A negative association between recruitment of the eastern oyster *Crassostrea virginica* and the brown tide *Aureoumbra lagunensis* in Mosquito Lagoon, Florida

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Abstract Within the Indian River Lagoon (IRL), blooms of the marine microalga *Aureoumbra lagunensis* in excess of 1×10^6 cells ml^{-1} have occurred on three occasions after first being locally identified in 2012. All blooms coincided with times of peak reproduction and growth of the eastern oyster *Crassostrea virginica* in Mosquito Lagoon (northern IRL). Over six years of field data on monthly recruitment of *C. virginica* were compared to bi-monthly cell counts of *A. lagunensis*. In addition to expected temperature and salinity-driven variations in oyster recruitment, cell counts of *A. lagunensis* in excess of 10,000 ml^{-1} reduced oyster recruitment. A second method of analysis similarly found a negative association with oyster recruitment when cell counts exceeded 9658 ml^{-1}. Understanding the impact of blooms of *A. lagunensis* is essential for managing harvesting, aquaculture, and restoration of this foundation shellfish species.

Keywords Algal bloom, Bivalve, Indian River Lagoon, Mosquito Lagoon, Pelagophyceae, Shellfish

Introduction

Dramatic declines of bivalve reefs over the past century have been reported globally (Beck et al. 2011). An overall decrease of 85% in bivalve acreage has been attributed to overharvesting, diseases, and loss of habitat (e.g. Newell 1988, Rothschild et al. 1994, Beck et al. 2011). Additional sources of declines include boat wakes, sedimentation, and invasive species (e.g. Wall et al. 2005, Yuan et al. 2016, Bilkovic et al. 2019). Algal blooms are another potential threat to shellfish populations growing in both intertidal and subtidal waters. Rising sea surface temperatures, hurricanes and cultural eutrophication have led to an increase in harmful algal blooms in estuaries (Cloern 2001, Heisler et al. 2002, O'Neil et al. 2012, Wetz and Yoskowitz 2013, Phlips et al. 2020). Subsequent laboratory studies have documented that high densities of certain phytoplankton species reduce bivalve filter-feeding and can lead to their death (e.g. Bricelj and Kuenstner 1989, Bricelj et al. 2001, Gobler et al. 2013). However, there is limited information on the

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impact of long-term exposure of bivalves to algal blooms in the natural environment.

Brown tides, composed of nanoplanktonic phytoplankton species from the algal group Pelagophyceae, are one algal bloom threat to bivalve populations (Gobler et al. 2013). Blooms often occur in shallow nutrient-rich estuaries with long water residence times (Buskey et al. 1996, 1998, 2001, Bricelj and Londsdale 1997, Gobler and Sunda 2012). In the southeastern United States and Gulf of Mexico, the dominant brown tide species is *Aureoumbra lagunensis* D.A. Stockwell, DeYoe, Hargraves et P.W. Johnson 1997. This species was first recorded in Laguna Madre, Texas during an extended bloom that lasted from 1990-1997 (Buskey et al 1998, 2001). Major blooms have since been recorded in the Indian River Lagoon (IRL), Florida (Gobler et al. 2013, Phlips et al. 2015), and Guantanamo Bay, Cuba (Koch et al. 2014). Transport of *A. lagunensis* via ballast and bilge water of ships and recreational vessels has likely occurred because this algal species has a resilient resting stage (Doblin et al. 2004, Kang et al. 2017) that also promotes recurrent blooms (Kang et al. 2017).

Blooms of *A. lagunensis* have not yet been observed to produce toxins; however, they have been shown to degrade coastal ecosystems directly or indirectly through extensive fish kills, reduced feeding of planktonic grazers, and loss of seagrass communities (Buskey and Hyatt 1995, Onuf 1996, Liu and Buskey 2000, Kang et al. 2015, Phlips et al. 2015). Gobler et al. (2013) showed that filtration rates of both hard clams (*M. mercenaria*) and oysters (*C. virginica*) were significantly lower under both low (4×10^5 cells ml^{-1}) and high (1×10^6 cells ml^{-1}) concentrations of *A. lagunensis* when compared to the control alga *Isochrysis galbana*. Complementing this result was reduced field growth of recruits of the eastern oyster *C. virginica* within first six months post-settlement during bloom versus non-bloom conditions (Gobler et al. 2013).

In this study, we examined the impact of brown tide blooms on the eastern oyster *Crassostrea virginica* in Mosquito Lagoon. We provide 6.5 years of monthly data on oyster recruitment on intertidal reefs in this shallow estuary and compare recruitment patterns to salinity, temperature, and cell counts of *A. lagunensis*. Additionally, we examine the relationships between cell counts of *A. lagunensis* and negative impacts on recruitment. This information is important not only to understand the interactions between these two organisms, but also to manage regional oyster aquaculture, harvesting and restoration efforts in areas where blooms may occur.

Materials and Methods

Study site. The IRL is a subtropical system and is considered one of the most biologically diverse estuaries in the United States (Provancha et al. 1992). It is comprised of three shallow-water estuaries: Mosquito Lagoon, Indian River, and Banana River. Mosquito Lagoon is a region characterized by extensive development along shorelines in its northern half, and limited development along southern shorelines connected to Canaveral National Seashore and Merritt Island National Wildlife Refuge (Cannon et al. 2020). Mosquito Lagoon had a limited history of algal blooms prior to 2012, but is characterized by wind-driven circulation, is microtidal (10 cm tidal range where intertidal oysters are

located), and is subject to long water residence times (mean water half-life: 76 days), high salinities, and, more recently, high nutrient loadings (Smith 1983, Phlips et al. 2015). Water temperatures range from 4 – 33° C, salinities range from 22.6 – 45.2 ppt, and vertical stratification is limited due to the shallowness of the lagoon (Phlips et al. 2015).

Brown tide pelagophyte *Aureoumbra lagunensis*. Cells of *A. lagunensis* are 4-5 μm in length and non-motile (Buskey et al. 1998). This species can grow in salinities ranging from 10 – 90 ppt, with optimal growth occurring in 20 – 70 ppt at 25° C (Buskey et al. 1998). Kang et al. (2017) additionally documented that *A. lagunensis* has a resting stage that enables this pelagophyte to resist high as well as low temperature extremes. This non-native species was first reported in Mosquito Lagoon waters in the summer of 2012 (Gobler et al. 2013). Phlips et al. (2015) defined the lower bloom threshold for *A. lagunensis* to be approximately 300,000 cells ml^{-1}, roughly equivalent to a biomass of 2 μg carbon ml^{-1}. The threshold represents the top 20% of all biomass observations for individual taxa in the northern IRL since 1997.

Eastern oyster *Crassostrea virginica*. The eastern oyster *C. virginica* is a foundation species in Mosquito Lagoon and the importance of these intertidal reefs has been well-documented for promoting biodiversity (Boudreaux et al. 2006, Barber et al. 2010), protecting shorelines from erosion (McClenachan et al. 2020), nutrient sequestration (Chambers et al. 2017), and trapping microplastics (Waite et al. 2018). Garvis et al. (2015) documented 2542 oyster reefs, primarily patch reefs, in Mosquito Lagoon waters using 2009 aerial photographs. Twenty-four percent of the coverage of *C. virginica* was lost between 1943 and 2009, based on earliest available aerial imagery, and these losses were primarily the result of intensive recreational boating activity eroding live oyster clusters from seaward reef edges in this shallow-water, microtidal habitat (Grizzle et al. 2002, Garvis et al. 2015, Walters et al. 2021). Restoration efforts have been underway since 2007 to reverse these wake-induced losses (Walters et al. 2021).

This bivalve is a protandric hermaphrodite with synchronized spawning and external fertilization that occurs in Florida waters when temperatures exceed 26° C and salinity exceeds 5-6 ppt (Kennedy et al. 1996). A single female *C. virginica* can produce 2-45 million eggs in a single spawning (Kennedy et al. 1996). Larvae are more sensitive to abiotic parameters than adults, with successful development and growth between 6 and 33 ppt and between 19 and 35° C (Kennedy et al. 1996). Laboratory studies with Gulf Breeze, FL oyster populations found that at 24° C, larval duration was 19 days (Forbes 1967). *C. virginica* requires a hard surface for settlement (Bayne 2017). As adults, *C. virginica* have broader tolerances for both salinity and temperature, surviving, for at least short periods of time in the laboratory, in salinities ranging from 0 – 42.5 ppt (Ingle and Dawson 1950) and temperatures ranging from -2 to 36° C (Kennedy et al. 1996).

Recruitment of *C. virginica*. Recruitment of the eastern oyster *Crassostrea virginica* in Mosquito Lagoon was monitored for 6.5 years to determine if there was an association between recruitment, salinity, temperature, and blooms of *Aureoumbra lagunensis*. Monthly sampling began in May 2013 and ended in September 2019. The sampling unit (oyster mat) consisted of 0.25 m^2 of aquaculture mesh with 36 disarticulated adult oyster shells (mean length: 78 mm) drilled near the umbo and attached via zip ties (Walters et al. 2021). Shells on the mesh were oriented perpendicular to the benthos; this is the orientation of individual live oysters and oyster clusters on reefs in Mosquito Lagoon (Stiner and Walters 2008). One oyster mat was deployed on the edge of 10 randomly selected restored intertidal patch reefs of *C. virginica* where recruitment had been observed during each of the previous three years and reefs had over 500 oysters m^{-2}. Reefs used for recruitment monitoring spanned approximately four km in Mosquito Lagoon (Figure 1). Each month, oyster mats were collected and replaced with new mats. Exposed mats were transported to the laboratory under dry, climate-controlled conditions where all recruited oysters were counted with the aid of a dissecting microscope (magnification: 20X). For each mat, all oysters were counted on both sides of each shell and then summed to provide a total number of *C. virginica*.

Collection of brown tide *Aureoumbra lagunensis*. Brown tide data were collected monthly from the nearby (< 5 km away) long-term sentinel sampling site at Oak Hill (Phlips and Badylak 2015, Figure 1). In July of 2013, additional *A. lagunensis* cell count data was collected during a bloom along a transect of five sites from the sentinel site at Oak Hill to a site in the city of New Smyrna, to compare central
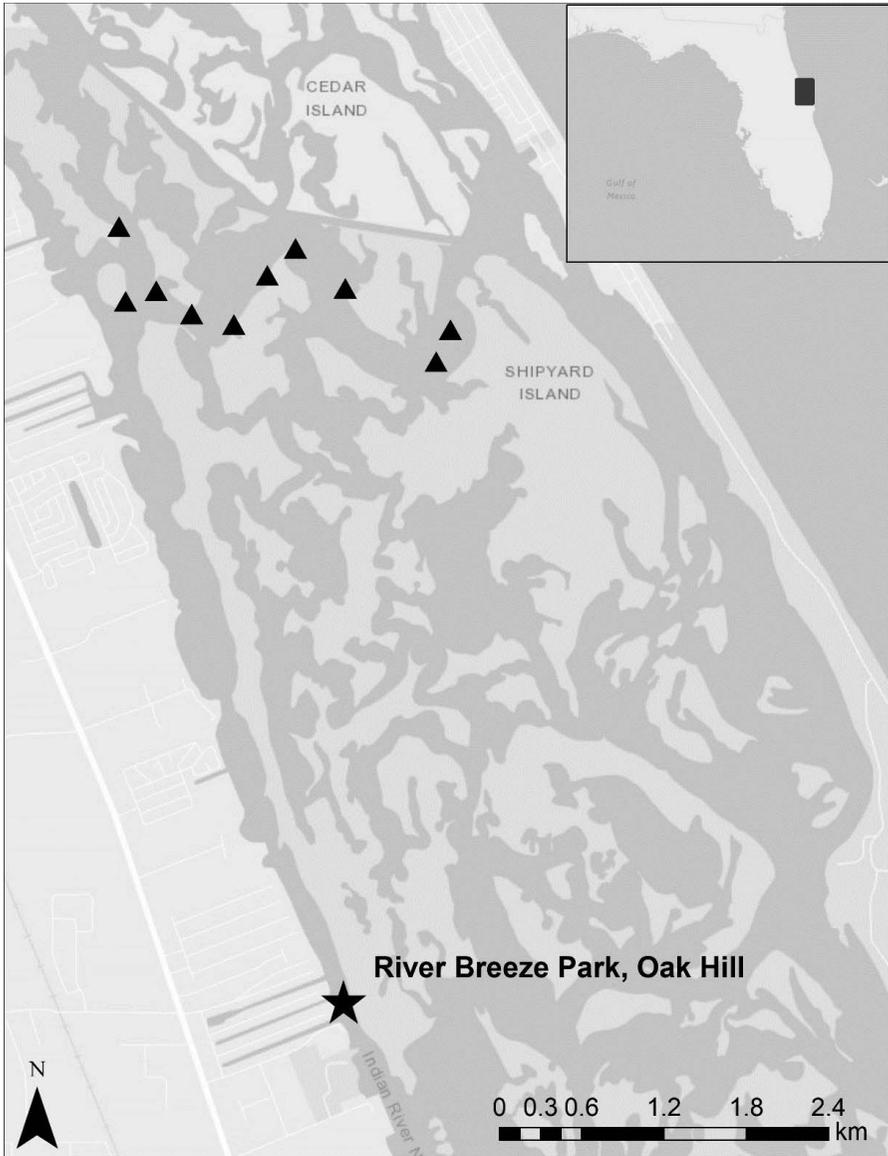
Figure 1. Map of Mosquito Lagoon collection sites. Locations of monthly recruitment monitoring are shown with triangles, and the location of microalgal sampling at Riverside Park in Oak Hill, Florida is shown with a star.

Mosquito Lagoon to the sentinel site cell counts. The sites included locations near the oyster research areas. The results demonstrated similar cell densities of *A. lagunensis* at all locations, including at the sentinel site. Cell counts along this transect varied by 27%, ranging from 405,340 and 555,868 cells ml^{-1}. Similar broad distributional characteristics have been observed in the northern Indian River Lagoon (Phlips et al. 2015).

Water was collected at the sampling sites using a vertical integrated sampling tube that captures water evenly from the surface to within 0.1 m of the bottom. Split phytoplankton samples were preserved on site, one with Lugol's iodine solution and the other with glutaraldehyde in 0.1-M sodium cacodylate buffer. Phytoplankton composition and densities were identified and counted at 100X and 400X in the laboratory with the Utermöhl method (Utermöhl 1958) and picoplanktonic phytoplankton were counted with autofluorescence microscopy at 1000X, as described in Phlips et al. (2015). Voucher specimens are stored at the University of Florida.

Collection of abiotic data. Abiotic data over the 6.5 years were collected from a variety of sources. Water temperature data were downloaded from the St. John's River Water Management District (SJRWMD) continuous water quality monitoring site (33814526-IRL S ML IRLML02). Values were averaged in RStudio by year and month from March 2014 – October 2019 (R Core Team 2015). Water temperature data from May 2013-February 2014 were collected once a month at reef locations with a digital thermometer. Salinity data were collected via weekly grab samples with a portable refractometer from the UCF Field Research Laboratory (28° 54′ 24.46″ N; 80° 49′ 16.55″ W) throughout the duration of the project. These data were combined with monthly salinity grab readings from 2013-2015 taken at reef locations and averaged by year and month in RStudio (R Core Team 2015).

Statistical analyses. Main effects of *A. lagunensis* cell counts (categorized based on observed counts: 0 cells; 1-1000; 1000-5000; 10,000-50,000; 50,000-200,000; 200,000-400,000; 500,000-10,000,000; >10,000,000), month, and year on oyster recruitment were evaluated using a generalized linear model (GLM) with Poisson distribution (R Core Team 2015). Interactions were not included due to extensive temporal variation in cell counts observed over the timeframe of this study. To further investigate threshold densities of cell counts with negative impacts on recruitment of *C. virginica*, a regression tree analysis was conducted in R using the "rpart" package (R Core Team 2015).

Results

Oyster recruitment showed significant variation based on *A. lagunensis* cell counts in addition to monthly and yearly fluctuations (GLM, p < 0.001 for all main effects). Blooms of *A. lagunensis* in the IRL system first appeared during the summer of 2012 (June - October), returned in 2013 (May - August), and remained for an extended period from June 2015 through August 2016 (Phlips et al. 2015, 2020). Peak oyster recruitment varied throughout our study, with yearly peaks occurring between April and September (Figure 2). Some years had one large peak while others had multiple peaks (Figure 2). As expected, the lowest recruitment occurred during winter months when water temperatures dropped below 26° C. Recruitment peaks were highest in summer months without brown tide, with monthly densities always exceeding 800 individuals m^{-2} and surpassing 2000 individuals m^{-2} in 2017 (Figure 2). During summers with brown tide, monthly recruitment was < 400 individuals m^{-2} and these dampened peaks were always delayed until after algal cell counts subsided. Significant declines in recruitment occurred when cell counts of *A. lagunensis* exceeded 10,000 cells ml^{-1} (GLM: t = -6.9, p < 0.001). This threshold was similar to that identified with regression tree analysis, with significant reductions in oyster recruitment observed at cell counts over 9658 (p = 0.02; 100.0 ± 9.2 vs 304 ± 30.0 individuals m^{-2}).

Monthly variation in oyster recruitment is also associated with water temperature; temperatures in Mosquito Lagoon ranged from 14.0° C in December/January to 31.4° C in July during the monitoring period. The minimum temperature threshold for observing ≥ 5 spat per month on an oyster mat was 19.3° C. During our study, salinity in Mosquito Lagoon ranged from 26.5 to 45.1 ppt.
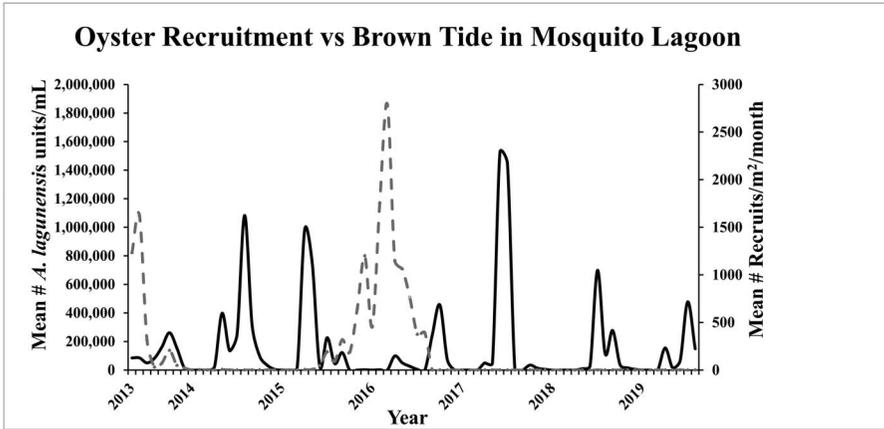
Figure 2. Oyster recruitment versus brown tide cell counts. Mean monthly values for recruitment of *Crassostrea virginica* (solid line) and cell counts of *Aureoumbra lagunensis* (dotted line) for Mosquito Lagoon, FL from May 2013 through September 2019.

Over these 78 months, mean monthly salinity exceeded 40 ppt during four months in summer 2012 and 2017, and was < 30 ppt during three months in summer 2014 and 2018. The overall mean salinity (± S.E.) was 35.1 (± 0.2) ppt. No recruitment occurred when salinity exceeded 41 ppt.

Discussion

Our study is the first to present a long-term field data set on the interactions between the eastern oyster *C. virginica* and the brown tide pelagophyte *A. lagunensis* in the natural environment. Results of laboratory studies (Gobler et al. 2013) and anecdotal reports by the public have suggested that all shellfish species in Mosquito Lagoon perished as a consequence of the intense *A. lagunensis* blooms that occurred between 2012 and 2016. Our results show that adult oysters did not perish during these blooms, but rather recruitment of new oysters was delayed until cell counts of *A lagunensis* decreased below a threshold level of 10,000 cell ml^{-1}. In fact, oyster densities on nearby restored reefs exceeded 1000 individuals m^{-2} in 2015 and 2016, followed by a sharp decline in 2017, and another increase that leveled off near 1000 individuals m^{-2} by 2018 (Walters et al. 2021). This study provides a starting point for asking important questions about the interaction between *C. virginica* and *A. lagunensis*: 1) Which pre- and post-settlement early life stage(s) are most vulnerable to *A. lagunensis* and result in reduced recruitment of *C. virginica*?, 2) Is the impact of *A. lagunensis* on *C. virginica* similar to other harmful algal bloom species?, and 3) Did *A. lagunensis* negatively impact spatial competitors of *C. virginica*?

Significantly fewer spat of *C. virginica* were observed when cell counts of *A. lagunensis* were high across a range of environmental factors. Some rebounding occurred post-bloom in late summer-fall in 2013 and 2016 when cell counts dropped. This suggests that the oysters may have delayed gamete production or

release until conditions improved for gamete, larval or spat success. Other possible alternatives for lower rates of recruitment during blooms include reduced larval survival and rapid mortality of spat once attached to shell substrate. Although distinguishing among these alternatives is beyond the scope of this research, it has long been known that *C. virginica* will delay reproduction when conditions, especially planktonic food availability, are not suitable, which is followed by rapid increases in gametogenesis and spawning under improved conditions (Kennedy et al. 1996). Additionally, nutritive stress results in skewing the sex ratio of *C. virginica* from unity to an excess of males, which may reduce fertilization success (Kennedy et al. 1996). Gobler et al. (2013) found that spat in Mosquito Lagoon were significantly smaller when measured after the first brown tide bloom and suggested this result was associated with delayed gamete production. Makris (2016) tested 6-hour survival of cultured larvae of *C. virginica* with zero, low, and high cell densities of *A. lagunensis* under replicated lab conditions using flumes. No significant differences among treatments were observed (Makris 2016). Moreover, Gobler et al. (2013) tested clearance rates of oyster spat from Mosquito Lagoon (6.1 mm length) and hatchery-reared adults (35 mm length) for 1 h. They found that feeding was significantly reduced with *A. lagunensis* relative to *Isocrysis galbana*, a microalga commonly used in oyster aquaculture. In this case, reduced feeding rates did not equate to oyster mortality. Finally, in aerated laboratory tanks, Makris (2016) exposed one-week spat collected from Mosquito Lagoon to 0, low, and high densities of *A. lagunensis* for seven days. All spat survived, were returned to Mosquito Lagoon, and placed on intertidal reefs in locations where natural cell counts were near or at zero. Survival and growth of these spat were tracked bi-weekly for 4 weeks. Post-exposure oyster survival over the 28-day period of this study was approximately 90% for juveniles exposed to *I. galbana* (control alga) and 80% for *A. lagunensis*. Combined, these results suggest high spat survival in Mosquito Lagoon and that gamete production or release is the stage at which *C. virginica* was most likely to be impacted by *A. lagunensis*.

Aureococcus anophagefferens, another ecosystem-disruptive brown tide species was first identified in the northeast US in 1985, where Bricelj and MacQuarrie (2007) documented that the hard clam *Mercenaria* experienced significantly higher mortalities and lower growth rates when cells were present. Similar to our oyster results, hard clam larvae did not die from contact (Padilla et al. 2015) and juveniles were able to recover after short-term (2-week) exposures (Bricelj and MacQuarrie 2007). Harke et al. (2011) showed a significantly decreased clearance rate of *Mercenaria* after exposure to *A. anophagefferens*. Although there have been no published studies on the physiological effects of *A. lagunensis* on the larvae of *C. virginica*, studies have shown that *A. anophagefferens* significantly depressed development and growth of larval *C. virginica* by decreasing lipid content and delaying metamorphosis (Talmage and Gobler 2012). It is important to note that toxic strains of *A. anophagefferens* have been identified, while no toxin has been identified for *A. lagunensis* (Gainey and Shumway 1991, Bricelj et al. 2004).

Oyster declines in Mosquito Lagoon prior to the arrival of brown tide in 2012 have been correlated with recreational boat wakes rather than high salinities (Grizzle et al. 2002, Garvis et al. 2015). This suggests that the Mosquito Lagoon oyster population has a salinity optimum at the high end of the known survival range of $0 - 42$ ppt (Kennedy et al. 1996, Ingle and Dawson 1950). Barnacles are known competitors of oysters for space and food as well as known predators of larval oysters (e.g. Osman et al. 1989). Boudreaux et al. (2009) showed that settlement, growth, and survival of *C. virginica* in Mosquito Lagoon was significantly reduced by the presence of *Balanus eburneus* and *Balanus amphitrite* when separated by a few millimeters on hard substrates. Coincident setting of barnacles and oysters occurred during warm water months in Mosquito Lagoon. Interestingly, barnacles were not negatively impacted by either high salinity or *A. lagunensis* (Makris 2016). Mean ($\pm$ S.E.) recruitment density during the peak of the longest bloom (summer 2016) was 1488.0 ± 531.2 individuals m^{-2} on the same oyster recruitment mats (Makris 2016). Algal blooms may thus provide a competitive advantage to barnacles in this substrate-limited environment.

While many biotic and abiotic variables simultaneously influence oysters, our field results suggest *A. lagunensis* reduces recruitment of *C. virginica* when present during warm months. Moreover, Walters et al. (2021) found that reduced densities on nearby intertidal reefs can last for many months or years post-bloom. Thus, care should be exercised by resource managers responsible for harvesting, restoration, and aquaculture if their operations are happening in locations experiencing brown tide. Above a 10,000 cells ml^{-1} threshold, extraction or deployment activities may need to be halted until the bloom subsides and recruitment resumes to avoid damage to the system, costly losses, or competing species covering the substrate deployed for oyster recruitment.

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