ROLE OF GENOTYPIC AND PHENOTYPIC (ALLELOPATHY) DIVERSITY OF HARMFUL ALGAL BLOOMS (HABS): CASE OF THE DINOFLAGELLATE ALEXANDRIUM TAMARENSE

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The adverse effects of harmful algal blooms (HABs) in marine ecosystems have focused attention on defining the role of factors that determine their increasing abundance and distribution. The dinoflagellate *Alexandrium tamarense* blooms regularly in cold temperate waters and can produce a suite of potent neurotoxins that cause paralytic shellfish poisoning (PSP) in humans, and have serious deleterious impacts on public health, ecological and economic resources. These toxins can accumulate to dangerous levels in shellfish and affect plankton food webs. Chemical interactions (allelopathy) among *A. tamarense* and other marine protists are an important biological factor for the dominance of *Alexandrium*, not solely restricted to the competition for nutrients under laboratory conditions. We tested the ecological significance of allelopathy in the regulation of *Alexandrium* population dynamics at a larger scale (15 x 2.5 m³ mesocosms) in the semi-enclosed bay of Hopavågen, Norway, during July 2009. Mesocosms bags were filled with filtered seawater to eliminate large zooplankton (< 200 µm in 12 bags) and both grazers and zooplankton (< 0.2 µm in 3 bags). Two different isolates of *A. tamarense* (lytic and non-lytic) were inoculated (100-200 cells L⁻¹) separately (2 x 3 bags, < 200 µm) and together (1 x 3 bags, < 200µm and 1 x 3 bags, < 0.2µm). Three bags were left without *A. tamarense* as controls. Nutrients (nitrate and phosphate) were added daily to all mesocosms. Multi-variable sampling revealed that both *A. tamarense* grew for two weeks in all inoculated bags (1000 cells L⁻¹) but never formed a dense bloom. The coccolithophorid *Emiliania huxleiyi* outcompeted other phytoplankton after 2 weeks and bloomed in all bags, favored by the disappearance of appendicularians. Important results include gene expression profiles of *Alexandrium* and the prevalence of two different genotypes in mixed communities, PSP toxin profiles in different trophic levels meta-, micro-zooplankton, phytoplankton, protistan grazing on *A. tamarense* and the impact of lytic *A. tamarense* of nanoflagellate bacterivory, and large datasets on both marine eukaryotic microplankton and prokaryotic diversity. Our study provides a platform for further studies on allelopathy in aquatic systems and its implication in ecological processes.

1. INTRODUCTION

Phytoplankton can produce a wide range of biologically active organic compounds, including allelochemicals. Those, as defined here, are secondary metabolites produced by one phytoplankton species, affecting the growth or physiological function of another microbe (protist or bacteria). Allelopathy is also an adaptation by some phytoplankton to achieve a competitive advantage over other species, and hence should be included as a relevant factor in competition in addition to traditional abiotic and biotic factors (Legrand et al. 2003). Also, allelochemicals with antibacterial,
antifungal and antiviral properties may play an indirect role in algal succession (Ianora et al. 2006). It has been shown that members of one plankton genus e.g. *Alexandrium* (Fig.1) can have different allelochemical properties (Tillmann & John 2002, Tillmann et al. 2007, 2008, Alpermann et al. 2009) combined with diverse genotypes and this has a strong impact on the population success of a given species (Yoshida et al. 2003).

![Image](image-url)

Figure 1. *Alexandrium tamarense*, lytic strain, isolated from the North Sea by U. Tillmann. Photo: C. Legrand.

The adverse effect of harmful algal blooms in marine ecosystems has focused attention on defining the role of factors that influence their abundance and distribution. There is no study about the ecological relevancy of allelopathy in bloom development of HAB species (but see Rijssel et al. 2007). Furthermore, the impact of phenotypic and genotypic diversity in bloom formation and success has not been investigated in large-scale experiments to our knowledge.

2. SCIENTIFIC OBJECTIVES

The overall objective of this experiment is to test the ecological significance of genetic and phenotypic (allelopathy) diversity in the regulation of *Alexandrium* population dynamics in mesocosms.

The measurable scientific objectives of this project are to

- Investigate the changes of the gene expression profiles of *Alexandrium* over the bloom formation in order to understand the regulation of toxicity and growth
- Quantify the role of allelopathy on pelagic microplankton (eukaryotes and prokaryotes) communities during the development of an artificially induced bloom of *A. tamarense* in presence and absence of allelochemicals
- Test the effect of allelochemicals on phenotypic and phylogenetic changes in bacterial communities and their interactions with nanoflagellate grazers during a bloom of *A. tamarense*
- Test the direct and indirect effects on allelochemicals on protistan species (grazers and competitors) and communities.
- Investigate the trophic links between different size fractions of plankton and how these trophic links are affected by allelochemicals
- Provide a platform for further studies on allelopathy in aquatic systems and its implication in ecological processes
3. METHODOLOGY

Two strains of *Alexandrium tamarense* (A2 lytic and A5 non lytic), isolated from the North Sea, producing Paralytic Shellfish Poisoning toxins (saxitoxins) were used to test the ecological role of allelopathy in bloom development of a HAB species in mesocosms.

Mesocosm studies (15 x 2.5 m³ mesocosms, Fig. 2) were carried out in the semi-enclosed bay of Hopavågen, Norway, during July 2009. Mesocosms bags were filled with filtered seawater to eliminate large zooplankton (< 200 µm in 12 bags) and both grazers and zooplankton (< 0.2 µm in 3 bags). Two different isolates of *A. tamarense* (lytic and non-lytic) were inoculated (100-200 cells L⁻¹) separately (2 x 3 bags, < 200 µm) and together (1 x 3 bags, < 200µm and 1 x 3 bags, < 0.2µm). Three bags were left without *A. tamarense* as controls. Nutrients (nitrate and phosphate) were added daily to all mesocosms.

Samples for nutrients and chlorophyll analyses were taken daily. Identification and enumeration of the plankton communities (protists, nanoflagellates, bacteria) including *Alexandrium* and PSP toxins analyses were done every 3rd day. Samples were taken both for microscopic determination and molecular diversity analyses of both eukaryotes and prokaryotes. The relative abundance of different strains of *Alexandrium* was monitored through qPCR/AFLP based methods every 3rd day. Samples (100L) for RNA extraction (transcriptome) were taken once a week.

4. PRELIMINARY RESULTS

Multi-variable sampling revealed that both *A. tamarense* grew for two weeks in all inoculated bags (1000 cells L⁻¹) but never formed a dense bloom. The coccolithophorid *Emiliania huxleyi* outcompeted other phytoplankton after 2 weeks and bloomed in all bags, favored by the disappearance of appendicularians. Chlorophyll a ranged 1-10 µg L⁻¹ during the study period. Important results include gene expression profiles of *Alexandrium* and the prevalence of two different genotypes in mixed communities, PSP toxin profiles in different trophic levels meta-, micro-zooplankton, phytoplankton), protistan grazing on *A. tamarense* and the impact of lytic *A. tamarense* of nanoflagellate bacterivory, and large datasets on both marine eukaryotic microplankton and prokaryotic diversity. Our study provides a platform for further studies on allelopathy in aquatic systems and its implication in ecological processes.

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