Dr. Natalia Servetto

Tittle of the project: **Southern Ocean acidification: potential effect on the Antarctic mollusk *Neobuccinum eatoni.***

- Differences between the proposed project and actual results:

The experiment proposed was successfully carried out at Potter Cove (62º14'S, 58º40'W; King George/25 de Mayo Island, South Shetlands) in an Antarctic Summer Campaign, as it is shown in Fig. 1. The Gene Expression through RNA-Seq were realized on the bivalve *Aequiyoldia eightsii* and the ascidia *Cnemidocarpa verrucosa* sp. A, however, it was not done in the mollusk *Neobuccinum eatoni* because RNA extraction was not of good quality.

We implemented a pH-manipulative experimental system using a design as shown in Fig. 1. Samples of both species were taken at final time (66 days of experiment) and tissue was snapped frozen in liquid nitrogen, stored at -80°C and transported to the Alfred Wegener Institute (AWI) (Bremerhaven, Germany) where the RNA-seq was realized.

Fig. 1: Overview of the experimental design used in the differential expression. Animals were collected in the Antarctic summer campaign and acclimated until the start of experimentation. Subsequently, exposure experiments were conducted for a long-time period of 66 days for control and Low pH condition. After exposure, tissue samples were taken and total RNA was extracted for analyses for differential expression analysis by DESeq at final time in *Cnemidocarpa verrucosa* sp. A and *Aequiyoldia eightsii* and for enzymatic activity at all times for the ascidia.

Total RNA was extracted from approximately 25 mg of *C. verrucosa* sp. A branchial basket using the RNeasy Mini Kit (Qiagen, Germany) according to the standard tissue protocol. For *A. eightsii,* gill tissue (15 - 20 mg) were homogenized in Trizol reagent (SIGMA) using a Precellys homogenizer (Precellys24, Bertin Technologies, France). Total RNA was extracted using the Direct-zol TM RNA MiniPrep Kit (ZYMO Research Corp., USA). Libraries were normalized and pooled to sequence them in the NextSeq 500 sequencer (AWI) as paired-end reads with the High output sequencing kit PE 300 (2 x 150 bp).

These results are in preparation for publication. In preliminary results we observed 224 up-regulated genes and 111 down-regulated genes (FC ≥ 2; p value ≤ 0.05) in the ascidian. In particular, the decrease in pH caused an up-regulation

of genes involved in the immune system and antioxidant response. While in the infaunal bivalve less differentially expressed genes were observed, (34 genes were up-regulated and 69 genes were down-regulated (FC ≥ 2; p value ≤ 0.05)) in response to Ocean Acidification (OA).

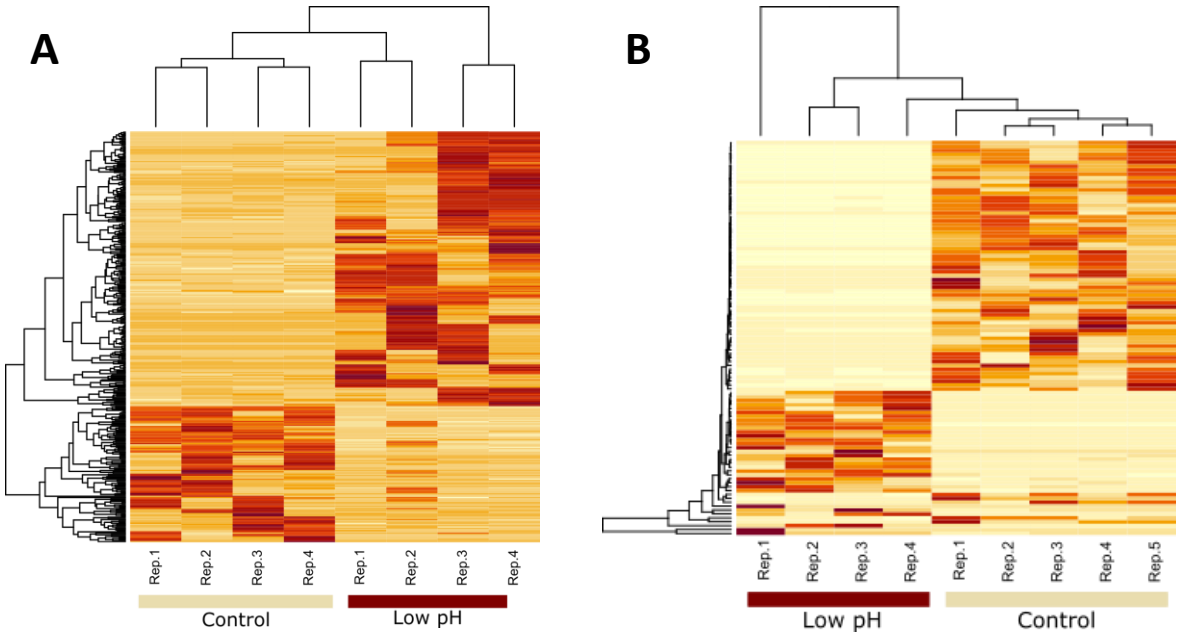

Fig. 2: Heatmap of differentially expressed genes in the A) ascidian *Cnemidocarpa verrucosa* sp. A and B*) Aequiyoldia eightsii*. Positive (yellow) values represent up regulated genes, while negative (red) values represent down regulated genes. Each row represents a differential expressed gene. Each column represents a replicate: Low pH, and control. Hierarchical clustering analysis is represented; distances between clusters are recomputed by the Lance–Williams dissimilarity update formula.

This work addresses the effect of OA in two common and widely distributed Antarctic benthic species, showing that the non-calcifying species showed a more pronounced response to Low pH condition.

Carbonate chemistry seasonal cycles in the Southern Ocean: the fjord Potter Cove.

On the other hand, **the Antarctic Science Bursary helped us to buy a pH SENSOR (πSAMI – Sunburst).** With the main aim to perform a seasonal and daily monitoring of carbonate chemistry, in order to evaluate the ambient local conditions and variability, as well as their potential causes (e.g., algae production, salinity oscillation by glacier melting, etc.). In this context, we are carrying out a water sampling in the fjord from 2019. This is being done monthly at three different points (according to different algal abundance and glacier influence), and at two depths (1 and 15 m). The pH determination is being carried out in Carlini station through spectrophotometric measurements, following the method described by Dickson et al (2007). AT measurements were measured in France in collaboration with Dr. Frédéric Gazeau. The collocation of the πSAMI in Potter Cove, thanks to the Antarctic Science support, will allow us to build a more comprehensive picture of the likely effect of climate change and OA on the benthic community of the fjord. This device is on the way to Antarctica, and we propose to put it in the fjord next Antarctic Summer Campaign (January-February 2023). It will measurement throughout the year (until next summer 2024) at 15 meters of depth.