# Cellular and molecular characterization of short- and long-term hyposaline acclimation in a marine diatom: insights into the noncoding realm

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## 1. Introduction

Long non-coding RNAs (lncRNAs) have gathered growing interest in the past decades, particularly in development and disease in multicellular organisms, leaving a gap of knowledge in other species, namely in marine protists such as diatoms. Many lncRNAs have been shown to be associated with Pi (1) and $CO_2$ (2) fluctuations in *P. tricornutum*, but their functions remain largely unknown.

In this work, we initially focused on investigating the presence and expression patterns of lncRNAs in the 3 morphotypes of the pleiomorphic *P. tricornutum* (oval, triradiate and fusiform). This led us to a second study investigating the effect (morphological and molecular) of salinity shifts in *P. tricornutum* cultures. In parallel, to enable lncRNA functional studies, we developed a novel CRISPR-Cas9 approach based on the uLoop system (4), multiplexing gRNAs and conjugation transformation.

## 2. Initial results

### LncRNAs show lower expression levels than mRNAs but are more specifically expressed

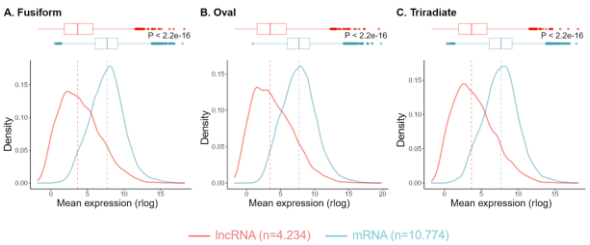


Figure 1. Expression level differences between coding and noncoding transcripts in *P. tricornutum* morphotypes (3)

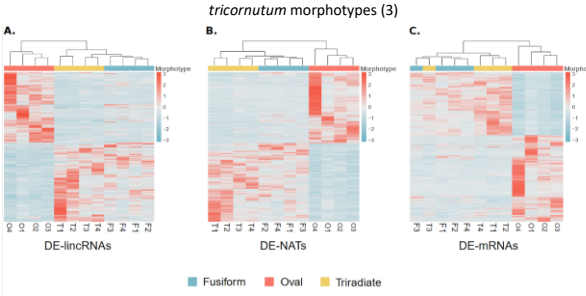


Figure 2. Clustering heatmaps of long noncoding (lincRNA and NAT) and coding transcripts from all three pairwise comparisons (TF, OF, OT) (3)

### Hyposaline variations overshadow the morphotype

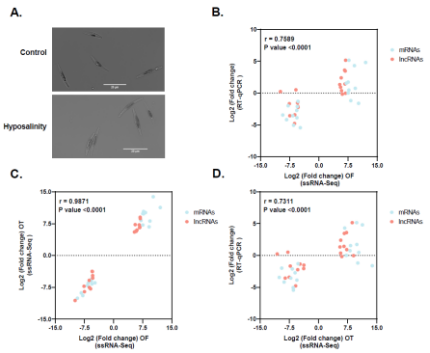


Figure 3. Analysis of the hyposaline effect compared to the impact of the morphotype in Pt1 (3)

## 3. Ongoing work

To follow up on these findings, we sought to investigate the short- (days) and long- (months) term response of *P. tricornutum* to drastic changes in salinity in the media.

### 3.1. Methodology

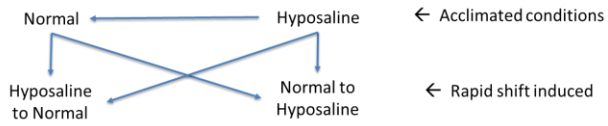


Figure 4. Schematic representation of the methodologies used for the RNA-seq analyses comparisons

### 3.2. Results

#### *P. tricornutum* cells are longer and their growth is slightly affected under hyposaline conditions

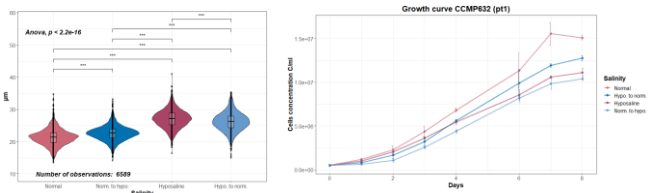


Figure 5. Phenotypic characterization of *P.tricornutum* acclimation to hyposalinity

#### LncRNAs expression is highly condition-specific

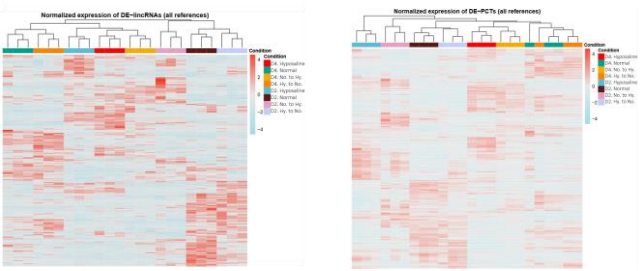


Figure 6 . Clustering heatmaps of lncRNAs and coding transcripts in hyposaline acclimatization

## Generation of bi-allelic CRISPR-Cas9 KO lncRNAs

Using the uLoop (4) and the CRISPR-Cas9 multiplexed system, we generated the first lncRNA mutant bank in *P. tricornutum.*

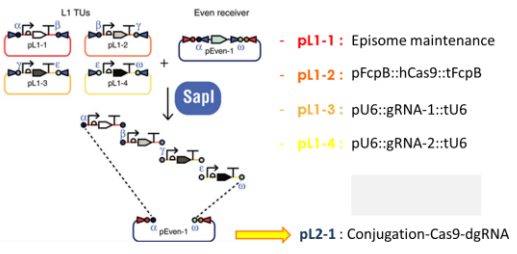


Figure 7. Schematic representation of the uLoop assembly and plasmids used to perform mutagenesis by conjugation in *P.tricornutum*

## 4. Conclusion

Taken together, *P. tricornutum* lncRNAs are particularly responsive to unfavorable conditions (nutrients, $CO_2$, salinity), suggesting a putative regulatory role in the stress response/ acclimation. Our recent studies revealed that after a long term acclimation to a hyposaline environment, *P. tricornutum* exhibits a specific transcriptome suggesting an extensive reprogramming of the cell associated with small changes in terms of length size and growth. In parallel, a highly efficient CRISPR-Cas9 protocol for the ablation of entire lncRNA loci, by multiplexing gRNAs, was developed to generate a KO mutant bank which is currently being used to validate lncRNA functions in *P.tricornutum.*

## Reference

1. Cruz de Carvalho et al. (2016). New Phytologist
2. Huang et al (2019). Frontiers in Microbiology
3. Debit*, Charton* et al (2023). Scientific Reports
4. Pollak et al. (2020) Synthetic Biology