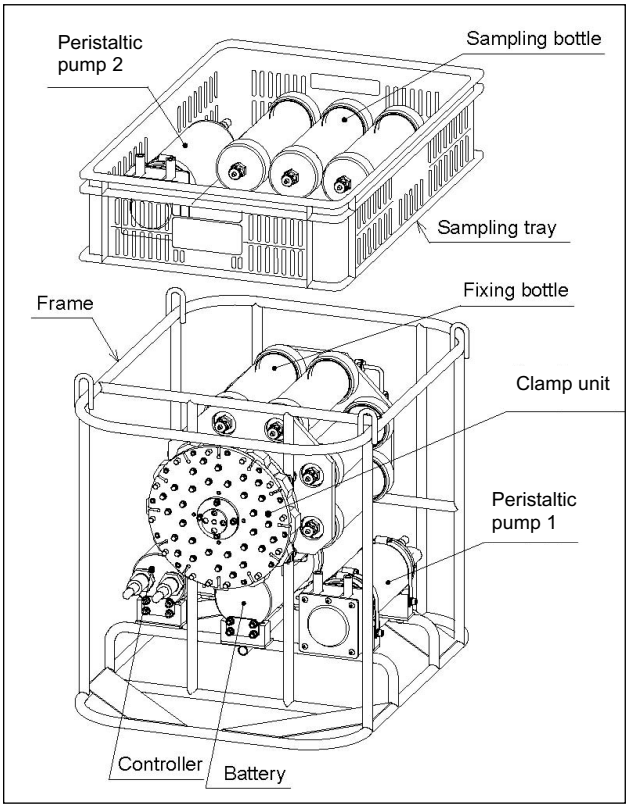


Fig. 1: *The ISMI setup. The main parts are the peristaltic pumps 1 & 2, the incubation and the fixation bottles, the clamp unit, the controller and the battery system. The tray as well as all cylindrical bottles can be removed for preparation. The pumps can be removed for maintenance and cleaning.*

Prior to the deployment of the device both, the incubation and fixation bottles were washed with 0.5 N HCl and rinsed with Milli-Q water, subsequently with pre-filtered deep seawater collected in advance from the same sampling depth with a Niskin bottle mounted on a conductivity-temperature-depth (CTD) rosette. The incubation bottles held ^{3}H-leucine (3,4,5-^{3}H labeled L-Leucine, specific activity of 100-150 Ci/mmol, final concentration 5 nM), and the fixation bottles contained formaldehyde (final concentration 2%). The ISMI was programmed prior to each deployment to sample a defined volume of water at a defined time of the day. At depth, sampled water was first transferred into the incubation bottles in triplicates where the water mixed with the substrate. Then, sub-samples were transferred into the fixation bottles in triplicate and fixed with formaldehyde *in situ* at the beginning and the end of the incubation period (Fig. 2).

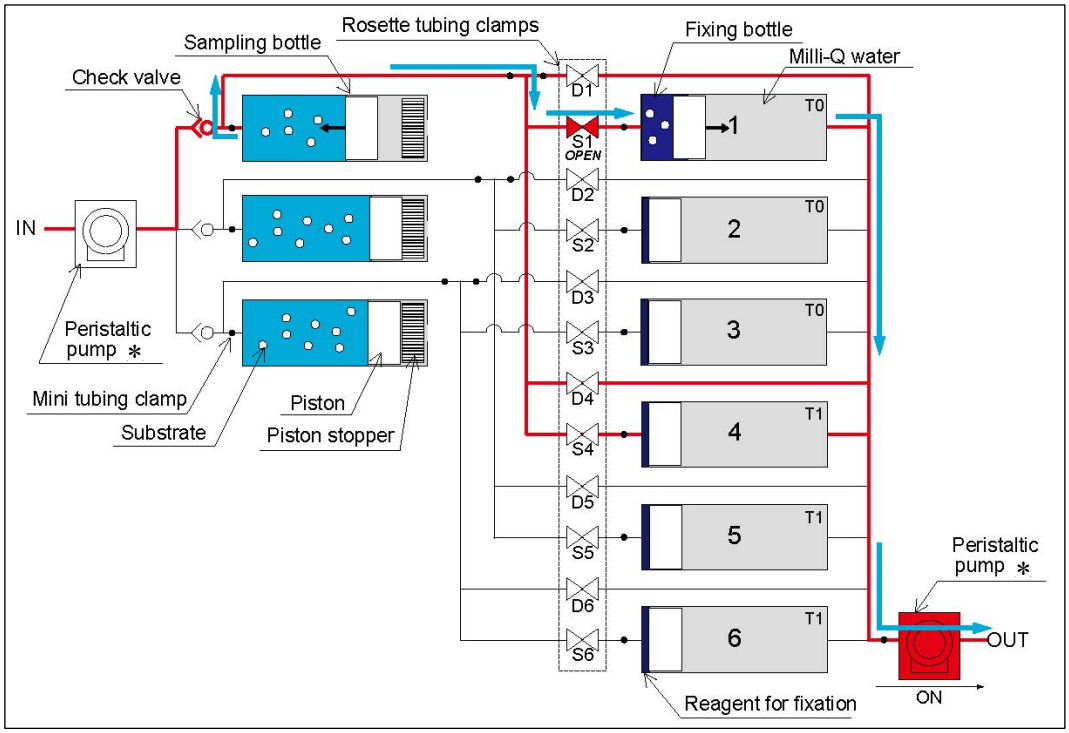


Fig. 2: *Sampled seawater is transferred into the incubation bottles. It mixes with the substrate (^{3}H-Leucine) then, sub-samples are transferred into the fixation bottles at the beginning (t0) and the end (t1) of the deployment. The check valves block the sample in one-way, all bottles are filled with Milli-Q water to withstand the hydrostatic pressure. The lines indicate the connection of the tubing between the devices and the bottles.*

Sampling

To characterize the marine prokaryotic activity at *in situ* pressure condition, the ISMI was deployed at three stations (A1, A3, and A6) in the North Atlantic during the cruise M139 on the research vessel *Meteor*, in July and August 2017 (Fig. 3 and Table 1). This study covers the examination of the samples obtained at Station A6, taken from the mesopelagic (450 m) and the bathypelagic zone (3000 m and 4000 m) (Table 1). After recovering the ISMI, the samples were filtered onto 0.2 µm pore size polycarbonate filters for bulk leucine incorporation measurements on board and stored at -80°C for later analyses. In parallel to the incubation at depth, water samples were taken with a Niskin bottle from the same depth and incubated in ISMI bottles under atmospheric pressure conditions for comparison. The atmospheric pressure samples were kept in the dark for the same incubation time as their *in situ* counterparts, maintaining the *in situ* temperature conditions.

Table 1: *Station plan of* Meteor *cruise M139*

Station	Cast No.	Latitude	Longitude	Depth (m)
A1	939	15° 54.08 N	68° 54.36 W	3750
A1	941	15° 53.25 N	68° 55.67 W	2000
A3	955	23° 33.23 N	48° 05.04 W	4000
A3	957	23° 33.23 N	48° 05.03 W	2000
A6	969	10° 20.40 N	36° 57.75 W	4000
A6	973	10° 20.40 N	36° 57.75 W	3000
A6	975	10° 20.40 N	36° 57.75 W	450

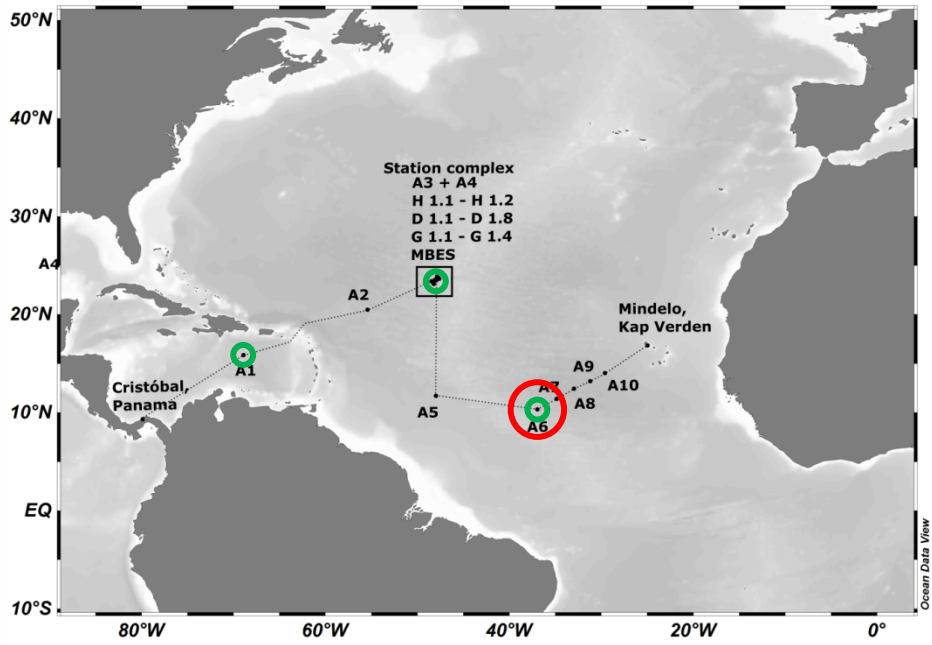


Fig. 3: *Map of sampling stations during the research cruise M139 on R/V* Meteor *in the North Atlantic. Sampling with the ISMI took place at Station A1, A3 and A6 (green circles), the samples examined in this study are from Station A6 (red circle).*

Catalyzed reporter deposition fluorescence *in situ* hybridization combined with microautoradiography (MICRO-CARD-FISH)

After fixing both, the *in situ* samples (*in situ*) and samples held under atmospheric pressure conditions (ATM) with formaldehyde (final concentration 2%), they were filtered onto 0.2 µm white polycarbonate filters (GTTP; Millipore) supported by a 0.45 µm cellulose

nitrate filter (HAWP; Millipore). MICRO-CARD-FISH was carried out according to the protocol of Sintes and Herndl (2006) with some alterations. In brief, the filters were cut in sections for hybridization with horseradish peroxidase-labeled oligonucleotides probes listed in Table 2. Hybridization was performed at 35°C for 15 h, if not indicated otherwise, followed by a washing step at 37°C. Signal amplification was carried out by adding H_2O_2 and tyramide-Alexa488, followed by incubation at 46°C for 15 min.

Microautoradiography was performed with the hybridized filters, placing them on slides coated with a photographic emulsion (Ilford nuclear emulsion K5 mixed with Sigma water 1:1). The slides were placed in light-tight boxes with silica gel as a drying agent and exposed at 4°C for 14 d. Subsequently, the filters were developed with Ilford Phenisol Developer (4 min) and fixed with Ilford Hypam Fixer (6 min). Cells were then counterstained with a DAPI mix (5.5 parts Citifluor, 1 part Vectashield and 0.5 parts of phosphate-buffered saline with DAPI at a final concentration of 2 µg ml^{-1}).

Table 2: *Overview of the probes and hybridization conditions applied (hybridization temperature 35°C; washing temperature 37°C)*

Probe	Target organisms	Sequence (5'→3')	FA[a] (%)	Reference
NON338	Negative control *Bacteria*	ACTCCTACGGGAGGCAGC	55	Wallner et al. (1993)
EUB338	*Bacteria*	GCTGCCTCCCGTAGGAGT	55	Amann et al. (1990)
EUB338-II	Supplement to EUB3338	GCAGCCACCCGTAGGTGT	55	Daims et al. (1999)
EUB338-III	Supplement to EUB3338	GCTGCCACCCGTAGGTGT	55	Daims et al. (1999)
SAR202-104	SAR202 clade	GTTACTCAGCCGTCTGCC	55	Morris et al. (2004)
SAR202-312	SAR202 clade	TGTCTCAGTCCCCCTCTG	55	Morris et al. (2004)
SAR406-97*	SAR406 clade	CACCCGTTCGCCAGTTTA	40	Fuchs et al. (2005)
Alt1413	*Alteromonas/Colwellia*	TTTGCATCCCACTCCCAT	55	Eilers et al. (2000)
Cren554	*Thaumarchaeota* Marine Group I	TTAGGCCCAATAATCMTCCT	20	Massana et al. (1997)
Cren537	*Thaumarchaeota* Marine Group I	TGACCACTTGAGGTGCTG	20	Teira et al. (2004)
Eury806	*Euryarchaeota* Marine Group II	CACAGCGTTTACACCTAG	20	Teira et al. (2004)

a Formamide (FA) concentration in CARD-FISH hybridization buffer
* Hybridization temperature 46°C; washing temperature 48°C

Image analysis of cells incorporating leucine

The slides were examined with an epifluorescence microscope (Axio Imager M2, Carl Zeiss) with filter sets for DAPI and Alexa488. The silver grain area surrounding the cells was determined using the transmission mode of the microscope. The size of the halo area surrounding a cell can be related to the leucine incorporation and thus, indicates the rate of

activity. At least 10 pictures with a total of more than 1000 DAPI-stained cells were counted per filter section. The number of prokaryotes and the number and area of silver grain halos of a given sample were determined with the software Zeiss KS300 for quantifying DAPI-stained cells, probe-positive cells and calculating the area of the silver grain surrounding the active cells.

Measurement of bulk leucine incorporation

Bulk leucine incorporation of the heterotrophic prokaryotic community was measured using ^{3}H-leucine at a final concentration of 5 nM in triplicate in 20-40 mL vials and triplicate fomaldehyde fixed blanks. The disintegrations per minute (DPM) were measured on board with a liquid scintillation counter. From the mean DPM values of the life sample, the mean of the blanks was subtracted and converted to leucine incorporation rates given in pmol leucine L^{-1} h^{-1}.

Results and Discussion

Bias test

To check for potential biases when measuring prokaryotic activity with the ISMI such as potential contamination of the water when deployed at depth including pumping, instrument tubing, and the remaining ISMI setup, seawater from 2000 m depth was taken to determine leucine incorporation. The bias test was performed under atmospheric pressure, comparing the full setup of ISMI on board and only the ISMI bottles. The bias test was conducted at 7°C for 12 h. During incubation both, the whole ISMI setup and the bottles were kept in the dark. Using a Welch two-sample t-test, we found no significant difference between the full set up of the ISMI and the ISMI bottles ($p = 0.79$). The full ISMI setup yielded 0.085 ± 0.005 pmol leucine incorporated L^{-1} h^{-1}, seawater incubated solely in the bottles yielded 0.082 ± 0.019 pmol leucine incorporated L^{-1} h^{-1} (n=3). Thus, the result obtained from the bias test gave confidence in the ISMI working reliably without contamination.

Bulk leucine incorporation into the heterotrophic prokaryotic community

Bulk leucine incorporation was significantly higher at atmospheric than at *in situ* pressure conditions (Fig. 4). When incubating water from 450 m depth at atmospheric

pressure, leucine incorporation rates were 0.336 ± 0.022 pmol L^{-1} h^{-1}, whereas *in situ* incubation revealed 0.205 ± 0.018 pmol L^{-1} h^{-1}. Thus, leucine incorporation rate at 450 m depth amounted to only 61% of that under atmospheric conditions. At 3000 m depth, leucine incorporation rates under atmospheric pressure conditions were 0.013 ± 0.001 pmol L^{-1} h^{-1} while *in situ* leucine incorporation rates were 0.004 pmol L^{-1} h^{-1}. Hence, leucine incorporation rate at 3000 m depth was only about 29% of that under atmospheric conditions. At 4000 m depth, leucine incorporation rates amounted to about 13% of that measured under atmospheric conditions with leucine incorporation rates of 0.033 ± 0.002 pmol L^{-1} h^{-1} and *in situ* leucine incorporation rates of 0.004 pmol L^{-1} h^{-1}. Leucine incorporation rates from mesopelagic water were one order of magnitude higher than those from bathypelagic waters. Our results indicated an increase in the difference in leucine incorporation between atmospheric and *in situ* pressure conditions with increasing depth. Similar results were also reported by Jannasch and Wirsen (1982).

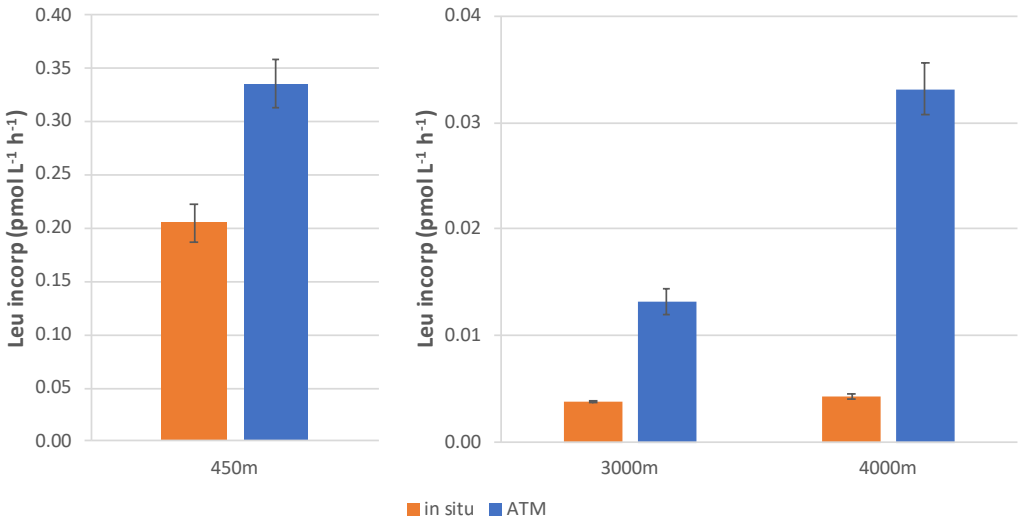


Fig. 4: *Leucine incorporation (Leu incorp) rates (pmol L^{-1} h^{-1}) of Station A6 during the M139 cruise in the North Atlantic under atmospheric pressure (ATM) and at* in situ *conditions using water collected at 450 m, 3000 m and 4000 m depth.*

Fraction of prokaryotic cells taking up leucine

DAPI-stained cells with at least three cell-associated silver grains were considered taking up leucine hence, being active. As depicted in Fig. 5, cells incorporating leucine at *in situ* conditions at 450 m depth made up 36 ± 1% of DAPI-stained cells. Under atmospheric

pressure conditions, leucine-positive cells amounted to 48 ± 3% of DAPI-stained cells. At 3000 m and 4000 m depth, leucine-incorporating cells made up 13 ± 1% of DAPI-stained cells under *in situ* conditions, whereas under atmospheric pressure condition it amounted to 19 ± 2% and 22 ± 2%, respectively (Fig. 5). Independent of the depth layer sampled, the fraction of leucine-positive cells was always higher under atmospheric than under *in situ* pressure conditions (Fig. 5). The percentages of metabolically active cells under atmospheric conditions from the mesopelagic and bathypelagic waters were similar to that reported by Herndl et al. (2005).

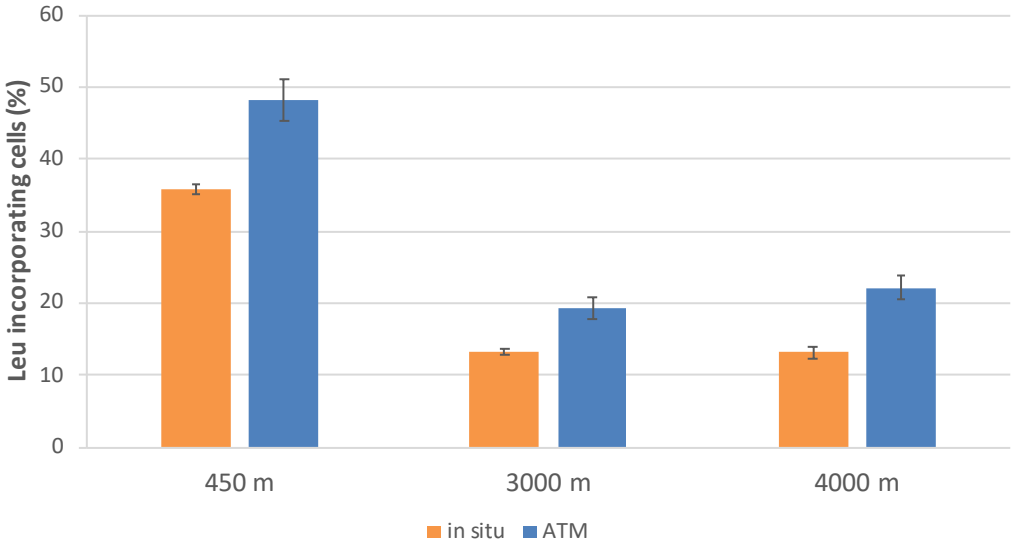


Fig. 5: *Relative contribution of counted DAPI-stained cells associated with a silver grain area to the overall number of DAPI-stained cells at* in situ *and atmospheric pressure conditions (n=6).*

Abundance of specific prokaryotic taxa

The abundance of specific prokaryotic taxa was determined by the relative abundance of DAPI-stained oligonucleotide probe-positive cells (Fig. 6). As a negative control, all samples were also hybridized with the antisense probes EUB388, NON388. Unspecific binding was only observed in three samples and less than 1% of counted cells.

The prokaryotic community was dominated by *Bacteria*, which were detected by the probe mix of EUB388, EUB388-II and EUB388-III (Table 2). The relative abundances of *Bacteria* varied with depths ranging from 26% to 45% of DAPI-stained cells. Bacterial abundance at 450

m depth at *in situ* conditions and from 3000 m at atmospheric pressure conditions was rather low (26% and 32% of DAPI-stained cells, respectively).

Archaea as the other major prokaryotic domain besides *Bacteria*, was much less abundant compared to *Bacteria*. Marine Group I *Thaumarchaeota* was identified by the probe mix of Cren554 and Cren537 and was generally more abundant than *Euryarchaeota* Marine Group II, identified with the probe Eury806. *Thaumarchaeota* made up 11 ± 2% of DAPI-stained cells, *Euryarchaeota* accounted for 3 ± 1% of DAPI-stained cells.

SAR202, SAR406, and *Alteromonas* were also targeted as representatives of major bacterial groups found in the deep sea. We observed an increase of relative abundances of SAR202 and SAR406 with depth. For example, the relative abundance of SAR202 increased with depth in both, under *in situ* and atmospheric conditions. At 3000 m and 4000 m depth, SAR202 and SAR406 made up 6% and 7% under *in situ* conditions and 10% and 11% under atmospheric pressure conditions, respectively. This trend has been shown also by previous studies (Varela et al., 2008; Nagata et al., 2010). *Alteromonas* is among the most abundant bacterial taxa present in the dark ocean (Salazar et al., 2016). In our study, *Alteromonas* was generally low in abundance and only minor variations in its relative contribution to DAPI-stained cells were found under both pressure conditions contributing 4 ± 2% averaged over all samples (Fig. 6).

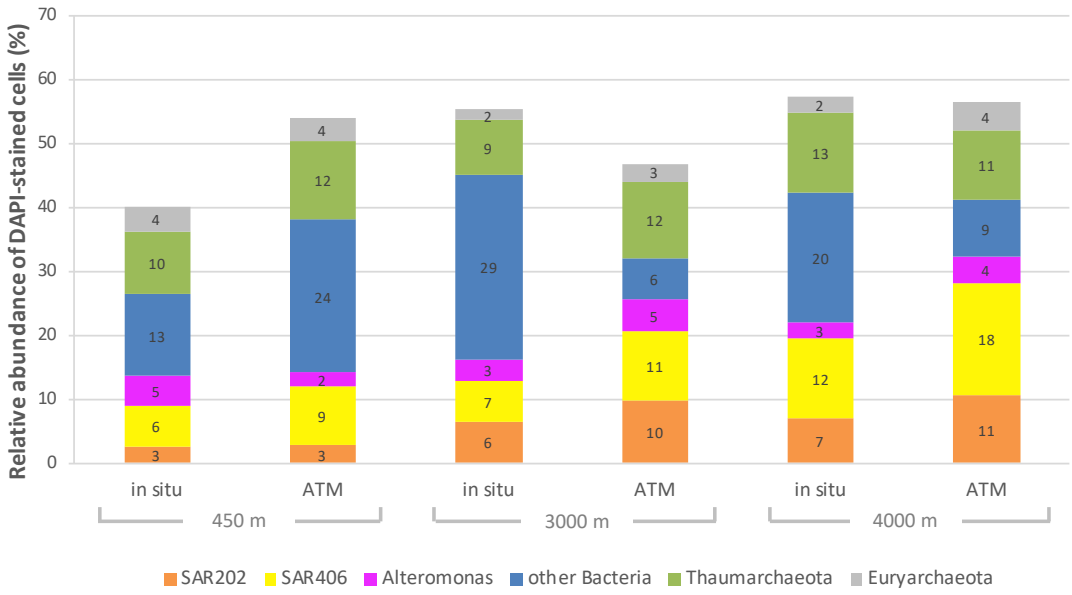


Fig. 6: *Relative contribution of selected bacterial taxa, the domains* Bacteria *and* Archaea *at Station A6 to the total number of DAPI-stained cells.* Archaea *included Marine Group I* Thaumarchaeota *and Marine Group II* Euryarchaeota. Bacteria *included SAR202, SAR406,* Alteromonas *and other* Bacteria *that remained unidentified.*

Relating silver grain area obtained by MICRO-CARD-FISH to bulk leucine incorporation

Active cells developed a halo area around the cells, the size of the halo area indicates the rate of activity. Following the protocol of Sintes and Herndl (2006), the average halo area of all cells per sample is related to the bulk leucine uptake of this particular sample. The total silver grain area of all samples was linearly related to the bulk leucine incorporation rate (r^2 = 0.98, Fig. 7). By keeping the incubation time as well as development time of the samples constant, this relation of the silver grain area to bulk leucine uptake allows to estimate the single-cell activity of individual prokaryotic groups in a given sample.

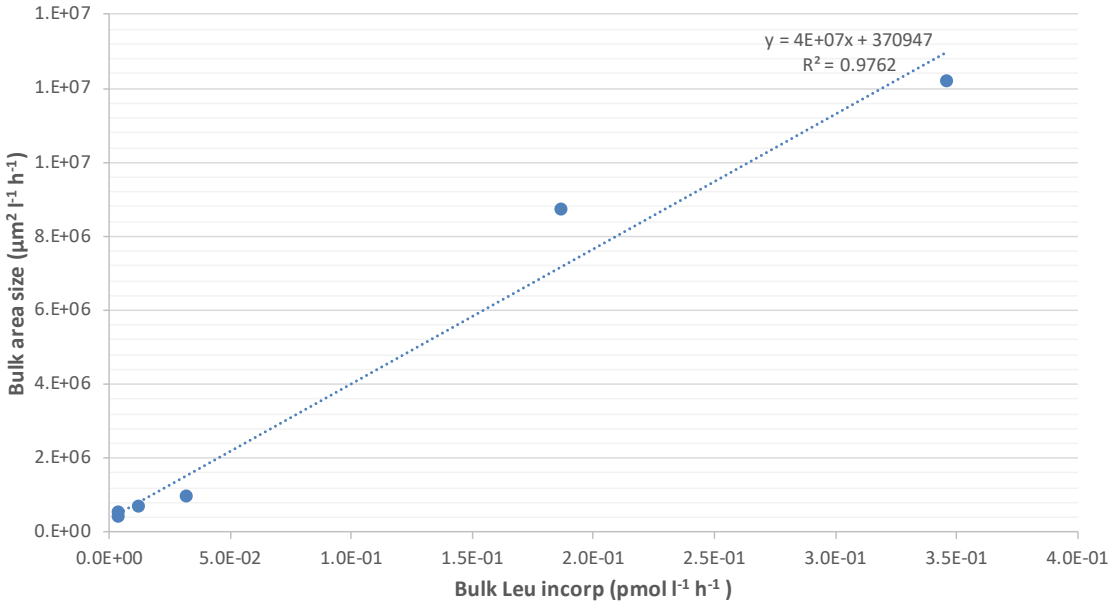


Fig. 7: *Relationship between bulk leucine incorporation and bulk silver grain area. Data were obtained from 450 m, 3000 m and 4000 m depth from samples incubated at* in situ *and atmospheric pressure conditions.*

Cell-specific activity of selected prokaryotic groups under *in situ* and atmospheric pressure conditions

The number of cells taking up leucine differed between depth layers and prokaryotic groups. Fig. 8 shows the percentage of cells belonging to different phylogenetic groups taking up leucine at *in situ* versus atmospheric pressure conditions of samples collected at the three depth layers.

Generally, the number of *Bacteria* incorporating leucine was higher under atmospheric than under *in situ* pressure conditions in all three depth layers. Under *in situ* conditions, *Bacteria* taking up leucine amounted to 52% of all bacterial cells at 450 m depth, whereas under

atmospheric pressure conditions 70% of all bacterial cells were taking up leucine (Fig. 8). In bathypelagic waters, leucine-positive cells amounted to 31% at 3000 m and 27% at 4000 m depth under atmospheric pressure, whereas under *in situ* pressure conditions 18% and 13% were leucine-positive at 3000 m and 4000 m depth, respectively (Fig. 8).

A similar tendency as for the domain *Bacteria* was observed in *Chloroflexi*-type SAR202 albeit with a less pronounced increase in the percentage of SAR202 leucine-positive cells than obtained for the domain *Bacteria* (Fig. 8). In contrast to the domain *Bacteria* and in SAR202, the percentage of SAR406 leucine-positive cells did not differ substantially between atmospheric and *in situ* pressure conditions regardless the depth layer sampled (Fig. 8). *Alteromonas* exhibited, however, a higher fraction of leucine-positive cells under *in situ* conditions in all depth layers (excluding the 450 m sample due to an insufficient number of leucine-positive cells obtained under atmospheric pressure conditions). In both bathypelagic samples of 3000 m and in 4000 m depth, the fraction of leucine-positive *Alteromonas* cells were 10% higher under *in situ* than under atmospheric pressure condition (Fig. 8).

Similar to the domain *Bacteria*, also the fraction of leucine-positive *Thaumarchaeota* was higher under atmospheric (by 4 - 11%) than *in situ* pressure conditions and in *Euryarchaeota* the fraction of leucine-positive cells was 5 - 6% higher under atmospheric pressure conditions (Fig. 8).

Taken together, the deep-water prokaryotes collected at this station in the North Atlantic exhibited different sensitivity towards hydrostatic pressure. The majority of the domain *Bacteria*, SAR202 and *Thaumarchaeota* seems to be predominately piezosensitive while SAR406 is partly and *Alteromonas* is apparently mainly piezophilic. The existence of an *Alteromonas macleodii* ecotype with specific adaptations to deep-sea conditions has been previously shown (López- López et al., 2005; Ivars-Martinez et al., 2008).

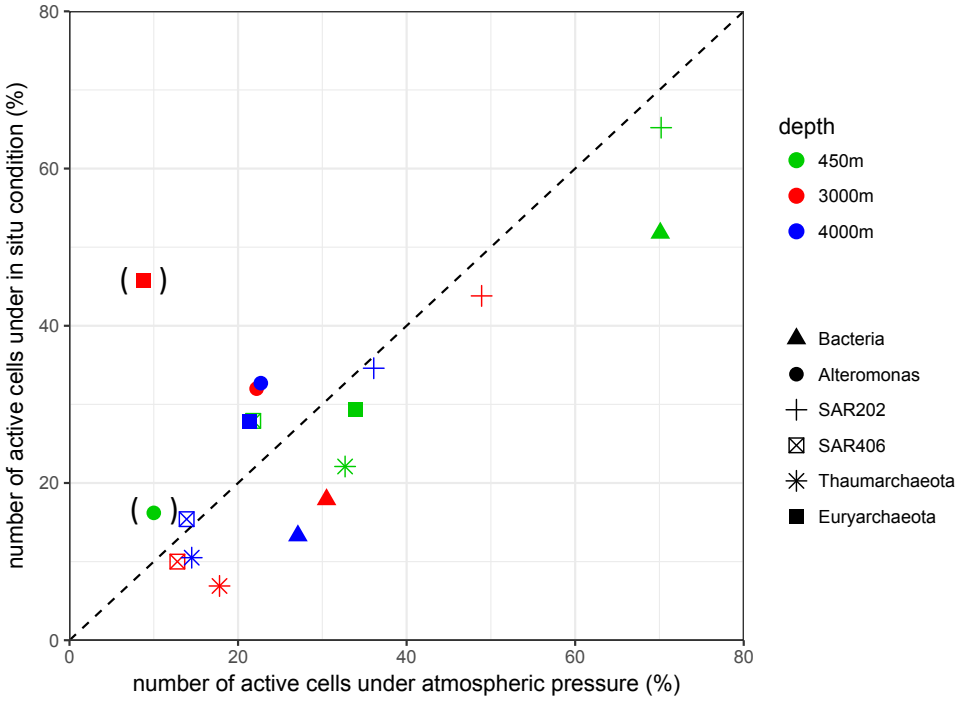


Fig. 8: *Fraction of leucine-positive cells under atmospheric versus* in situ *pressure conditions. The dashed line indicates the 1:1 line. Brackets denote outliers and indicate data points that are excluded due to insufficient sample size (n < 10).*

Using the quantitative MICRO-CARD-FISH approach (Sintes and Herndl, 2006), the contribution of the specific prokaryotic groups to bulk leucine incorporation under *in situ* and atmospheric pressure conditions was determined (Fig. 9). Overall, group-specific prokaryotic leucine uptake was always higher under atmospheric pressure conditions than under *in situ* (Fig. 9). In the mesopelagic realm, bulk leucine incorporation into the heterotrophic prokaryotic community was on average one order of magnitude higher than in bathypelagic waters.

At 450 m depth, bacterial leucine uptake rates amounted to 8.71 pmol L^{-1} h^{-1} under *in situ* and was about twice as high (18.43 pmol leucine incorporated L^{-1} h^{-1}) under atmospheric conditions (Fig. 9). At 3000 m depth, leucine uptake under *in situ* conditions amounted to 0.255 pmol L^{-1} h^{-1} while under atmospheric pressure conditions leucine uptake was 0.753 pmol L^{-1} h^{-1}, hence 3-times higher. At 4000 m depth, bacterial leucine uptake amounted to 0.278 pmol L^{-1} h^{-1} under *in situ* conditions and 2.56 pmol L^{-1} h^{-1} under atmospheric conditions, thus about 10-times higher at atmospheric than under *in situ* pressure conditions (Fig. 9). In terms of cell-specific activity rate, SAR202, SAR406 and *Alteromonas* exhibited similar trends under *in situ* and atmospheric pressure conditions as the domain *Bacteria* (Fig. 9).

Thaumarchaeota followed the trend from *Bacteria* samples but exhibited generally a lower activity than *Bacteria* (Fig. 9). At 450 m, *Thaumarchaeota* took up 1.0 pmol leucine L^{-1} h^{-1} under *in situ* and 2.98 pmol leucine L^{-1} h^{-1} under atmospheric pressure conditions. In deeper layers, leucine uptake of *Thaumarchaeota* was roughly ten times higher than under *in situ* conditions (Fig. 9). The effect of pressure changes was generally lower in *Euryarchaeota* than in the other prokaryotic groups tested (Fig. 9). For example, at 4000 m depth, leucine uptake under *in situ* conditions amounted to 0.038 pmol L^{-1} h^{-1} and was only about 3 times lower than the leucine uptake under atmospheric conditions (0.133 pmol L^{-1} h^{-1}; Fig. 9).

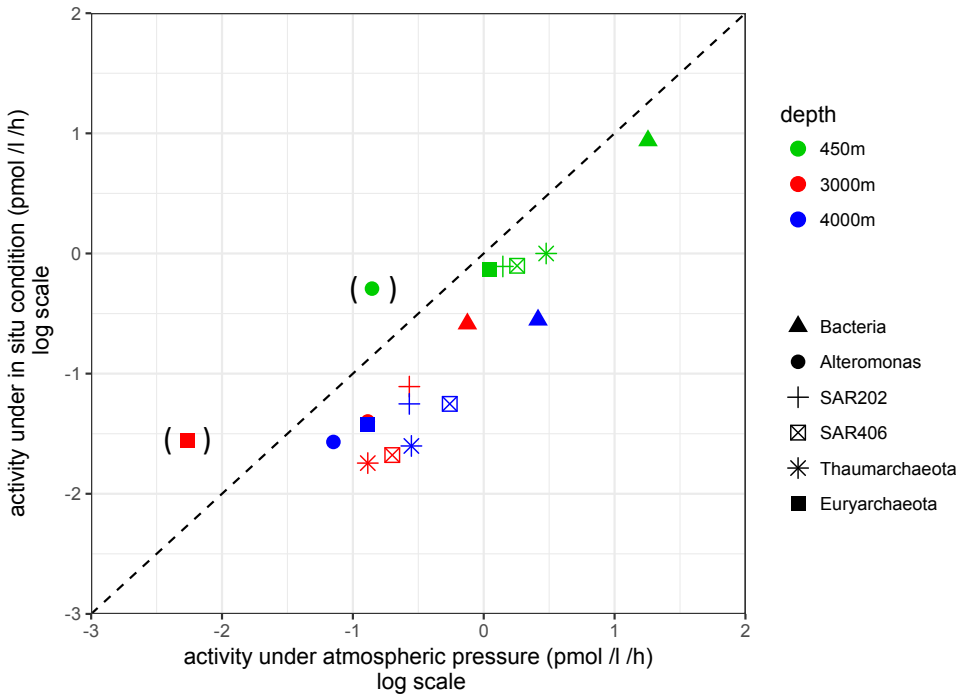


Fig. 9: *Relation between leucine uptake rates (pmol L^{-1} h^{-1}) under atmospheric versus* in situ *pressure conditions (logarithmic scale). The dashed line indicates the 1:1 relation. Brackets denote outliers and indicate data points that are excluded due to insufficient sample size (n < 10).*

The distribution of leucine uptake within the individual prokaryotic groups under *in situ* and ambient pressure conditions were also investigated (Fig. 10). Under *in situ* conditions, most cells developed halo area sizes between 0.18 – 0.30 µm² at 450 m and 0.06 – 0.18 µm² in both bathypelagic depth layers (Fig. 10). Halo area sizes > 0.84 µm² indicate highly active cells. These highly active cells (taking up leucine) were predominantly found under atmospheric pressure conditions making up 6% of the total silver grain area in the sample

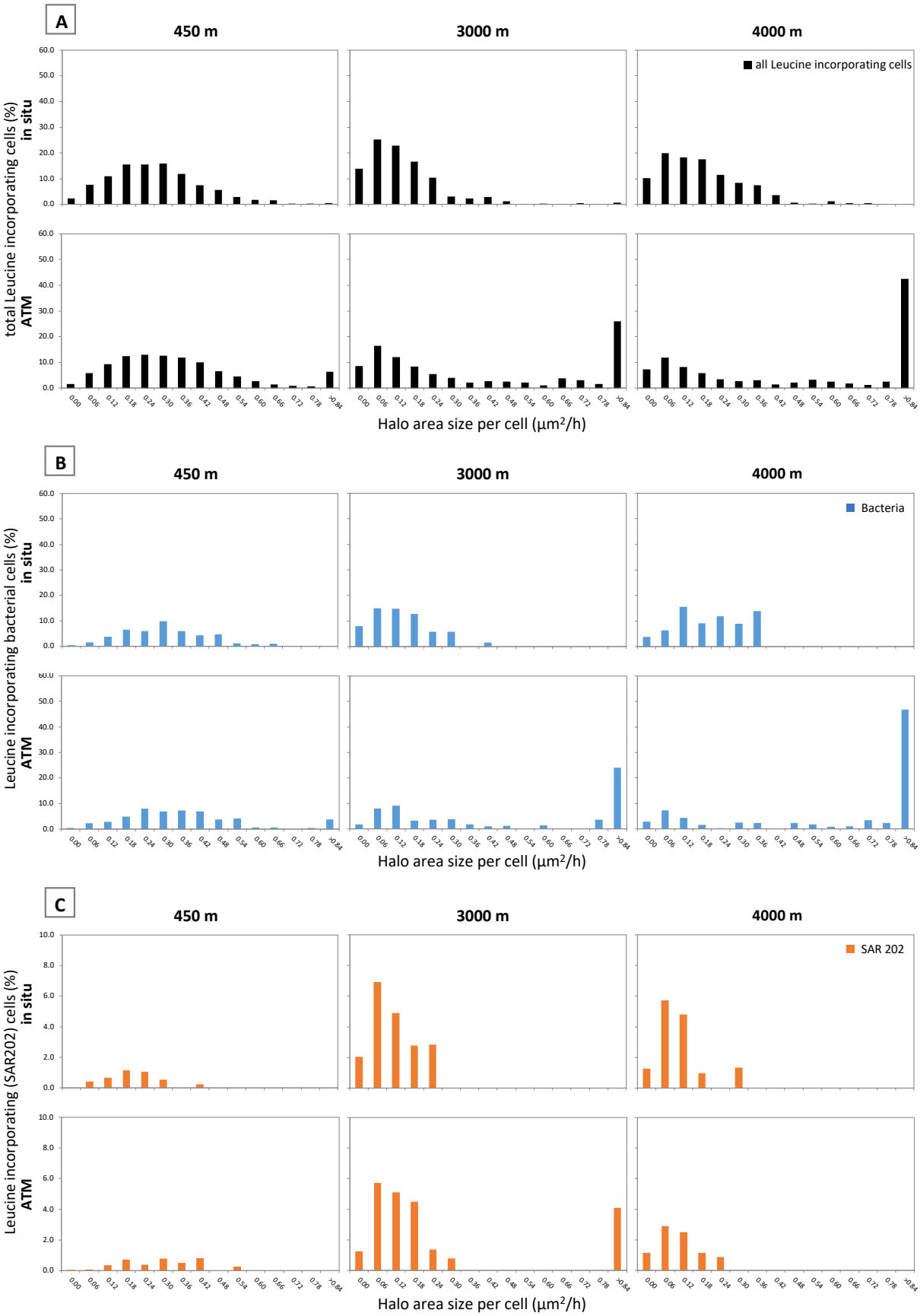
from 450 m, 26% at 3000 m and 43% in samples from 4000 m depth of the total leucine uptake under atmospheric conditions (Fig. 10 A).

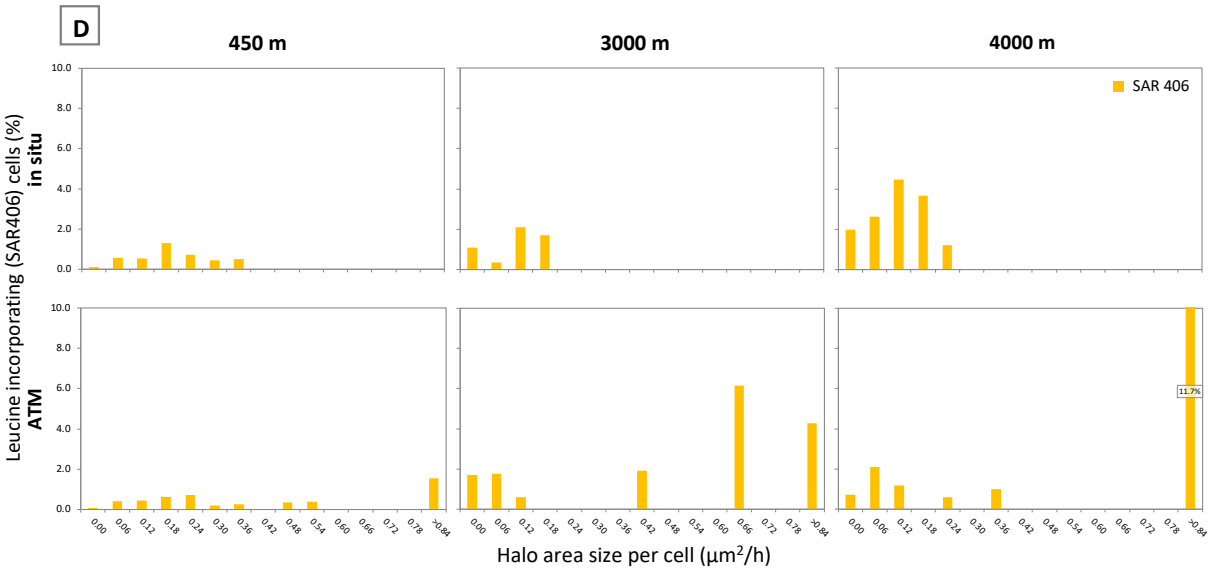
The major fraction of cells taking up leucine was *Bacteria*. The distribution pattern of leucine uptake under atmospheric pressure conditions was skewed towards large halo area size categories in all three depth layers (Fig. 10 B).

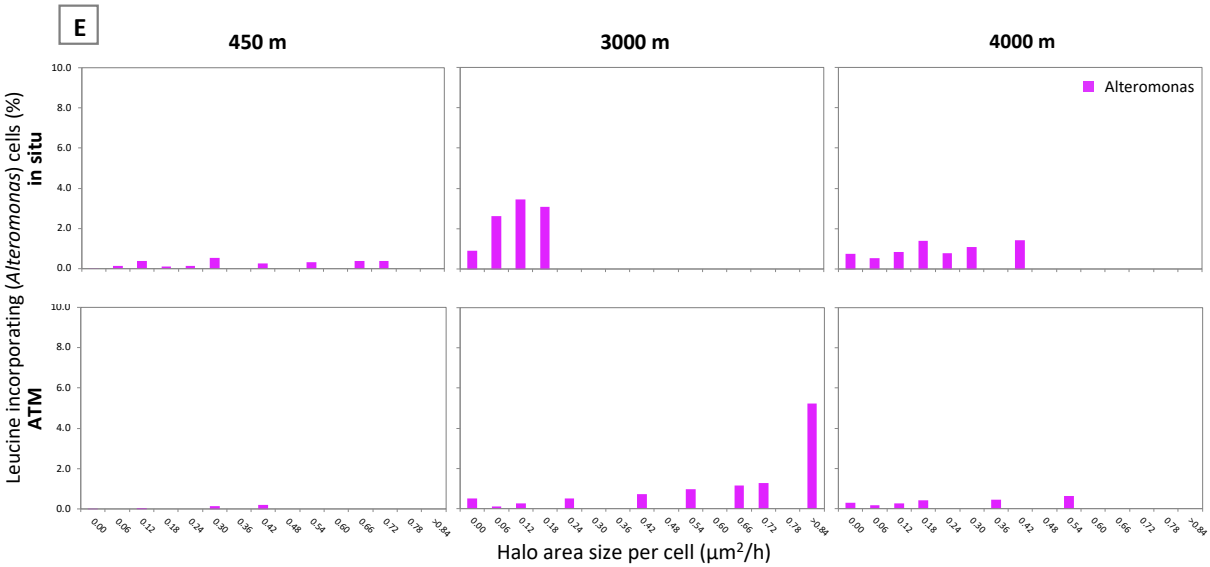
Individual groups of *Bacteria* showed a variable distribution pattern of leucine uptake (Fig. 10 C-E). In SAR202, more high active cells were observed under atmospheric pressure conditions in both, 450 m and 3000 m. This trend was reversed in 4000 m where slightly more high active cells were found under *in situ* pressure condition (Fig. 10 C). Under *in situ* pressure conditions, SAR406 exhibited a lower activity pattern on a single cell level (up to 0.36 μm^2), while under atmospheric pressure, the activity pattern shifted towards more active cells in all three depths (Fig. 10 D). An increase of highly active cells under atmospheric pressure conditions compared to *in situ* conditions in *Alteromonas* was observed in all depths, except for 450 m depth which was excluded due to a lack of sufficient number of active cells under the atmospheric pressure conditions (Fig. 10 E).

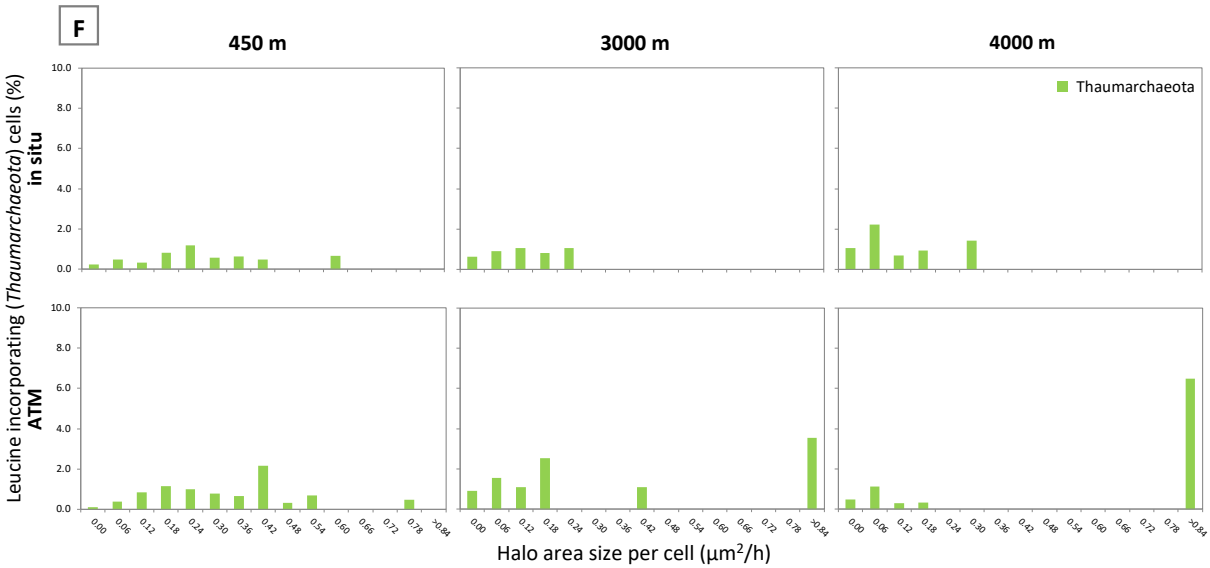
When comparing the distribution of *Thaumarchaeota* taking up leucine between *in situ* and atmospheric pressure conditions, more highly active cells were found under atmospheric than *in situ* pressure conditions. This trend became more pronounced with depth (Fig. 10 F). *Euryarchaeota* cells were low in abundance throughout the water column. In mesopelagic waters, a few high active *Euryarchaeota* cells were detected under atmospheric pressure conditions (Fig. 10 G).

These results contrast the findings of Tamburini et. al (2013), who concluded that deep-sea microorganisms are adapted to high pressure conditions and, when brought to the surface, exhibit lower activity than under *in situ* hydrostatic pressure. Our results correspond, however, to the studies of Jannasch and Wirsen (1982) concluding that increased hydrostatic pressure results in a reduction of activity (Jannasch et al., 1976; Jannasch and Wirsen, 1982).


A
450 m
3000 m
4000 m
all Leucine incorporating cells
total Leucine incorporating cells (%)
in situ
ATM
Halo area size per cell (µm²/h)
B
Bacteria
Leucine incorporating bacterial cells (%)
C
SAR 202
Leucine incorporating (SAR202) cells (%)


D
450 m
3000 m
4000 m
SAR 406
Leucine incorporating (SAR406) cells (%)
in situ
ATM
11.7%
Halo area size per cell (µm²/h)


E
450 m
3000 m
4000 m
Alteromonas
Leucine incorporating (Alteromonas) cells (%)
in situ
ATM
Halo area size per cell (µm²/h)


F
450 m
3000 m
4000 m
Thaumarchaeota
Leucine incorporating (Thaumarchaeota) cells (%)
in situ
ATM
Halo area size per cell (µm²/h)

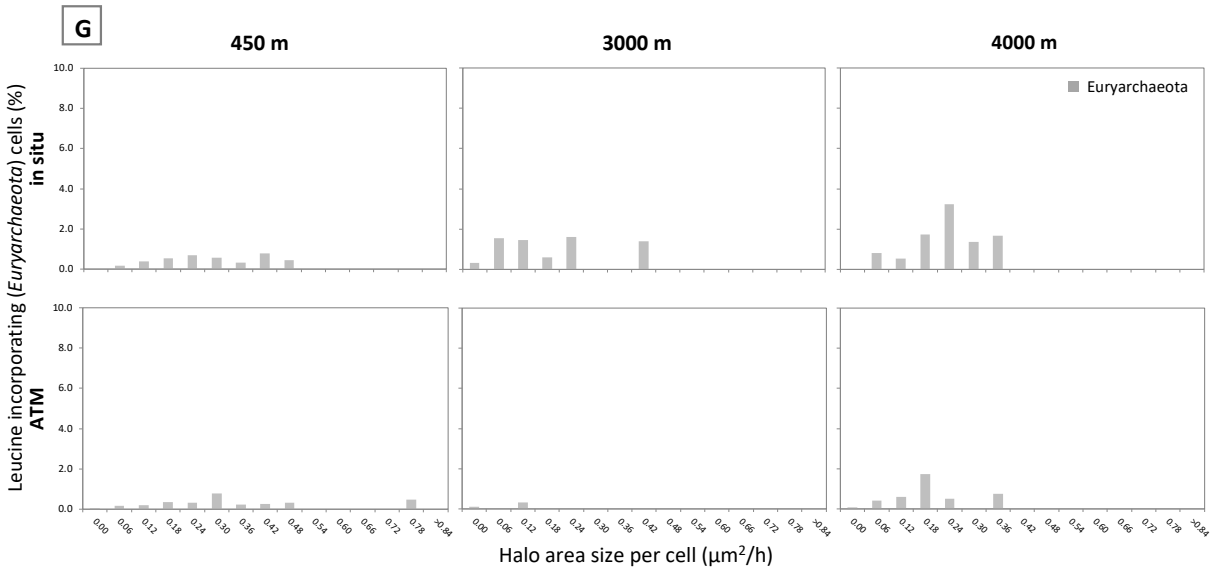


Fig. 10: *Distribution of the abundance of prokaryotic cells taking up leucine from 450 m, 3000 m and 4000 m depth under* in situ *(*in situ*) and atmospheric pressure (ATM) conditions. Halo area size per cell (µm²/h) correlates with leucine incorporation rates and indicate the rate of activity, increasing from left to right. (A) contains all DAPI-stained, leucine incorporating cells that were found in all samples throughout the water column. The relative contribution of (B)* Bacteria*, (C) SAR202, (D) SAR406, (E)* Alteromonas*, (F)* Thaumarchaeota*, (G)* Euryarchaeota *cells to the total leucine incorporating cells is shown.*

Conclusion

The main aim of this study was to investigate how hydrostatic pressure affects activity of marine deep-sea prokaryotes and how different groups respond to changes in pressure. Throughout this study, our results indicated that most of investigated deep-sea prokaryotic groups seem to be piezosensitive, thus growing faster under atmospheric pressure conditions. We also observed, however, that specific prokaryotic groups reacted very individually. At least some of the *Alteromonas* and SAR406 seem to be piezophilic or at least piezotolerant, while almost all the other deep-sea prokaryotic groups appear to be piezosensitive.

References

Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R. and Stahl, D. A. (1990) “Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations.” *Appl. Environ. Microbiol*. 56(6): 1919-1925.

Arístegui, J., Gasol J. M., Duarte, C. M., Herndl, G. J. (2009) “Microbial oceanography of the dark ocean’s pelagic realm,” *Limnology and Oceanography*, 54(5), pp. 1501–1529. doi: 10.4319/lo.2009.54.5.1501.

Bartlett, D. H. (2002) “Pressure effects on in vivo microbial processes,” *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, 1595(1), pp. 367–381. doi: 10.1016/S0167-4838(01)00357-0.

Daims, H., Bruhl, A., Amann, R., Schleifer, K. H., Wagner, M. (1999) "The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: Development and evaluation of a more comprehensive probe set." *Syst. Appl. Microbiol.* 22(3): 434-444.

Delong, E. F., Preston, C. M., Mincer, T., Rich, V., Hallam, S. J., Frigaard, N., Martinez, A., Sullivan, M. B., Edwards, R., Brito, B. R., Chisholm, S. W., Karl, D. M. (2006) “Community Genomics among microbial assemblages in the Ocean’ s Interior,” *Science*, 311(January), pp. 496–503. doi: 10.1126/science.1120250.

Eilers, H., Pernthaler, J., Glockner, F. O. and Amann, R. (2000) "Culturability and in situ abundance of pelagic bacteria from the North Sea." *Appl. Environ. Microbiol.* 66(7): 3044-3051.

Elvert, M., Suess, E., Greinert, J., Whiticar, M. J. (2000) "Archaea mediating anaerobic methane oxidation in deep-sea sedi- ments at cold seeps of the eastern Aleutian subduction zone." *Org Geochem* 31:1175–1187

Fang, J., Zhang, L., Bazylinski, D.A. (2010) "Deep-sea piezosphere and piezophiles: geomicrobiology and biogeochemistry." *Trends Microbiol* 18: 413–422.

Fuchs, B. M., Woebken, D., Zubkov, M. V., Burkill, P., Amann, R. (2005) "Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea." *Aquat. Microb. Ecol.* 39(2): 145-157.

Hawley, A. K., Nobu, M. K., Wright, J. J., Durno, W. E., Morgan-Lang, C., Sage, B., Schwientek, P., Swan, B. K., Rinke, C., Torres-Beltrán, M., Mewis, K., Liu, W., Stepanauskas, R., Woyke, T., Hallam, S. J. (2017) “Diverse Marinimicrobia bacteria may mediate coupled biogeochemical cycles along eco-thermodynamic gradients,” *Nature Communications*. Springer US, 8(1), pp. 1–9. doi: 10.1038/s41467-017-01376-9.

Herndl, G. J., Reinthaler, T., Teira, E., Aken, H., Veth, C., Pernthaler, A., Pernthaler, J. (2005) “Contribution of,” *Applied and environmental microbiology*, 71(5), pp. 2303–2309. doi: 10.1128/AEM.71.5.2303.

Ivars-Martinez, E., Martin-Cuadrado, A., D’Auria, G., Mira, A., Ferriera, S., Johnson, J., Friedman, R., Rodriguez-Valera F. (2008) “Comparative genomics of two ecotypes of the marine planktonic copiotroph *Alteromonas macleodii* suggests alternative lifestyles associated with different kinds of particulate organic matter” *ISME Journal* vol. 2, p. 1194–1212

Jannasch, H. W. and Wirsen, C. O. (1982) “Microbial activities in undecompressed and decompressed deep-seawater samples.,” *Applied and environmental microbiology*, 43(5), pp. 1116–1124.

Jannasch, H. W., Wirsen, C. O. and Taylor, C. D. (1976) “Undecompressed microbial population from the deep sea,” *Applied and Environmental Microbiology*, 32(3), pp. 360–367.

Kato, C. (2011). Distribution of piezophiles. In Extremophiles Handbook. Horikoshi, K. (ed.). Tokyo, Japan: *Springer Japan*, pp. 643–655.

Landry, Z., Swa, B. K., Herndl, G. J., Stepanauskas, R., Giovannoni, S. J. (2017) “SAR202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter,” *mBio*, 8(2), pp. 1–19. doi: 10.1128/mBio.00413-17.

Lauro, F. M. and Bartlett, D. H. (2008) “Prokaryotic lifestyles in deep sea habitats,” *Extremophiles*, 12(1), pp. 15–25. doi: 10.1007/s00792-006-0059-5.

Lekunberri, I., Sintes, E., de Corte, D., Yokokawa, T., Herndl, G. J. (2013) “Spatial patterns of bacterial and archaeal communities along the Romanche Fracture Zone (tropical Atlantic),” *FEMS Microbiology Ecology*, 85(3), pp. 537–552. doi: 10.1111/1574-6941.12142.

López-López, A., Bartual, S. G., Stal, L., Onyshchenko, O., Rodríguez-Valera, F. (2005) "Genetic analysis of housekeeping genes reveals a deep-sea ecotype of Alteromonas macleodii in the Mediterranean Sea." *Environ Microbiol* 7: 649–659. Martín-Cuadrado

Massana, R., Murray, A. E., Preston, C. M. and DeLong, E. F. (1997) "Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel." *Appl. Environ. Microbiol.* 63(1): 50-56.

Morris, R. M., Rappe, M. S., Urbach, E., Connon, S. A., Giovannoni, S. J. (2004) "Prevalence of the Chloroflexi-related SAR202 bacterioplankton cluster throughout the mesopelagic zone and deep ocean." *Appl. Environ. Microbiol.* 70(5): 2836-2842.

Nagata, T., Tamburini, C., Arístegui, J., Baltar, F., Bochdansky, A. B., Fonda-Umani, S., Fukuda, H., Gogou, A., Hansell, D. A., Hansman, R. L., Herndl, G. J., Panagiotopoulos, C., Reinthaler, T., Sohrin, R., Verdugo, P., Yamada, N., Yamashita, Y., Yokokawa, T., Bartlett, D. H. (2010) “Emerging concepts on microbial processes in the bathypelagic ocean - Ecology, biogeochemistry, and genomics,” *Deep-Sea Research Part II: Topical Studies in Oceanography*, 57(16), pp. 1519–1536. doi: 10.1016/j.dsr2.2010.02.019.

Prieur, D., Erauso, G., Jeanthon, C. (1995). Hyperthermophilic life at deep-sea hydrothermal vents. *Planet Space Sci* 43:115–122

Reinthaler, T., van Aken, H. M. and Herndl, G. J. (2010) “Major contribution of autotrophy to microbial carbon cycling in the deep North Atlantic’s interior,” *Deep-Sea Research Part II: Topical Studies in Oceanography*. Elsevier, 57(16), pp. 1572–1580. doi: 10.1016/j.dsr2.2010.02.023.

Salazar, G., Cornejo-Castillo, F. M., Benítez-Barrios, V., Fraile-Nuez, E., Álvarez-Salgado, X. A., Duarte, C. M., Gasol, J. M., Acinas, S. G. (2016) “Global diversity and biogeography of deep-sea pelagic prokaryotes,” *ISME Journal*, 10(3), pp. 596–608. doi: 10.1038/ismej.2015.137.

Schattenhofer, M., Fuchs, B. M., Amann, R., Zubkov, M. V., Tarran, G. A., Pernthaler, J. (2009) “Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean,” 11, pp. 2078–2093. doi: 10.1111/j.1462-2920.2009.01929.x.

Sintes, E. and Herndl, G. J. (2006) “Quantifying substrate uptake by individual cells of marine bacterioplankton by catalyzed reporter deposition fluorescence in situ hybridization combined with microautoradiography,” *Applied and Environmental Microbiology*, 72(11), pp. 7022–7028. doi: 10.1128/AEM.00763-06.

Smith, C. R., Baco, A. R. (2003). Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology*, vol 41. pp 311–354

Tamburini, C., Boutrif, M., Garel, M., Colwell, R. R., Deming, J. W. (2013) “Prokaryotic responses to hydrostatic pressure in the ocean - a review,” *Environmental Microbiology*, 15(5), pp. 1262–1274. doi: 10.1111/1462-2920.12084.

Teira, E. Reinthaler, T., Pernthaler, A., Pernthaler, J. and Herndl, G. J. (2004) “Combining catalyzed reported deposition-fluorescence in situ hybridization and automicrography to detect substrate utilization by Bacteria and Archaea in the Deep Ocean,” *Applied and Environmental Microbiology*, 70(7), pp. 4411–4414. doi: 10.1128/AEM.70.7.4411.

Varela, M. M., Van Aken, H. M. and Herndl, G. J. (2008) “Abundance and activity of Chloroflexi-type SAR202 bacterioplankton in the meso- and bathypelagic waters of the (sub)tropical Atlantic,” *Environmental Microbiology*, 10(7), pp. 1903–1911. doi: 10.1111/j.1462-2920.2008.01627.x.

Wallner, G., Amann, R. and Beisker, W. (1993) "Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms." *Cytometry* 14(2): 136-143.

Wright, J. J., Mewis, K., Hanson, N. W., Konwar, K. M., Maas, K. R., Hallam, S. J. (2014) “Genomic properties of Marine Group A bacteria indicate a role in the marine sulfur cycle,” *ISME Journal*. Nature Publishing Group, 8(2), pp. 455–468. doi: 10.1038/ismej.2013.152.

Yayanos, A.A. (1995) “Microbiology to 10,500 meters in the deep-sea.” *Annual Rev. Microbiol* 49: 777–805.

Zaballos, M., López-López, A., Ovreas, L., Bartual, S. G., D'Auria, G., Alba, J. C., Legault, B., Pushker, R., Daae, F. L., Rodríguez-Valera, F. (2006) “Comparison of prokaryotic diversity at offshore oceanic locations reveals a different microbiota in the Mediterranean Sea.” *FEMS Microbiology Ecology*, Vol. 56, p. 389–405

Zusammenfassung

Hydrostatischer Druck als physikalisch, ökologischer Parameter hat eine signifikante Wirkung auf die Lebensweise von Tiefseemikroben. Die meisten Studien über Stoffwechselraten von Prokaryoten aus der Tiefsee jedoch vernachlässigen diesen Faktor, weshalb viele Ergebnisse weitgehend unter atmosphärischen Druckbedingungen bestimmt wurden. Das Ziel dieser Forschungsarbeit ist es herauszufinden, wie sich zunehmender hydrostatischer Druck auf Prokaryoten in der Tiefsee auswirkt und ob sich Unterschiede in Produktivität so wie Aktivität, im Vergleich zu Versuchen unter atmosphärischem Druck, aufzeigen lassen. Zu diesem Zweck wurde ein „*in situ* microbial incubator“ (ISMI) im Nordatlantik an Bord des Forschungsschiffes *Meteor* eingesetzt. Das ISMI dient dazu, Mikroorganismen aus definierten Wassertiefen unter den jeweilig vorherrschenden Umweltbedingungen (speziell auf Druck und Temperatur bezogen) *in situ* inkubieren zu können. Mit Hilfe der Methode „Catalyzed reporter deposition fluorescence *in situ* hybridization“ in Kombination mit Mikroautoradiographie konnten wir in den daraus resultierenden Proben Abundanzen von spezifischen, prokaryotischen Gruppen bestimmen und deren Aktivitätsrate visualisieren. Insgesamt zeigten unsere Ergebnisse eine generell verringerte Aktivität in tieferen Gewässern (*in situ*) gegenüber einer allgemein höheren Aktivität unter atmosphärischen Druckbedingungen. Darüber hinaus war die prokaryotische Gemeinschaft aktiver in mesopelagischen als in bathypelagischen Gewässern. Im Laufe dieser Studie haben unsere Ergebnisse die Theorie, dass ein größerer Anteil der untersuchten Mitglieder der prokaryotischen Tiefseegruppen piezosensitiv zu sein scheint, und somit atmosphärische Druckbedingungen bevorzugt, kontinuierlich unterstützt. Es gilt jedoch zu erwähnen, dass spezifische prokaryotische Gruppen teilweise sehr individuell reagierten. Diese Beobachtungen gaben Hinweis auf mögliche piezophile oder zumindest piezotolerante Mitglieder in Tiefsee-Prokaryoten, die in dem Genus *Alteromonas* zu finden sind. Zusammenfassend deuten unsere Daten darauf hin, dass Aktivitätsmessungen von Mikroorganismen in der Tiefsee unter hydrostatischen Druckbedingungen *in situ* erfolgen sollten, um realistische Werte von Stoffwechselraten der Tiefsee-Prokaryoten zu erhalten.