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## SHORT COMMUNICATION

# IS TEMPERATURE, DISSOLVED OXYGEN, OR SALINITY DRIVING OYSTER MORTALITY ON BREAKWATERS? §

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**KEY WORDS:** living shoreline, predation, *Crassostrea virginica*, tidal elevation, *Stramonita haematoma*

## INTRODUCTION

Coastal human settlements are threatened by coastal erosion and flooding, and historically, these hazards were managed by hardening shorelines with structures such as seawalls, bulkheads, and revetments (Dugan et al. 2011). Hardening shorelines can produce negative ecological outcomes, so coastal managers may prefer shoreline protection that incorporates natural features, e.g., living shorelines (Bilkovic et al. 2016). For example, many living shorelines attempt to seed oyster reefs by incorporating hard structures as substrate for oyster settlement (Morris et al. 2019). Once established, oyster reefs provide a suite of ecological benefits including shoreline protection, improvement of water quality, habitat provision, and more (Grabowski et al. 2012). In this context, oyster reefs are especially prized as a living breakwater that can protect the shoreline and grow in pace with sea level rise (Morris et al. 2019).

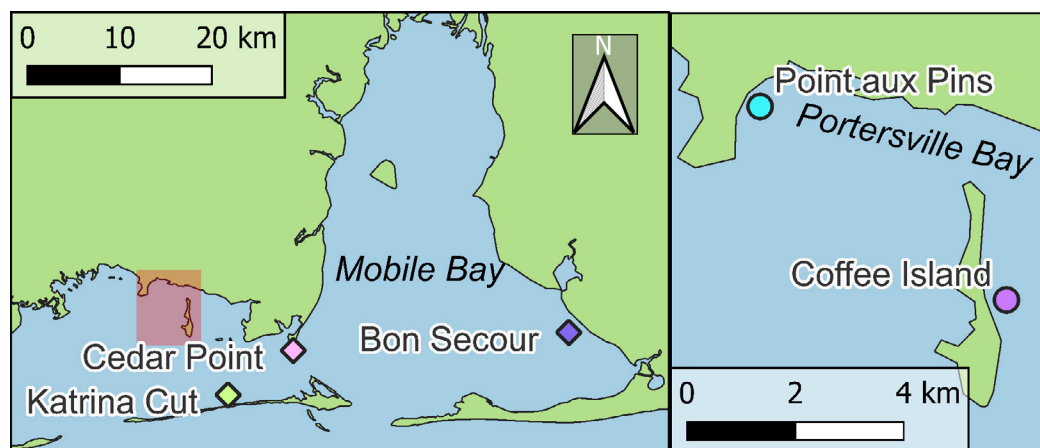
In practice, the successful development of oysters (*Crassostrea virginica*) at living shoreline sites varies regionally and from project to project (Morris et al. 2021; Wellman et al. 2021). Many ecological factors could affect whether oysters fail to settle or survive on breakwaters. A better understanding of these ecological factors could lead to more successful oyster development in future living shoreline projects (Morris et al. 2019). Here,

we report on the early stages of a field experiment examining potential factors limiting adult oyster development on living shoreline breakwaters in coastal Alabama. Specifically, we focus on whether water quality could be limiting oyster development on breakwaters. Water quality affects virtually every aspect of oyster biology; for example, unfavorable water quality can prevent larval development or kill settled spat and adults (Shumway 1996). Therefore, the main objective of this study was to see if water temperature, dissolved oxygen (DO), or salinity potentially affects the mortality of oysters on breakwaters.

## MATERIALS AND METHODS

### Study site

The study site included 2 living shoreline restoration sites in Portersville Bay, AL (Figure 1). The Point aux Pins (PaP) living shoreline was constructed in November 2020 and consists of 15 segments of emergent detached breakwaters composed of rows of large precast concrete structures (Wave Attenuation Devices, WADs). The Coffee Island (CI) living shoreline was constructed in April 2010 and consists of 9 subtidal detached breakwater segments; 3 segments composed of bagged oyster shell, 3 segments of ReefBlk (Coastal Environments, Inc.), and



**FIGURE 1.** Study site in Mobile Bay, AL. Left: Map of the ARCOS stations selected for continuous water quality data (diamonds). The red square indicates the extent of the inset map on the right. Water quality site colors correspond to data displayed on Figure 3.

Right: Map of the living shoreline study sites (circles) where tiles were deployed.

§The first author conducted this research as part of the Dauphin Island Sea Lab's Research Experience for Undergraduates in the coastal and nearshore marine science program.

3 segments of Reef Balls (Reef Ball Foundation).

#### Preparation and deployment of seeded tiles

Oysters were seeded onto the rough surface of 80 standard ceramic wall tiles (15.24 cm x 15.24 cm) at the Auburn University Shellfish Laboratory in early May 2023. Oyster spat were reared in a closed system at the Auburn Lab for about one week, then transferred to flow-through holding tanks at the adjacent Dauphin Island Sea Lab for about 3 weeks. Prior to field deployment, 8 tiles with the lowest number of spat were removed, and the remaining 72 tiles were culled to standardize about 10 spat per tile (range: 8–12 spat; ~430 spat/m<sup>2</sup>).

Seeded tiles were deployed in the field on 12 newly constructed deployment poles designed to maintain tiles at a

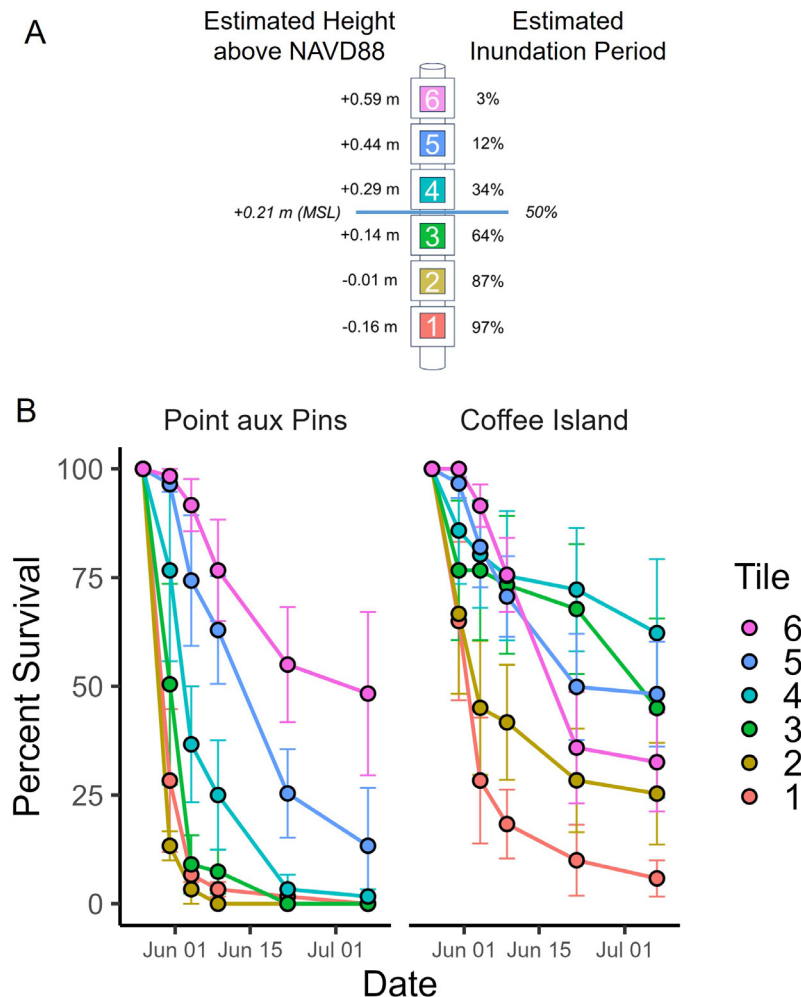
gradient of fixed intertidal elevations. Each deployment pole consisted of a base pole and a removable segment. The base pole (3.175 cm diameter PVC) was pounded into the sediment to the point of refusal and cut near the water line at the time of deployment (at about mid tide). The height of this base pole was measured in meters above the North American Vertical Datum of 1988 (NAVD88) using a Real Time Kinematic (RTK) Global Navigation Satellite System (GNSS) receiver (Emlid Reach RS2). The removable segment (5.08 cm diameter PVC) was drilled and tapped such that 6 tiles could be fixed to the pole using bolts and washers. In addition, a stopping bolt was installed in the segment based on the height of the corresponding base pole such that, when the segment

bolt rested on the base pole, the middle of the tiles was at a target elevation of +0.21 m NAVD88; this elevation was estimated to be the height of 50% inundation based on 3 years of water level data at the National Oceanic and Atmospheric Administration (NOAA) Dauphin Island Station (8735180) (Figure 2A). Therefore, 3 tiles (tiles 1–3) on each pole were expected to be inundated greater than 50% of the time, 3 tiles (tiles 4–6) were expected to be inundated less than 50% of the time, and tile height and exposure treatments were maintained throughout the experiment (Figure 2A).

Six seeded deployment poles were installed at the PaP site immediately shoreward of the 3 WAD segments as pairs of 2; each pair of poles was treated as pseudo replicates and combined into one “pole” for statistical analyses. Six poles constructed with tiles without oyster spat were installed to the south of the site away from breakwaters, also in pairs, to observe wild oyster settlement at the broader site. The deployment scheme at CI differed from that at PaP: deployment poles were installed in pairs of seeded and unseeded poles. Three deployment pole pairs were installed immediately shoreward of the Reef Ball segments; these breakwaters were selected since the other breakwater types (bagged oyster shell and ReefBlk) have noticeably degraded since their installation over a decade ago (Judy Haner, The Nature Conservancy, pers. Comm.). Three additional pairs of deployment poles were installed away from breakwater structures. This scheme was chosen to investigate whether the existing breakwaters at Coffee Island influenced oyster settlement or mortality.

#### Data collection

We frequently visited the deployment poles to count the live and dead oysters that were on each tile; the first 3 visits were within 5 days of the initial deployment (26 May 2023) or previous visit, while the fourth and fifth visits were done on a biweekly interval to conclude the monitoring period (7 July 2023). Water quality (temperature, DO, and salinity) parameters were measured prior to and after



**FIGURE 2.** Tile deployment configuration and survivorship of oyster spat. **A.** Diagram of the deployment pole configuration. The estimated height (m above NAVD88) and an estimated inundation period based on 3 years of water level data (2020 to 2022, from the National Oceanic and Atmospheric Administration (NOAA) Dauphin Island Station 8735180) for each deployed tile (1 to 6). **B.** Mean  $\pm$  se survivorship data for seeded oyster tiles, expressed as a percentage of surviving oysters from the initial count on each tile. Circle points represent actual sampling dates, with each tile number represented by differently colored lines, corresponding to **A**, above. Left: Point aux Pins. Right: Coffee Island.

sampling each site using a YSI handheld multimeter (ProSolo ODO/CT). We also leveraged Alabama's Real-Time Coastal Observing System (ARCOS, [arcos.disl.org](http://arcos.disl.org)) to obtain continuous data for temperature, DO, and salinity during the experiment. We selected ARCOS stations to represent a range of conditions across coastal Alabama (stations Cedar Point, Katrina Cut, and Bon Secour, Figure 1) for as long as the sensor data was available. ARCOS data were downloaded for the period of 22 May 2023 (4 days pre-deployment) to 10 July 2023 (3 days following the fifth visit).

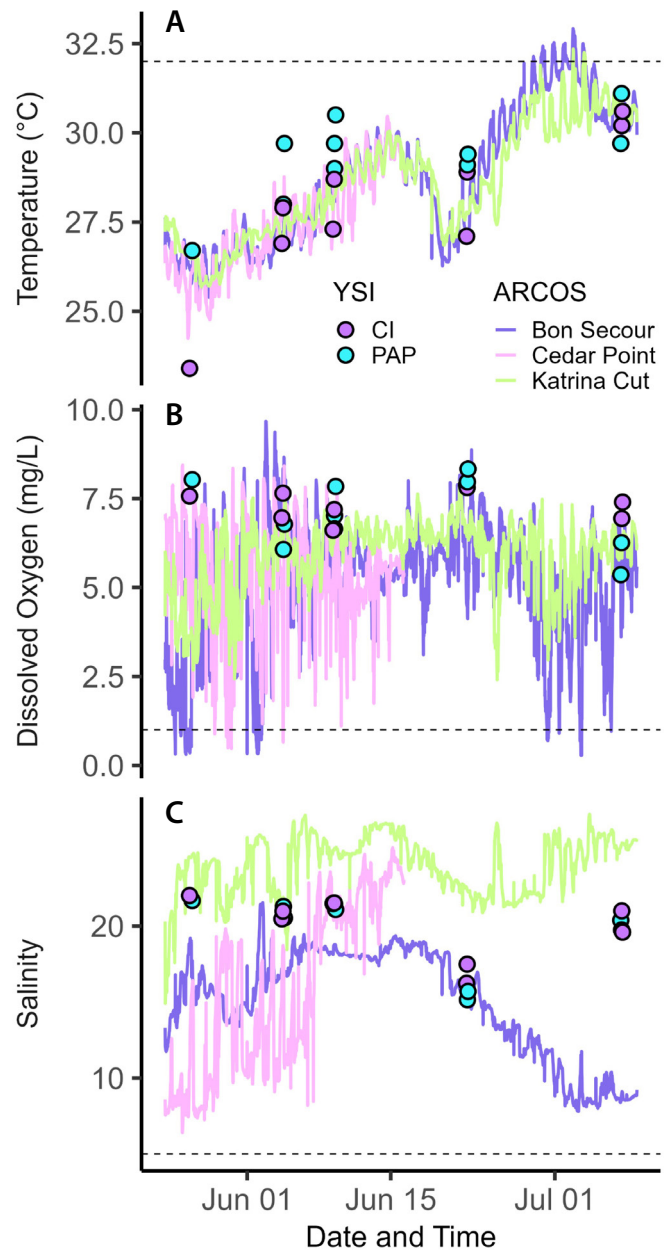
#### Statistical analyses

To determine whether oyster mortality on tiles varied across sampling locations, we performed 2 generalized linear mixed models using a logit link ("MASS" package, `glmmPQL` (family = "binomial"), Bates et al. 2015) on the status of deployed oyster spat (live or dead) across the factors of Site, Tile Number as an ordered factor, and Pole as a random factor. One regression was performed on the data collected 9 d post-deployment, and the second regression was performed on the data 42 d post deployment. To determine whether water quality parameters were likely driving oyster mortalities, we examined how continuously measured water temperature, DO, and salinity compared to known oyster tolerance thresholds, particularly during periods of significant oyster mortality on the tiles. Statistical analyses were conducted in the R software for statistical programming version 4.1.1. (R Core Team 2021).

#### RESULTS AND DISCUSSION

We observed significant oyster mortalities on tiles, with some tiles experiencing 100% mortality in as little as 5 days of field deployment (Figure 2B). On visit 2 (9 days post deployment, 06/04/2023), there was not a statistically significant effect of Site ( $t_7 = 2.191$ ,  $p = 0.065$ ), although there appeared to be marginally greater oyster mortality at PaP than at CI (Figure 2B). There was a significant effect of Tile number ( $t_{40} = 6.472$ ,  $p < 0.001$ ), as the oysters on the lower tiles died faster at both sites. On visit 5 (42 days post deployment, 07/07/2023), there were significant effects of both Site ( $t_7 = 2.611$ ,  $p = 0.034$ ) and Tile Number ( $t_{40} = 3.069$ ,  $p = 0.004$ ). Overall oyster mortality was greater at PaP by the end of the study period (Figure 2B). For tiles, there was a difference in survival across the tidal elevation range at each site. At PaP, oyster survival increased with increasing height and exposure period (Figure 2B). At CI, oyster survival appeared to maximize on Tile 4, and decreased on tiles below and above Tile 4. Additionally, no new oyster settlement was observed on either the seeded tiles or the unseeded tiles during this monitoring period.

Water quality was variable over the monitoring period and between sampling sites, but the onsite measurements were generally in the range of the continuous measurements (Figure 3). In this study, our aim was to determine whether measured water quality values likely represented extreme values that could lead to the oyster mortalities observed in this study. Therefore, we compared measured water quality to water quality values that have been reported as being relevant for oyster mortality.



**FIGURE 3.** Water quality data collected from onsite YSI readings (points) and continuously from ARCOS stations (lines). A. Water temperature. B. Dissolved oxygen. C. Salinity. Dotted lines indicate values considered to represent potential lethal limits for oysters (temperature: 32°C, dissolved oxygen: 1 mg/L DO, salinity: 5, see text for details).

ties. Rybovich et al. (2016) observed significant mortalities in spat and seed oysters subject to combinations of extreme temperatures (32°C) and low salinities (1, 5). Although oysters can occasionally survive at these extreme temperatures or salinities, they are more likely to become stressed and may die, especially if exposed to a combination of stressors (Rybovich et al. 2016). Therefore, we considered 32°C to represent a conservative lethal upper limit for oysters (Figure 3A), and salinity 5 to represent a conservative lethal lower limit for oysters (Figure 3C). Temperature was observed to briefly exceed 32°C in early



July only, while salinity was never observed below 5 during this monitoring period (Figures 3A and 3C). For DO, oysters have been observed withstanding concentrations of 1 mg/L DO for 5 d (Sparks et al. 1958). Hypoxic conditions were occasionally observed over the monitoring period at various sites, including 1 mg/L DO; however, such conditions never lasted more than a few days (Figure 3B). Therefore, we infer that neither water temperature, DO, nor salinity could account for the significant mortalities observed on the oyster tiles during this monitoring period. In fact, water quality appeared to be within oyster tolerance limits during the early stages of the experiment, during which significant oyster mortalities were observed shortly after deployment (Figure 2).

Besides water quality, there are multiple potential drivers of the oyster mortalities observed in the early stages of this experiment. It is likely that a significant amount of the mortality was due to predation. Southern oyster drills (*Stramonita haemastoma*), an important oyster predator in the Gulf of Mexico (Butler 1985), were observed on several deployment poles and settlement tiles during each site visit. Furthermore, oyster drills were occasionally found resting on top of living oysters, likely in the process of feeding. In addition to oyster drills, there are several oyster predators common in estuarine environments including mud, stone, and blue crabs (O'Connor et al. 2008) and fish (Anderson and Connell 1999). In fact, the general trend observed at both sites of increasing oyster mortality with decreasing tile height is consistent with oyster predation as a driver; greater inundation times permit predators to have greater access to subtidal oysters than intertidal oysters (Johnson and Smee 2014). At CI, but not at PaP, mortality was lowest on Tile 4 and increased for more exposed tiles. The upper limits of oyster survival could be explained by exposure and desiccation stress (Ridge et al. 2015); at CI, this may have resulted in a local optimal elevation around Tile 4 between subtidal and supratidal stressors. We cannot rule out the possibility of oyster disease contributing to mortality; however, it is not likely to explain the initial wave of losses which occurred quickly after deployment.

Although water quality did not likely directly drive oyster mortality observed during this experiment, water quality could have had important indirect effects on oyster biology. For example, oyster larvae are much more sensitive to low DO concentrations than adults, failing to settle and dying more quickly under hypoxic conditions (Baker and Mann 1992). If the occasional hypoxia measured at some of the ARCOS stations also occurred at the monitoring sites, the hypoxia might explain why no new oyster settlement was observed. Oyster spawning can also be inhibited by prolonged exposure to fresh water (Butler 1949), though this was not observed during our

study (Figure 3C). Furthermore, the distribution of many oyster predators and prevalence of diseases can be dramatically influenced by water temperature and salinity (Shumway 1996). Oyster drill feeding on oysters may be inhibited at salinities <15 (Manzi 1970), but we did not directly observe salinities <15 at our sites (Figure 3B). Regional and interannual variations in salinity may dramatically alter the prevalence of oyster predators in the Mobile Bay area (Park et al. 2014); therefore, the intense predation likely observed during this study may not necessarily reflect dynamics in other locations or in different years with different salinity regimes. Furthermore, while water quality may have been within oyster tolerance thresholds during this study period, it is possible that water quality will exceed oyster tolerances or promote predation or disease at other times of the year (Wadsworth et al. 2019).

It will be important to monitor the survivability of the remaining oysters as the tiles remain in the water. For oysters to develop on breakwaters, form self-sustaining reefs (Morris et al. 2019), and grow in pace with sea level rise (Rodriguez et al. 2014), reefs must recruit at least as many individual oysters as are lost each year. That means oysters must survive at least one year, given that the young-of-the-year are not usually reproductive, and fecundity increases with size (Cox and Mann 1992). If most of the remaining oysters continue dying over the next few months, this may indicate significant barriers in the environment to oysters developing on breakwater structures, particularly if there is negligible wild settlement. In this case, oyster restoration on breakwaters may require additional interventions such as remote setting and predator exposure (Belgrad et al. 2021). On the other hand, if the remaining oysters can mostly survive to a reproductive age, this may indicate potential refuge locations for oysters to exist at some sites or intertidal elevations; future living shoreline projects could apply this information when deciding on siting or structure design. Regardless, these findings may depend on conditions at the sites and particular conditions during the monitoring period; additional work is needed to determine if these findings are generalizable as environmental conditions change over time, and whether they apply to other locations within and beyond this region.

Our evidence suggests that water temperature, DO, and salinity may not have affected oyster mortality at these living shoreline sites during our study period. Other factors like predation and exposure should be further investigated as potentially limiting oyster development on breakwater structures. These findings improve our understanding of oyster ecological processes at living shoreline sites, helping move us toward more effective implementation of oysters at restoration sites.

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