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

**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.*

**Cell size characteristics of life cycle stages**

<table>
<thead>
<tr>
<th><em>Pseudo-nitzschia australis</em></th>
<th><em>Pseudo-nitzschia pungens</em></th>
<th><em>Pseudo-nitzschia fraudulenta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell apical length (µm)</td>
<td>Cell apical length (µm)</td>
<td>Cell apical length (µm)</td>
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<tr>
<td>Vegetative cells</td>
<td>Vegetative cells</td>
<td>Vegetative cells</td>
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<tr>
<td>Gametangia</td>
<td>Gametangia</td>
<td>Gametangia</td>
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</table>

- 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
  - \( \mu = 0.3228 \pm 0.003534 \) Size \((p < 0.05)\)
- *P. fraudulenta*: influence of age
  - \( \mu = 0.6093 \pm 0.00481 \) Age \((p < 0.001)\)
- *P. pungens*: influence of cell size and age
  - \( \mu = 0.4563 \pm 0.0051 \) Age + 0.0014 Size \((p < 0.001)\)

**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
- *P. australis* gametangia size range

**Conclusions**
- 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?

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

- **Batch experiments**
  - 16 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 EUSA kit (Biosemse)

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