High-frequency valvometry reveals *Crassostrea virginica* behavior in the presence of microcystin-producing cyanobacteria and low salinity

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Background

Louisiana estuaries experience periods of high freshwater inflow that can sharply reduce salinity[1,2] and can serve as a conduit for freshwater algae that originate in upstream sources, such as ponds and lakes[3].

Eastern oysters from Louisiana better tolerate lower salinity compared to other populations[4], but they can then be exposed to freshwater cyanobacteria[5]. In fact, *Microcystis aeruginosa* and its associated toxin, microcystin (MC), have been detected in LA estuaries[1] and shellfish[6].

Valve closure is a common response of oyster exposed to environmental stressor, which can hinder their energy budget. With freshwater events occurring more frequently, potential effects of toxic cyanobacteria on oysters will increase.

Therefore, we ask

How do toxic and nontoxic cyanobacteria effect oyster behavior?

Methods

- Oysters were obtained from the LA Sea Grant's oyster hatchery in Barataria Bay (n = 220).
- *Microcystis aeruginosa* (toxic, UTEX LB3037 and nontoxic, UTEX LB2386) strains cultured in BG-11 media.
- Salinity slowly adjusted to 5 for oysters and algae
- Experimental setup included a toxic (n = 5) and nontoxic (n = 5) treatment, each with a no-oyster control aquaria (Fig. 1). Each treatment tank had 20 oysters.
- A total of 16 oysters were equipped with valvometers (Fig. 2., n = 8 per treatment) recording at a rate of 10 measurements per second.
- Experiment ran for 72 hours with water samples taken every 12 hours and 1 L treatment algae added every 24 h.
- Endpoints include valve opening amplitude (VOA) and intra-and extracellular toxins in water using ELISA.

Fig. 2. Valvometers being set on oysters.

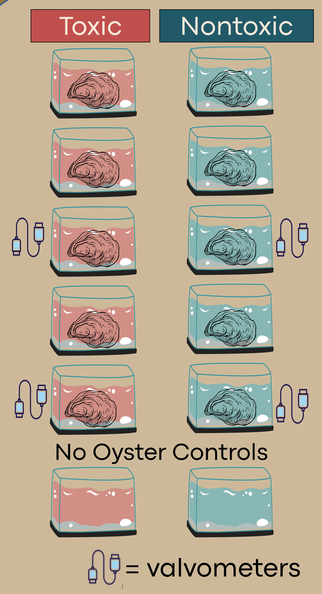

Fig. 1. Diagram of experiment setup. Each oyster represents n = 20; valvometer represents n = 4 measured oysters.

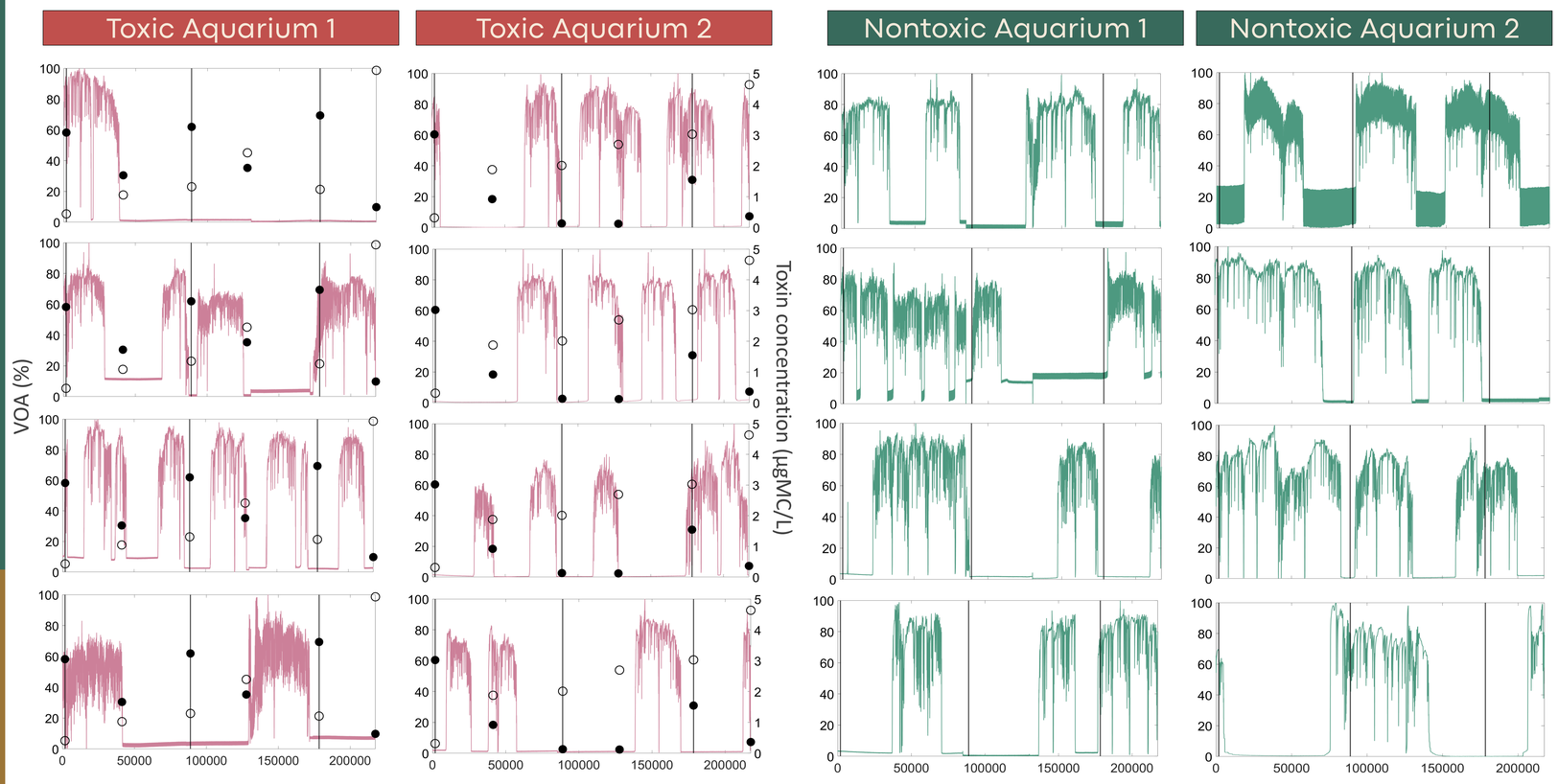

Fig. 3. Continuous valvometry data for all oysters measured (n = 16). Lines show percentage of max valve opening amplitude (VOA; left y-axis) collected 10 times every 1 second from oysters fed toxic (pink lines, left) and nontoxic (green lines, right) *M. aeruginosa*. Microcystin concentrations (µgMC/L; right y-axis) for the toxic treatment are represented by filled (intracellular) and empty (extracellular) circles. Vertical lines indicate feeding timepoints

Results & Discussion

Oysters in the toxic treatment (n = 8) did not spend significantly more time closed (*i.e.*, <10% VOA; $M = 55.86$, $SD = 15.78$) than oysters in the nontoxic treatment (n = 8, $M = 36.87$, $SD = 19.36$) as shown by a t-test between the treatment groups ($t(14) = 2.15$, $p = 0.06$). Across all timepoints in the toxic treatment aquaria (n = 5) microcystin concentrations were higher in the extracellular fraction ($M = 2.28$, $SD = 1.48$) than intracellularly ($M = 1.40$, $SD = 1.08$). All nontoxic treatment aquaria (n = 6) were confirmed to be nontoxic (below method detection limit). These results indicate that oysters may not close more often in the presence of microcystin, but more sample and data analysis needs to be conducted for these results to be conclusive.

Future Directions

Further processing of samples will include clearance rate of cyanobacterial cells and toxin concentration in oyster tissue, as well as more data analysis on the valvometry data. Results of this study will allow us to better understand the sublethal effects of a cyanobacteria diet at lowered salinities on oyster behavior and biology.

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