



Bivalve Feeding on the Brown Tide *Aureoumbra lagunensis* in a Shallow Coastal Environment

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Brown tides formed by *Aureoumbra lagunensis* decrease light penetration in the water column and are often followed by hypoxic events that result in the loss of fish and shellfish. To understand the ability of bivalve filter feeders to control and prevent *A. lagunensis* blooms, we exposed eastern oysters (*Crassostrea virginica*), hooked mussels (*Ischadium recurvum*), and hard clams (*Mercenaria mercenaria*) to a naturally co-occurring brown tide in the Indian River Lagoon (IRL), Florida, United States. Bivalves were exposed in the laboratory to multiple concentrations (10^4 to 10^6 cells mL^{-1}) of isotopically labeled (^{13}C and ^{15}N) *A. lagunensis* cells. The standard clearance rate (herein clearance rate) of each bivalve species was calculated using flow cytometry to quantify *A. lagunensis* cell removal. The highest clearance rates were at 10^4 cells mL^{-1} , but values varied across bivalve species (2.16 ± 0.30 , 3.03 ± 0.58 , and 0.41 ± 0.12 L h^{-1} for *C. virginica*, *I. recurvum*, and *M. mercenaria*, respectively). Although clearance rates decreased with increasing bloom concentrations, bivalves were still consuming algal cells at all concentrations and were retaining and assimilating more cells at the highest concentrations, as revealed by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. We highlight interspecific differences among bivalve species in the removal of *A. lagunensis*, supporting the importance of healthy and diverse filter feeding communities in estuaries, especially as threats of brown tides and other HABs are increasing in the Anthropocene.

Keywords: algal bloom, stable isotopes, clearance rate, Indian River Lagoon, filter feeding, bivalves, *Aureoumbra lagunensis*

INTRODUCTION

The proliferation of harmful microalgae in aquatic ecosystems, commonly known as Harmful Algal Blooms (HABs), have been recorded since ancient times (Moore, 1977; Hallegraeff, 2003). These HABs can be comprised of diverse microalgal species and may be toxic or non-toxic. Toxic blooms alter the health of or cause death in many organisms (Landsberg, 2002; Backer and McGillicuddy Jr., 2006), while non-toxic HABs change abiotic conditions (Hallegraeff, 2003; Gobler and Sunda, 2012). These different mechanisms result in large ecosystem level effects. For instance, hypoxic events caused by bacteria degrading dead algal cells and depleting dissolved oxygen levels are often associated with non-toxic blooms and found in bottom waters that are isolated from surface waters by density gradients (Hallegraeff, 2003; Gobler et al., 2013). Brown tides are examples of HABs caused by two different species of marine microalgae from the class

Pelagophyceae, *Aureoombra lagunensis* and *Aureococcus anophagefferens*. These species are small (2–5 μm), spherical, non-motile cells with simple life cycles and contain the unique pigment 19'-butanoyloxyfucoxanthin (DeYoe et al., 1997). Both species have the ability to disrupt and damage coastal ecosystems but only *A. anophagefferens* may express toxicity (Bricelj and Lonsdale, 1997). However, the ecological effects caused by *A. lagunensis* cannot be underestimated.

The non-toxic brown tide, *A. lagunensis*, has been responsible for disrupting coastal lagoons and endangering their health and sustainability. These brown tides can substantially decrease light penetration in the water column, preventing adequate sunlight for seagrass growth and reducing seagrass abundance dramatically, with dangerous cascading effects for the ecosystem including fish and shellfish kills (Onuf, 1996; Gobler et al., 2013; Lapointe et al., 2020). Moreover, *A. lagunensis* blooms are often persistent within a system; for example, a brown tide persisted for over 8 years without interruption in the Laguna Madre, Texas (Buskey et al., 1998). In 2012, a brown tide ($\sim 10^6$ cells mL^{-1}) was reported in the Indian River Lagoon, the first time outside Texas (Gobler et al., 2013), and reappeared in 2013, 2015, 2016, and 2018 (Galimany et al., 2017b; Philips et al., 2019). Brown tides have also been reported in Guantánamo Bay, Cuba (Hall et al., 2018). The proliferation and recurrence of *A. lagunensis* may be related to eutrophication (Lapointe et al., 2015); however, other factors including extreme climatic conditions or suppression of grazer populations should not be ignored (Philips et al., 2015).

Population dynamics of HABs are regulated by both “bottom-up” abiotic factors such as light and nutrients affecting their development, and “top-down” factors controlling their loss rates including pathogens and grazers (Buskey, 2008). For example, the lack of filter feeding grazers, such as bivalves, may have played an important part in brown tide formations in Laguna Madre, Texas (Montagna et al., 1993; Buskey et al., 1997). The ecosystem services offered by bivalves, including the maintenance of water quality through filtration and assimilation of phytoplankton, are key to sustaining coastal ecosystems. Nonetheless, bivalve populations are decreasing worldwide because of overharvesting and ecosystem degradation (Beck et al., 2011; FAO, 2018). When bivalve populations are depleted, instead of helping to mitigate bloom formation, they may be negatively affected by brown tides, further perpetuating water degradation. For example, hard clams (*Mercenaria mercenaria*) and eastern oysters (*Crassostrea virginica*) decreased their clearance rates when feeding during an *A. lagunensis* bloom in the Indian River Lagoon (Florida, United States) (Gobler et al., 2013; Galimany et al., 2017b). This altered feeding behavior may be a result of *A. lagunensis* cells producing exopolymer secretions that may affect the motility of cilia on the bivalves' gills (Liu and Buskey, 2000) or, simply, a physiological response to an excess of organic matter clogging their gills (Ward and Shumway, 2004). However, both bivalve species (*M. mercenaria* and *C. virginica*) fed on *A. lagunensis* (Gobler et al., 2013; Galimany et al., 2017b, 2020), unlike other HABs, which generally cause bivalve closure and non-feeding (Shumway, 1990). Importantly, bivalves feed more efficiently on lower, pre-bloom densities of the brown tide, i.e., 10^4 cells mL^{-1} , and the hooked mussel (*Ischadium*

recurvum) even increased its feeding on *A. lagunensis* over time when the density was sustained at pre-bloom conditions (Galimany et al., 2020).

Bivalves provide important ecosystem services including water clarification through the removal of phytoplankton (Galimany et al., 2013, 2017b; Cranford, 2019). Previous studies in the IRL demonstrate that bivalves may remove *A. lagunensis* at low concentrations (Galimany et al., 2020) and some may even clear fully developed brown tides (Galimany et al., 2017b). However, there is a lack of information on the assimilation of this microalga by bivalves, including non-commercial species, to potentially ameliorate brown tides. To address this research gap we investigated how co-occurring bivalve species could potentially mitigate *A. lagunensis* cells from pre-bloom (the beginning of the bloom formation) to bloom concentrations (brown tide), conducting laboratory feeding assays with three bivalve species naturally found on the East coast of North America, including the Indian River Lagoon: eastern oysters (*Crassostrea virginica*), hooked mussels (*Ischadium recurvum*), and hard clams (*Mercenaria mercenaria*). The clearance rate of each bivalve species was calculated using flow cytometry as a proxy for algal cell removal. In addition, algal cells were isotopically (^{13}C and ^{15}N) labeled to verify that they were ingested, and algae-derived nutrients assimilated. We hypothesized that, although the clearance rates of these bivalve species would decrease with increasing concentrations of brown tide, all three species would be capable of continuing to feed on and assimilate *A. lagunensis* at bloom concentrations. This work aims to elucidate the important role of bivalves in coastal shallow ecosystems and highlight any species-specific differences in their potential to ameliorate *A. lagunensis* brown tides.

MATERIALS AND METHODS

Brown Tide Collection

In December 2015, a brown tide caused by the pelagophyte *Aureoombra lagunensis* D. A. Stockwell, DeYoe, Hargraves & P. W. Johnson, 1997, occurred in all the basins of the Indian River Lagoon and persisted for several months (Galimany et al., 2017b; Philips et al., 2019). Water containing a natural brown tide (*Aureoombra lagunensis*) was collected from the dock of the Banana River Resort, Indian River Lagoon (Florida, FL, United States) in Cocoa Beach ($28^{\circ} 16' 20.54'' \text{N}$; $80^{\circ} 36' 29.07'' \text{W}$). The brown tide was immediately transported to the Smithsonian Marine Station at Fort Pierce (SMSFP) and kept in aerated buckets in the laboratory at 23°C . *Aureoombra lagunensis* cells were isotopically labeled by incubation with inorganic tracer compounds enriched in the heavy isotopes of C and N (98 atom percent [at%] ^{13}C or ^{15}N). These compounds ($\text{NaH}^{13}\text{CO}_3$ [1.18 mM] and $\text{Na}^{15}\text{NO}_3$ [0.117 mM]) were added directly into buckets holding *A. lagunensis* 24 h prior to the start of experiments following methods in Freeman et al. (2013). We have shown previously that *A. lagunensis* assimilates $\text{Na}^{15}\text{NO}_3$ (Galimany et al., 2020), but dual labels (both ^{13}C and ^{15}N) were still

used for these experiments to ensure that *A. lagunensis* cells were adequately enriched in at least one isotope to allow for evidence of trophic transfer (^{13}C and/or ^{15}N enrichment in bivalve tissue).

Brown Tide Identification

To characterize the phytoplankton bloom during the brown tide, we collected water samples from Cocoa Beach, FL. Water samples were taken on 25, 28 and 29 January and 5, 8 and 27 February 2016 to track the bloom in Banana River (FL). Water samples were analyzed through a C6 flow cytometer (BD Biosciences, San Jose, CA, United States) for 30 s on the slow flow rate setting ($14\ \mu\text{L}\ \text{min}^{-1}$) using plots contrasting side scatter (SSC) vs. red fluorescence (FL4) to identify and quantify the algal population. Plots from the bloom were compared with plots generated from a pure *A. lagunensis* culture provided by the University of Texas Marine Science Institute, which was isolated by the laboratory of Dr. Tracy Villareal, University of Texas, in April 1996 from the Laguna Madre (North Padre Island, Texas), as previously used in (Galimany et al., 2017b).

The presence of *A. lagunensis* was also confirmed by genetic identification using a fragment of the 16S ribosomal gene (16S rRNA) as a genetic marker. DNA was extracted from the cell pellet obtained by centrifuging a volume of 50 mL of water collected during the algal bloom. The cell pellet was freeze-dried and stored at -80°C for 48 h, transferred into a 1.5 mL tube, and powdered using a TissueLyser II (QIAGEN, DE). This powder was used to extract the total DNA using the extraction kit PowerPlant[®] Pro (Mo BIO, CA). The 16S rRNA fragment was amplified by PCR in 25 μL reactions with the following conditions: 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, 0.25 mM each dNTP, 0.8 μM of each primer. Primers used were CYA106F 5' CGG ACG GGT GAG TAA CGC GTG A 3' and equimolar mixture of CYA781R(a) 5' GAC TAC TGG GGT ATC TAA TCC CAT T 3' and CYA781R(b) 5' GAC TAC AGG GGT ATC TAA TCC CTT T 3' (Nübel et al., 1997). The PCR product was sequenced at the Smithsonian Laboratories of Analytical Biology, the sequence was checked for base call and aligned to compare to reference homologous sequences using BLAST search tool in GenBank.

Experimental Bivalve Species

Three local bivalve species were used for the experiments: eastern oysters [*Crassostrea virginica* (Gmelin, 1791)], hooked mussels [*Ischadium recurvum* (Rafinesque, 1820)], and hard clams [*Mercenaria mercenaria* (Linnaeus, 1758)]. *C. virginica* and *M. mercenaria* were purchased from Aquagem Farms Inc. in Sebastian, Indian River Lagoon, FL ($27^\circ\ 48'\ 25.00''\ \text{N}$; $80^\circ\ 28'\ 01.37''\ \text{W}$); *I. recurvum* was collected from Stuart, Indian River Lagoon, FL ($27^\circ\ 12'\ 04.34''\ \text{N}$; $80^\circ\ 15'\ 19.67''\ \text{W}$). All bivalves were cleaned of epiphytes and other encrusting organisms and kept in the laboratory in a flowing seawater system feeding on a mix of natural water and *Tetraselmis* sp. for a week before the experiments. Adult individuals were used for the experiments with mean shell lengths ($\pm\text{SE}$) of 53.79 ± 2.70 , 29.48 ± 0.60 , and 49.89 ± 0.42 mm for *C. virginica*, *I. recurvum*, and *M. mercenaria*, respectively.

Bivalve Clearance Rates

To quantify the removal of *A. lagunensis* cells by the three studied bivalve species we conducted a depletion experiment. Five concentrations (cells mL^{-1}) of *A. lagunensis* were tested: 10^4 , 5×10^4 , 10^5 , 5×10^5 , 10^6 , encompassing pre-bloom to bloom concentrations of *A. lagunensis*. The natural brown tide was diluted using water at the same salinity (26 ppt) filtered through Millipore PVDF Membrane Filters (0.22 μm pore size). For each *A. lagunensis* concentration, five of each bivalve species were haphazardly selected from the acclimation tank containing previously collected bivalves and placed individually in an aerated 500 mL jar ($N = 5$ bivalves per species and algal density). One extra jar with an empty shell of each bivalve species at each algal concentration was left as a control. Bivalves were fed using a similar method described in Galimany et al. (2020). Briefly, bivalves were fed ^{13}C - or ^{15}N -enriched *A. lagunensis* twice a day for four days: (1) early morning, each bivalve was transferred to a jar containing filtered estuarine water (26 ppt). Meanwhile, the experimental jar was quickly emptied, washed and filled with the corresponding *A. lagunensis* concentration. The bivalves were placed back in the experimental jars in less than 30 s; (2) in the afternoon, the algal concentration remaining in each jar was calculated using a C6 flow cytometer (BD Biosciences, San Jose, CA, United States) as described below. Then, the amount of *A. lagunensis* remaining was calculated and, if needed, more was added to each jar to reach the initial concentrations.

On the last *A. lagunensis* feeding (day four), water samples from each jar were taken every 5 min, starting at time 0 and continuing for 30 min, after which samples were taken every 10 min until 1 h had passed. Each of these 10 samples was collected with a pipette and preserved with 1% formalin (final concentration) until analysis. Following the termination of feeding experiments, all bivalve individuals were held in fresh, filtered seawater for 12 h to ensure that their guts were empty from ^{13}C or ^{15}N labeled algal cells. Bivalves were then dissected and dried at 60°C for 48 h to determine individual dry tissue weight. Dried soft tissue from all individuals was used for stable isotope analyses.

To calculate *A. lagunensis* concentration in each jar, water samples were processed on a C6 flow cytometer (BD Biosciences, San Jose, CA, United States) for 2 min on the fast flow rate setting ($35\ \mu\text{L}\ \text{min}^{-1}$), as in Galimany et al. (2020). Algal cells were identified and quantified with plots contrasting side scatter (SSC) vs. red fluorescence (FL4) generated by the C6. Then, clearance rates (CR) were determined from the exponential decrease in algal cell concentration as a function of time using the formula (Riisgård, 2001):

$$\text{CR} = (V) \ln (C_0/C_t)$$

where C_0 and C_t are algal concentrations at time 0 and time t , and V is the volume of water (500 mL). All CR values were standardized (Y_s) to 1 g of dried bivalve flesh using the following equation:

$$Y_s = Y_e \times (1/W_e)^b$$

where, Y_e is the experimentally determined rate, and W_e is the dry body mass measured for each bivalve. For the predetermined feeding rate constant b values, 0.73 was used for *C. virginica* (Riisgård, 1988), 0.8 was used for *M. mercenaria* (Riisgård, 1988), and 0.67 for *I. recurvum* (Galimany et al., 2020). These values differ among species as previously demonstrated by Riisgård (1988); thus, using specific b values provides more accurate calculations. All clearance rates reported are standardized and the unit for standard is $L\ h^{-1}\ gDW^{-1}$ but, as commonly reported in feeding behavior studies, the units written will omit the weight.

To verify that bivalves filtered and cleared *A. lagunensis* cells from natural water samples, the percent decrease in *A. lagunensis* cells was calculated for each experimental jar. This was calculated as (**Supplementary Figure 1**):

$$\begin{aligned} & \text{Decrease } A. \text{ lagunensis } (\%) \\ &= \frac{A. \text{ lagunensis cells } T0 - A. \text{ lagunensis cells } T60}{A. \text{ lagunensis cells } T0} \times 100 \end{aligned}$$

Stable Isotopes

Dried whole bivalve tissue was ground to a fine powder using a mortar and pestle and acidified by exposure to 12 N HCl fumes in a closed environment for 12 h to remove inorganic carbon. After drying at 60°C to remove residual acid, samples were weighed to the nearest 0.001 mg into tared silver capsules. Isotope analysis was carried out at the Stable Isotope Facility at UC Davis using a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). Isotope values are reported in δ notation in units of per mil (‰) following equations outlined in Fry (2006). The long term standard deviation for standards at the UC Davis isotope facility is 0.3‰ for ^{15}N and 0.2‰ for ^{13}C .

Statistical Analyses

Clearance rates and isotope values were compared using a two-way ANOVA with species and algal concentrations as factors. Data met assumptions for analysis; normality and homogeneity of variances were checked using Shapiro-Wilk and Levene's test, respectively. Tukey post hoc tests determined differences within factors. When interactions were found, a one-way ANOVA was performed to find differences for each bivalve at the different brown tide concentrations. Only bivalves that were open and actively feeding were used for the statistical analyses ($N = 4$ for 10^4 cells mL^{-1} and 5×10^4 cells mL^{-1} for *C. virginica*; $N = 5$ in all other cases). The relationships between feeding and algal depletion, and between tissue isotope value and number of cells removed for each species were established with non-linear exponential regressions. The statistical software used was SPSS Statistics 23.0 (IBM).

Scaling Up Restoration Projects

Based on numbers calculated from the laboratory filter-feeding experiments, we were able to scale up minimum and maximum clearance rates for *C. virginica* and *M. mercenaria* at the population level, based on restoration projects carried out in the Indian River Lagoon. Scaling up clearance rates of *I. recurvum*

to the population level was not possible because there are no estimates on the abundance of the species within the IRL nor any restoration projects with mussels. Examining mussel populations and their part in ecosystem health in the IRL is a potential next step in this research.

An oyster reef restoration project started in the Canaveral National Seashore (IRL) in 2007 to enhance the natural oyster population, which has severely decreased in the last decades¹. The restored oysters measured 37.1 mm in length in 2019 (Walters et al., 2021). This reported measure was converted into soft tissue dry weight (0.35 g) using a weight/length relationship obtained with the collected data from the laboratory and previous field data (Galimany et al., 2017b). Oyster density in the restored reefs was variable and, when oysters reached adult sizes, the density ranged from about 500 oysters m^{-2} in 2017 to about 1,000 oysters m^{-2} in 2020. Estimates of the reported reef area, densities, and oyster lengths, allow us to estimate that in 2017, the reef had approximately 183,000 individuals of adult *C. virginica*, whereas, in 2020, the population increased to 367,000 adult individuals. Using these population calculations, the estimated oyster dry weights, and the calculated clearance rates we estimated the potential clearance of brown tide when feeding on the different concentrations of *A. aureoanura* for the restored *C. virginica* reef. The IRL clam project emerged in 2019 with the goal to collect *M. mercenaria* that had survived consecutive brown tides. With the assumption that these would be resistant clams to new brown tides, the goal was to use these collected adults to obtain hatchery seed and release 2M 2.5 cm clams by summer 2021². Then, using a weight/length relationship obtained with the collected data from the laboratory and previous field data (Galimany et al., 2017b), 2.5 cm clams were estimated to weight 0.25 g. This measurement was used to estimate the clearance rates for a restored population of 2M individuals of *M. mercenaria* when feeding on the different brown tide concentrations.

RESULTS

Brown Tide Identification

The presence of *A. lagunensis* in the algal bloom was confirmed by genetic identification. The alignment of the 16S rRNA gene partial sequence obtained from the water sample collected on February 27 of 2016 (GenBank accession number MH523586) to the chloroplast genome (cpDNA) of *Aureoanura lagunensis* (GenBank accession number GQ231542.1) resulted in 100 % identity to homologous fragment at the 103 to 582 nucleotide positions of the cpDNA genome (**Supplementary Table 1**).

Bivalve Clearance Rates

Brown tide cells were removed by all three bivalve species as determined by % decrease in algal cells after 60 min (**Figure 1**), although % reduction decreased as algal concentrations increased. Clearance rates also showed decreases with increasing algal cell concentrations, and a significant interaction was

¹<https://shuckandshare.org/projects.html>

²<http://www.irlclamproject.com/>

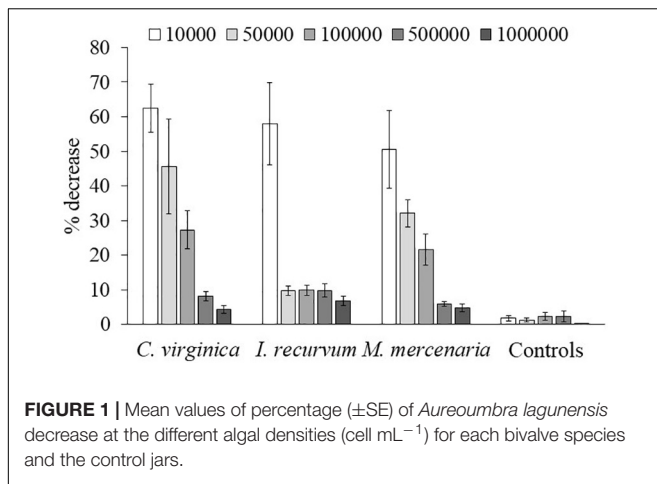


FIGURE 1 | Mean values of percentage (\pm SE) of *Aureobamba lagunensis* decrease at the different algal densities (cell mL^{-1}) for each bivalve species and the control jars.

TABLE 1 | Two-way ANOVA table analyzing the clearance rate of each studied species (*Crassostrea virginica*, *Ischadium recurvum*, and *Mercenaria mercenaria*) at each brown tide concentration.

| Source | Type III sum of squares | df | Mean square | F | Sig. |
|--------------------------------|-------------------------|----|-------------|---------|--------|
| Corrected model | 46.977 ^a | 14 | 3.356 | 18.384 | <0.001 |
| Intercept | 37.224 | 1 | 37.224 | 203.935 | <0.001 |
| Species \times concentration | 11.245 | 8 | 1.406 | 7.701 | <0.001 |
| Species | 11.117 | 2 | 5.559 | 30.453 | <0.001 |
| Concentration | 25.175 | 4 | 6.294 | 34.481 | <0.001 |
| Error | 10.587 | 58 | 0.183 | | |
| Total | 92.376 | 73 | | | |
| Corrected total | 57.564 | 72 | | | |

^a $R^2 = 0.816$ (Adjusted $R^2 = 0.772$).

found on clearance rates between bivalve species \times brown tide concentration ($F_{8,58} = 7.70$, $p < 0.001$) (Table 1 and Figure 2). To elucidate differences within species, clearance rates were analyzed for each bivalve species with a one-way ANOVA comparing the different brown tide concentrations. Each species had significantly different clearance rates among the concentrations of brown tide ($F_{4,18} = 21.68$, $p < 0.001$ for oysters; $F_{4,20} = 15.63$, $p < 0.001$ for mussels; $F_{4,20} = 4.77$, $p = 0.007$ for clams). As expected, clearance rates at 10^4 cells mL^{-1} were the highest (Figure 2). Non-linear exponential regressions between clearance rates and *A. lagunensis* concentrations for each bivalve clarified these results and all bivalves had a negative relationship (Table 2). The slope of the function (number before e) indicates that *C. virginica* and *I. recurvum* both rapidly reduce their clearance rate as the density of brown tide increases, but *M. mercenaria* had a slower response and overall lower clearance rates at all concentrations (Figure 2).

To elucidate differences between bivalve species, clearance rates including all brown tide concentrations were analyzed with a one-way ANOVA comparing the different bivalve species. The three species had significantly different clearance rates ($F_{2,70} = 7.96$, $p = 0.001$). Tukey post hoc tests showed that *C. virginica* and *I. recurvum* had higher clearance rates overall for brown tide than *M. mercenaria*. In detail, when studying

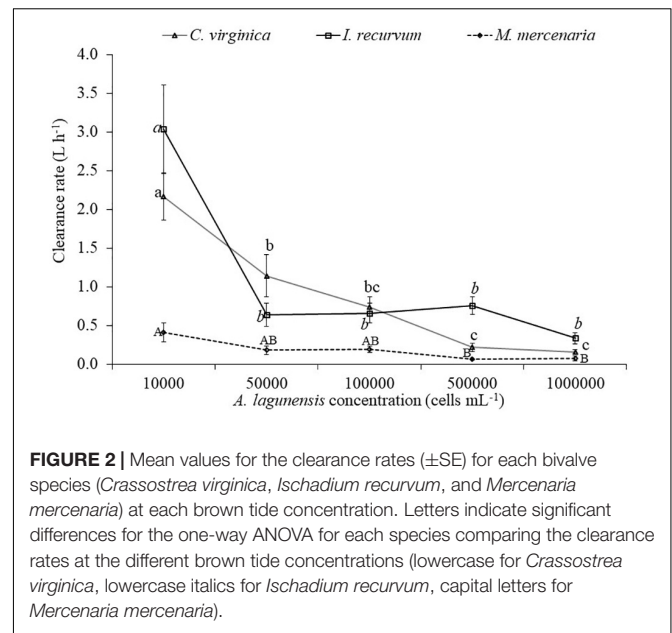


FIGURE 2 | Mean values for the clearance rates (\pm SE) for each bivalve species (*Crassostrea virginica*, *Ischadium recurvum*, and *Mercenaria mercenaria*) at each brown tide concentration. Letters indicate significant differences for the one-way ANOVA for each species comparing the clearance rates at the different brown tide concentrations (lowercase for *Crassostrea virginica*, lowercase italics for *Ischadium recurvum*, capital letters for *Mercenaria mercenaria*).

TABLE 2 | Nonlinear exponential regressions between clearance rates and *Aureobamba lagunensis* cell densities.

| Species | Calculation | Clearance rate |
|------------------------------|-------------|---|
| <i>Crassostrea virginica</i> | Calculation | CR = $1.12 (\pm 0.22) e^{-2.37 \times 10^{-6} \text{ cells}}$ |
| | F | 41.69 |
| | p | <0.001 |
| | R^2 | 0.649 |
| <i>Ischadium recurvum</i> | Calculation | CR = $1.11 (\pm 0.22) e^{-1.28 \times 10^{-6} \text{ cells}}$ |
| | F | 10.79 |
| | p | 0.03 |
| | R^2 | 0.29 |
| <i>Mercenaria mercenaria</i> | Calculation | CR = $0.20 (\pm 0.40) e^{-1.48 \times 10^{-6} \text{ cells}}$ |
| | F | 13.20 |
| | p | 0.001 |
| | R^2 | 0.34 |

The negative sign after the e indicates that all regressions are negative. p indicates significance; the adjusted regression coefficient is indicated by R^2 . Asterisks denote significant p-values ($p < 0.05$).

differences among bivalve species at each algal concentration, we obtained significantly different clearance rates throughout (Table 3). Tukey post hoc tests elucidated the differences among each bivalve species, indicating that clearance rates between *C. virginica* and *I. recurvum* did not differ at most concentrations; however, clearance rates between these two species and *M. mercenaria* differed across many algal concentrations (Table 3). Interestingly, clearance rates between *C. virginica* and *M. mercenaria* did not differ at the two highest concentrations of algal cells, probably because the clearance rates were quite low for both bivalves at these bloom concentrations.

Stable Isotope Analysis

Bivalve tissue from these experiments was enriched in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ compared to wild-caught bivalves from the IRL. For

TABLE 3 | One-way ANOVA table analyzing clearance rates differences among bivalve species at each brown tide concentration.

| Cell mL ⁻¹ | ANOVA | | Tukey (p value) | | |
|-----------------------|--------|---------|--|---|--|
| | F | p | <i>Crassostrea virginica</i> - <i>Ischadium recurvum</i> | <i>Crassostrea virginica</i> - <i>Mercenaria mercenaria</i> | <i>Ischadium recurvum</i> - <i>Mercenaria mercenaria</i> |
| 10 ⁴ | 12.163 | 0.002 | 0.320 | 0.027 | 0.001 |
| 5 × 10 ⁴ | 7.841 | 0.008 | 0.140 | 0.006 | 0.160 |
| 10 ⁵ | 7.857 | 0.007 | 0.858 | 0.008 | 0.022 |
| 5 × 10 ⁵ | 25.080 | < 0.001 | 0.001 | 0.314 | < 0.001 |
| 10 ⁶ | 7.151 | 0.009 | 0.067 | 0.472 | 0.008 |

Tukey post hoc test details significant differences between pairs of species; shaded boxes indicate significant differences.

instance, average (across all treatments) $\delta^{15}\text{N}$ values ($\pm\text{SE}$) from experimental samples were $357.41\text{‰} \pm 54.36$, $317.63\text{‰} \pm 27.97$, and $27.48\text{‰} \pm 2.66$ for *C. virginica*, *I. recurvum*, and *M. mercenaria*, respectively, while mean $\delta^{15}\text{N}$ values from wild-caught individuals were 6.75‰ for *C. virginica* and 8.62‰ for *M. mercenaria*. While we do not have natural abundance values for *I. recurvum*, other mussels from the IRL were also low: 6.22‰ for *Geukensia demissa* and 4.65‰ for *Mytella charruana* (Galimany et al., 2017a). Experimental $\delta^{13}\text{C}$ values ($\pm\text{SE}$) were lower than $\delta^{15}\text{N}$ values: $5.20\text{‰} \pm 4.54$, $-11.73\text{‰} \pm 1.31$, and $-15.77\text{‰} \pm 0.68$ for *C. virginica*, *I. recurvum*, and *M. mercenaria*, respectively, but were still high compared to $\delta^{13}\text{C}$ values of wild-caught bivalves: -19.32‰ for *C. virginica*, -21.22‰ for *M. mercenaria*, and -17.87 and -21.74‰ for the mussels *Geukensia demissa* and *Mytella charruana*, respectively (Galimany et al., 2017a).

A significant interaction was observed between bivalve species × brown tide concentration for both isotopes studied ($F_{8,58} = 5.22$, $p < 0.001$, and $F_{8,58} = 9.40$, $p < 0.001$, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) (Table 4). To elucidate differences within species, isotope values were analyzed for each bivalve species with a one-way ANOVA comparing the different brown tide concentrations. *C. virginica* and *M. mercenaria* had significantly different isotope values among the concentrations of brown tide ($\delta^{13}\text{C}$ $F_{4,18} = 7.52$, $p = 0.001$ and $\delta^{15}\text{N}$ $F_{4,18} = 17.12$, $p < 0.001$ for oysters; $\delta^{13}\text{C}$ $F_{4,20} = 9.10$, $p = 0.001$ and $\delta^{15}\text{N}$ $F_{4,20} = 7.27$, $p < 0.001$ for clams). *I. recurvum* only had significantly different isotope values for $\delta^{13}\text{C}$ ($F_{4,20} = 14.44$, $p < 0.001$) but not for $\delta^{15}\text{N}$ ($F_{4,20} = 2.14$, $p = 0.113$) (Figure 3).

To elucidate differences between bivalve species, isotope values including all brown tide concentrations were analyzed with a one-way ANOVA comparing the different bivalve species. The three species had significantly different isotope values ($F_{2,70} = 17.45$, $p < 0.001$ for $\delta^{13}\text{C}$ and $F_{2,70} = 28.27$, $p < 0.001$ for $\delta^{15}\text{N}$). *I. recurvum* and *M. mercenaria* had lower isotope values for $\delta^{13}\text{C}$ than *C. virginica*. However, *C. virginica* and *I. recurvum* had higher $\delta^{15}\text{N}$ isotope values than *M. mercenaria*.

After the algal exposures, $\delta^{13}\text{C}$ isotope values for all three bivalves were positively related to the number of cells removed by the bivalves in one hour (Figure 4 and Table 4). For $\delta^{15}\text{N}$, only *C. virginica* and *M. mercenaria* had a significant positive relationship (Figure 5 and Table 5).

Scaling Up

The scaling up estimates for *C. virginica* were based on both the low density restored reef from 2017 (500 oysters m⁻²) and the high density restored reef from 2020 (1,000 oysters m⁻²). Considering the low-density values, the potential of the oysters to clear the lowest concentration of brown tide would range between $8.2 \cdot 10^6$ and 10^7 L day⁻¹ (Figure 6A). When feeding on the highest concentration of brown tide, these values would decrease to $5.7 \cdot 10^5$ and $8.3 \cdot 10^5$ L day⁻¹ (Figure 6A).

Considering the reef with high density, the potential to clear the lowest concentration of brown tide would increase up to $16.4 \cdot 10^6$ to $21.7 \cdot 10^6$ L day⁻¹ (Figure 6B). However, when feeding on the highest concentration of brown tide, these values would range between $1.1 \cdot 10^6$ and $1.7 \cdot 10^6$ L day⁻¹ (Figure 6B).

The 2M individuals of restored *M. mercenaria* could filter an estimated maximum of $6.3 \cdot 10^6$ L day⁻¹ when feeding on the lowest concentration of brown tide studied, whereas the minimum amount of *A. lagunensis* cleared from the water could be estimated in $540,000$ L day⁻¹ when feeding on bloom concentrations (Figure 7). Considering that *M. mercenaria* can reach lengths up to 13 cm,³ the potential for water quality sustainability of the 2M restored clams through filter-feeding could greatly increase, with rough estimates of $107 \cdot 10^6$ L day⁻¹ when feeding at 10^4 cells mL⁻¹, a value that would decrease to $19 \cdot 10^6$ L day⁻¹ when feeding on a fully developed brown tide.

DISCUSSION

All studied bivalve species cleared *A. lagunensis*. Clearance rates decreased with increasing microalgal concentrations, but in all cases, the number of cells removed increased with higher cell concentrations. Thus, although each species adjusts their feeding behavior to high loads of brown tide concentrations (Barillé et al., 1997; Velasco and Navarro, 2002), their utility as consumers of HAB cells remains high even at full bloom concentrations. *C. virginica* and *I. recurvum* were physiologically able to maintain active filtration at high concentrations of particulates (i.e., 10^4 cells mL⁻¹), probably because when the material captured in the gills exceeds the ingestive capacity, the excess particles are eliminated as pseudofeces

³<https://www.sealifebase.ca/summary/Mercenaria-mercenaria.html>

TABLE 4 | Two-way ANOVA table analyzing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of three different bivalve species studied (*Crassostrea virginica*, *Ischadium recurvum*, and *Mercenaria mercenaria*) at each brown tide concentration.

| Source | Dependent variable | Type III sum of squares | df | Mean square | F | Sig. |
|-------------------------|-----------------------|--------------------------|----|-------------|---------|--------|
| Corrected model | $\delta^{13}\text{C}$ | 13318.804 ^a | 14 | 951.343 | 12.863 | <0.001 |
| | $\delta^{15}\text{N}$ | 2918299.766 ^b | 14 | 208449.983 | 18.852 | <0.001 |
| Intercept | $\delta^{13}\text{C}$ | 4557.339 | 1 | 4557.339 | 61.617 | <0.001 |
| | $\delta^{15}\text{N}$ | 3713045.804 | 1 | 3713045.804 | 335.798 | <0.001 |
| Species × concentration | $\delta^{13}\text{C}$ | 3090.216 | 8 | 386.277 | 5.223 | <0.001 |
| | $\delta^{15}\text{N}$ | 831334.491 | 8 | 103916.811 | 9.398 | <0.001 |
| Species | $\delta^{13}\text{C}$ | 4900.279 | 2 | 2450.139 | 33.127 | <0.001 |
| | $\delta^{15}\text{N}$ | 1463477.933 | 2 | 731738.966 | 66.177 | <0.001 |
| Concentration | $\delta^{13}\text{C}$ | 4827.796 | 4 | 1206.949 | 16.319 | <0.001 |
| | $\delta^{15}\text{N}$ | 560816.766 | 4 | 140204.192 | 12.680 | <0.001 |
| Error | $\delta^{13}\text{C}$ | 4289.787 | 58 | 73.962 | | |
| | $\delta^{15}\text{N}$ | 641328.135 | 58 | 11057.382 | | |
| Total | $\delta^{13}\text{C}$ | 22027.339 | 73 | | | |
| | $\delta^{15}\text{N}$ | 7448090.181 | 73 | | | |
| Corrected total | $\delta^{13}\text{C}$ | 17608.591 | 72 | | | |
| | $\delta^{15}\text{N}$ | 3559627.901 | 72 | | | |

^a $R^2 = 0.756$ (Adjusted $R^2 = 0.698$).

^b $R^2 = 0.820$ (Adjusted $R^2 = 0.776$).

(Shumway et al., 1985; Hawkins et al., 1998). However, other bivalves, like *M. mercenaria*, are not as efficient at selecting particles prior to ingestion, therefore lowering their clearance rates as particle concentrations increase is their main strategy to cope with high seston loads (Prins et al., 1991; Hawkins et al., 1998; Galimany et al., 2017b). Similarly, in another study, when feeding on a *A. lagunensis* brown tide at 10^6 cells mL^{-1} , clearance rates decreased to 0.06 L h^{-1} for *C. virginica* and 0.01 L h^{-1} for *M. mercenaria* (Gobler et al., 2013; Galimany et al., 2017b),

allowing the bivalves to prevent gill collapse and filter the algal bloom. Behavioral differences may account for the low clearance rates recorded for *M. mercenaria*. While oysters and mussels attach to hard substrate and remain within the water column, hard clams bury within sediments to avoid predation and we did not provide any sediment for the clams to bury within, as

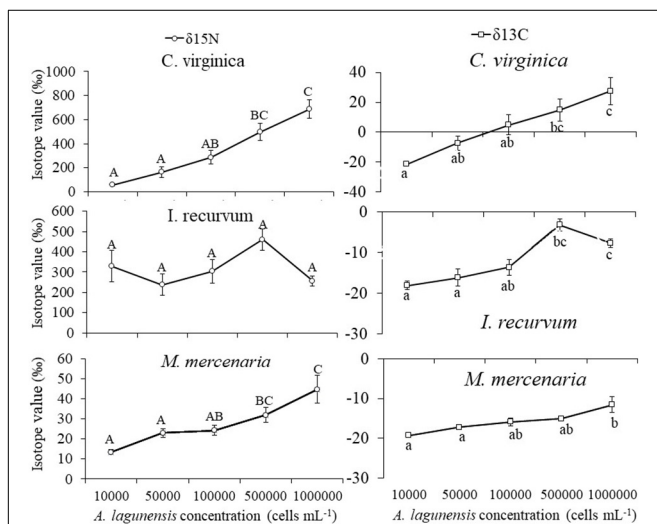


FIGURE 3 | Mean isotope (\pm SE) value of $\delta^{15}\text{N}$ (left) and $\delta^{13}\text{C}$ (right) for each bivalve species (*Crassostrea virginica*, *Ischadium recurvum*, and *Mercenaria mercenaria*) at each brown tide concentration. Letters indicate significant differences among concentrations for the one-way ANOVA.

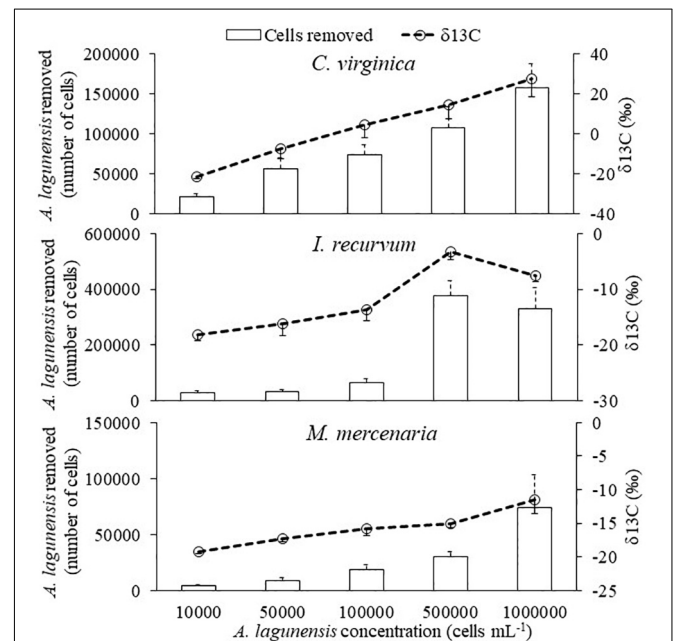
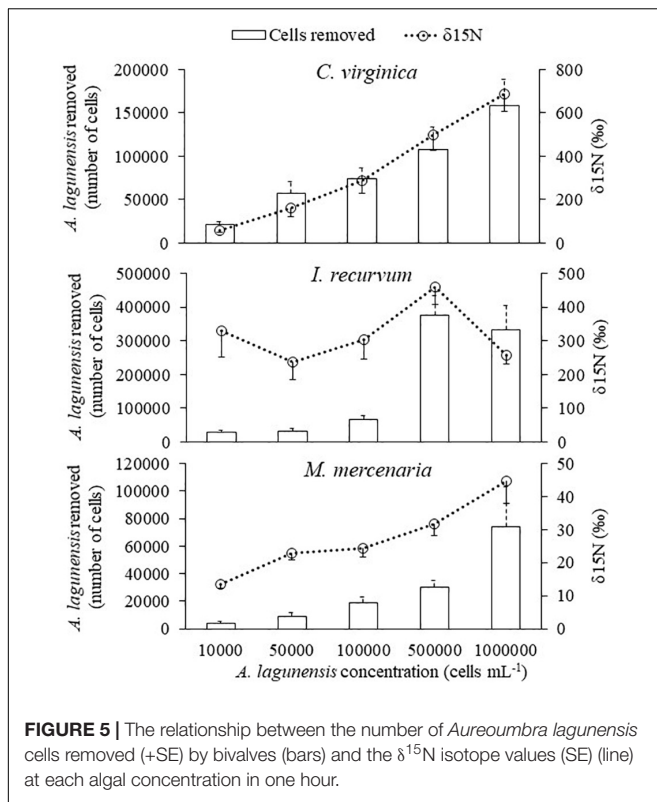


FIGURE 4 | The relationship between the number of *Aureoumbra lagunensis* cells removed (\pm SE) by bivalves (bars) and $\delta^{13}\text{C}$ isotope values (SE) (line) at each algal concentration in one hour.



resuspension could adversely influence our results. Therefore, our results may be underestimated. However, laboratory studies comparing the feeding of different clam species held without sediment report that *M. mercenaria* had lower clearance than the other clams thus it seems to be a bivalve with low feeding rates (Riisgård, 1988). Changes in clearance rates may disguise the fact that when feeding on high seston loads, such as an algal bloom, bivalves, not only increase pseudofeces production but may also pack undigested phytoplankton cells in their feces (Riisgård et al., 2011). Thus, the particles cleared from the water column and retained in the gills may not always be used as a food source (Kjørboe and Møhlenberg, 1981; Ward and Shumway, 2004).

Providing bivalves with algal cells enriched in the heavy atoms of carbon and nitrogen (^{13}C and ^{15}N) allows us to determine whether retained cells are actually consumed and assimilated into bivalve tissue (Galimany et al., 2020). Similar work labeling food items with ^{15}N *in situ* has been carried out with bivalves in other systems (Raikow and Hamilton, 2001). Based on ^{15}N enrichment, *A. lagunensis* cells were consumed and assimilated by all bivalves over the course of exposure to the bloom. Additionally, positive relationships between isotope values and cell removal indicate that while bivalves are altering their feeding behavior to accommodate bloom conditions, they are retaining and consuming more cells from the water column as cell concentrations increase. Therefore, while bivalves were most likely producing more pseudofeces, especially at the highest algal concentrations, they were also assimilating the brown tide at all concentrations. Although we used both ^{13}C - and ^{15}N -tracer compounds for this experiment, we found higher enrichment

of ^{15}N within bivalve tissue, suggesting that *A. lagunensis* cells assimilated exogenous nitrate more rapidly than they fixed carbon. *A. lagunensis* has an extremely high tolerance to phosphorus (P)-limited conditions, but an increase of nitrogen in the water seems to trigger the algal bloom (Liu et al., 2001), which might explain why the algae assimilated the ^{15}N -labeled NO_3 with a greater efficiency than fixing ^{13}C .

Differences in clearance rates and ^{15}N enrichment across bivalve species suggest that *C. virginica* and *I. recurvum* are more efficient at clearing and assimilating nutrients from *A. lagunensis* than *M. mercenaria*. Interestingly, the $\delta^{15}\text{N}$ values of *C. virginica* and *M. mercenaria* tissue increased with the concentration of *A. lagunensis*, implying that, even with a reduced clearance rate, these bivalves were assimilating more algal cells as they were increasingly available at higher (up to bloom) concentrations. However, the $\delta^{15}\text{N}$ values of *I. recurvum* were high but similar across treatments and were not related to the number of *A. lagunensis* cells removed. The clearance rate for this species was much higher at the lowest cell concentration but decreased and remained similar at concentrations higher than 10^4 cells mL^{-1} . It is reasonable to suggest, then, that *I. recurvum* may utilize similar amounts of microalgae as a food source despite being exposed to higher concentrations. Thus, as brown tide concentration increased, the cells removed are most likely packed as undigested cells in feces instead, as observed for other bivalves when feeding on phytoplankton blooms (Hégaret et al., 2007). Taken together with the clearance rates, these data support the consumption of *A. lagunensis* cells at bloom concentrations, but also highlight that this removal varies across bivalve species.

The fact that bivalves consumed brown tide cells even at bloom concentrations supports the idea that bivalves play a key role in shallow ecosystems where brown tide blooms occur, such as Laguna Madre (TX) (Montagna et al., 1993) and the Indian River Lagoon (FL) (Galimany et al., 2020), potentially ameliorating *A. lagunensis* brown tides. Bivalves provide many ecosystem services including turbidity reduction by filtration (Dame, 2011). However, interspecific variation in filtration efficiency may determine their ability to feed during algal blooms. For instance, we found that clams had the lowest clearance rates and enrichment in ^{15}N and ^{13}C , suggesting that fewer cells are removed and assimilated compared to the other bivalve species. Clearance rates for *C. virginica* and *M. mercenaria* feeding on natural phytoplankton are about 1 to 3 L h^{-1} and 0.5 to 1 L h^{-1} , respectively (Galimany et al., 2017b; Gray and Langdon, 2018), similar to our laboratory observations when the bivalves were exposed to pre-bloom concentrations (10^4 cells mL^{-1}).

Understanding and quantifying the feeding of bivalves is important to estimate their use as a Nature-based solution to improve water quality in degraded ecosystems. It is important to consider, when using laboratory estimates, that factors such as predator interactions and changes in environmental parameters can affect feeding behaviors and reduce rates obtained in a controlled system (Ruesink, 2000). Nonetheless, while laboratory experiments may not fully represent field data, they are a good proxy to study the feeding behavior of bivalves (Velasco and Navarro, 2005). By using clearance rates found in the

TABLE 5 | Nonlinear exponential regressions between the number of *Aureobamba lagunensis* cells removed by the bivalves from the water after 60 min exposed to the experimental algal concentrations and isotope values of the whole bivalve flesh.

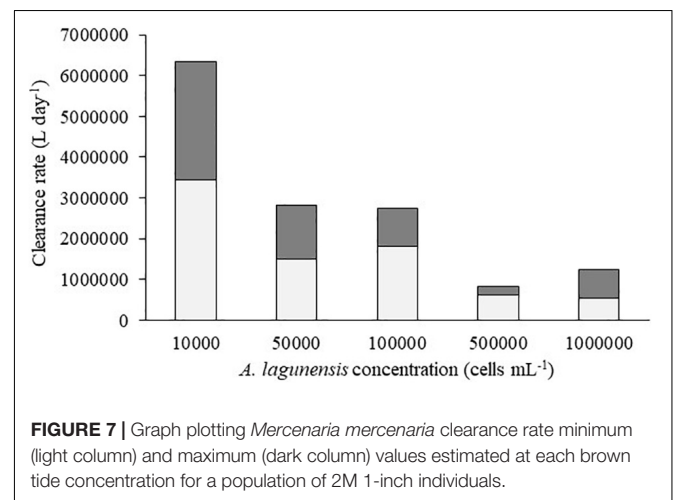
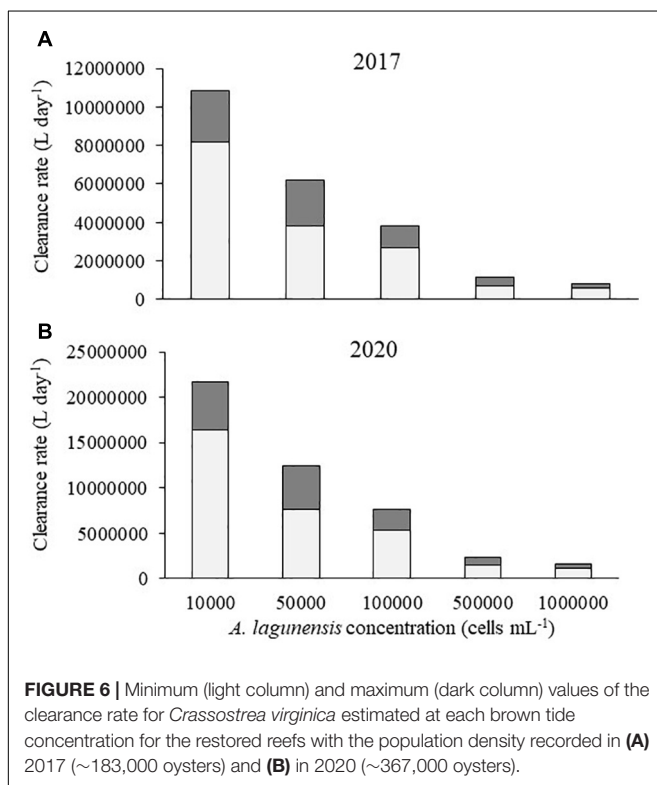
| Species | | $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ |
|------------------------------|-------------|---|---|
| <i>Crassostrea virginica</i> | Calculation | $\delta^{13}\text{C} = 7.36 (\pm 2.91) e^{1.09 \times 10^{-8} \text{ cells}}$ | $\delta^{15}\text{N} = 108.86 (\pm 31.63) e^{9.46 \times 10^{-9} \text{ cells}}$ |
| | F | 8.76 | 12.13 |
| | p | 0.007 | 0.002 |
| | R^2 | 0.26 | 0.34 |
| <i>Ischadium recurvum</i> | Calculation | $\delta^{13}\text{C} = 7.45 (\pm 1.32) e^{2.34 \times 10^{-9} \text{ cells}}$ | $\delta^{15}\text{N} = 261.83 (\pm 37.63) e^{4.98 \times 10^{-10} \text{ cells}}$ |
| | F | 10.20 | 0.7073 |
| | p | 0.04 | 0.409 |
| | R^2 | 0.28 | 0.03 |
| <i>Mercenaria mercenaria</i> | Calculation | $\delta^{13}\text{C} = 7.25 (\pm 0.40) e^{6.78 \times 10^{-9} \text{ cells}}$ | $\delta^{15}\text{N} = 19.37 (\pm 1.55) e^{9.05 \times 10^{-9} \text{ cells}}$ |
| | F | 31.22 | 26.53 |
| | p | <0.001 | <0.001 |
| | R^2 | 0.56 | 0.515 |

The positive sign after the e indicates that all regressions are positive. P -values indicate significance; the adjusted regression coefficient is indicated by R^2 .

laboratory, we estimated the potential effect of restoration by scaling the laboratory values to actual restoration projects. Considering that the IRL has a water surface area of 914 km² and an average depth of 1.22 m, the two species of bivalves used in restoration projects mentioned herein may be clearing about 0.002% of the water volume when feeding on the lowest brown tide concentration. Several oyster restoration efforts have been undertaken in recent decades to improve bivalve populations and recover bivalve ecosystem services, including improved water quality (McClenachan et al., 2020). Monitoring restored oyster reefs is not a common a practice because of funding limitations, but if we use the densities reported in

the Canaveral National Seashore, we can estimate that the oysters from the additional 89 restored reefs (McClenachan et al., 2020), only in one area of the IRL, could be clearing about 1% of the IRL volume daily. This estimate of total volume cleared is conservative, as it only takes into account the restoration projects within Mosquito Lagoon area and does not consider natural reefs, filtration of other species, or additional restoration projects throughout the IRL. Moreover, bivalve restoration actions tend to focus on commercial species, such as *C. virginica* and *M. mercenaria*, whereas non-commercial species (e.g., *I. recurvum*), are traditionally understudied (zu Ermgassen et al., 2020). Diversification of bivalve species may provide new opportunities to achieve greater outcomes for water quality and several bivalves are reported to filter water at rates that merit restoration attention (Kreeger et al., 2018), including *I. recurvum* based on our results. This study, and the population level estimates we have generated provide a working baseline to understand the potential role bivalves can play in mitigating harmful algal blooms both before and during a bloom.

In conclusion, this research provides evidence that the bivalves *C. virginica*, *I. recurvum*, and *M. mercenaria* can effectively consume *A. lagunensis* at different concentrations,



from pre-bloom to bloom concentrations. In Florida, as many areas in the world, populations of bivalve mollusks of both recreational and commercial importance are stressed by a variety of factors, including habitat degradation, overfishing and development (Arnold, 2001). However, resident bivalve populations clear and assimilate bloom-forming algal species and may therefore play a role in the mitigation and prevention of HABs in coastal ecosystems (Edebo et al., 2000; Galimany et al., 2017b, 2020). Thus, the use of natural solutions, such as shellfish restoration and establishment of bioremediation strategies should be encouraged to help mitigate the effects of eutrophication (Galimany et al., 2017c; Locher et al., 2020) that may lead to *A. lagunensis* brown tides. We highlight the importance of suspension feeder biodiversity in the ecosystem function of coastal ecosystems, and suggest that diversification of bivalve restoration projects could be an important step in restoring ecosystem health.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

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AUTHOR CONTRIBUTIONS

EG conceived and planned the experiments. EG, JL, CF, AD, and JH carried out the experiments. All coauthors contributed to sample preparation and analysis. EG, JL, CF, IS-G, JH, and VP contributed to the interpretation of the results. EG took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.714816/full#supplementary-material>

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