Title of the Master Thesis

“Temperature-dependent response of microbial communities in the meso- and bathypelagic Atlantic Ocean”

Author

Pavla Debeljak BSc.

Desired academic degree

Master of Science (MSc)

Wien, 2014

Study number as per study sheet: A 066 833

Study direction as per study sheet: Master's degree in Ecology

Supervisor:

Univ.-Prof. Dr. Gerhard J. Herndl
To my grandfather, a beautiful mind.

“For whatever we lose (like a you or a me) it’s always ourselves we find in the sea.”

E. E. Cummings
# Table of content

Abstract 4

1. Introduction 5
2. Material and Methods 8
3. Results 16
4. Discussion 19
5. Conclusion 24
6. Tables and Figures 25
7. Acknowledgments 36
8. References 37

Zusammenfassung 43

Curriculum Vitae 44
Abstract

Marine microbial communities are essential contributors to global biomass, nutrient cycling, and biodiversity since the early history of Earth and their stability is influencing biogeochemical processes. In the present study, the temperature response (in situ temperature vs. 20°C) of the meso- and bathypelagic prokaryotic community of the North Atlantic and the Ross Sea in terms of dissolved inorganic carbon (DIC) fixation and community composition was determined. The prokaryotic community composition was assessed by 16S rRNA fingerprinting and 454 Tag sequencing. At a temperature of 20°C, DIC fixation was up to 230-times higher than at in situ temperature. This response was observed for the North Atlantic, in the Ross Sea, however, the increase in DIC fixation at 20°C remained insignificant. Although significant differences in prokaryotic community composition were observed between the in situ temperature and the 20°C incubations, the selective pressure applied as temperature increase, did not lead to a convergence of the prokaryotic communities of the different water masses incubated at 20°C. Sequencing revealed a high contribution of the gammaproteobacterial order Oceanospirillales to the total sequence abundance in the 20°C incubations. Our results indicate a high metabolic plasticity among members of this order and the potential of autotrophic DIC fixation indicated by the presence of genes for sulphur oxidation and carbon fixation, present in some Oceanospirillales (Swan et al. 2011). The temperature response is apparently not ubiquitously present in all the world’s oceans albeit widely distributed. The energy sources for this inorganic carbon fixation of the deep-sea microbial community remain enigmatic.

Key words: Microbial community, DIC Fixation, Dark Ocean, Chemoautotrophy, Bacteria
1. **Introduction**

The availability of resources, selective loss factors such as grazing and viral lysis, and physical parameters such as temperature and salinity influence the community structure of marine microbes resulting in distinct patterns with depth and latitude (Agogué et al. 2011). Although most major prokaryotic groups are cosmopolitan, studies across the world’s oceans have described latitudinal variation in community composition identified through 16S rRNA gene phylogeny and DNA fingerprinting (Pommier et al. 2006; Fuhrman et al. 2008; Schattendorfer et al. 2009; Giovannoni & Vergin 2012). The past decade has brought further insights into these community dynamics by applying large-scale parallel pyrosequencing that allow for efficient and deep molecular sampling of the microbial community (Sogin et al. 2006; Gilbert et al. 2009; Agogué et al. 2011). However, little information is available connecting community composition and biogeochemical parameters. Microbial communities are sensitive to perturbations, as often observed by the “bottle effect”, i.e., a rapid change in microbial community composition when natural waters are confined (Hammes et al. 2010; Giovannoni & Vergin 2012). The stability of microbial communities is directly relevant to a number of environmental processes. Of particular interest is whether biogeochemical functions are maintained in response to changing inputs (Giovannoni & Vergin 2012).

Metabolic strategies characterize microorganisms with regard to their ability to obtain carbon and energy. The ability to assemble cell material from inorganic carbon is restricted to autotrophic organisms. Therefore, autotrophic processes are a crucial component of the global carbon cycle transforming inorganic carbon into organic carbon and thereby providing nutrients for heterotrophic organisms (Hügler & Sievert 2007). The global redox balance of the Earth as well as the CO₂ and O₂ concentrations in the atmosphere are regulated by the balance between autotrophy and heterotrophy. Based on our current knowledge of marine ecosystems it can be assumed that a large fraction of the organic matter synthesized by
primary producers is converted to dissolved organic matter (DOM) and is taken up almost exclusively by bacteria and archaea (Azam & Malfatti 2007). This fraction can be seen as the original source of organic material in an ecosystem that is due to carbon dioxide fixation by photosynthetic bacteria, multicellular plants, unicellular algae, or chemosynthetic microorganisms.

On average, one half of global primary production takes place in the oceans (Falkowski et al. 1998; Field et al. 1998). Therefore, it is important to explore how organisms use this carbon to establish spatio-temporal patterns of carbon and energy flux (Azam & Malfatti 2007). A fraction of DOM is assimilated and re-introduced to the classical food chain; however, the bulk is respired to carbon dioxide by bacteria and archaea through the so-called microbial loop, which is crucial for the cycling of carbon (Azam et al. 1983). Although these processes are known to occur in the photic zone, there is only fragmentary knowledge on how they function in the dark ocean (below 200m) and what the fuelling energy sources are. In the past decade, chemoautotrophy has been identified as a significant metabolic pathway in which energy is obtained from the oxidation of electron donors in the environment and all necessary organic compounds are synthesized from carbon dioxide and inorganic nutrients (Swan et al. 2011; Anantharaman et al. 2013; Herndl & Reinthaler 2013). Some of the identified energy sources fuelling this reaction in form of reduced compounds might be ammonia, urea, sulphide, hydrogen, iron and methane but the extent and availability of in situ energy sources for chemoautotrophy in the dark ocean is not yet fully understood nor quantified (Herndl et al. 2005; Anantharaman et al. 2013; Herndl & Reinthaler 2013). Recent studies suggest that the fixation of dissolved inorganic carbon (DIC) in the dark ocean is of the same order of magnitude as heterotrophic biomass production (Alonso-Saez et al. 2010; Reinthaler et al. 2010; Herndl & Reinthaler 2013). According to this high DIC fixation, new organic matter could potentially be synthesized in the dark ocean fuelling the heterotrophic food web (Herndl & Reinthaler 2013).
During the Microbial Ecology of the Deep Atlantic (MEDEA) cruise from October to November 2011 (MEDEA-I) on board the R/V *Pelagia* an increase in DIC fixation rates was observed after an accidental incubation of the microbial community from deep waters at elevated temperatures. Consequently, measurements of DIC fixation were also performed on the subsequent MEDEA-II cruise from June to July 2012 and the Tracing the fate of Algal Carbon Export in the Ross Sea (TRACERS) cruise from February to April 2013 to further investigate this temperature response. The increase in temperature in incubation experiments resulted in high $Q_{10}$ values for DIC fixation. Based on these results, we investigated differences in the microbial community composition and diversity in incubations performed at 20°C and compared to those at in situ temperature. We used two microbial fingerprinting techniques to elucidate changes in the community response. Additionally, samples were for 454 Tag Sequencing (Roche) sequenced to gain more in-depth phylogenetic information on the community composition. The community response was investigated to determine whether changing one environmental factor such as temperature could lead to a convergence of communities, regardless of their origin, i.e., different deep-water masses. With this approach we also tried to identify taxa responsible for the increase in DIC fixation at elevated temperature.
2. **Material and Methods**

**Sampling**

Seawater from specific water masses was collected from the meso- and bathypelagic eastern North Atlantic Ocean during the MEDEA-I and -II cruises with RV *Pelagia* (MEDEA-I, October-November 2011; MEDEA-II, June-July 2012), and from the Ross Sea during the TRACERS cruise on the RVIB *Palmer* (February-April 2013) and incubated at either in situ temperature or at 20°C for 72 h (Figure 1, Table 1).

Sampling was performed with a CTD (conductivity-temperature-depth; Seabird, Bellevue, WA, USA) rosette sampler equipped with 24 25-L Niskin bottles and sensors for chlorophyll fluorescence, turbidity, photosynthetic active radiation and oxygen.

**Experimental setup**

A tremendous increase in DIC fixation rates was observed after the accidental incubation of 20 L$^{-1}$ of seawater at 20°C during the MEDEA-I cruise. This serendipical observation led to incubation experiments on this research cruise. Incubation experiments were set up with 20 L$^{-1}$ of North Atlantic Deep Water (NADW) and Mediterranean Sea Outflow Water (MSOW) incubated at in situ temperature and at 20°C for 72 h. On the MEDEA-II cruise, experiments with four water masses (oxygen minimum zone [OMZ], Labrador Sea Water [LSW], North Atlantic Central Water [NACW], and Iceland-Scotland Overflow Water [ISOW]) were performed, again incubated at in situ temperature and at 20°C for 72 h (Table 1).

To determine the composition of the microbial community in the in situ temperature incubations and that at 20°C, microbial biomass was collected by filtering 20 L$^{-1}$ of sample through a 147 mm 0.2 µm pore size polycarbonate filter (GTTP Millipore) using a peristaltic
pump. The filters were preserved in RNaLater solution (Ambion) and kept at -80°C. In total, 14 samples each of 20 L\(^{-1}\) were obtained from the three cruises.

**Determining prokaryotic abundance and DIC fixation**

To determine prokaryote abundance, samples were fixed with glutaraldehyde (2 % final concentration), flash-frozen in liquid nitrogen, stained with SYBR Green and enumerated on a FACSARia II flow cytometer (Becton Dickinson).

For DIC fixation measurements, seawater samples (40 mL of live samples in triplicate and formaldehyde-fixed blanks in duplicate) were incubated in the dark with \(^{14}\)C-labeled bicarbonate (3.7 x 10\(^{6}\) Bq, Amersham) at either in situ temperature or 20°C for 72 h. The incubation was terminated by adding formaldehyde to the live samples and filtering the water through 0.2 µm polycarbonate filters (Millipore). After rinsing the filters with 0.2 µm filtered seawater, filters were exposed to fuming HCl for 12 h and then transferred to scintillation vials, 8 mL of scintillation cocktail (Filter Count, Perkin-Elmer) was added and the radioactivity counted in a liquid scintillation counter (Perkin Elmer, TriCarb 2910TR) after 18 h. For replicate samples, the disintegrations per minute (DPMs) were averaged, blank-corrected, and converted into DIC fixation rates.

**DNA extraction**

Portions of the preserved filters were treated with proteinase K (1.25 µg mL\(^{-1}\) final volume) at 50°C for 15 min, and subsequently with lysozyme (1.25 µg mL\(^{-1}\) final volume) at 37°C for 15 min. The nucleic acids were extracted with phenol (pH 7.9) and phenol-chloroform-isoamyl alcohol (25:24:1, by vol.). The residual phenol was removed by extracting with chloroform-isoamylalcohol (24:1, v/v). Nucleic acids were purified, desalted and concentrated with UV-irradiated ultrapure Milli-Q water. The quality and quantity of
DNA was checked on a NanoDrop 2000c (ThermoScientific) by measuring the absorbance at 260 nm wavelength.

For efficient PCR amplifications, a second precipitation step was introduced in which the salt concentration was adjusted to 0.2 M with 5 M NaCl and isopropanol was used to precipitate DNA. In order to improve this process, a DNA carrier was used (0.5 µL of 20 mg mL\(^{-1}\) solution of glycogen). Samples were incubated overnight at 4°C and then purified with 70% ethanol. The samples were kept at -80°C until further analysis.

**PCR conditions**

For Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis, PCR amplification of the 16S rRNA gene was performed with fluorescently labelled primers. The Archaea-specific primers 21F-6FAM and 958R-VIC yielded an amplicon of approximately 915 bp and the Bacteria-specific primers 27F-6FAM and 1492R-VIC yielded an amplicon of approximately 1500 bp (Table 2). Each PCR reaction (50 µL) was comprised of 2 µL of DNA template, 28.8 µL of UV-irradiated ultrapure water (PCR Grade), 0.2 µL of Taq polymerase, 5 µL of the corresponding buffer, 4 µL of dNTPs, 1 µL of BSA, 8 µL MgCl\(_2\) and 0.5 µL (50 pmol final concentration) of both primers. PCR was performed in duplicate under the following conditions: initial denaturing step at 95°C (for 3 min), followed by 30 amplification cycles of denaturing at 95°C (for 30 sec), annealing at 55°C (for 30 sec), and elongation at 72°C (for 1.5 min). Cycling was completed by a final elongation step at 72°C (for 10 min), followed by cooling at 4°C until further analysis. The amplicons were checked on a 1% agarose gel and stained in a freshly prepared 250 mL 1x TAE buffer containing SYBR Gold (1:10,000) for 30 min. Duplicate PCR reactions were pooled and purified using the PCR Extract MiniKit (5 Prime) following the manufacturer’s instructions. The purified PCR amplicons were spectrophotometrically quantified with a NanoDrop and stored at -20°C for further analysis.
For Denaturing Gradient Gel Electrophoresis (DGGE), only bacterial 16S rRNA genes were amplified using Bacteria-specific primers 341F-GC and 907R yielding an amplicon of approximately 600 bp (Table 2). Each PCR reaction (50 µL) consisted of 2 µL of DNA template, 28.8 µL UV-irradiated ultrapure water (PCR Grade), 0.2 µL of Taq polymerase, 5 µL of the corresponding buffer, 4 µL of dNTPs, 1 µL of BSA, 8 µL MgCl₂ and 0.5 µL (50 pmol final concentration) of both primers. A touchdown PCR was performed in duplicates under the following conditions: initial denaturing step at 95°C (for 4 min), followed by 5 amplification cycles of denaturing at 95°C (for 30 sec), annealing at 60°C (for 30 sec), and elongation at 72°C for 1 min followed by 25 amplification cycles of denaturing at 95°C (for 30 sec), annealing at 55°C (for 30 sec), and elongation at 72°C for 1 min. Cycling was completed with a final elongation step at 72°C for 10 min, followed by cooling at 4°C until further processing. The amplicons were checked on a 1% agarose gel and stained in a freshly prepared 250 mL 1x TAE buffer containing SYBR Gold (1:10,000) for 30 min. Duplicate PCR reactions were pooled and stored at -20°C for further analysis. All PCR reactions were performed on an Eppendorf Mastercycler Pro S.

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

The amplified and purified 16S rRNA was used for microbial community fingerprinting by T-RFLP (Moeseneder et al. 2001). VIC and FAM-labelled PCR products were digested overnight at 37°C on an Eppendorf Mastercycler Pro S. Each digest contained 100 ng of purified PCR product, 0.2 µL of the restriction enzyme HhaI, and the recommended buffer (20 µL final reaction volume). For T-RFLP analysis, the product of the restriction digest was desalted with Sephadex (GE Healthcare Life Sciences, Sweden) and further denatured in the presence of 10 µL Hi-Di formamide (Invitrogen) per reaction at 95°C for 3 min. Additionally, each sample contained 0.3 µL of GeneScan 1200 LIZ (Applied Biosystems) marker for size determination of VIC and FAM fragments. For detection, the
samples were run on a capillary sequencer (Applied Biosystems) following the method of Moeseneder et al. (2001). The obtained electropherograms were first evaluated using the PeakScanner Software (Applied Sciences) and then analysed with GelCompar Software (Applied Maths, v.6.5) by converting electropherograms to virtual gel bands while normalizing and linking them to the internal standard (1200 LIZ). Any peaks smaller than 50 bp or larger than 1200 bp were considered as remains of the primer or undigested fragments and were therefore eliminated from further analyses. The resulting peaks of the labelled 16S rRNA fragments were binned according to the fragment length. Thresholds for assigning individual operational taxonomic units (OTUs) were as follows: within 1 bp difference for fragments shorter than 700 bp, ± 3 bp for fragments between 700-1000 bp, and ± 5 bp for fragments longer than 1000 bp (Hewson et al. 2006). Data was exported as presence/absence matrices (p/a) and further analysed with Primer6 software.

**Denaturing Gradient Gel Electrophoresis (DGGE)**

DGGE was performed using an INGENYphorU-2 System (Ingeny International BV) according to the manufacturer’s instruction manual. Glass plates were cleaned with 96% ethanol before gel casting. Depending on the concentration of the PCR products, 15-20 µL of the amplified sample was loaded on a 6% polyacrylamide gel containing gradients of 20% - 80% denaturants or 30% - 70% denaturants both polymerised with 50 µL of 20% ammonium persulphate and 5 µL of tetramethylethylenediamine. The gradients depended on the volume of 80% ureaformamide used while casting the gel. Electrophoresis was run at a constant voltage of 100 V at 60°C for 16 h in a 1x TAE buffer. The gels were scanned by a Gel Doc XR+ Molecular Imager (Bio Rad). Individual bands were excised, re-amplified and inspected by DGGE alongside the original sample. If the bands were at the correct height as the original samples, they were sequenced on a capillary sequencer (Applied Biosystems).
**Cloning and Sequencing**

Clone libraries of the 16S rRNA gene were established for two samples to obtain more detailed taxonomic information. PCR reactions were performed in duplicate, amplifying the bacterial 16S rRNA gene using the same conditions as described above (Table 2). Cloning reactions were performed with the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) using TOP10 *Escherichia coli* cells according to the manufacturer’s instructions. Transformed cells were plated on freshly prepared lysogeny broth (LB) plates containing 100 mg L\(^{-1}\) ampicillin and 40 µL of X-Gal (40 mg mL\(^{-1}\)) and incubated overnight at 37°C. After blue-white screening, 8 clones per sample were randomly picked with sterile toothpicks, transferred onto a LB plate (master-plate) and re-grown overnight at 37°C. For screening the correct insert size, M13 PCR was performed using vector-binding M13F and M13R primers (Table 2) under the following conditions: one initial denaturing step at 95°C (for 4 min), followed by 30 amplification cycles of a denaturing step at 95°C (for 30 sec), annealing at 60°C (for 30 sec), and elongation at 72°C (for 1 min), and a final elongation step at 72°C (for 10 min). Amplicons were visualized on a 1% agarose gel (SYBRGold staining), excised and sequenced on a capillary sequencer (Applied Biosystems). The sequences were aligned using MEGA 5 and compared to known sequences using the basic local alignment search tool (BLAST, [http://www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST), Altschul et al. 1990).

**454 Tag Sequencing**

Samples for tag pyrosequencing were sent to Research and Testing Laboratories (Lubbock, Texas, USA) where bacterial primers 28F and 519R were used for amplification of the highly variable regions V1-3 of the 16S rRNA gene (Table 2). Subsequent sequencing was performed on a Roche 454 FLX/FLX+ platform following the company’s standard protocol. Sequences were retained as standard flowgram format files (sff) and
further processed using two standard operation pipelines for 16S rRNA sequences. Following the QIIME protocol, the sequence reads were assigned to their nucleotide barcode and quality filtered by removing any low quality or ambiguous reads (Carporaso et al. 2010). In order to improve the data quality, a maximum of two primer mismatches was allowed and the minimum average quality score in one read was lowered to 23. For calculating diversity indices and the analysis of shared OTUs between water masses and incubations, the Mothur pipeline for 16S rRNA was used (Schloss et al. 2009). Again, a maximum of two primer mismatches was allowed.

**Calculations and Statistical Analysis**

\[ Q_{10} \]

The \( Q_{10} \) temperature coefficient for DIC fixation was calculated following the formula:

\[
Q_{10} = \left( \frac{R_2}{R_1} \right)^{\frac{10}{(T_2 - T_1)}}
\]

where \( R \) is the rate of the reaction and \( T \) is the temperature in degree Celsius. \( Q_{10} \) is unit-less as it is the factor by which a rate changes.

**Variability of OTUs per water mass**

The deviation (in %) was calculated for each community of a treatment pair (20°C versus in situ temperature incubations) and for each OTU as follows:

\[
\text{deviation(\%)} = \left( \frac{\bar{x}_{\text{WM}}}{\bar{x}_{\text{T}}} \times 100 \right) - 100
\]

where \( \bar{x}_{\text{WM}} \) is the relative abundance of a OTU in the 20°C incubation and \( \bar{x}_{\text{T}} \) is the mean relative abundance of this OTU in both incubations (Agogué et al. 2011).
Statistical analyses were performed with PRIMER 6 (NMDS, ANOSIM, SIMPER; Primer-E, Ltd) and using the vegan software package implemented in R (PCA; The R Foundation for Statistical Computing).
3. Results

Temperature dependency of DIC fixation

Prokaryotic chemosynthesis, as determined by DIC fixation, significantly increased at an incubation temperature of 20°C with values up to 230-times higher than at in situ temperature (ranging from 1-3°C). These high DIC fixation rates were measured for seawater samples collected throughout the water column in the eastern North Atlantic (Figure 2). Typically, $Q_{10}$ values range from 2-3 for biological systems. For the North Atlantic, $Q_{10}$ values for DIC fixation rates ranged from 3.8 to 292 with an average of $48.5 \pm 56.4$ (Figure 3).

In contrast, $Q_{10}$ values of DIC fixation rates in the Ross Sea averaged 2.01 ± 0.94 (range: 0.3 to 4.4, n=45, Figure 3). Neither in the Atlantic nor in the Ross Sea, the increase in DIC fixation was accompanied by a significant increase in prokaryote abundance over the 3-day period.

Prokaryotic community response to elevated temperature determined by T-RFLP or DGGE and cloning

Generally, the number of OTUs was lower for archaea as compared to bacteria in both the in situ temperature and the 20°C incubation experiments performed during the MEDEA-I and -II cruises. Several OTUs were identified which were notably abundant in the 20°C but absent in the in situ temperature incubations (Figure 4). No specific response of any of the archaeal OTUs, however, was detected in the 20°C incubations as compared to the in situ temperature incubations. Based on these results, the bacterial community composition was investigated in more detail by 454 tag sequencing (see below).

Using DGGE, the bacterial community of the Ross Sea off Antarctica clearly differed in the band pattern from the bacterial community of the Atlantic (Figure 5). For the MEDEA-I samples, some bacterial OTUs were present in 20°C incubations which were absent in the in situ temperature incubations.
situ temperature incubations. Bands from the incubations of NADW and MSOW of the 20°C treatment were excised and directly sequenced. When blasted, these sequences showed 99% similarity to an uncultured 16S rRNA clone of *Alcanivorax borkumensis*, an alkane-degrading Proteobacterium (Kasai et al. 2002). Clone libraries were created from these two samples, and eight clones were picked and sequenced for each water mass. Clone sequencing revealed a number of sequences affiliated to SAR324 (99% sequence similarity), *Chloroflexi* (98%), *Alcanivorax* sp. clone (97%) and *Oleiphilus* sp. (96%).

**454 Tag Sequencing**

After processing 454 sequence data with two standard pipelines for 16S rRNA (QIIME and Mothur), the trimmed sequence reads were assigned to OTUs (Table 4). The Chao and Ace estimators and the Shannon and Inverse Simpson diversity indices were calculated. Generally, there is a clear decrease in alpha-diversity of the bacterial community in the 20°C incubations compared to in situ temperature incubations. The Ross Sea bacterial community exhibited a low diversity in the in situ temperature incubations and an even lower diversity in the 20°C incubation. For the experiments with water collected from different water masses of the North Atlantic, the highest diversity was observed for the Mediterranean Sea Outflow Water for both incubation temperatures (Table 4).

As revealed by Bray-Curtis similarity analyses and visualized in a non-metric multidimensional scaling plot (Figure 6), the bacterial communities changed during the course of the incubation in the 20°C treatment leading to a distinctly different community than in the in situ temperature incubations at the end of the incubation period of 72 h (p=0.006, ANOSIM). In addition, the mean difference in Bray-Curtis similarities of the bacterial communities incubated at in situ temperature was 67.3 ± 7.3 and thus significantly different (p<0.001, Wilcoxon signed rank test) to the mean difference (47.1 ± 22.9) of the communities incubated at 20°C (Figure 7). When related to each other, the Bray-Curtis similarities showed
a significant correlation indicating that the underlying patterns of the observed community shift was similar in the incubations at in situ temperature and at 20°C (Rho=0.74, p=0.004, Mantel test). The main factors responsible for the shifts in the bacterial community were temperature (p=0.021) and DIC fixation rates (p=0.007) as revealed by principal component analysis.

Similarity percentage (SIMPER) was used to determine which individual sequence contributed most to the dissimilarity between the treatments. The taxa mainly responsible for the shift in the bacterial community in the 20°C treatment were identified as *Pelagibacteracea* (contributing 38.6% to the observed dissimilarity), *Oleiphilacea* (contributing 17.1%), SAR324 (7.4%), *Alcanivorax* (3.8%), and SAR202 (3.1%) (Table 3). Thus, these species together accounted for a change of 70% from the mean relative abundance and were therefore taken into further consideration (Table 3). The % deviation of the relative OTU abundance in the 20°C incubations calculated from all sequence abundances of both treatments was +76.8-100% in relative abundance of *Oleiphilacea* in the bacterial community of all the water masses, as well as an increase of 90.4-98.7% in *Alcanivorax* in all samples except in the bacterial community of the oxygen minimum zone (OMZ) and the Labrador Sea Water in the 20°C treatments. In contrast, SAR324 and SAR202 decreased in relative abundance by 23.0-99.6% and 1.8-100%, respectively, in all the bacterial communities collected from all the water masses incubated at 20°C. The relative abundance of *Pelagibacteracea* decreased in the 20°C incubations in the bacterial communities collected in all the water masses except for a slight increase in the OMZ (0.5%) and Mediterranean Sea Outflow Water (1.8%). The bacterial community of the Ross Sea exhibited generally larger fluctuations in the 20°C incubations than the bacterial communities of the North Atlantic water masses (Figure 8).
4. Discussion

Changes in the microbial community upon increasing the temperature

The clear differences observed between the in situ temperature and the microbial communities incubated at 20°C as obtained by both fingerprinting techniques and sequence data, are partially due to the loss in alpha diversity as seen by the Inverse Simpson Index (Table 4). Interestingly, this did not lead to a significant change in prokaryotic abundance. Previously active microbes might become inactive under these specific conditions while previously inactive ones might become active. This would lead to a stable microbial abundance while inducing shifts in the community composition.

While elevated DIC fixation rates and high $Q_{10}$ values (average 48) were obtained for the microbial communities throughout the North Atlantic, similar responses were not detected for the microbial community of the Ross Sea. The Ross Sea bacterial community was very different from the North Atlantic bacterial communities (Figure 5) and was generally lower in diversity in both treatments (the in situ temperature and the 20°C incubations) than the North Atlantic bacterial communities. Distinct meridional gradients in surface properties separate waters of the Southern Ocean around Antarctica from the warmer and saltier waters of the subtropical circulations (Orsi et al. 1995). The eastward flow of the Antarctic Circumpolar Current south of the Subtropical Front is driven by the world’s strongest westerly winds (Trenberth et al. 1990). Thus, the Antarctic Circumpolar Current efficiently isolates the waters around Antarctica from the waters further north, extending into the bathypelagic zone (Trenberth et al. 1990). Hence, these (sub)polar Antarctic waters exhibit physical, chemical and (micro)biological characteristics distinctly different from the waters adjacent to the north (Piola et al. 1982, Orsi et al. 1995).
Beta-diversity of communities

Although the composition of the bacterial communities changed when incubated at 20°C, this did not lead to a convergence of these communities collected from different water masses (Figure 7). By relating those two incubations to each other, it becomes clear that the distance between the communities decreased but the structure of the bacterial communities incubated at in situ temperature remained similar, indicating that the communities that were distant at in situ temperature remained distant at 20°C. Temperature is generally considered to be a major selection factor of microbial communities. The commonly observed depth stratification of microbial communities reported for the global ocean is assumed to be mainly mediated by temperature in addition to substrate availability (DeLong et al. 2006, Giovannoni et al. 2005). As the bacterial assemblages are water mass-specific, different bacterial taxa might respond to elevated temperature in the different water masses explaining our observations.

Taxa-specific response to elevated temperature

Five taxa were responsible for 70% of the shift in the bacterial community at elevated temperature (Table 3). *Pelagibacteraceae*, the SAR11 clade, contributed most to these changes (38.6%). The SAR11 clade is a lineage ubiquitously present in the marine environment, estimated to account for 25-50% of all prokaryotic cells in the ocean (Morris et al. 2002). The relative sequence abundance of the SAR11 clade decreased in all the incubations with water from nearly all water masses (Figure 8). SAR324 and SAR202 are both typical deep-water bacteria increasing in their relative contribution to the overall bacterial abundance with depth (Varela et al. 2008; Agogué et al. 2011; Lekunberri et al. 2013), indicating their adaptation to low temperature. This might explain their decrease in relative sequence abundance in the 20°C incubations.
Oleiphilacea and Alcanivorax are both Gammaproteobacteria belonging to the same order of Oceanospirillales. Analyses of single amplified genomes from the mesopelagic Atlantic Ocean, screened for several key genes mediating DIC fixation and chemolithoautotrophic energy acquisition, indicated that Oceanospirillales possesses genes for both DIC fixation and sulphur oxidation (Swan et al. 2011). Sequences coding for the large subunit of ribulose-1,5-bisphosphate-carboxylase-oxygenase (RuBisCO), the key enzyme mediating the Calvin-Benson-Bassham cycle, were recovered in 25% of Gammaproteobacteria. RuBisCO-containing bacterial lineages are found throughout the dark oxygenated water column. The energy source of these chemoautotrophs is reduced inorganic sulphur, which is rare in the water column. Therefore, it is not clear what the sources of reduced sulphur compounds are for chemolithotrophic energy acquisition (Walsh et al. 2009, Swan et al. 2011). Our Oleiphilacea sequence obtained from the incubations at 20°C is only 87% similar to the sequence described in Swan et al. (2011). However, when compared to sequences from the Oleiphilacea family that are known to be heterotrophic (Golyshin et al. 2002), the similarity varies between 84-89%. Judging from these results our obtained sequences indicate high metabolic plasticity in the order of Oceanospirillales as some members show potential for chemoautotrophy while Alcanivorax sp. clearly shows a heterotrophic metabolism. This could indicate that some species of this order are mixotrophs and have the potential for autotrophic CO₂ fixation with coupled oxidation of reduced sulphur compounds (Swan et al. 2011).

Although of the Q₁₀ value for DIC fixation of the microbial community of the Ross Sea varied only from 2-3 and thus shows a commonly observed temperature dependency of biological activity, sequence data indicate an increase in the same taxa as for the North Atlantic water masses. The relative abundance of Oceanospirillales increased from 17.8% in the in situ temperature incubation to 60.8% at 20°C. From these 60.8%, 53.1% are due to an
increase in the relative abundance of *Alcanivorax* and 3.0% due to an increase in *Oleiphilus*. This provides further evidence for a significantly different bacterial community composition in the Ross Sea compared to the North Atlantic incubations. Additionally, the increase in the relative abundance of *Oleiphilus* in the 20°C incubations was substantially lower in the Ross Sea than in the Atlantic water masses (17.3% ± 11.3) highlighting the potential for DIC fixation by this group.

**Archaea**

Archaea constitute a significant component of the prokaryotic plankton in the ocean (DeLong et al. 1992; Fuhrman et al. 1992) and quantitative studies revealed that *Thaumarchaeota* might account for ~20% of all prokaryotic cells in the global ocean (Karner et al. 2001). Several key features for autotrophy such as ammonia oxidation in *Nitrosopumilus maritimus* (Könneke et al. 2005) and the presence of ammonia monooxygenase in *Cenarchaeum symbiosum* (Hallam et al. 2006) have been found in archaea affirming the potential of inorganic carbon fixation in this domain. However, there is evidence that some *Thaumarchaeota* are likely heterotrophs or mixotrophs, utilizing organic carbon such as amino acids (Ouverney & Fuhrman 2000; Teira et al. 2006; Varela et al. 2008; Reinthaler et al. 2010). Studies in the dark ocean of the Atlantic have shown that even though *Thaumarchaeota* contribute up to 20% to the prokaryotic abundance, they might contribute less to the bulk DIC fixation than bacteria (Varela et al. 2008). Additionally, growth rates of archaea are about one magnitude lower than those of the total prokaryotic community in the meso- and bathypelagic North Atlantic (Herndl et al. 2005). When applied to our results of the incubation experiments, this might explain why there were no significant changes in the archaeal community identified by T-RFLP during the course of the incubations.
Anaplerotic pathway

Bacterial uptake of inorganic carbon might indicate the presence of chemoautotrophic bacteria as discussed above or an anaplerotic metabolism responsible for the uptake of CO₂. Laboratory studies have shown that the anaplerotic uptake of CO₂ might be responsible for 1-8% of the cellular carbon in bacteria if easily metabolizable substrate is available (Roslev & Larsen 2004; Reinthaler et al. 2010). Since the DOM pool in the dark ocean is considered refractory, anaplerotic reactions likely play only a minor role in the DIC uptake of bacteria in the dark ocean (Ashworth et al. 1966; Roslev & Larsen 2004; Herndl et al. 2005; Reinthaler et al. 2010). Presumably, anaplerotic metabolism is also to some degree temperature-dependent. Taken together, it appears unlikely that anaplerotic metabolism strongly influenced the measured DIC fixation rates in our experiments.

DIC fixation and energy acquisition

The high DIC fixation rates measured at elevated temperature in our experiments require energy sources to fuel the associated metabolism. Based on our incubation experiments, we conclude that there are energy sources available in the deep ocean fuelling these high levels of DIC fixation at elevated temperatures which are only inefficiently used or not accessible at the in situ temperature of around 1-3°C. Q₁₀ values for the DIC fixation in our incubations averaged 48.5, which is substantially higher than the typical Q₁₀ values of 2-3 commonly reported for biological systems (Skyring 1987, Westrich 1988) supporting the view that reduced inorganic energy sources are accessible at elevated temperature. Overall, the nature of the energy sources thriving the observed high Q₁₀ values of DIC fixation remains enigmatic.
5. Conclusion

A significant shift in the bacterial community composition was observed after increasing the temperature to 20°C in incubation experiments with deep ocean microbial communities commonly thriving at in situ temperatures of around 2°C. This elevated temperature, however, did not lead to a convergence in the community composition in natural microbial communities collected from several major deep-water masses. Sequencing results indicate a high metabolic plasticity in the gammaproteobacterial group of *Oceanospirillales*. $Q_{10}$ values of around 50 for DIC fixation in deep ocean microbial communities have been obtained in this study throughout the Atlantic deep waters but not in the waters of the Ross Sea, Antarctica. These high $Q_{10}$ values indicate that the increase in temperature activated parts of the microbial community not active under in situ temperature conditions or alternatively, that anaplerotic reactions of the heterotrophic microbial community are greatly stimulated under elevated temperature conditions. Albeit the exact mechanisms driving this enhanced DIC fixation remain enigmatic, this newly discovered metabolic process of dark-ocean bacteria calls for more detailed studies on this new facet of dark-ocean microbes particularly on the differences in the $Q_{10}$ values of DIC fixation in microbial communities of deep Atlantic and the waters around Antarctica.
6. **Tables and Figures**

**Table 1** Depth and temperature of the water masses sampled for the incubation experiments under in situ temperature and at 20°C.


<table>
<thead>
<tr>
<th>Water mass</th>
<th>Depth (m)</th>
<th>In situ potential T (°C)</th>
<th>Cruise</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISOW</td>
<td>2000</td>
<td>3.0</td>
<td>Medea II</td>
</tr>
<tr>
<td>NACW</td>
<td>400</td>
<td>4.9</td>
<td>Medea II</td>
</tr>
<tr>
<td>LSW</td>
<td>1800</td>
<td>3.5</td>
<td>Medea II</td>
</tr>
<tr>
<td>OMZ</td>
<td>512</td>
<td>8.4</td>
<td>Medea II</td>
</tr>
<tr>
<td>MSOW</td>
<td>1200</td>
<td>7.0</td>
<td>Medea I</td>
</tr>
<tr>
<td>NADW</td>
<td>2750</td>
<td>2.5</td>
<td>Medea I</td>
</tr>
<tr>
<td>AAW</td>
<td>275</td>
<td>-3.0</td>
<td>Tracers</td>
</tr>
</tbody>
</table>
**Table 2** Archaeal and bacterial primers used for the amplification of 16S rRNA genes and the TOPO® TA® Cloning product.

<table>
<thead>
<tr>
<th>Method</th>
<th>Target</th>
<th>Primer</th>
<th>Sequence 5’ to 3’</th>
<th>Annealing temperature (°C)</th>
<th>Company</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Archaea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRFLP</td>
<td>16S rRNA</td>
<td>21F-6FAM</td>
<td>TCCGTTGTAGCCTCGGCCGGA</td>
<td>55</td>
<td>ThermoScientific</td>
<td>DeLong, 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>958R-VIC</td>
<td>YCCGGCTGGATCGTCAAATT</td>
<td>55</td>
<td>ThermoScientific</td>
<td>DeLong, 1993</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRFLP</td>
<td>16S rRNA</td>
<td>21F-6FAM</td>
<td>AGAGTTTGATCCTTGCTCAG</td>
<td>55</td>
<td>ThermoScientific</td>
<td>DeLong, 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1492R-VIC</td>
<td>GCTTACCTTTCTACGACTT</td>
<td>55</td>
<td>ThermoScientific</td>
<td>DeLong, 1993</td>
</tr>
<tr>
<td>DGGE</td>
<td>16S rRNA</td>
<td>341F-GC</td>
<td>CGCCCGCAGCGCCGAGGCGCCGAGCAG</td>
<td>65</td>
<td>ThermoScientific</td>
<td>Muyzer et al. 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>907R</td>
<td>CGCCGCTAGTTCTGAGTTT</td>
<td>65</td>
<td>ThermoScientific</td>
<td>Muyzer et al. 1994</td>
</tr>
<tr>
<td>Cloning</td>
<td>pCR8®/GW/TOPO® vector</td>
<td>M13F</td>
<td>GAAAACGACCCGCAG</td>
<td>60</td>
<td>Invitrogen</td>
<td>Invitrogen TO-PO®TA®Kit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M13R</td>
<td>CAGGAAACAGCTATGAC</td>
<td>60</td>
<td>Invitrogen</td>
<td>Invitrogen TO-PO®TA®Kit</td>
</tr>
<tr>
<td><strong>454 Tag Sequencing</strong></td>
<td>16S rRNA</td>
<td>28F</td>
<td>GAGTTGTATCCTTGGGCTCAG</td>
<td>PCR performed by company</td>
<td>Provided by Research and Testing Laboratories</td>
<td>Lane et al. 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>519R</td>
<td>GTNTACNGGGCGGKCTG</td>
<td>PCR performed by company</td>
<td>Provided by Research and Testing Laboratories</td>
<td>Turner et al. 1999</td>
</tr>
</tbody>
</table>
Table 3 Percentages of contribution to the dissimilarity of the bacterial community in the 20°C treatment, calculated by Similarity percentages (SIMPER) analysis. Cumulative percentages demonstrate the increasing cumulative % of dissimilarity.

<table>
<thead>
<tr>
<th>Bacterial taxa</th>
<th>Contribution%</th>
<th>Cumulative%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelagibacteraceae</td>
<td>38.6</td>
<td>38.6</td>
</tr>
<tr>
<td>Oleiphilaceae</td>
<td>17.1</td>
<td>55.6</td>
</tr>
<tr>
<td>SAR324</td>
<td>7.4</td>
<td>63.1</td>
</tr>
<tr>
<td>Alcanivorax</td>
<td>3.8</td>
<td>66.9</td>
</tr>
<tr>
<td>SAR202</td>
<td>3.1</td>
<td>70.0</td>
</tr>
</tbody>
</table>

Table 4 Sequence Information and Diversity indices obtained by 454 tag sequencing. Total reads were assigned to OTUs after trimming. The Chao and ACE estimate predict an average number of OTUs per sample. The Shannon and the Inverse Simpson Index are both measures of alpha-diversity.

Cluster distance 0.03

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Total Reads</th>
<th>Assigned OTUs</th>
<th>Chao</th>
<th>Ace</th>
<th>Shannon</th>
<th>Inverse Simpson</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMZ in situ</td>
<td>16491</td>
<td>1896</td>
<td>2812.11</td>
<td>3268.71</td>
<td>6.11</td>
<td>134.69</td>
</tr>
<tr>
<td>OMZ 20°C</td>
<td>6947</td>
<td>795</td>
<td>1126.24</td>
<td>1116.81</td>
<td>4.8</td>
<td>15.73</td>
</tr>
<tr>
<td>LSW in situ</td>
<td>2723</td>
<td>312</td>
<td>401.18</td>
<td>416.9</td>
<td>4.64</td>
<td>37.75</td>
</tr>
<tr>
<td>LSW 20°C</td>
<td>10848</td>
<td>873</td>
<td>1335.56</td>
<td>1602.08</td>
<td>4.84</td>
<td>23.4</td>
</tr>
<tr>
<td>NACW in situ</td>
<td>732</td>
<td>232</td>
<td>387.89</td>
<td>529.67</td>
<td>4.82</td>
<td>71.82</td>
</tr>
<tr>
<td>NACW 20°C</td>
<td>1462</td>
<td>385</td>
<td>740.11</td>
<td>1068.26</td>
<td>4.88</td>
<td>35.09</td>
</tr>
<tr>
<td>ISOW in situ</td>
<td>1743</td>
<td>407</td>
<td>589.68</td>
<td>565.77</td>
<td>5.22</td>
<td>71.07</td>
</tr>
<tr>
<td>ISOW 20°C</td>
<td>911</td>
<td>175</td>
<td>233.29</td>
<td>234.98</td>
<td>4.08</td>
<td>17.4</td>
</tr>
<tr>
<td>NADW in situ</td>
<td>1811</td>
<td>523</td>
<td>805.12</td>
<td>840.58</td>
<td>5.64</td>
<td>165.2</td>
</tr>
<tr>
<td>NADW 20°C</td>
<td>11892</td>
<td>1472</td>
<td>2247.89</td>
<td>2730.13</td>
<td>5.7</td>
<td>68.81</td>
</tr>
<tr>
<td>MSOW in situ</td>
<td>2209</td>
<td>566</td>
<td>822.48</td>
<td>802.93</td>
<td>5.76</td>
<td>181.55</td>
</tr>
<tr>
<td>MSOW 20°C</td>
<td>1641</td>
<td>520</td>
<td>853.88</td>
<td>864.44</td>
<td>5.58</td>
<td>120.81</td>
</tr>
<tr>
<td>AAW in situ</td>
<td>8893</td>
<td>437</td>
<td>885.76</td>
<td>1119.42</td>
<td>3.84</td>
<td>12.53</td>
</tr>
<tr>
<td>AAW 20°C</td>
<td>4242</td>
<td>121</td>
<td>169</td>
<td>181.45</td>
<td>1.93</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Figure 1. Location of samples collected at the MEDEA-I cruise (indicated by full circle) in October and November 2011 and MEDEA-II cruise (indicated by full triangles) in June and July 2012.
Figure 2. Distribution of DIC Fixation rates throughout the water column of the Atlantic Ocean measured at in situ temperature and at 20°C. Water samples were collected during the MEDEA I and II cruise.
Figure 3. $Q_{10}$ values for DIC fixation rates of all cruises sampled. The grey bar indicates a $Q_{10}$ of 3 representing typical values for biological systems.
Figure 4. Presence/absence matrix of bacterial OTUs obtained by T-RFLP of 16S rRNA gene in incubations at in situ temperature and at 20°C of microbial communities collected from different water masses in the Atlantic during the MEDEA-I and -II cruise. Highlighted in red are OTUs present in the 20°C incubations but absent in those at in situ temperature. For abbreviations of water masses see Table 1.
Figure 5. Band pattern obtained by DGGE gel of bacterial 16S rRNA genes from the community obtained in incubations at in situ temperature and at 20°C. AA#3 represents Antarctic Mode Water.
Figure 6. Ordination of bacterial communities by non-metric Multidimensional scaling analysis of 454 tag sequence data based on Bray Curtis similarities. Communities are plotted according to water masses and incubations; blue symbols represent communities incubated at in situ temperature, red symbols indicate bacterial communities obtained from 20°C incubations. The water mass from where the bacterial community has been collected is also indicated. For abbreviations of the water masses see Table 1.
Figure 7. Similarity of communities plotted as the mean distance of similarity to each other compared to mean distances in communities incubated 20°C.
Figure 8. Taxa-specific response to the incubation temperature for the 5 bacterial taxa contributing most to the shift in the community from in situ temperature to 20°C. The deviation (in %) from the mean relative abundance for the 20°C incubation was calculated for the individual water masses. For abbreviations of the water masses see Table 1.
7. Acknowledgements

I would like to thank my supervisor Gerhard J. Herndl for giving me the opportunity to be part of his team. Thank you very much for always having an open door for us students. I would like to thank Roberta L. Hansman for guidance throughout my thesis and many fruitful discussions about this interesting project. Many thanks to Christian Baranyi, for answering all my, sometimes very abstract, questions concerning lab work or statistics, thank you for that! Thanks to Daniele De Corte for helping me with my sequence data. Thanks to Alex Frank and Thomas Reinthaler for sending my samples with their batch for sequencing. Thanks to everyone else in the Department of Limnology and Bio-Oceanography for the great time in the lab or at lunchtime, I always enjoyed the nice atmosphere with you.

I would also like to thank the persons who made this all possible, my parents for their unconditional support in everything I do. I would like to thank my sister for her critical approach to science and her very helpful comments. I would also like to thank my friends for being there for me and for sharing this great year with me.

Thanks to my best ecologist Jörg for being as sophisticated and inspirational. This would not have been as much fun without Anna T. who was always there for a quick coffee and a stand-up-talk.

Financial support for this MSc thesis work was provided by the European Research Council under the European Community’s Seventh Framework Program (FP7/2007-2013) / ERC grant agreement No. 268595 (MEDEA project) to GJH and the Austrian Science Fund (FWF) projects: I486-B09 and P23234-B11 to GJH.
8. References


Zusammenfassung

Curriculum Vitae

Pavla Debeljak

Education and Academic degree

2011 – 2014 Master Studies Ecology at the University of Vienna, specialization on Microbial Oceanography


2012 – Master Studies in Gender studies specializing on Science, Technology and Society at the University of Vienna

2011 – 2012 Erasmus-Exchange semester in Rennes, France

2007 – 2011 Bachelor Studies of Biology at the University of Vienna specialization on Ecology. Bachelor thesis “Effect of nutrients on the respiration of aquatic sediment microorganisms”

04.06.2007 Graduation at the Gymnasium Biondekgasse Baden

1999 – 2007 Gymnasium BG & BRG Baden Biondekgasse

1995 – 1999 Primary school in Baden

Training and courses followed

February 2013 Experimental Design and Statistical Methods in Limnology, Introducing the R Project

September 2012 Field Course in Marine Ecology at the Royal Netherland Institute of Sea Research (NIOZ), Texel, The Netherlands

July 2012 Course in Phylogeny of Prokaryotes, Introduction to ARB

July 2011 Introductory course to Mediterranean ecosystems at the Ruder Bošković Institute, Rovinj, Croatia

February 2011 Fluorescence-in-situ Hybridisation Course at the Department of Microbial Ecology, University of Vienna

December 2012 Course in Stable Isotope Probing at the Department of Terrestrial Ecosystem Research, University of Vienna

May 2010 Bachelor thesis at the former Department of Limnology, University of Vienna. Field work at Weidlingbach, Lower Austria

April 2010 Introduction to Methods of Marine Biology at the Marine Biology Station in Piran, Slovenia
August 2009  Field Course in Western Siberia “From the steppe zone to tundra”, exploring carbon and nitrogen cycle in arctic ecosystems

Additional research experience

Since March 2013  Teaching assistant for the undergraduate lecture “Introductory lecture in Biology” in the field of Ecology

July 2013  Summer School on the Diversity of Marine Plankton (DIMAPLAN), Villefranche-sur-Mer, France

June 2012  Workshop “Epigenetics, Society and Gender”, University of Vienna

2010 – 2011  Laboratory work at the Department of Ecosystem-Ecology, University of Vienna

July 2010  Introduction to Methods in Ichthyology at the Institute of Oceanography and Fisheries in Split, Croatia

Skills and Qualifications

- It-Skills: Good knowledge of Microsoft Office
- Statistics: Basic knowledge of SPSS and R
- Language qualification:
  Croatian, German: Mother tongues (bilingual)
  English: fluent spoken and written English
    • CAE, Cambridge Certificate of Advanced English, 2007
    • October 2010 - January 2011: Writing and Speaking Scientific English Course (Timothy Skern), University of Vienna
  French: fluent spoken and written French (European C2-Level)
  Italian: basic knowledge (European A2-Level)