Temperature rules growth but salinity rules toxicity in a *Alexandrium minutum* culture from an estuarine area in NW Spain

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POSTER Nº B-P-8

Introduction

The dinoflagellate *Alexandrium minutum* is responsible for paralytic shellfish poisoning (PSP) episodes in Western Europe[1], commonly in areas directly affected by significant freshwater inputs, as the Ría de Vigo (NW Spain), a coastal embayment with estuarine influence characterized by seasonal upwelling. Because pronounced environmental short-term changes characterized these ecosystems, tolerance windows for *A. minutum* against temperature (T) and salinity (S) stress were studied using a factorial approach.

Material & Methods

A gradient matrix with five temperatures (12.5; 14.4; 19; 23.6; and 25ºC) and five salinities (5; 9.7; 21; 32.3 and 37) were assayed on a clonal *A. minutum* strain. Samples to estimate cell densities were collected every 2-3 days during 42 days, fixed with acidic Lugol's solution (0.5%) and two aliquots of each sample counted in a 1 ml Sedgwick-Rafter counting chamber. Growth rates were calculated following the equation $\mu = (\ln N2 - \ln N1)/(t2 - t1)$. The impact of these environmental conditions in toxin production (HPLC-FLD-PCOX method[2] modified by[3]) and cell biovolume (Imaging Flow Cytometry, Amnis FlowSight), was also checked on selected conditions according with the previously determined highest and lowest growth points (temperatures 15ºC and 25ºC, and salinities 10 and 30). Samples were taken once a week during a period of 4 weeks. For biovolume measures the cells were classified according to their DNA content (stained with propidium iodide) as "C" (basic content of the species) or "2C" cells with double DNA content (cells in mitosis). Statistical analyses were performed using R software (RStudio 1.2.1335 version). Ideas 6.0 (Amnis) was used to measure cell volumes and to identify C cells and 2C cells.

Results

The optimal values of temperature for growth were determined in the range of 19.0-20.6ºC, while salinity tolerance covered all the range tested, with limited influence for growth. Clonal toxin profile was characterized by pre-dominantly production of GTX4, followed by GTX3 and smaller amounts of GTX1 and GTX2, but toxin production was focussed only on GTX4 as another gonyautoxins could not always be quantified.

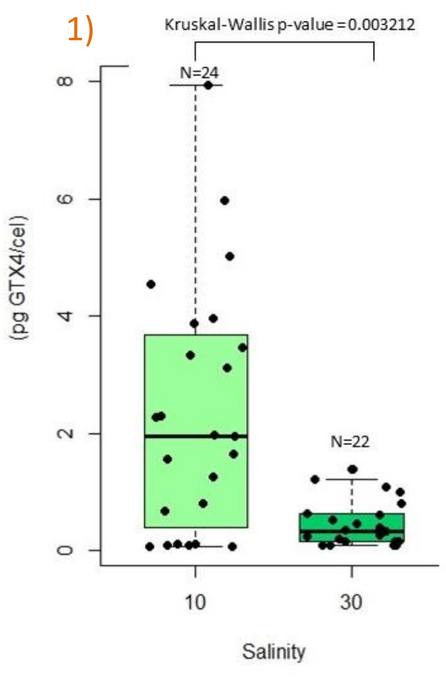

Figure 1.- Boxplot (with all data points, median, minimum, maximum and interquartile range) and Kruskal-Wallis test results showing a significant effect of salinity on cellular toxicity (pg GTX4/cel).

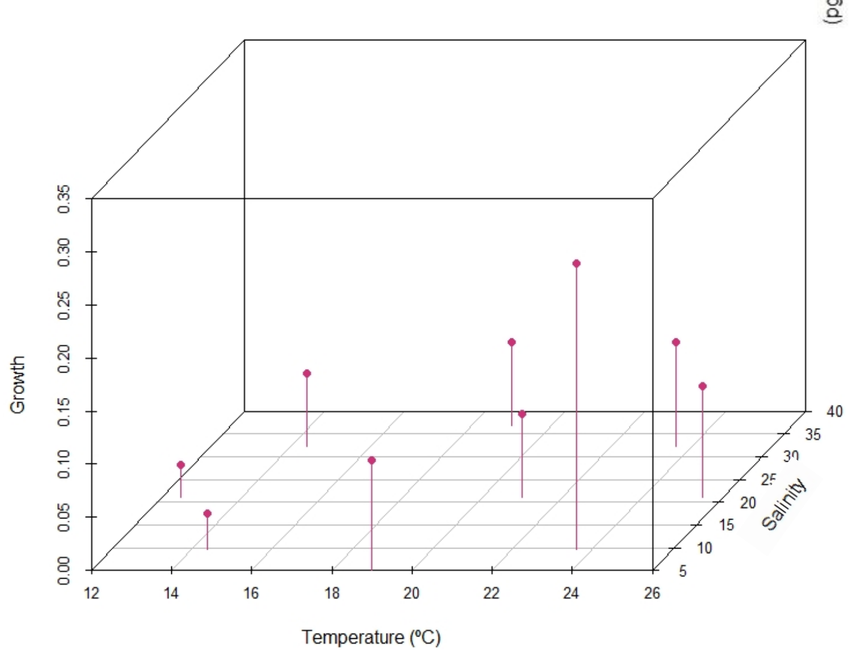

Figure 2.- Growth rates (μ) for each temperature: salinity combination studied.

Toxin results showed a significant increasing content of GTX4 per cell when decreasing salinity and no significant response to temperature. Biovolume remained stable throughout temperature and salinity conditions.

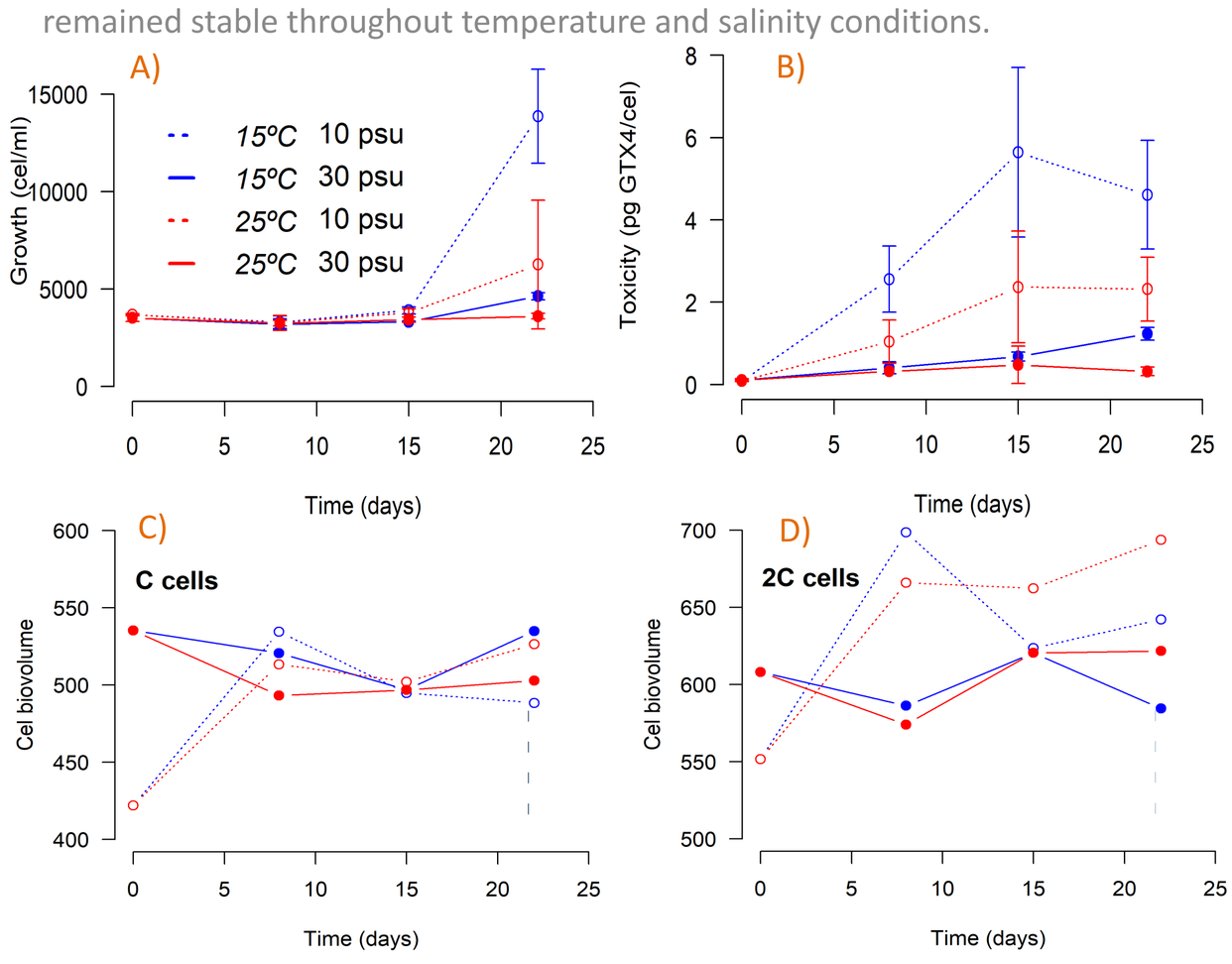

Figure 3. (A) Growth rates (cel/ml); (B) cell toxicity (pg GTX4/cel); (C) cel biovolume (μm³/cel) in C cells and (D) cel biovolume (μm³/cel) in 2C cells.

Conclusions & Discusion

A. minutum toxicity was not affected within the range of temperatures studied (12.5-25.5ºC). Neither temperature nor salinity ranges (10-30 psu) lead to significant differences over the rates of population growth and cell biovolume. However, an important effect over the toxicity of cells (pg GTX4 per cell) was detected depending on the salinity values tested. These results confirm *A. minutum* as an euryhaline and eurythermic species, an adaptive advantage to outcompete other species[4]. In a global warming scenario, this characteristic could help this species to expand and be present in an increasing number of locations[1]. Although a saline-gradient relationship has been reported before for the *A. minutum* vegetative cell distribution[5], our study highlights the relevance of salinity also on bloom toxicity, suggesting that blooms could be more toxic in estuarine locations with important freshwater inputs.

Acknowledgements

Sixto, M. acknowledges the pre-doctoral contract from the Spanish Ministry MINECO linked with DIANAS project (CTM2017-86066-R). This work is a contribution to the "Unidad Asociada de Microalgas Nocivas, IEO-CSIC". Thanks to the support of the EU-INTERREG Atlantic Area project PRIMROSE (EAPA 182/2016); GAIN grant GRC-VGOHAB IN607A-2019/04 for Galician Networks of Excellence, Innovation Agency (Xunta de Galicia) and the CCVIEO culture collection of microalgae (IEO Vigo).

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