

Exposure of the eastern oyster, *Crassostrea virginica*, to *Alexandrium monilatum*: toxicity pathway, histopathology and gene expression

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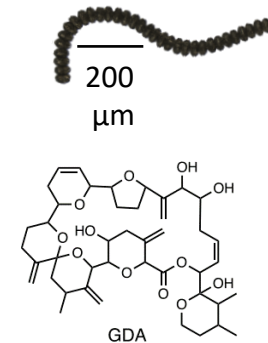
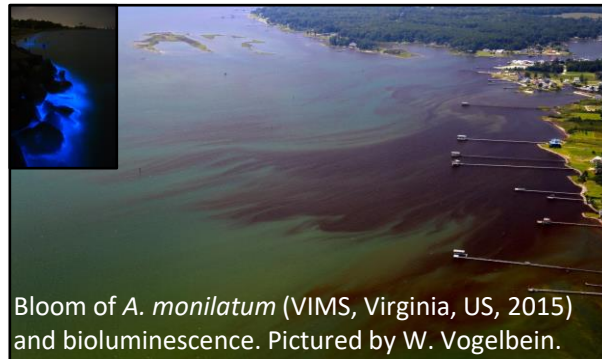
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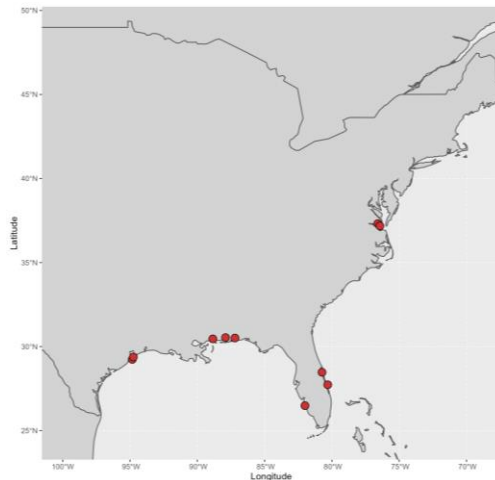
Virginia HAB Task Force meeting
February 24th 2023

Introduction – *Alexandrium monilatum*

- Bioluminescent dinoflagellate ***A. monilatum*** has tropical and sub-tropical distribution (Howell, 1953; Halim, 1967; Viquez and Hargraves, 1995; Mackiernan, 1968)
- Dense and large **blooms** in the South of the **Chesapeake Bay**, almost **annually since 2007** in the York River (Wolny et al. 2020)



- Produces toxigenic **compounds**, the **goniodomins**, negatively impact aquatic organisms:



- Fish and shellfish mortality exposed from days to a week to bloom (10^3 cells · mL⁻¹ in Florida and Gulf of Mexico) (see May et al. 2010)

- Epithelial erosion in *Crassostrea virginica* in the Chesapeake Bay (days, 10^3 cells · mL⁻¹) (Pease 2016)

- Accumulation of goniodomin A (2 to 8770 µg · kg⁻¹) in bivalves (months, 40×10^3 cells · mL⁻¹) (Harding et al. 2009)

Introduction – *Alexandrium monilatum*

- Laboratory exposure studies:

- **Mortality** in fish

- Larvae, juvenile and adults (hours, 10^2 cells · mL⁻¹ culture in senescent phase or lysed cells) (Gates and Wilson 1960; Sievers 1969; Ray and Aldrich 1976; May et al. 2010)

- **Negative effect** in **shellfish** (Sievers 1969, Ray and Aldrich 1976, May et al., 2010)

- Shell closure, decrease in clearance rate and valve gape (*P. viridis* and *C. virginica*) and **mortality** (*M. mercenaria*) (days, 10^{2-3} cells · mL⁻¹ senescent phase)

- Mortality in larval *C. virginica* and *M. mercenaria* (hours, 10^2 cells · mL⁻¹ lysed)

- Effect of extracellular compounds (goniodomins?)

- What effect does a low-concentrated, exponentially growing culture of ***A. monilatum*** in "clean" culture medium have on the **physiology** and **gene expression** of *Crassostrea virginica*?

- What are the kinetics of **accumulation**, **compartmentalization** and **depuration** of GDs?

Materials and methods – 96h exposure

- Monoclonal *A. monilatum* (York River 2007 strain)
 - Producer of **GDA** ($> 1000 \text{ pg} \cdot \text{cell}^{-1}$ in **intra.**; $< 1 \text{ pg eq. cell}^{-1}$ in **extra.**), GDB, GDC and **GDA_{sa}** ($50 \text{ pg} \cdot \text{cell}^{-1}$ in **intra.**; $25 \text{ pg eq. cell}^{-1}$ in **extra.**)
 - Culture in **exponential growth** phase and resuspended in **clean culture medium**
- Adult **triploids** *C. virginica* (75 x 51 mm) provided by VIMS Hatchery (ABC) – acclimated and starved for 5 days before the experiment



Materials and methods – 96h exposure

- Experimental design

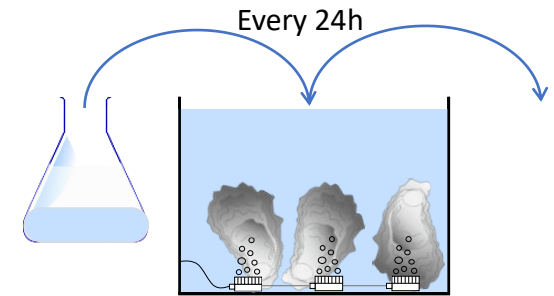
- **Exposure** (96h) followed by **depuration** (96h) - daily renewal of sea water and algae

- Three treatments:

- **Fed** with *A. monilatum* ($614 \text{ cells} \cdot \text{mL}^{-1}$)

- Fed with **non-toxic** phytoplankton

- **Unfed**



- Water sampling for phytoplankton **cell enumeration**, **clearance rate** calculation and **feces** or pseudo-feces observations

- Assessment of oyster **mortality** (response to physical stimulus)

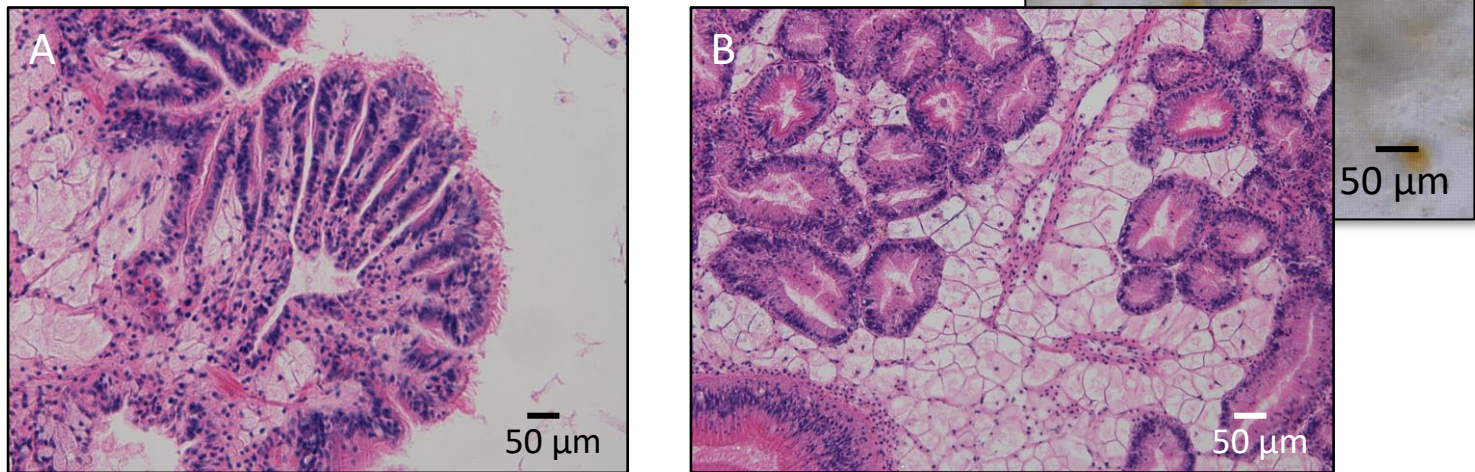


- Oyster **sampling** ($t = 0, 6, 24$ and 96 h of exposure and $t = 24$ and 96 h of depuration) for weight and measurements, **dissection** (gills, digestive glands and remaining tissues) for **toxin quantification** (IFREMER), **gene expression** analysis and paraffin **histology**



Results – Ecotoxicology of *C. virginica* exposed to *A. monilatum*

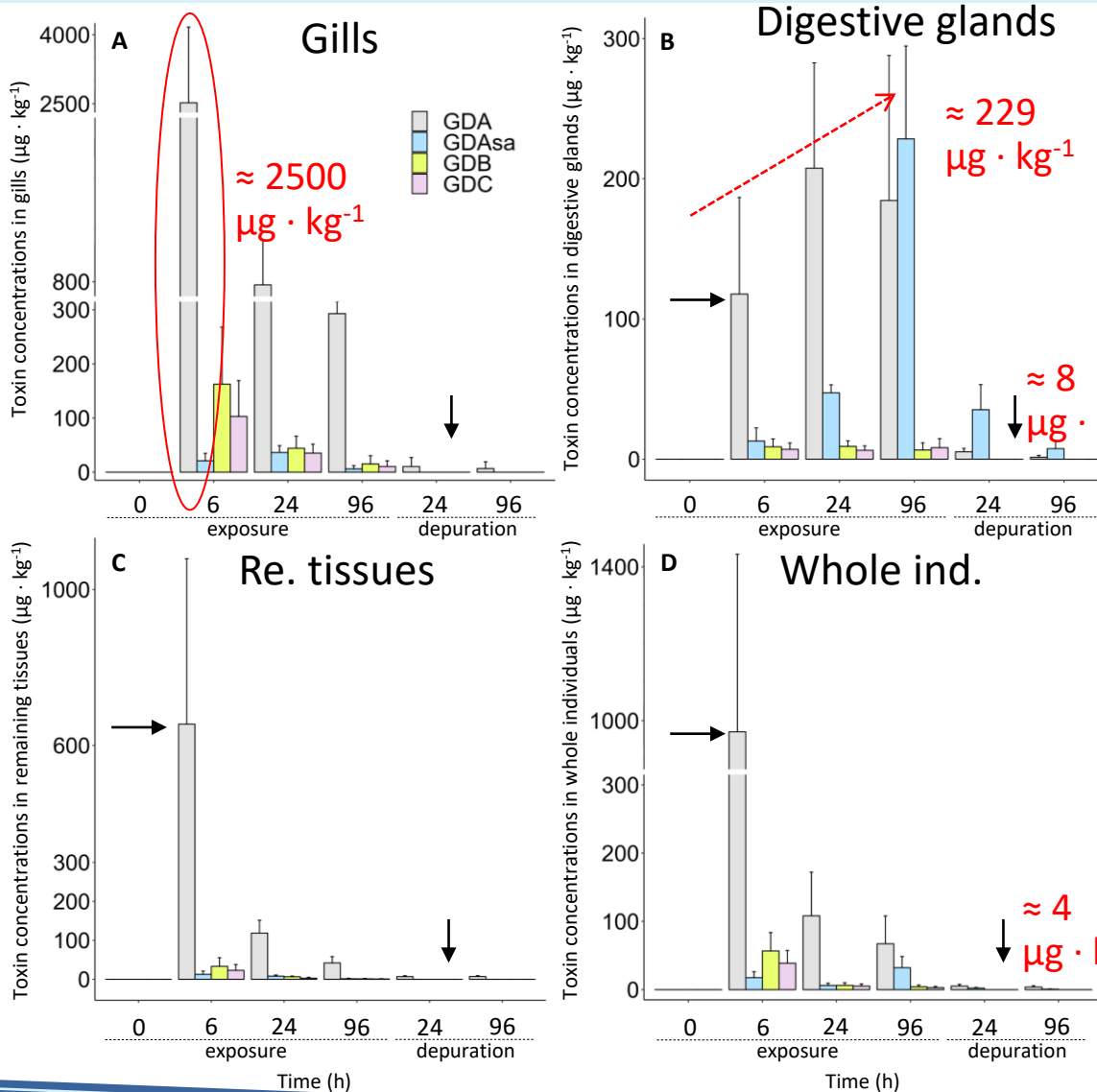
- **No mortality** in none of the 3 treatments – Positive clearance rate → **oysters filtered *A. monilatum*** (live cells in feces and pseudo-feces)
- No tissue pathology was observed:



A. Gill and B. digestive gland tissues of *Crassostrea virginica* exposed to $614 \text{ cells} \cdot \text{mL}^{-1}$ of *Alexandrium monilatum* (YR2007) for 96 h, exhibiting healthy histology.

- **Toxin quantification** in oyster tissues:

Results – Ecotoxicology of *C. virginica* exposed to *A. monilatum*



- **Uptake** (≤ 6 h) of goniodomins by oysters, while variability between individuals.

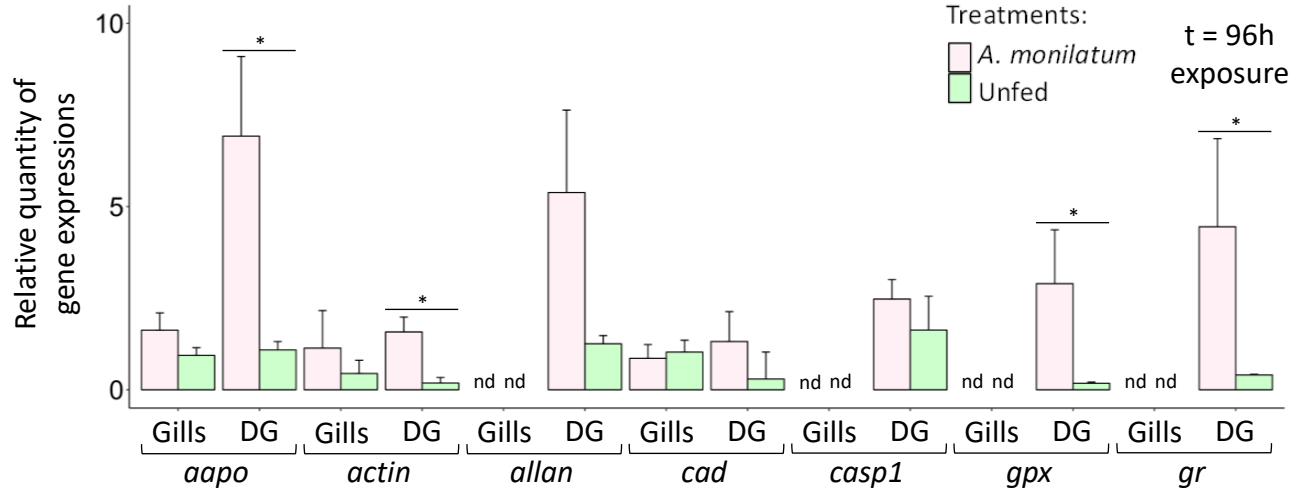
- **Max. [GDA]** in gills after 6 h; 21 and 4 times less in digestive glands and remaining tissues; 3 times less when normalized by weight

- After 24 h, \searrow in [goniodomins] in all tissues, but trend of **accumulation of GDA and GDAsa in digestive glands (biotransformation)**.

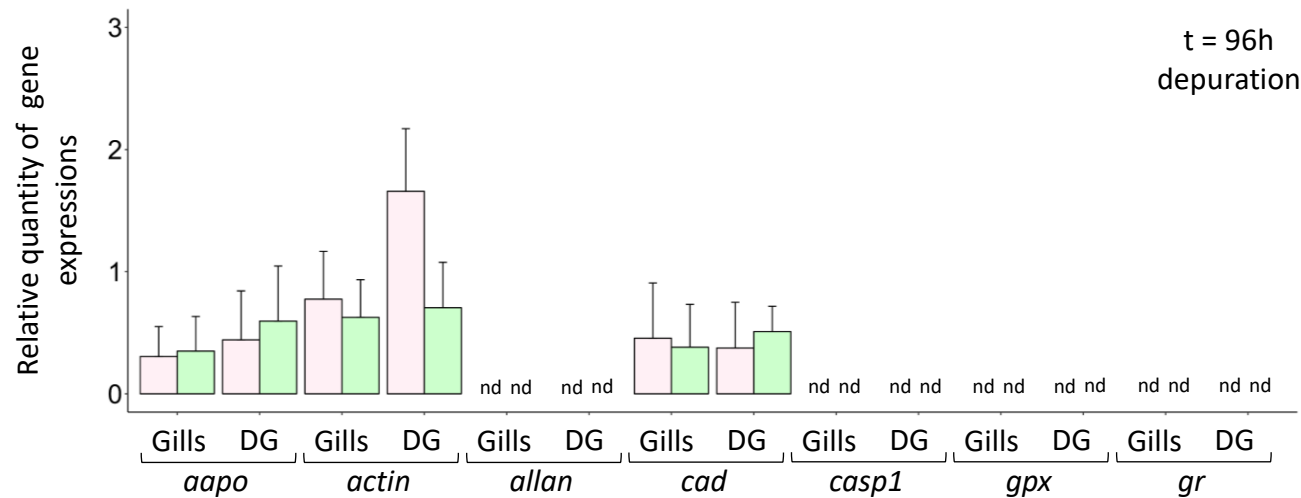
- Depuration: GDB and GDC non-detected after 24 h. **GDA detected after 96 h of depuration** in whole compartments and GDAsa detected only in digestive glands

Results – Ecotoxicology of *C. virginica* exposed to *A. monilatum*

• Gene expression



- **Overexpression** of genes involved in redox cycling (*gpx* and *gr*) → oxidative stress; actin synthesis (target of goniodomins) and apoptosis (*aapo*)



- **Down-expression** of redox genes → low antioxidant capacity - vulnerability
- **Low anti-inflammatory** and stress genes → detoxification

Conclusion – Ecotoxicology of *C. virginica* exposed to *A. monilatum*

- Toxin profile of a local strain of *A. monilatum* → **high GDA producer** (> 1 ng per cell)
 - **No mortality and visible physiological stresses** when exposed to low concentration of *A. monilatum* - Lower toxicity of the cells compared to extracellular metabolites
 - Observation of **uptake, compartmentalization, accumulation, biotransformation** and **depuration** of several goniodomins (GDA and isomers) by oysters during *in vitro* exposure (up to **2.5 ng · kg⁻¹**) – Trace of goniodomins after 4 days of depuration
 - Effects of exposure on expression of genes involved in important physiological process:
 - **Redox cycling** – Exposure caused oxidative stress - Vulnerability of oysters to oxidative stress (DNA damages, cellular damages) – Similar to oyster exposed to PST producer (i.e. *A. tamarense*; Qiu et al. 2013)
- Implications: physiological effects on commercially important species during dense and large bloom in the Chesapeake Bay
- Perspectives: Long term effect of chronic exposure? Transfer of toxicity through the food-web? Effect on sensitive early life stages?

Thank you for your attention

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