From sea to human health: exploring the molecular basis of dinoflagellate toxicity.

Acronym: Sea2Health

Supervisors: S. Castagnetti (LBDV/UMR7009) and R. Léeme (LOV/UMR7093)

Dinoflagellates are cosmopolitan unicellular eukaryotes found in both marine and freshwater habitats. Due to their taxonomic position and their abundance, dinoflagellates hold an interest from a scientific standpoint, as well as from a health and economic prospective. Dinoflagellates are one of the major agents causing Harmful Algal Blooms (HAB), explosive population expansions events that, because of their toxicity, severely jeopardize aquaculture industry and pose a serious health concern for humans as their toxins, which can accumulate in edible fish/shellfish or being aerosolized, are often poisonous for humans. It is generally accepted that the onset of HAB will become more frequent and acute in the coming years, being amplified by climate changes and warming of seawater (Gobler, 2020).

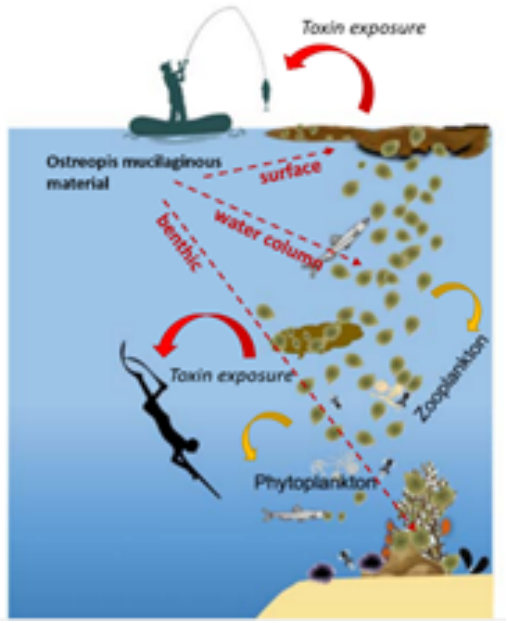

Figure 1: Schematic depiction of the MBM dynamics in the water column, benthos and surface waters and potential harmful interactions with phytoplankton, zooplankton and humans (through toxin exposure).

In the Mediterranean Sea, HAB involving the dinoflagellate *Ostreopsis.* cf. *ovata* have occurred in summer with increasing regularity in the last 20 years [2] and since 2020 outbreaks of *Ostreopsis* have been reported also in the Bay of Biscay (Basque country), causing several hundred intoxications and great concern to French health authorities (cf. ANSES report, Lemée et al., 2023). This benthic dinoflagellate (about 50 microns in size) thrives in coastal shallow waters. *O*. cf. *ovata* benthic cells are embedded in a mucilaginous extracellular matrix which can be dispersed in the water column and at the sea surface (Berdalet et al., 2022) and then aerosolized by waves and wind, carrying a toxic mixture into the atmosphere which can cause acute poisoning, skin irritation and respiratory problems in humans (Figure 1), as reported at several locations in France, Spain, Italy and Algeria (Berdalet et al., 2022; Tichadou et al., 2010; Illoul et al., 2012). Indeed, *O.* cf. *ovata* produces palytoxin and analogues, named ovatoxins (Ciminiello et al., 2012), highly toxic substances that can have a detrimental impact on co-existing fauna and flora, as well as less potent families that possess anti-cancer activity (Ternon et al., 2022a) particularly expressed at the onset of the exponential growth phase (Ternon et al., 2022b).

Several field and lab-based studies have shown that environmental conditions are major contributors to *Ostreopsis* proliferation and toxicity. Proliferation is favoured by high temperature, salinity and stability in the water columns, conditions observed in the summer periods (Pistocchi et al. 2011). Recent studies performed by Team 2 indicate that toxin synthesis is modulated by dinoflagellate cells at key phases of their proliferation (Brissard et al. 2014, Ternon et al. 2022b), or when they are submitted to biotic (chemical interactions; Ternon et al., 2018) and abiotic (temperature, hydrodynamics; Gemin et al., 2021) stressors. For instance, chemical interactions with co-occurring benthic diatoms and bacteria stimulate the biosynthesis of phycotoxins in *Ostreopsis* cells (Ternon et al., 2018, and unpublished data from team 2).

Despite the extensive investigations into the ecological and cellular basis of HABs, **the molecular processes underpinning *Ostreopsis* proliferation and its toxicity** still remain largely elusive. The aim of the proposed PhD project is to identify the molecular pathways involved in cell proliferation and toxin biosynthesis in *Ostreopsis*.

More specifically in the proposed project the main aims are:

1. **To identify genes involved in cell proliferation** (mitosis, cytokinesis, trophic mode) **and toxin biosynthesis**) by analysing transcriptomic data sets generated from monoclonal *Ostreopsis* cultures established in team 2. Transcriptomic datasets for cultured *O.* cf. *ovata* are already available in team 1. The student will identify orthologues of candidate genes involved in cell proliferation, trophic modes and toxicity in the two species. For some of the key conserved genes, the student will then analyse the transcription profile using qPCR and the the spatio-temporal distribution of the encoded protein, using specific antibodies (custom-made or commercially available), at different cell cycle stages and during bloom development in the Bay of Villefranche and correlate it with changes in proliferation and with the toxicity profile of the culture.

2. **To obtain a snapshot of the active gene repertoire** associated with different bloom stages and define the changes in transcriptional landscape associated with the development of a *O.* cf. *ovata* bloom. Team 1 has already generated meta-transcriptomic data corresponding to different stages of *O.* cf. *ovata* bloom (quiescent, bloom establishment, stationary phase and post-bloom senescence, Figure 2) occurring in the Bay of Villefranche. *Ostreopsis* reads have been identified from the meta-transcriptome by mapping to reference transcriptomes obtained from monoclonal *Ostreopsis* cultures. Using these datasets, the student will initially analyse the expression profiles of the candidate genes identified in aim 1 to determine which genes undergo changes in transcriptional level that could influence the change in toxicity and proliferative state observed during the bloom.
In a parallel unbiased approach, the student will then identify all genes whose expression level varies when comparing different proliferative stages and that could therefore be involved in the switch in proliferative mode, in the synthesis of toxins and in a possible change in trophic status which might influence bloom development (auto- vs mixo-trophy). The expression profile of candidate genes will then be confirmed by qPCR in samples to be collected during *Ostreopsis* bloom in Villefranche (sampling and analysis of bloom dynamics with team 2).

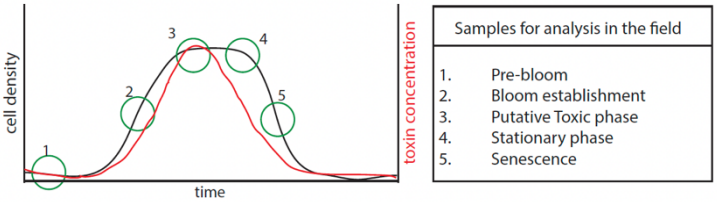

Figure 2: Variation in cell abundance (green) and toxicity (red) during bloom development and senescence. Numbers indicate sampling time for metatrascriptomic analysis during bloom development.

3. **To analyse changes in the expression of biosynthetic pathways associated with changes in proliferation mode and/or toxicity.** For these functional studies, the student will use clonal populations of *O.* cf. *ovata* available in team 2, and will analyze, under standardized culture conditions, the impact of single biotic (presence/absence of bacteria, competitors, predators) and abiotic (different temperature, salinity, hydrodynamic) stressors on cell cycle progression, growth rate, gene expression profiles and toxicity to determine how changes in any of these environmental parameters affect the machinery controlling toxin biosynthesis and how these changes are translated in differences in population growth and toxicity. Recent findings obtained by Team 2 indicate that the bacterial consortium occurring in culture with *O.* cf. *ovata* favors cell proliferation while it reduces toxin synthesis (Delie, 2022). The effects of bacteria on *Ostreopsis* metabolism were shown to be effective after 5 days of co-culture. The student will repeat these co-culture experiments to generate transcriptomic data set (cell culture, total RNA extraction for outsourced library generation and sequencing, *de novo* assembly of reads) from both control and treated cultures to determine changes in the gene expression profile. Sampling will be performed at the beginning of the interaction (+2 days), at the onset of the interaction (+ 5 days) and at the senescence of the culture (+ 10 days). Samples for the chemical content will also be collected at the same time points to correlate toxins cellular content to gene expression.

Deciphering the molecular machinery controlling dinoflagellate proliferation will provide important insight into the mechanisms underlying bloom formation and toxicity. Moreover, dissecting cell cycle

control in a distantly diverging eukaryotic group, such as the dinoflagellate, which has so far remained largely undescribed, will provide important information to our understanding of the evolution of cell cycle control in eukaryotes. The identification of molecular signatures associated with pre-bloom and bloom formation will also provide potential molecular tools for more precise and effective monitoring of bloom development. Finally, the bioactivity of phycotoxins also constitutes an **incredible opportunity in biomedicine** with many phycotoxins being currently investigated as treatment for modern diseases, including cancer (Assunçao et al., 2017 Cho et al., 2020). A molecular understanding of the biosynthetic pathways involved in phycotoxins production will pave the way for the implementation of their *de novo* synthesis, an essential step to allow for future opportunities/exploitation in **clinical research**.

The project will be carried out at the Institute de la mer in Villefranche (IMeV) in the teams of Stefania Castagnetti (UMR7009) and of Rodolphe Lemée (UMR7093). The two teams have complementary expertise respectively in cell biology/bioinformatics and ecology/chemistry, which are combined and necessary for the completion of the project. The student will carry out the bioinformatic analysis (aims 1-3), candidate validation (immunofluorescence and qPCR, aim 1-2) and cell cycle analysis (aim 1) in team1. Team 2 will be in charge of microalgal *in situ* collection and cultivation under different ecological conditions (aim 1-3), as well as chemical analysis and metabolite synthesis pathways (aim 1 and 3) under the supervision of Dr Ternon, an expert in toxin chemistry.

References:

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