CHAPTER 6

SYNTHESIS

Anouk M.-T. Piquet

Despite their microscopic size, which might suggest that marine microbes only fulfill a modest role in marine ecosystems, nothing less is true. These tiny organisms are the motors of all global biogeochemical cycles. On the one hand photoautotrophs ("light utilizing, inorganic carbon, feeders") are responsible for most of the oceanic primary productivity and on the other hand heterotrophs ("organic carbon, feeders") are responsible for "sustained activity" by efficiently recycling a large fraction of the produced organic material and simultaneously releasing limiting elements back into the system. The first studies that described the composition of marine micro-eukaryotes made use of morphologic traits in order to discriminate them by microscopy analyses. Studies devoted to the analysis of prokaryotes used cultivation based approaches using mainly physiological traits as taxonomic determinants. Since two decades DNA is being used to identify species by sequencing the conserved small subunit ribosomal RNA gene: consisting of 16S rRNA and 18S rRNA for the prokaryotes and eukaryotes, respectively. Although many studies have described the micro-eukaryotic and prokaryotic community composition of marine environments, the number of publications on polar marine microbes is yet quite scarce and is concentrated on a few polar locations. For example: the Antarctic Peninsula and the Canadian Arctic have deserved much attention, while other regions completely lack data. The first step to study any ecosystem or location is to describe the organisms present: the community. Therefore we still desperately need to provide information on the community composition of other polar locations, in particular now that climate change is strongly affecting these regions.

Recent scientific publications reveal that climate change is occurring much faster than was first expected. This is especially true in polar regions, where the observed climate related temperature changes have been most pronounced. Rising temperatures are expected to cause increased glacier calving and melting rates. These effects are currently already being observed. Increased temperatures will therefore increase an inflow of freshwaters in the marine environment causing a salinity gradient and stratification of the water column. As a result, organisms, in particular the less motile micro-organisms, trapped in the upper water layer will also have to cope with enhanced solar radiation levels, including the deleterious ultraviolet radiation (UV-R). So, how will climate change affect marine microbes, their community composition, herewith potentially affecting their productivity and functioning? Questions that demand answers as these might have serious repercussions at the ecosystem and global scale. Rapid climate related changes occurring in the polar regions also demand an inventory of the actual marine microbial community composition that will provide essential baseline information for proper interpretation of near or further future changes.

In this thesis we used molecular tools to analyze and describe micro-eukaryotic and bacterial community composition from three polar sites. We analyzed fragments of the 16S and 18S rRNA genes by denaturing gradient gel electrophoresis (DGGE), which yields a community fingerprint and provides a rapid visual tool to detect shifts in community structure. We also sequenced environmental clone libraries generated from sea water samples collected at these locations in order to identify species composing the community. Herewith we contribute to the knowledge on marine microbes inhabiting polar seas.

Next to merely describing the marine microbial community composition of these polar sites we also attempted to study some of the potential effects of global climate change on natural

microbial communities. The effects studied in this thesis included the impact of solar UV-R on Antarctic marine micro-eukaryotes and bacteria, the effects of water column stratification on Antarctic marine microbial communities (eukaryotes and bacteria), and finally the effects of sediment rich glacial meltwater inflow on marine microbes from two Arctic fjords. These three issues will be addressed separately in the following paragraphs.

1. Does ultraviolet radiation shape natural Antarctic marine microbial communities?

The effects of differentially attenuated natural solar radiation on marine micro-eukaryotic communities from Prydz Bay, a coastal Eastern Antarctic location, were assessed in **Chapter 2.** The consequences of UV irradiation on the bacterial fraction of the community were studied in **Chapter 3**. Natural marine microbial communities smaller than 200 µm were incubated for 14 days in six 650L polyethylene tanks. Each tank was exposed to differently attenuated natural solar radiation. The treatments ranged from PAR (> 400nm) only, one PAR + UV-A (315 to 400 nm) and four PAR+ UVA+ UVB (280 to 315 nm) conditions (low, intermediate 1, -2 and high). The minicosm incubation experiments were repeated four times from October 2002 to January 2003. In the first 3 experiments, the natural marine microbial community was sampled from under the sea-ice; while the last experiment was conducted after sea ice had broken out. Next to assessing the shaping potential of UV-R on the communities, our sampling strategy also provided an insight in the natural succession of marine microbes from Prydz Bay. Herewith we provided the first molecular characterization of marine micro-eukaryotes and Bacteria from this Antarctic site.

Band patterns, generated for 18S rDNA fragment by DGGE, are reflective of the micro-eukaryotic community composition and revealed shifts over time and between size fractions. A significant contribution of natural UV irradiation on micro-eukaryotic community composition could not be detected (**Chapter 2**). In contrast, community changes associated with time of sampling were well resolved by DGGE analysis. Microscopy analysis performed by Thomson *et al.* (2008) on the samples from experiments 2 to 4 revealed that UV-R had variable effects on phytoplankton community composition. Nor the taxonomic approach, nor the molecular approach did provide uniform results on the shaping effects of UV-R radiation on micro-eukaryotic communities. Only the abundance of the haptophycean flagellate *Phaeocystis antarctica*, as determined by microscopy, seemed to be promoted by UV-B in all experiments. This suggests that under stratified and enhanced UV-R radiation conditions *Phaeocystis antarctica* would have a selective advantage within the marine micro-eukaryotic community. The observed increase in *Phaeocystis antarctica* was not detected by DGGE, nor were the quantitative shifts described microscopically for the other taxa. It appeared that DGGE had sufficient resolution potential in order to reveal major micro-eukaryotic community composition shifts over time, but minor and quantitative shifts at the taxon level were too subtle to be detected by the DGGE approach. Furthermore, universal primers used to target the 18S rRNA gene might have been too general. Thus, primers targeting specific micro-eukaryotic groups (s.a. haptophecean group) might have had more resolution potential in the analysis of UV-R related effects on community composition. Given the earlier described

disadvantages of classical microscopy (problems with identification of small cells, and relatively small sample volume analyzed) it remains to be investigated which method would be more accurate for observing minor quantitative shifts within communities.

DGGE patterns generated for the bacterial fraction of the marine microbial community (**Chapter 3**) were significantly shaped by time, while UV-R only had a significant shaping effect on two out of the four experiments, namely experiments 1 (October) and 3 (December). In both experiments band patterns of communities exposed for 14 days to the two highest UV-B irradiation treatments revealed a diversity loss.
Sequencing data on the shifts in bacterioplankton composition after incubation under either PAR or highest UV-B irradiation treatment revealed a few trends. The relative abundance of *Gamma-Proteobacteria* sequences decreased following incubation and the decrease was more pronounced after exposure to UV-B irradiation. A shift in the relative abundance of *Alpha-Proteobacteria* related sequences following incubation appeared to depend on the initial community composition. In the first two experiments *Alpha-Proteobacteria* were initially relatively abundant, but after incubation under UV-B irradiation their relative abundance decreased. In the last two experiments *Alpha-Proteobacteria* were initially scarce but became more abundant after incubation under PAR and UV-B. Overall, our study in coastal Antarctic waters does indicate that the UV-R effect can be significant and affects the composition of natural Antarctic bacterioplankton communities but these changes are not profound and are frequently subtle.

2. Describing the community composition and succession of Antarctic marine microbes from Prydz Bay (Eastern Antarctic).

DGGE patterns generated for the Prydz Bay marine micro-eukaryotic community in **Chapter 2** revealed clear differences between samples collected directly under the ice (during austral spring) and samples collected after the sea ice break-up (early austral summer). Moreover, differences among the sub-sea ice samples were apparent: band patterns from the first sample (October) differed strongly from the band patterns generated for the samples collected in November and December, which were highly similar to each other. The shifts in micro-eukaryotic community composition unveiled by DGGE were confirmed by cloning and sequencing data. Throughout the sampling period, three communities were distinguished: a post-winter/early spring community (October sample) comprising dinoflagellates, ciliates, cercozoans, stramenopiles, viridiplantae, haptophytes and metazoans; the November and December samples consisting of a dinoflagellate dominated community, and finally a diatom dominated community that developed after the sea ice breakup in January. Taxonomic analysis of the samples from experiments 2 to 4 analyzed in Chapter 2 was performed by Thomson *et al*. (2008). Microscopy data revealed similar shifts in general community composition throughout the sampling period with dinoflagellates dominating the community in November and December, followed by a diatom dominated community in January. Phylogenetic analysis of 472 sequenced clones comprised 47 phylotypes, belonging to the *Dinophyceae*, *Stramenopiles*, *Choanoflagellidae*, *Ciliophora*, *Cercozoa* and *Metazoa*. Among these

sequences two dinoflagellate, one ciliate and one metazoan clones potentially represented novel strains with less than 93% similarity to any sequence reported in the NCBI database. Overall, sequences related to the dinoflagellates dominated our samples, which suggests that they fulfill an important role in Antarctic coastal marine ecosystems preceding ice breakup as well as between phytoplankton bloom events. The sequencing data approach also revealed that Prydz Bay harbored a diverse group of dinoflagellates composed of several phylotypes. Dinoflagellates are sensitive to fixation steps and have cryptic taxonomic features. They are consequently very challenging to identify by microscopy. For these organisms molecular identification provides additional resolution and is therefore very valuable.

A similar cloning and sequencing approach was performed in **Chapter 3** providing information on the identity of the Bacteria within the Prydz Bay marine microbial community. Phylogenetic analysis of 671 partial clone sequences of the 16S rRNA gene (~750bp) revealed that Prydz Bay harbored a diverse bacterioplankton community, including several bacterial phyla and classes that had only sporadically been recovered from other polar marine sites. The sequences included members related to the *Cytophaga-Flavobacteria-Bacteroidetes* (CFB), *Gamma-Proteobacteria*, *Alpha-Proteobacteria*, *Delta-Proteobacteria*, *Planctomycetes*, *Gemmatimonadetes*, *Verrucomicrobia* and one yet unclassified bacterium.

Samples collected from under the ice in October and November harboured clones related to *Cytophaga-Flavobacteria-Bacteroidetes* (CFB), *Gamma-Proteobacteria* and *Alpha-Proteobacteria*. The sequences were initially evenly distributed over the three dominant phyla/classes, but at the end of spring a shift occurred towards a CFB dominated community. This shift was likely due to the onset of a springtime phytoplankton bloom. This relative CFB dominance persisted after the sea ice break-up when diatoms dominated the Prydz Bay micro-eukaryotic community. Members of the CFB are known to respond rapidly to phytoplankton blooms by their capacity to degrade complex organic matter of microalgal origin. Here the shift towards CFB dominance occurred before phytoplankton bloom initiation, suggesting the complex organic material might have been advected from the sea ice edge bloom, or released from the sea ice microalgal brines during the progressive degradation and melting of the surrounding sea ice. The second most dominant bacterioplankton group belonged to the SAR86 cluster from the *Gamma-Proteobacteria*, which is also known to be associated to phytoplankton blooms. Overall the Prydz Bay bacterioplankton community composition matched findings from previous studies performed at polar marine sites and the bacterioplankton response to the onset of phytoplankton bloom also fitted the general observations.

3. Does natural water column stratification and stratification break-up shape Antarctic marine microbial communities?

Antarctic marine microbes live under extreme and highly variable physical conditions. The average underwater solar irradiation is strongly reduced by wind driven deep vertical mixing or sea ice cover. However in summer, coastal and marginal ice zone Antarctic waters undergo major physical alterations with the return of light. The increased solar radiation and temperatures induce sea ice melting, which in turn cause salinity stratification of the water column. Stratification generally persists as long as strong wind events responsible for vertical mixing remain absent.

Changes in marine microbial community composition, including the micro-eukaryotic and bacterial fraction, were investigated in **Chapter 4** by DGGE analysis and subsequent rDNA fragment cloning and sequencing during a stratified and a post stratified (wind driven) period in Antarctic coastal waters. Samples from surface up to 10 m depth were collected in the summer of 1998 from Ryder Bay (Adelaide Island) located on the western side of the Antarctic Peninsula.

Surface salinity and wind speed data recorded during the survey revealed two distinct periods. The first period was characterized by low wind speeds (average 3 $m.s^{-1}$) and low surface salinity (average 31.2 *psu*). A high wind event (11.7 $m.s^{-1}$) marked the transition to a wind mixed period sustained by high wind speeds (av. 6.2 $m.s^{-1}$), characterized by increased surface salinity (av. 32.4 *psu*). DGGE revealed two major patterns for the micro-eukaryotic community that coincided with the stratified and wind mediated mixed periods. During the stratified period the micro-eukaryotic community fingerprints unveiled depth related differences, while the bacterial community only revealed weak depth related differences. The high wind event caused a rapid shift in the micro-eukaryotic community fingerprint. Sequencing data divulged a shift from an *Actinocyclus* sp. to a *Thalassiosira* sp. dominated micro-eukaryotic community. A shift in the bacterial community followed with a delay of a few days, suggesting a secondary response to changes in the micro-eukaryotic community rather than to the mixing event itself. Sequencing of partial 16S rDNA fragments revealed that changes in the bacterial community fingerprints corresponded to a shift from an *Alpha-* and *Gamma- Proteobacteria* to *Cytophaga-Flavobacterium-Bacteroidetes* dominated community under mixed conditions.

The results on natural marine microbial community shifts presented in **Chapter 4** showed that stratification and transition to less-stable Antarctic surface waters have an important shaping effect on the micro-eukaryotic and bacterial communities. As mentioned previously climate related changes are occurring faster than expected, especially in the polar regions. Models predict decreased sea ice cover and glacial melting with altered salinity and temperature stratification, as well as altered average wind speeds. Stratification events as well as non-stabilized surface waters are likely to significantly affect the composition of marine microbial communities, as shown in our study as well as elsewhere.

4. Describing the natural marine microbial community composition from two Arctic fjords at the onset of the summertime glacial melting period.

In this chapter we address a number of questions regarding the fjord's microbial ecosystem:

- ***Does the community composition differ between sampling locations, in particular between the two fjords?***
- ***Does the community composition of a single location show temporal variation?***
- ***Which environmental factors correlate most strongly with shifts in community composition?***

Kongsfjorden and Krossfjorden are semi-open glacial fjords that share a common mouth to the open sea on the Western coast of Spitsbergen. The fjords are influenced by glacier-meltwater as well as the inflow of warm saline transformed Atlantic water. This current flows northwards to the West of Spitsbergen and is responsible for mediating relatively mild temperatures at a latitude of 79°N. The mild temperatures and Arctic-Atlantic boundary location of both fjords have promoted this system to being considered as a model system and a natural indicator for climate-related changes.

The influx of glacial meltwater is maximal in summer. Freshwater enriched in sediment particles strongly affects both the surface water salinity and the underwater light climate. These physical alterations of the water column correspond to the average predicted climate related changes in polar regions. Therefore, understanding the dynamics that shape marine microbes in the Kongsfjorden and Krossfjorden during summer are of considerable value.

Summertime surface micro-eukaryotic and bacterial community dynamics were studied in **Chapter 5** by application of molecular tools including community fingerprinting, cloning and sequencing. Herewith we provided the first sequencing data for marine microbes for both fjords. Virtually no information existed on the composition of micro-eukaryotes from the Krossfjorden, while information on the bacterial fraction was lacking for both fjords.

DGGE band patterns generated from samples collected from various marine and freshwater locations revealed that the Kongsfjorden and Krossfjorden harbored distinct marine microbial communities, in spite of sharing a common mouth to the open ocean and both being subdued to glacial meltwater input. Community fingerprints generated for surface samples collected from the central Kongsfjorden over a month period revealed a stable micro-eukaryotic community, while the bacterial community fingerprints varied over time. The shifts in bacterial band patterns coincided with a period of reduced salinity and increased sediment load. Ordination analysis on DGGE band pattern variation and the environmental variables revealed that the composition of the marine microbial community varied according to the sampling location and that it was partly shaped by changes in salinity. Additionally the observed increase in sediment influx correlated significantly with the relative abundance of several eukaryotic and bacterial DGGE bands. Sediment was negatively correlated to eukaryotic bands, while the correlation was positive for the relative abundance of bacterial bands.

Ribosomal RNA sequences recovered from Kongs- and Krossfjorde micro-eukaryotic communities were related to the *Dinophyceae*, *Ciliophora*, *Cercozoa*, *Choanoflagellida* and

Chlorophyta. It also unveiled a community largely dominated by heterotrophs and bacteriovores, except for the photoautotrophic *Chlorophyta*. The lack of sequences related to typical marine phytoplankton (diatoms or *Phaeocystis* spp.) either indicated that the spring bloom had passed due to nutrient depletion or that members of these groups avoided the less saline surface waters. The bacterial community of the Kongs-Krossfjorden consisted of groups expected for marine polar sites with sequences related to the *Alpha-Proteobacteria*, *Gamma-Proteobacteria* and *Cytophaga-Flavobacterium-Bacteroidetes* (CFB). Surprisingly, sequences related to the typical freshwater *Beta-Proteobacteria* were also recovered from the fjords, showing that meltwater functions as an input of biodiversity into the fjord system. It remains to be determined whether these organisms can adapt to the extant salinity and form an active fraction of the bacterial community, or contribute to the genetic diversity by bringing novel DNA into the gene pool.

Both an increased freshwater input into the marine system resulting from melting glaciers and snow/ice fields plus an increase in sediment runoff from the land are likely to occur as a consequence of the currently observed global temperature rise. Our data show that both parameters are important determinants for marine microbial communities in the Kongs-Krossfjorden system. The recovery of "non-marine" bacteria from marine locations also suggests that meltwater is a source of Prokaryotes. Overall our data suggest that global warming expressed in increased glacial calving and melting rates can affect marine microbial community composition and therewith potentially the ecosystem.

CONCLUDING REMARKS

The first chapter presented in this thesis included a review on the publications and sequencing data on polar marine microbes that were published so far. This revealed that diversity studies characterizing polar marine microbes are still relatively scarce.

This thesis substantially contributes to this knowledge by providing bacterial and micro-eukaryotic sequences for three yet uncharacterized polar locations: Prydz Bay (Eastern Antarctic), Ryder Bay (Western Antarctic Peninsula) and for the Arctic Kongsfjorden-Krossfjorden (Spitsbergen). Our micro-eukaryotic sequencing data included some potential novel strains. From Prydz Bay we recovered novel sequences related to two dinoflagellates, one ciliate and one metazoan; the Ryder Bay sequences included one diatom, one ciliate and one metazoan novel sequences. Moreover the sequencing data unveiled that dinoflagellates constitute a dominant fraction of the community (Prydz Bay and Kongs-Krossfjorden) outside of phytoplankton bloom periods. The dinoflagellate groups proved to be diverse as they were composed of several operational taxonomic units, which would have remained undetected by microscopy approach due to their cryptic morphological features. This shows that molecular tools have more resolution potential with regard to some specific plankton groups, as compared with classical identification methods like microscopy.

The bacterial sequences revealed that the three polar sites were home to bacterial sequences expected for marine polar waters, with members of the *Cytophaga-Flavobacteria-Bacteroidetes*, *Gamma-* and *Alpha-Proteobacteria* dominating the bacterial community. We

did recover a few surprising sequences: the Prydz Bay sequences revealed the presence of *Delta-Proteobacteria*, *Planctomycetes*, *Gemmatimonadetes*, *Verrucomicrobia* and one yet unclassified bacterium. These sequences are only sporadically recovered from polar marine sites, which supports the idea that polar marine sites may still be source of rare and yet undiscovered microbial species. The Kongsfjorden-Krossfjorden unveiled the presence of unexpected *Beta-Proteobacteria* sequences that were related to sequences from a subglacial and freshwater system. This suggests that glacier meltwater is an important source of non-marine species. Yet, whether these can survive in a marine system and form an active component of the community demands additional and other analytical approaches like Micro-CARD-FISH. Catalyzed reporter deposition (CARD) fluorescence in situ hybridization (FISH) combined with microautoradiography (Micro) provides the possibility to identify specific groups of microbes that are actively consuming a radioactively labelled food source. This yields quantitative data on community composition, active fractions of the community, and in particular which specific groups are active.

Throughout this thesis we also determined possible impacts of climate change on marine microbial community structure. DGGE proved to be a valuable tool in revealing shifts over time and in space. *In situ* changes observed for the Ryder Bay micro-eukaryotic and bacterial community were easily obtained and revealed that water column stratification versus mixing events can shape the community composition. Similarly changes in the Kongs-Krossfjorden surface marine microbial communities were efficiently exposed by the shifts in the DGGE band patterns. These shifts proved to be partly explained by changes in the surface salinities. Also, relative abundances of some DGGE bands were significantly correlated with the sediment input of meltwater origin. Although DGGE did reveal some UV-R related shifts for the Prydz Bay bacterial community, it lacked resolution to reveal significant shifts for the micro-eukaryotic fraction of the community, in spite of some quantitative shifts that had been observed by microscopy. This reveals that although DGGE can efficiently expose large changes in community composition, the more subtle and quantitative shifts are not easily detected. Using group specific primers that target specific eukaryotic groups may provide higher resolution and should be considered in future studies.

Molecular methods proved to be very valuable in the analysis of polar marine microbial diversity. This approach has the potential of rapidly revealing composition shifts as compared to the more laborious and time consuming microscopy analysis, especially when analysing the smaller plankton species (flagellates, protozoa and prokaryotes). Yet, describing the community composition is a first step in understanding a polar marine microbial system. A second step is to attempt to link diversity to environmental variables and then unravel the function of the different microbial entities constituting the community.

The approach used here merely says something on the presence or absence of DNA from organisms. However, these might be present in a passive state. Therefore application of novel techniques like Micro-CARD-FISH is highly recommended. By now this technique is widely used for the prokaryotic community, but it still desperately needs development and optimization for micro-eukaryotes. An alternative approach to determine the active fraction of the community is to sequence and analyze the rRNA fraction, which allows identification of

micro-organisms that are actively transcribing their DNA. Comparison of a DNA based clone library with a RNA based clone library would then provide similar kind of data: total community composition versus the composition of the active community.

Then, in order to determine and understand the function of marine microbial communities, complementary data on the ecosystem abiotic properties are required. In stead of searching for a needle in a haystack, data on the abiotic properties of the system would provide a search direction. To this extent multidisciplinary approaches would be very valuable and substantially increase ecosystem understanding. For example glacial meltwater is often enriched in sediment. Geochemical analysis of the sediment would reveal which elements (such as iron) are being fed into the system. For example, if iron is being fed into the system one might search for functional genes involved in iron oxidation as starting point for understanding the energetic pathways fuelling the microbial system.

Moreover, combining molecular analysis with cultivation dependent approach would also provide valuable information about the identity and function of organisms hidden behind the 16S rRNA environmental sequences. Without that information one can only guess about their function based on similarity of the sequences with sequences from the still low number of cultivated species. Other techniques that would be of benefit to unravel ecosystem function are to be found in the specific identification of functional genes through (quantitative) gene amplification and via high throughput analysis of total DNA (meta-genomics), RNA (meta-transcriptomics) or protein (meta-proteomics). Currently the field of molecular ecology is advancing fast by the introduction of whole genome sequencing, meta-genomics, transcriptomics and proteomics. These techniques are also becoming less expensive, therefore in the near future, analysis of complex microbial communities will include these techniques. This will dramatically expand the amount of data generated and further contribute to our goal to understand ecosystem functioning in relation to its ever changing environment.

Within this thesis several questions were addressed regarding the potential effects of climate change on marine polar marine microbes. The data presented for the Kongsfjorden/Krossfjorden system does indicate that effects of increased glacier calving and melting caused by increased temperatures and resulting in water column stratification will affect the composition of the marine microbial communities. Among the meltwater induced water column stratification two scenarios should be considered. First scenario is the stratification of clear waters, where organisms trapped in the upper surface layer become exposed to higher solar irradiance, including UV-R. Our data from Prydz Bay revealed that UV-R had significant but minor and subtle shaping effect on the bacterial community, while the response of the micro-eukaryotes was not conclusive. In addition, we performed microcosm experiments in the Kongsfjord in 2005, during which effects of natural UV exposure on microbial community structure was studied. The outcomes of these experiments did not reveal clear UV related changes (results not incorporated in this thesis). The second scenario consists of enhanced calving and melting of grounded glaciers or retreated glaciers, where sediment rich meltwater flows into the marine system. This results in a salinity gradient, with fine sediments being trapped within the low-salinity layer, resulting in a murky surface layer. Our data showed that salinity and sediment load were factors that affected the composition of the

microbial community and fed the system with non-marine bacteria. We therefore conclude that the second stratification scenario will also affect marine microbial communities. As a turbid surface layer will also strongly affect the underwater light climate we therefore recommend that additional analysis should be performed on the microbial organisms confined below this murky surface layer. Also productivity analyses should be included in order to determine whether the primary producers that require light to perform photosynthesis and fuel the system with organic carbon suffer of light limitation and therefore reduced productivity.

In conclusion data presented in this thesis demonstrate that molecular tools provide a great approach for the analysis of marine microbes, either to determine microbial diversity or to detect community structure shifts. Molecular community fingerprinting tools proved to be very efficient, for both micro-eukaryotes as well as bacteria. However, fine tuning is still required in order to be able to unveil subtle shifts. Diversity studies through molecular approaches provide rapid and new insights in species composition and are highly efficient in divulging novel species. However if molecular techniques are combined with other techniques either classical or cultivation based, this would generate even stronger and more informative data sets. Therefore, I would advise future research planning to use molecular tools, as presented in this thesis, for the characterization of natural marine microbes, but to also consider alternative analytical methods.

References

Thomson, P.G., A.T. Davidson, and N. Cadman 2008. Temporal changes in effects of ambient UV radiation on natural communities of Antarctic marine protists. *Aquat Microb Ecol 152: 131-147.*