Initially discovered in the Bay of Marennine in northern France, the “greening” of oysters has become a well-known occurrence. *Haslea ostrearia* is pennate-shaped, benthic, or free-living and grows on other plant surfaces/algae. Typically diatoms produce a golden-brown pigment, but *Haslea* produces a unique blue-green pigment called marennine. This blue pigment is water-soluble and is usually expelled into the seawater by the diatom during growth, creating “blue water” (BW). This BW dyes the gills of oysters green, becoming an economic benefit because they create a higher price at market. Marennine has been shown to have antibiotic properties and may function as an immunostimulant for bivalves. However, other research on this pigment suggests that there may also be negative impacts on physiology, particularly at higher concentrations.

Green gills have recently been found worldwide, including in North Carolina. Although, whether this algae is a new species that produces this colored pigment or if it is a subspecies of *Haslea ostrearia* is hard to determine. Marennine gathers in the apical area of the cell (intracellular or IMn) and can be synthesized into the water (extracellular or EMn), but the mechanism that creates the synthesis and accumulation of marennine is yet to be identified. Marennine is still not well-known chemically or functionally, so its impact on bivalves and humans still needs to be explored.

**Figure 1:** Left: Eastern oyster without green gill (Chris Jewett) Right: Wild green gill eastern oyster from Stamp Sound

**Figure 2:** Live cell of *Haslea* under light microscopy (Guohtcine et al. 2014)

**Figure 3:** *Haslea* sp. at Cedar Island

**Methods: Collection, Isolation, and Growth**
- Samples were collected in Cedar Island on February 9, 2022, and in Stamp Sound on March 22, 2022.
- The samples were taken back to the Hatchery/the Algal Resource Center (ARC) in MarBionic at CMS, where the cells were isolated.
- Following observation of cell division, the diatoms will be cultured following previously developed protocols (Alves de Souza, pers. comm.).
- Blue water will be harvested, filtered, and concentration will be estimated using spectrophotometry.

**Methods: Exposure Experiments**
- For each exposure trial, there will be three different treatment groups: a control (no exposure to BW), low BW, and high BW, each consisting of 24 animals (<30mm shell height) per treatment
- Before exposure, the experimental animals will be measured (shell height, length, width, wet weight), and an additional 24 animals will be put in the freezer to quantify the initial condition according to the protocols in Lawrence and Scott.
- The bivalves will then be exposed to the BW treatment in aerated buckets for a period of 24 hours then moved into the flow-through zebrafish rack system.
- The rack system is connected to the main hatchery water system and receives food-laden, filtered, temperature-controlled seawater at 25°C.
- The animals will be in this system for a total of 12 weeks, with a mid-point (6 week) conditioning sample of 8 animals per treatment.
- Growth (Shell height, length, width and wet weight) will be assessed monthly.

**Analyses**
- Condition index analysis following Lawrence and Scott, will be quantified at the beginning, at 6 weeks, and at 12 weeks.
- Clearance rates (difference in particle counts between inflow and outflow of chambers) will be determined at biweekly intervals.
- The data on growth and survival will be analyzed through statistical tests evaluating interaction and significance.

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**References**

