Summary

Archaea form one of the three domains of life on Earth and together with the bacteria form the prokaryotes. The application of molecular techniques such as ribosomal DNA or lipid analyses revealed that Archaea appear widespread and abundant. In the marine environment planktonic Archaea consist of two major groups, the Crenarchaeota and the Euryarchaeota of which the former appears to be the most abundant and may account for ca. 20% of all prokaryotic cells in the global ocean. Despite the fact that marine Crenarchaeota constitute a substantial fraction of the picoplankton in the world oceans, little is known about their basic physiology. Results of stable isotope and radiocarbon analyses of the marine crenarchaeotal membrane lipids suggested that marine Crenarchaeota may utilize bicarbonate as their carbon source. Microautoradiography investigations performed in the neritic waters of the Mediterranean Sea, the Californian Coast and the deep Atlantic Ocean showed that some marine Archaea are capable to take up amino acids and some deep sea marine Crenarchaeota and Euryarchaeota are capable to utilize bicarbonate or CO_2 as their carbon source. Field studies in the Santa Barbara Channel and the Arabian Sea showed a positive correlation of the abundance of Crenarchaeota and nitrite concentrations. In Arctic waters a positive correlation of marine Crenarchaeota with particulate organic nitrogen was reported. This suggested that marine Crenarchaeota may be involved in the marine nitrogen cycle but the metabolic requirements of pelagic Archaea remain still enigmatic.

The membrane lipids of Crenarchaeota are unique and consist of glycerol dibiphytanyl glycerol tetraethers (GDGTs). Recent studies done on core top sediments showed that the distribution of sedimentary crenarchaeotal GDGTs from different geographic locations varies with sea surface temperature (SST). The change of the GDGT distribution of marine Crenarchaeota was expressed in an index of GDGT isomers, which was named the Tetraether Index of lipids with 86 carbon atoms, the TEX_{86}. GDGTs are preserved upon sedimentation and were found in sediments up to 140 million years old. Therefore this new index was considered as a new temperature proxy for reconstruction of SST in paleoenvironments. However, the observed correlation of the GDGT distribution in core top sediments with SST does not provide direct evidence that marine Crenarchaeota adjust their membrane lipids to

temperature or that temperature is the only controlling factor. Salinity and nutrient concentrations can vary to a substantial degree in marine systems and may also influence the GDGT distribution. Furthermore, marine Crenarchaeota occur highly seasonal in surface waters and are generally relatively more abundant in the deeper zones of the ocean. However, the new temperature proxy TEX_{86} correlates best with annual mean SST. Therefore, it is as yet not clear during which season and at which depth in the water column the lipid signal used for TEX_{86} paleothermometry is biosynthesized.

In this thesis we focused on two major objectives. The first objective was to shed more light on the basic physiology of marine Crenarchaeota and addreses the ecological and physiological aspects of the marine Crenarcheota (**Chapter 2**, **3** and **4**). The second objective was to validate the newly introduced temperature proxy TEX_{86} (**Chapters 5**, **6** and **7**). This part deals with factors which determine the relative GDGT distribution in marine Crenarchaeota and the GDGT signal found in the water column and surface sediment.

In **Chapter 2** an *in situ* ^{13}C bicarbonate labeling experiment to study the carbon acquisition mechanism of marine Crenarchaeota is described. Fully labeled $H_2{}^{13}CO_3$ was added to a mesocosm tank filled with coastal North Sea Water and incubated for a week. The experiment was performed in the dark to eliminate photosynthetic activity. The distribution of the label in the various lipids was studies and revealed that very low ^{13}C incorporation was detected in lipids derived from bacterial and plant material. Especially the *anteiso*-C_{15} fatty acid, a compound exclusively attributed to bacteria, showed minor ^{13}C enrichment with $\Delta\delta^{13}C$ values of 10‰. In contrast, the crenarchaeotal membrane lipids were heavily labeled in ^{13}C with $\Delta\delta^{13}C$ values between 400-440‰ for the crenarchaeotal biphytanes. About 70% of the detected ^{13}C incorporation into lipids is accounted for by the crenarchaeotal biphytane membrane lipids. This showed that marine Crenarcheota are actively incorporating $H_2{}^{13}CO_3$ or $^{13}CO_2$ derived thereof and that at least some pelagic marine Crenarchaeota are thus autotrophic organisms.

In **Chapter 3** a combined study on an enrichment culture of a marine Crenarchaeota and a field time series observation was conducted to gain more information about the energy source of marine Crenarcheota and their possible involvement in the marine nitrogen cycle. In the enrichment culture an increase in crenarchaeotal abundance was observed when the ammonia level dropped and the nitrite concentration strongly increased. This data suggested

that the marine crenarchaeotal species which proliferated under laboratory conditions is capable of ammonia oxidation. Further evidence for archaeal nitrification was obtained by quantification of an archaeal *amoA*-like gene with quantitative polymerase chain reaction (Q-PCR). A strong correlation (r=0.99) between the crenarchaeotal abundance and the archaeal *amoA*-like gene copy number abundance was observed during the course of the experiment. The involvement of Crenarcheota in nitrification processes was further investigated in coastal North Sea waters. In the winter season the marine Crenarchaeota dominate the archaeal community and show a strong positive correlation with nitrite concentrations. In contrast, the cell abundances of beta-and gamma-proteobacteria, the known nitrifying bacteria, was invariant and did not show a correlation with the generation of nitrite. Thus, the data obtained from the enrichment experiment and from the field studies indicate that marine Crenarchaeota are active in the marine nitrogen cycle as nitrifiers.

In **Chapter 4** the seasonal dynamics of marine Archaea in costal North Sea surface waters during a time series is described by using a wide array of molecular approaches. The identity of the marine Crenarchaeota was determined by PCR using a general archaeal primer par followed by denaturing gradient gel electrophoresis (DGGE) in combination with sequencing. Three different quantification methods were used, catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH), Q-PCR and GDGT lipid analyses, to determine the abundance of the marine Archaea and to compare the relative robustness of the methods. The abundance pattern of CARD-FISH and Q-PCR were similar and indicated that one archaeal cell contains on average 0.9 ± (0.6) copy numbers of 16S rRNA genes. GDGT concentrations and crenarchaeotal cell abundances determined by CARD-FISH indicated the source organisms for GDGT membrane lipids in oxygenated waters are indeed restricted to marine Crenarchaeota. Comparison of the cell counts and the lipid analyses revealed that one crenarchaeotal cell contains on average 3 (± 0.5) pg GDGTs. A distinct seasonal distribution pattern of pelagic marine Crenrchaeota was observed with the CARD-FISH, GDGT and DGGE approach. In the winter season, when nutrient levels are elevated in the water, the marine Crenarchaeota dominate the archaeal community whereas the marine Euryarchaeota are more abundant during summer and fall when nutrient levels are lower. Different metabolic requirements of both archaeal groups probably cause this seasonal succession. The data

suggests that at least some marine pelagic Crenarchaeota are chemoautotrophic while some pelagic Euryarchaeota are probably heterotrophic organisms.

Chapter 5 describes a study which shows that temperature is the major controlling factor in the marine crenarchaeotal GDGT distribution. Mesocosm studies with marine Crenarchaeota incubated at temperature ranging from 5 to 35ºC and salinities of 27‰ and 35‰ were performed to test the validity of the TEX_{86} proxy. During the course of the experiment an increase in the concentration of GDGTs was measured with amounts up to 3.4 $\mu g\ l^{-1}$, indicating substantial growth of marine Crenarchaeota. With increasing temperature an increase of cyclopentane-ring containing GDGTs was observed. Different salinities did not have an effect on the GDGT distribution. The obtained TEX_{86} values of the different incubation temperatures showed a linear correlation which had a similar slope to the core top correlation, but the intersection of the correlation line with the Y-axis was different. This was probably caused by the differences between the crenarchaeotal species enriched under laboratory conditions and the more complex crenarchaeotal communities in the field. These mesocosm experiments indicated that water temperature is indeed the major controlling factor for the GDGT distribution of marine Crenarchaeota, and confirms that the TEX_{86} reflects temperature.

In **Chapter 6** suspended particulate organic matter (POM) samples from surface and deep waters at different oceanic settings were analyzed for their GDGT distribution to find out at which season and at which depth the GDGT signal for the TEX_{86} is produced. This investigation showed that GDGTs occur in higher abundance during the winter and early spring in surface waters. The depth distribution showed that GDGTs are generally more abundant below 100 m depth. A weak correlation of the TEX_{86} from below the photic zone with in situ temperature was observed. However, the TEX_{86} from deeper parts of the water column correlated best with SST. The TEX_{86} from POM in the upper 100m of the water column showed a linear correlation with in situ temperatures which was nearly identical to the core top equation. All POM samples correlated best with SST and were similar to the core top equation but with a lower correlation coefficient probably due to scatter of a mixed signal from living suspended Crenarchaeota and fossil sedimenting GDGTs. These data suggest that the GDGT signal which is transported to the sediment mainly reflects temperatures of the upper 100 m. In the upper water column an active food-web exists and therefore the GDGTs

from the upper parts of the ocean are probably more effectively transported to the sediment by grazing and repackaging

In **Chapter 7** a study on sedimenting particles from the northeast Pacific and the Arabian Sea was performed to analyze the seasonal and spatial distribution of the GDGT fluxes and the TEX_{86} signal transported to the sediment. At the northeastern Pacific studies were done on GDGT fluxes in different depth and size classes and reveled that the TEX_{86} stays relatively invariant in different size fractions of descending particles. This suggests that alteration due to grazing and re-packaging of sedimentary GDGTs by zooplankter do not substantially influence the GDGT distribution and thus the TEX_{86}. In both settings the TEX_{86} signal in the sediment traps at all depths reflect SST. In the shallow trap at 500 m in the Arabian See time series a strong correlation of the TEX_{86} with seasonal SST was observed. The TEX_{86} signal in the deeper traps was relatively invariant during the annual cycle and reflects annual mean SST. In the deeper traps the TEX_{86} signal is probably strongly influenced by lateral transport of particles. This suggests that the TEX_{86} in deeper waters or sediments probably reflects an integrated annual SST rather than a seasonal temperature signal.

The experimental work and the field studies presented in this thesis brought new insights in the physiology of marine Crenarchaeota and the new paleothermometer TEX_{86}. With the ^{13}C label experiment, the enrichment culture and the field observation in the North Sea it has been shown that at least some marine Crenarchaeota are chemolithoautotrophic organisms involved in nitrification. Since marine Crenarchaeota are one of the most abundant classes of picoplankton in the world ocean and occur over a large depth range, marine crenarcheotal chemolithoautotrophy may be a general and quantitatively important oceanic process. Marine crenarchaeotal nitrification may play an important role in the biochemical cycling of nitrogen in the ocean. Furthermore, marine Crenarchaeota are probably a significant global sink for inorganic carbon and may represent an important but as yet unrecognized component of the global carbon cycle.

In case of the TEX_{86}, the experimental work and the field studies validate the newly introduced temperature proxy. The mesocosm experiments demonstrated that temperature is the major controlling factor in the crenarchaeotal GDGT distribution, thus the TEX_{86} reflects temperature. The field studies done on POM and trap samples clearly showed that the GDGT signal which reaches deep waters or the sediment reflects SST or temperature from the upper

100 m of the water column due to grazing and repackaging processes. Therefore it should be possible to use the newly introduced paleothermometer TEX_{86} for reconstruction of the upper water column temperatures in ancient environments. Since GDGTs are found in sediments up to a 140 million years old this new temperature proxy may have a great potential as a tool for ancient climate reconstruction.