

# Cell size changes linked to life cycle may influence toxin (DA) production in three *Pseudo-nitzschia* species

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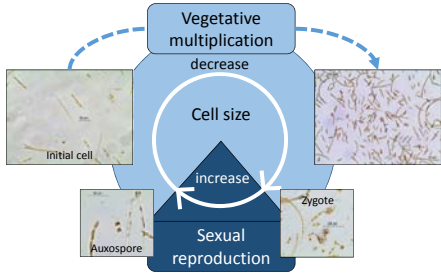
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## Introduction

- Three diatoms that produce a **neurotoxin, domoic acid (DA)**: *Pseudo-nitzschia australis*, *Pseudo-nitzschia pungens* and *Pseudo-nitzschia fraudulenta*.
  - Responsible for the ASP syndrome in humans (Amnesic Shellfish Poisoning).
  - Severe sanitary and socio-economic consequences for shellfisheries or fisheries.
- Life cycle**: reduction in cell size during vegetative multiplication and obligatory sexual reproduction to restore large size cells.



## Objectives and methods

Characterization of *Pseudo-nitzschia* cell size changes during life cycle (gametangia\* and initial cell).

### Mating experiments

76 for *P. australis*, 61 for *P. pungens* and 120 for *P. fraudulenta* at different cell sizes.

#### Before each experiment:

- Acclimation to experimental conditions
- Parental strains size measurement

#### During experiment:

- Microscopic observations to detect the different sexual stages

#### When sexual reproduction succeeded:

- Initial cells size measurement

\*Cells were considered at the « gametangia stage » when they were able of sexual reproduction.

Several strains (≥ 9) for each species.

### Experimental conditions

16°C – 100 μmol photons.m<sup>-2</sup>.s<sup>-1</sup>

Study of the influence of cell size (linked to life cycle) and age of strain on growth rate and DA production

### Batch experiments

36 for *P. australis*, 32 for *P. pungens* and 30 for *P. fraudulenta* at different cell sizes.

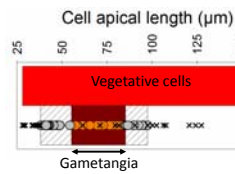
- Silicate or phosphate limitation
- To induce DA production in stationary phase

#### On the second day of the stationary phase:

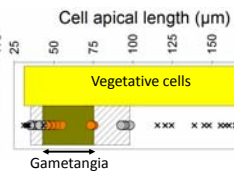
- Cellular DA (cDA) measurement by ASP ELISA kit (Biosense)

## Cell size characteristics of life cycle stages

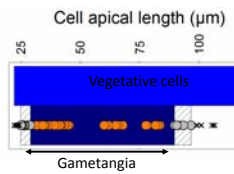
### *Pseudo-nitzschia australis*



### *Pseudo-nitzschia pungens*



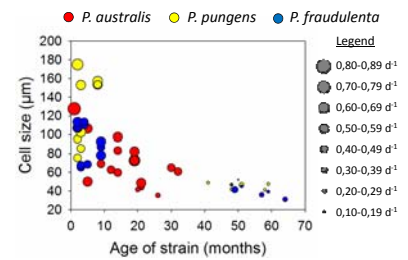
### *Pseudo-nitzschia fraudulenta*



- Sexual size range narrower in *P. australis* and *P. pungens*

Possible consequences on the timing of sexual reproduction in natural population and thus on population dynamics during blooms (D'Alelio et al. 2010).

## Cell size and/or age of strain influence growth rate



*P. australis*: influence of cell size

$$\mu = 0.3228 + 0.003534 * \text{Size} \quad (p < 0.05)$$

*P. fraudulenta*: influence of age

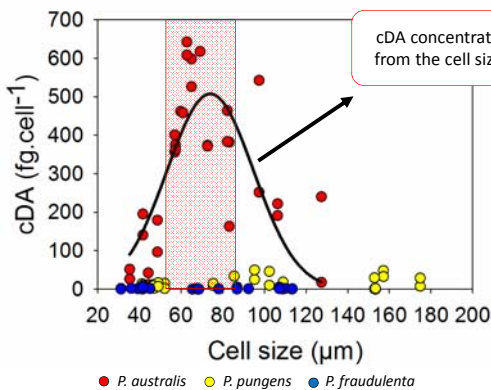
$$\mu = 0.6093 - 0.00481 * \text{Age} \quad (p < 0.001)$$

*P. pungens*: influence of cell size and age

$$\mu = 0.4563 - 0.0051 * \text{Age} + 0.0014 * \text{Size} \quad (p < 0.001)$$

## Life cycle stages influence toxin (DA) production in *P. australis*

9 *P. australis* strains, 14 *P. pungens* strains and 11 *P. fraudulenta* strains at different cell sizes

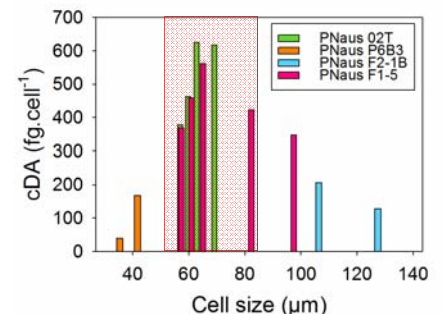


cDA concentration in *P. australis* can be predicted from the cell size by a **Gaussian model** ( $p < 0.001$ )

This model predicts max cDA concentration at 71 μm.

*P. australis* is particularly more toxic in the size range where cells are capable of sexual reproduction

4 *P. australis* strains tested at different cell sizes during size reduction



## Conclusions

Age of strain

Growth rate of *P. pungens* and *P. fraudulenta*

Cell size / life cycle

Growth rate and toxin production of *P. australis*

- Sexual reproduction during blooms may influence their toxicity.
- Modification of cellular metabolism that favors DA production when cells are capable of sexual reproduction.
- Link DA – sexual reproduction ?