SUMMARY

Diatoms (Bacillariophyceae) represent a powerful tool for assesing environmental changes and therefore are routinelly used in water quality monitoring all over the world (Kelly & Whitton 1995, Lenoir & Coste 1996, Potapova et al. 2004, Van Dam et al. 1994, Wu 1999). In the Antarctic region, diatoms are one of the most numerous and diverse microalgae (Jones 1996, Van de Vijver & Beyens 1999), while globally they dominate in marine microphytobenthic communities (Schlie & Karsten 2017). Marine ecosystems, and especially those in the Antarctic peninsula area, are greatly impacted by the climate change: glacier melting leads to higher freshwater and sediment inflow into the sea, lowering the salinity and increasing the turbidity in the surface sea water in coastal areas, the latter leading to a lower light penetration while sediment inflow may increase the nutrients into the water column - all these are factors known to affect the benthic marine communities (e.g. Zacher et al. 2009, Sahade et al. 2015, etc). In addition, new substrata become available for colonization (Passoti et al. 2015). The lack of knowledge and data, which we can use as a base for monitoring changes in benthic communities leads to underestimation of the effects of climate change over the marine benthos (Sahade et al. 2015). Although diatoms have been a subject of a number of studies in Antarctica, starting from mid 19th Century (Ehrenberg 1844, see also Zidarova et al. 2016), still the knowledge of entire ecological groups in the region, such as the epilithic marine diatom communities remains scarce.

The project aims to (1) study the colonization processes and development of epilithic diatom communities of marine benthos on newly submerged substrata in two contrasting environments, as well as (2) to provide new and fundamental data for marine epilithic diatom community structure and species diversity on new substrata, while attempting to answer the following questions: what is the colonization rate and epilithic marine diatoms communities development on new substrata in Antarctica; which are the first benthic diatom species able to colonize new substrata; what is the effect of climate change (i.e. increased freshwater and sediment inflow) on the development of marine epilithic diatom communities (and their species composition); and finally, can we use marine epilithic diatoms as first indicators for climate induced environmental changes in Antarctica. During the study we will also test for first time in Antarctica a method for benthic marine diatom DNA preservation, with further DNA extraction and PCR amplification which could be used in future metagenetics biodiversity studies.

In order to study colonization processes of marine epilithic diatoms on newly submerged substrata in two contrasting environmental conditions, 2 sets of ceramic tiles (each of 8 tiles in 3 replicas) will be submerged into the water column and sampled weekly for a period of up to 8 weeks. Sampled material will be stored in a known (equivalent) volume in order to get comparable results between the different weeks and sites. The two sampling sites, located in the South Bay of Livingston Island, will be chosen as being representative for two different conditions, one typical for climate change conditions, with increased inflow of freshwater and sediments during summer, and second, representing “normal” conditions (without freshwater and sediment inflow). For better characterization of the two sampling sites with each sampling measurements of water pH, oxygen concentration and salinity will be taken with a portable multimeter. Additionally, the concentrations of total P, phosphates, nitrates, nitrites and silica in the water will be assessed using a portable photometer. Secchi disk will be used to measure the water turbidity in the two sampling sites. During last sampling (week 8) a small sub-sample of live material will be stored in sterile vials with RNAlater® Solution (ThermoFisher Scientific). Further tests for DNA extraction and PCR amplification will be done in the lab. For genomic DNA extraction two approaches will be tested: phenol-chloroform extraction, according to Genomic DNA Preparation from RNAlater™ Preserved Tissues Protocol (ThermoFisher Scientific) and a commercial DNA extraction kit DNeasy PowerBiofilm Kit (Qiagen). For PCR amplification a set of primer pairs for the 18S rRNA gene targeting V4-5 hypervariable regions (Stoeck et al. 2010) will be used.

For diatom species identification both light (LM) and scanning electron microscope (SEM) will be used (Zidarova et al. 2016). For microscopic analyses, diatom valves will be prepared by the method of Hasle & Fryxell (1970) and mounted in Naphrax®. In order to trace the community development and possible changes in the community structure in the two sites over the 8 week period, on each slide 300 valves will

be identified up to species level and counted (e.g. Prygiel et al. 2002). Diversity and evenness indexes will be calculated. The differences between the different weeks of colonization will be tested by one-way ANOSIM using Bray-Curtis distance following Desrosiers et al. (2014). For assessment of colonization process both community structure and diatom cell density will be used. The latter will be obtained by counts of diatom cells in Sedgewick Rafter chamber on inverted microscope.

At the end of the project we expect:

(1) to be able to tell whether possible differences in the colonization process and communities structure between the two sites are likely to be related to the different environmental conditions (i.e. climate change included). The use of artificial substrata allows for precision and better comparisons between the sites (Lamberti & Resh 1985, Lane et al. 2003, Desrosiers et al. 2014), as we eliminate a large number of other factors, such as habitat microstructure, exposure, depth and light penetration, etc., that may influence the diatom communities structure in natural habitats. Secondly, it gives opportunity to understand which species might be the first colonizers of newly exposed substrata.

(2) to provide first data for the colonization rate and the development of epilithic diatom communities in marine microphytobenthos in Antarctica. Studies on the colonization of new substrata by algae in Antarctica and the development of their communities are rarity (Campana et al. 2018); no such experiments have so far been made with diatoms. The weekly observations will allow us to follow the colonization process and to provide data for the first diatom species able to colonize new substrata in Antarctica. In addition, we will help us to understand whether the Antarctic marine diatoms colonize new substrata with the same rate as marine diatoms from other latitudes, as these organisms might be adapted for faster colonization and community development (e.g. Ligowski et al. 2012), considering the short period with favorable environmental conditions in the Antarctic.

(3) as marine epilithic diatoms are currently studied as potential indicators for water quality assessment in coastal areas with the use of artificial substrata (Desrosiers et al. 2014), data obtained during this study could be useful in the development or refinement of methodology for routine sampling of marine benthic epilithic diatoms for other cold-water regions of the world.

(4) using both LM and SEM for morphological characterization of the species will lead to better knowledge on the morphology of the Antarctic marine benthic diatoms and will be useful in any further study requiring a trustful taxonomic work.

(5) Considering the low number of recently recognized and described from the Maritime Antarctic marine benthic diatoms (Fernandes & de Souza-Mosimann 2001, Fernandes et al. 2007, Al-Handal et al. 2008a, b, 2018), which is in contrast with the recent results on the Maritime Antarctic freshwater diatom flora (Zidarova et al. 2016), and the high number of unidentified or uncertainly identified taxa listed in Al-Handal & Wulff (2008), it is possible that during this study new diatom species are found.

Finally,

(6) for first time, for Antarctic marine benthic diatoms, methods for samples preservation, DNA extraction and PCR amplification will be tested. This would give us an opportunity for the development of a new approach for taxonomic and biodiversity studies in future, combining both morphological and molecular methods.