

# INVESTIGATING THE EXPRESSION OF miRNAs IN EXTRACELLULAR VESICLES OF ATLANTIC HALIBUT AS MARKERS FOR SPERM QUALITY

Jorge M. O. Fernandes<sup>1,2</sup>, Renan J. C. Appel<sup>2</sup>, Golam Rbbani<sup>2</sup>, Elvira Fernandez<sup>3</sup>, José Beirão<sup>2</sup>, Alexandros Tsakogiannis<sup>3</sup>, Sandra Ramos-Judéz<sup>3</sup>, Catarina Oliveira<sup>3</sup>, Rune Valaker<sup>4</sup>, Tanja Østerbø<sup>4</sup>, Carlos Marrero<sup>3</sup>, and Elsa Cabrita<sup>3</sup>

<sup>1</sup>Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain

<sup>2</sup>Nord University, Bodø, Norway

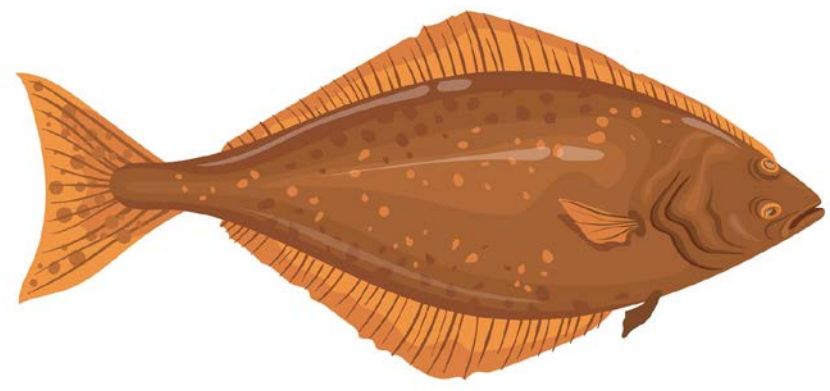
<sup>3</sup>University of the Algarve, Faro, Portugal

<sup>4</sup>Sogn Aqua, Bergen, Norway

## BACKGROUND

### Atlantic halibut

*Hippoglossus hippoglossus*

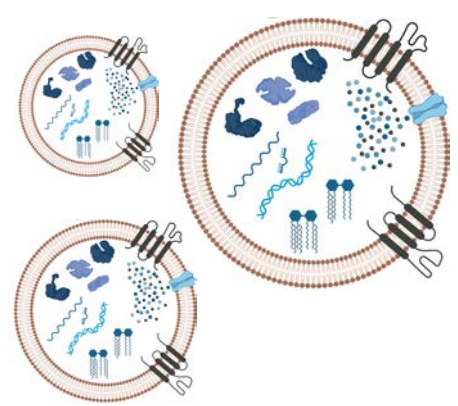


Marine species of high economic value

Reproductive obstacles that may be partly related to broodstock diet

### Extracellular vesicles (EVs)

Sperm maturation, egg activation and embryo development

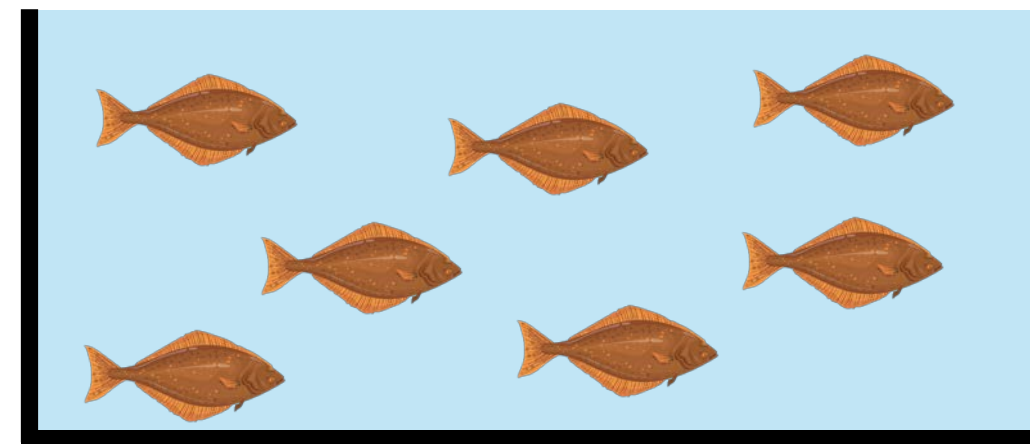


Enriched complex load of biological components, such as lipids, signaling proteins, regulatory and non-coding RNAs (ncRNAs)

Do miRNAs from EVs in semen contribute to reproduction?

Source: Rodriguez-Martinez and Roca, 2022

## METHODS

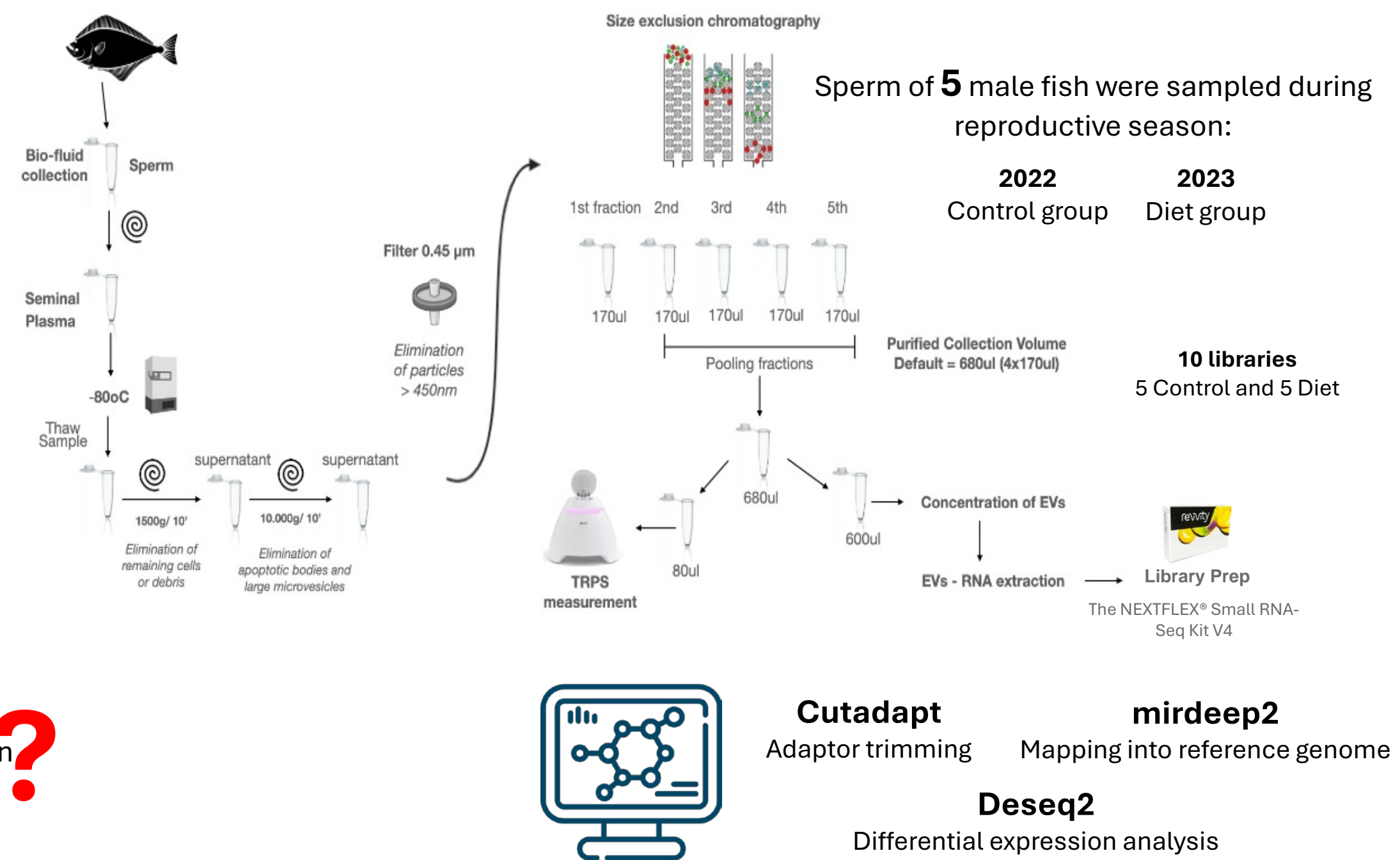


*Gracilaria gracilis*

*Phaeodactylum tricornutum*

Algal diet (DT)

Control diet (CT)



## RESULTS

- 12 significantly differentially expressed miRNAs 10 upregulated and 2 downregulated
- Differentially expressed miRNAs shows effective correlation with sperm quality parameter and antioxidant enzymes
- Upregulated miRNAs including miRNA-196, miRNA-196 -5p, miR-196a, miR-196a-5p showed negative correlation with DNA fragmentation, but a opposite with linearity (LIN).
- Almost all the upregulated miRNAs showed positive correlation with antioxidant enzymes including glutathione reductase (GSR), glutathione peroxidase (GPX), and superoxidase dismutase (SOD). Conversely, down-regulated miRNA miR-22b showed little or no correlation with and antioxidant enzymes.

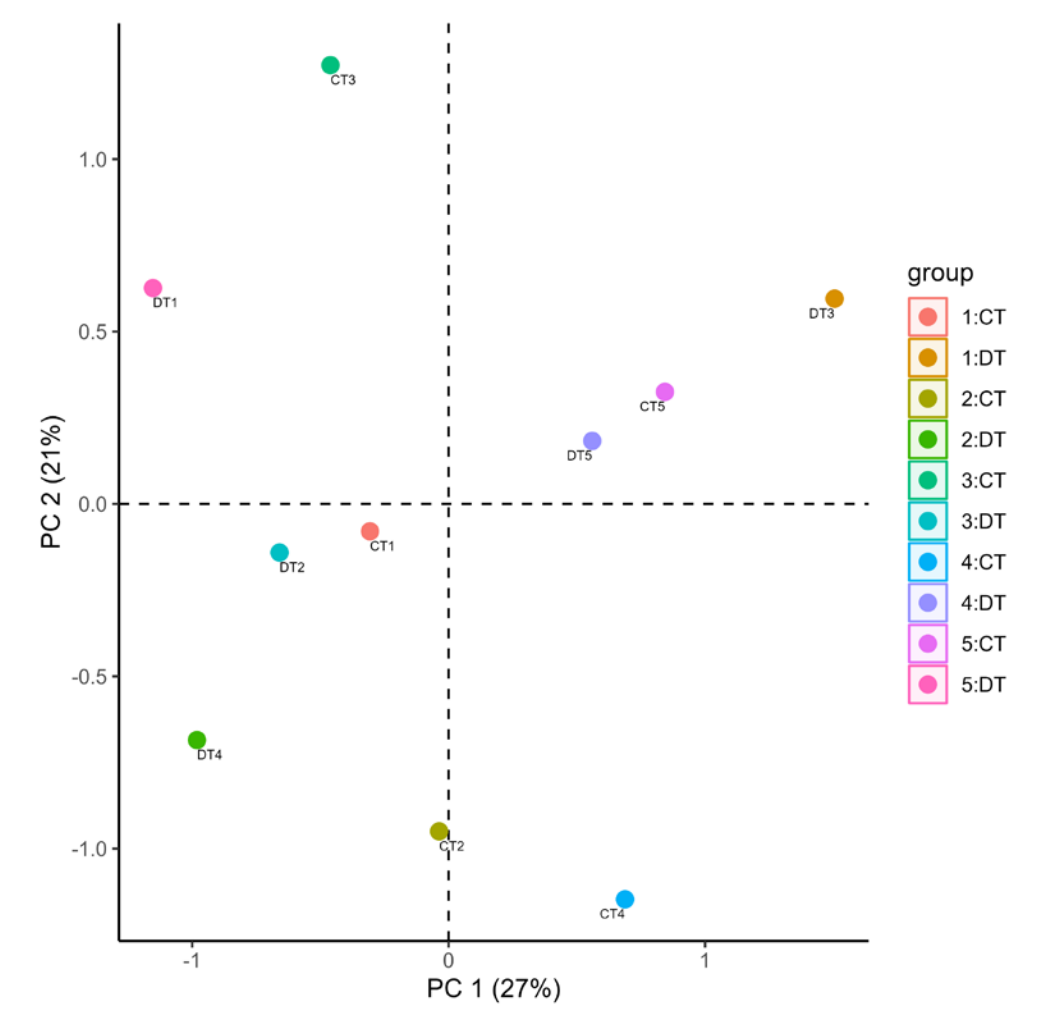


Figure 1. PCA plot shows the variability of sample between the groups. Here, PCA1 and PCA2 cover 27% and 21% variability, respectively.

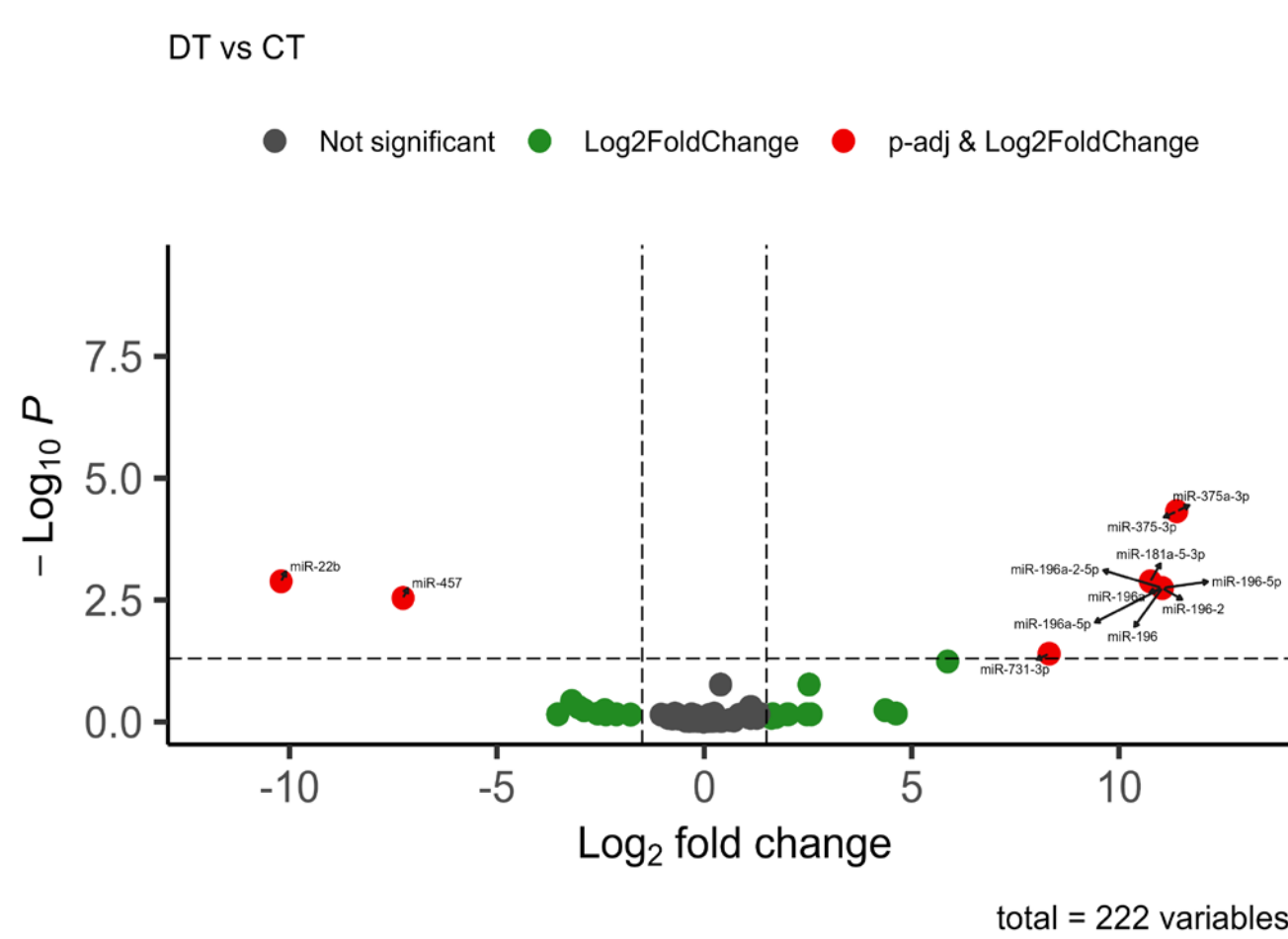


Figure 2. Volcano plot shows the distribution of miRNAs based on fold change between control and diet supplemented group.

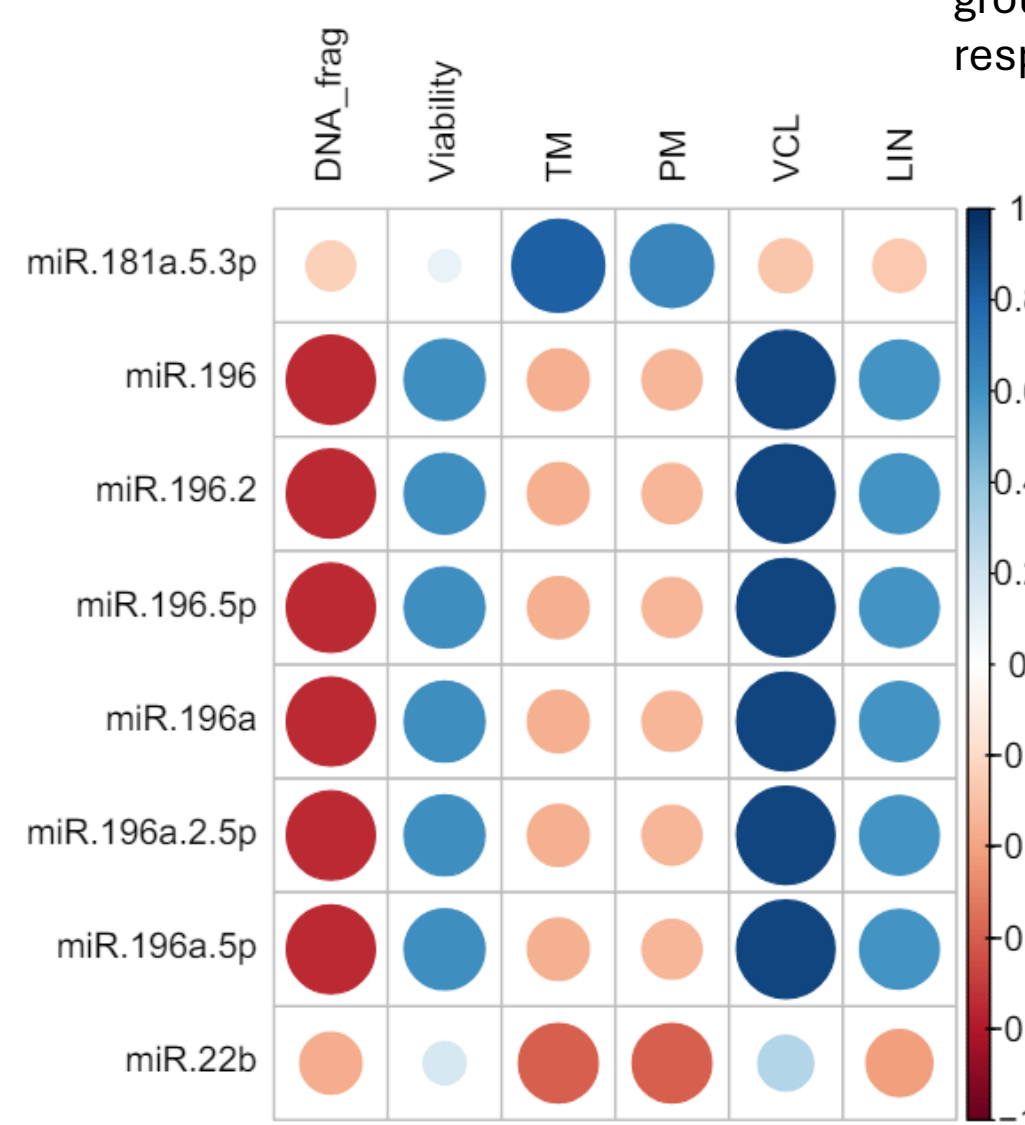


Figure 3. Pearson correlation of differentially expressed miRNAs and sperm quality

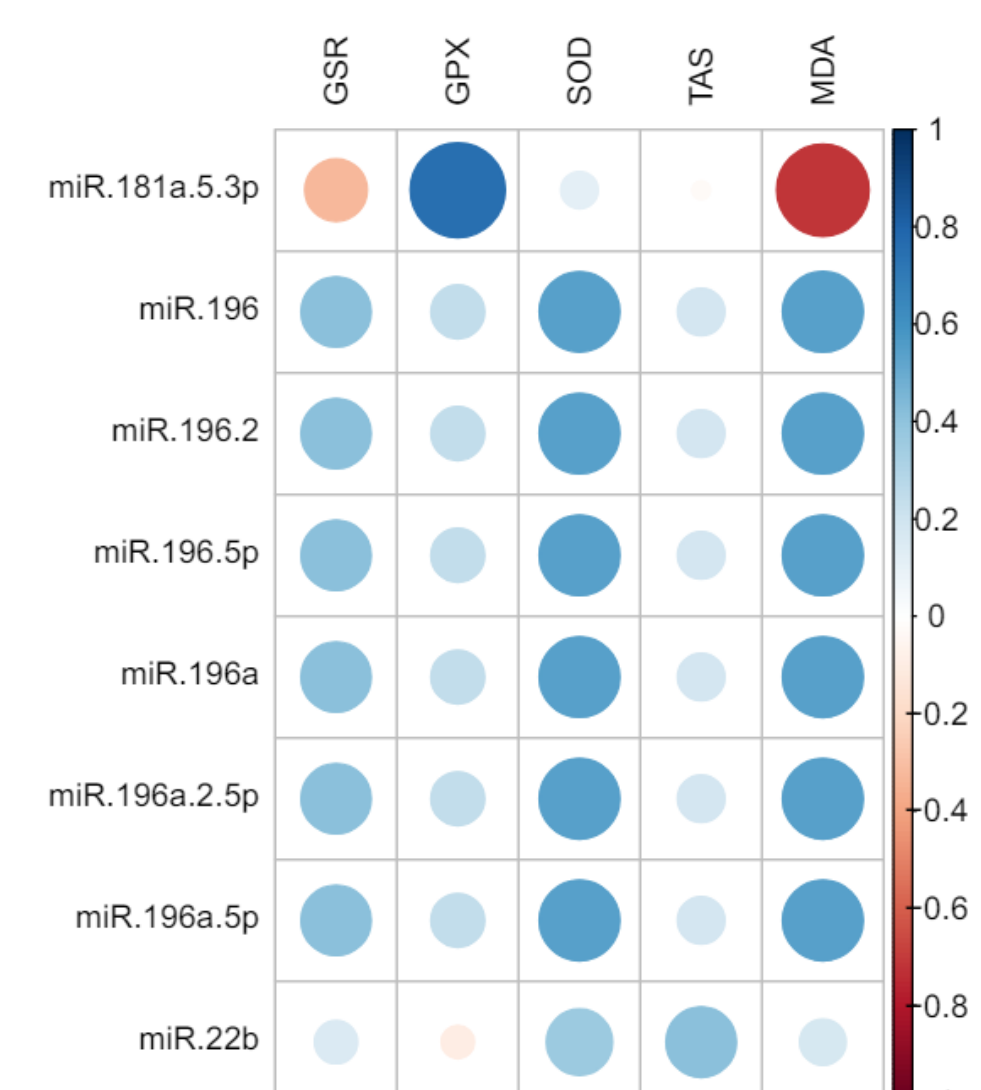


Figure 4. Pearson correlation of differentially expressed miRNAs and antioxidant enzyme activity.

## CONCLUSIONS

The upregulation of miR-375 indicates **enhanced metabolic processes** with the algae-enriched diet, which are crucial for maintaining reproductive activities and overall health during the reproductive season.

The upregulated miRNAs may promote **spermatogenesis and improve sperm quality**, contributing to better reproductive outcomes.

Validating these differentially expressed miRNAs presents the potential to utilize them as **markers for male reproductive health** in aquaculture

### Project Information

BREEDFLAT

EEA and Norway Grants, ref. PT-INNOVATION-0080