Feeding behavior of eastern oysters *Crassostrea virginica* and hard clams *Mercenaria mercenaria* in shallow estuaries

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ABSTRACT: Eastern oysters *Crassostrea virginica* and hard clams *Mercenaria mercenaria* are key organisms for both the ecosystem services they provide and for their commercial value, but their populations have declined greatly worldwide. In an attempt to understand the interaction between bivalve physiology and environmental conditions, filter-feeding assays were carried out in a shallow estuary, the Indian River Lagoon (IRL; Florida, USA). The feeding behavior of the bivalves was studied using *in situ* filter-feeding devices and the biodeposition method in the 3 basins of the IRL during March and August 2015. Water characteristics (temperature, salinity, dissolved oxygen, chl *a*, and total, organic, and inorganic particulates) were related to possible changes in the feeding physiology of the bivalves. Oysters had higher clearance rates, filtration rates, and rejection than clams. The high rejection of inorganic matter allowed oysters to increase the organic matter ingested, leading to high absorption efficiencies. In contrast, because clam rejection was low regardless of elevated levels of inorganic matter, their absorption efficiency only increased with higher organic matter content. Both species preferred higher salinities, and the amount of organic matter in the water had a negative relationship with some feeding parameters (filtration rate for both species, and rejection for oysters). Acute environmental change brought about by a brown tide (caused by the alga *Aureoumbra lagunensis*) also affected these 2 bivalve species differently, supporting the hypothesis that oysters and clams have different physiological capabilities that drive their ability to survive in dynamic estuarine ecosystems.

KEY WORDS: Indian River Lagoon · Brown tide · *Aureoumbra lagunensis* · Seston · Clearance rate · Bivalves

INTRODUCTION

Bivalves are an important foundational group known to provide a variety of ecosystem services, including improving water quality, reducing shoreline erosion, stabilizing estuarine sediments, and enhancing nutrient cycling (Newell 2004, Ward & Shumway 2004). Although their presence and high abundance are crucial to support healthy ecosystems, bivalve stocks have decreased worldwide as a consequence of overfishing, habitat loss, poor water quality, and other factors (Beck et al. 2011, FAO 2012). Restoration efforts have been conducted worldwide in an attempt to restore the ecosystem functions lost with bivalve decline. These efforts, however, have not always been successful for a variety of reasons, such as disease, poor water quality, or low larval recruitment to sustain the restored populations (Arnold et al. 2002, Mann & Powell 2007). Failed restoration efforts in areas where healthy populations once flourished are not surprising, as local conditions are likely no longer favorable for these species. Therefore, prior to restoration, an assessment of the water quality and species performance

seems crucial to prevent restoration failure (Mann & Powell 2007).

The filter-feeding ability of bivalves allows them to filter and consume most types of organic matter from the water column, ranging from detritus and bacteria to zooplankton (Gosling 2003). They also have the ability to sort particles from the seston and pack rejected particles into pseudofeces (Shumway et al. 1985). These mucus strings containing the rejected particles are expelled out of the gills prior to ingestion. Different physiological variables involved in the feeding behavior of bivalves, such as clearance rates and particle selection, can be influenced by the quality and quantity of various particle types in the seston (Widdows et al. 1979, Bayne et al. 1993, Galimany et al. 2013). The way that the physiological variables associated with feeding may be affected, however, can vary according to species or locations. For example, Pacific oysters *Crassostrea gigas* have faster filtration and selection rates than Sydney oysters *Saccostrea glomerata* when processing large amounts of particulate matter (Bayne 2002). Algal blooms can also influence the feeding behavior of bivalves (Bayne 2002), decreasing clearance rates as a response to their toxicity (Hégaret et al. 2007), or overloading their ingestive capacity for increased seston abundance (Beninger & St-Jean 1997). Environmental parameters such as water temperature and salinity can also influence bivalve physiology. Therefore, the study of the feeding behavior of bivalves and its relationship with water characteristics is essential to determine their potential to provide ecosystem services.

The Indian River Lagoon (IRL) is a narrow and shallow subtropical estuary along the central eastern coast of Florida (USA). The IRL has been suffering from a variety of threats such as eutrophication and freshwater releases (Dybas 2002). Recently, several phytoplankton blooms, such as brown tides caused by the microalga *Aureoumbra lagunensis*, have been responsible for seagrass loss and fauna mortality (Gobler et al. 2013). Some of these factors may have changed the abundance and distribution of several native bivalve species, including eastern oysters *C. virginica* and hard clams *Mercenaria mercenaria*. Oyster populations have severely decreased over the years in all areas of the IRL as a consequence of low salinity, overharvesting, and habitat degradation (Wilson et al. 2005, Garvis et al. 2015). Clam populations thrived in the IRL, and a clam fishery was sustained until 1985, when most clams died as a result of fresh water flowing into the clam beds from flood control canals (MacKenzie et al. 2001). Several oyster restoration projects are ongoing in an attempt to revitalize the IRL ecosystem (Garvis et al. 2015); however, as in other shallow estuaries, oyster restoration is not always successful. Likewise, hard clam restoration efforts began in 1997 (Arnold 2001), but the population has not yet recovered, and clams are naturally found in low abundances.

With the local focus of restoration efforts on bivalves, we aimed to investigate the link between bivalve physiology and environmental variables in order to identify suitable sites for restoration. To do this, *in situ* filter-feeding experiments were conducted at several sites within the IRL, a large, shallow estuary subject to re-suspension of particulate matter as a result of freshwater inflow and wind. As such, the IRL provides a diversity of water conditions and an ideal model system in which to study animal responses to changing environmental conditions in estuarine ecosystems. We hypothesized that bivalve feeding behavior would be influenced by environmental conditions and water characteristics and that these responses would differ between the 2 species. Thus, we provide fundamental information necessary for understanding the feeding ecology of 2 important bivalve species and their ability to function under different environmental conditions.

MATERIALS AND METHODS

Experimental sites and bivalve collection

The filter-feeding experiments were performed in the northern regions of the IRL (Fig. 1). The following sites were chosen from each body of water comprising the IRL system: Oak Hill in the Mosquito Lagoon (ML) (28° 53.782′ N, 80° 51.033′ W), the city of Cocoa in the Indian River (IR) (28° 21.157′ N, 80° 43.446′ W), and Cocoa Beach in the Banana River (BR) (28° 15.825′ N, 80° 36.482′ W).

Experiments were conducted in March and August 2015, representing the dry and wet seasons in Florida. Although assessment of seasonality was not the goal of this study, we wanted to ensure that we were capturing temporal and spatial variability in the seston throughout our study system. Three and 4 filter feeding experiments were run at each site during the first and second sampling periods, respectively, at ML and IR, and 2 and 3 filter feeding experiments were carried out in the BR during the first and second sampling periods, respectively. Each experiment was conducted with a group of adult eastern oysters (average length: 64.7 ± 1.6 [SE] mm) and hard clams (average length 42.1 ± 0.3 mm).
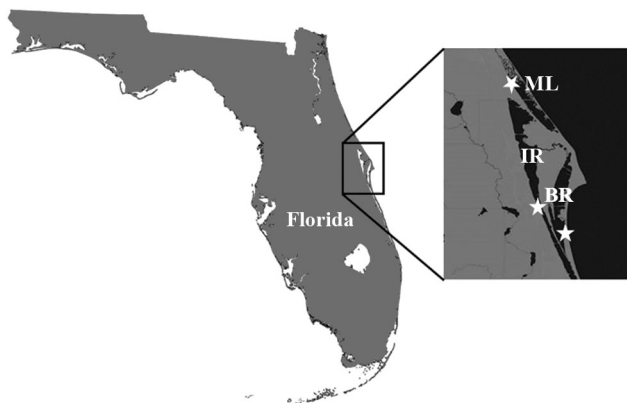
Fig. 1. Florida (USA) and detail of the North Indian River Lagoon with its 3 basins: ML, Mosquito Lagoon; BR, Banana River; IR, Indian River. Stars denote sampling sites

Oysters and clams were purchased from Aquagem Farms Inc. in Sebastian, FL. All bivalves collected were cleaned of epiphytes and other encrusting organisms and kept in the laboratory in a flowing seawater system for about 1 wk before the experiments. For the first experiment at each site and season, 10 bivalves of each species were hung in a mesh bag 3 d before the sampling. For the following experiments, bivalves had an acclimation period of 1 wk at each site. Controls were made by dissecting out the oyster and hard clam meat and gluing their shells back together (1 control per species per experiment).

Water characteristics and physiological feeding parameters

Temperature (°C), salinity (ppt), and dissolved oxygen (mg l^{-1}) were measured with a YSI meter at the beginning and end of each experiment. Three water samples were filtered through pre-weighed Whatman GF/C filters (25 mm Ø) until clogged to collect biomass for chlorophyll a (chl a) analyses. Filters for chl a analysis were frozen, lyophilized overnight, and extracted with 5 ml of 90% acetone at 4°C overnight. The concentration of chl a was quantified by measuring extract absorbance at 750, 664, 647, and 630 nm and with the equations of Parsons et al. (1984). Final values were corrected for the volume of water filtered through each filter and are reported in µg l^{-1}.

Two portable, filter-feeding, flow-through devices were designed to simulate *in vivo* conditions of bivalve feeding (Fig. 2). The devices were first described by Galimany et al. (2011). One portable filter-feeding device consisted of a common PVC tank that received lagoon water from an underwater pump at each experimental site. Aeration was added to the common tank to prevent particle settlement. A rubber tube connected the common tank to each of the 10 PVC chambers, each containing a single live bivalve except for the control for each species. Both bivalve species were positioned near the flow exit tube of the chambers, and clams were attached to the bottom with a piece of plastic hook and loop fastener to avoid movement. The flow of water was maintained at a constant rate of 12 l h^{-1} for all bivalves.

To determine characteristics of the seston, between 50 and 100 ml (enough to clog the pre-weighed GF/C filters) of lagoon water were collected from the chambers containing the controls every 15 min for 2 h, filtered, and rinsed with ammonium formate to dissolve salts from the samples on the filters. In the laboratory, all filters were dried at 60°C for 48 h and weighed to measure the total particulate matter (TPM). The filters were then ashed at 450°C for 4 h to obtain the particulate inorganic matter (PIM). The particulate organic matter (POM) was calculated as the weight loss between TPM and PIM. Average values for TPM, PIM, and POM for each day were used for feeding behavior calculations (n = 7 for ML and IR; n = 5 for BR).

To determine when to start feces and pseudofeces collection, the gut transit time (GTT) of both species was determined before each experiment. GTT was calculated using a method adapted from Hawkins et al. (1996). Three clams and 3 oysters were placed individually in beakers in a mixture of lagoon water and *Tetraselmis* sp. monoculture. The elapsed time between the ingestion of the mixture and the deposition of green-colored feces was considered to be the GTT (min).
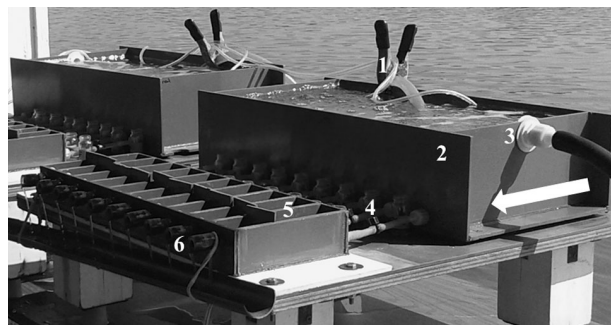

Fig. 2. Image detail of a portable, flow-through device used for the filter-feeding experiments. (1) Clamp holding the tube with water pumped from the environment; (2) PVC 'reservoir' tank; (3) overflow of the reservoir tank; (4) plastic tubes with valves to regulate flow connecting the reservoir tank with each individual chamber; (5) individual chambers that each hold a single bivalve; (6) overflow of the chamber. White arrow shows water flow direction

Nine oysters, 9 clams, and 1 control (empty shell) for each species were placed in the individual chambers of the feeding devices and allowed to recover for at least 1 h from any stress associated with handling. The number of bivalves that fed and were used to determine feeding parameters are as follows: 47 oysters and 37 hard clams in ML, 21 oysters and 20 hard clams in BR, and 30 oysters and 28 hard clams in IR (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m567p125_supp.pdf). The individual chambers were cleaned before the beginning of the experiment to remove biodeposits created during the recovery time and any silt that may have accumulated. In each chamber of the feeding device, feces and pseudofeces were collected with a pipette as soon as they were produced, and kept separately throughout the 2 h experiment. All samples of feces and pseudofeces for each chamber were filtered separately through pre-weighed Whatman GF/C filters (25 mm Ø) and rinsed with ammonium formate to dissolve salts from the samples on the filters. In the laboratory, all filters were dried at 60°C for 48 h and weighed to determine dry weights of feces and pseudofeces. The filters were then ashed at 450°C for 4 h to obtain ash weights of feces and pseudofeces.

The physiological parameters of the feeding behavior of the bivalves (Table 1) were then calculated according to the biodeposition method (Iglesias et al. 1998). This method is based on using the inorganic matter of the water as a tracer of the ingestion, egestion, and rejection feeding processes. These parameters were then standardized to 1 g of dried bivalve flesh using the following equation:

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

where Y_s is the standardized physiological rate, Y_e is the experimentally determined rate, and W_e is the dry body mass measured for each bivalve. We used a b value of 0.73 for oysters and 0.8 for clams, as described by Riisgård (1988). Each animal was only used in an experiment once because each bivalve was sacrificed to obtain its dry weight.

Brown tide

In December 2015, a brown tide (caused by the alga *Aureoumbra lagunensis*) occurred in all basins of the IRL and persisted until the end of March 2016 (K. Hubbard pers. comm., and see http://news.brevardtimes.com/2016/04/fwc-indian-river-lagoon-algae-dna-tests.html). The brown tide phytoplankton was characterized with water samples from the BR site where filter-feeding experiments occurred in 2015. Water samples were taken on 25, 28, and 29 January and on 5 and 8 February 2016 to track the bloom in BR. Water samples were processed through a C6 flow cytometer (BD Biosciences) for 30 s on the slow flow rate setting (14 µl min^{-1}) using plots contrasting side scatter vs. red fluorescence to identify and quantify the algal population. Plots from the bloom were compared with plots generated from a pure *A. lagunensis* culture (Texas Brown Tide) kept at the laboratory, which was provided by the University of Texas Marine Science Institute and was isolated from the Laguna Madre (North Padre Island, Texas) in April 1996 in the laboratory of Dr. T. Villareal.

The presence of *A. lagunensis* was confirmed by the positive amplification of a constitutive ribosomal

Table 1. Physiological components of absorptive balance for bivalves. TPM: total particulate matter (mg l^{-1}); PIM: particulate inorganic matter (mg l^{-1}); POM: particulate organic matter (mg l^{-1})

Parameter and units	Description	Calculation
Clearance rate (l h^{-1})	Volume of seawater passing through the gills per unit of time	(mg inorganic matter from both feces and pseudofeces per unit of time) / (PIM water)
Filtration rate (mg h^{-1})	TPM from the seawater retained in the gills per unit of time	Clearance rate × TPM water
Rejection (%)	TPM that has been retained in the gills but rejected prior to ingestion	[(mg organic and inorganic matter from pseudofeces per unit of time) / (filtration rate)] × 100
Organic ingestion rate (mg h^{-1})	POM retained in the gills and ingested by the bivalve per unit of time	(Clearance rate × POM water) − (mg organic matter from pseudofeces per unit of time)
Absorption rate (mg h^{-1})	POM ingested by the bivalve and not egested as feces per unit of time	Organic ingestion rate − (mg organic matter from feces per unit of time)
Absorption efficiency (%)	Efficiency of the feeding process	Absorption rate / organic ingestion rate

gene (18S rRNA). Total genomic DNA was extracted and purified from the total cells in a volume of 50 ml of water collected from the Banana River site using a PowerPlant® Pro (Mo BIO) extraction kit. A partial fragment of 18S rRNA (800 bp) was amplified by PCR in 25 µl reactions with the following conditions and primers previously used to identify *A. lagunensis*: 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl$_2$, 0.25 mM each dNTP, 0.8 µM of each primer (Euk A: 5′ AAC CTG GTT GAT CCT GCC AGT 3′; and 329 R: 5′ TGA TCC TTC YGC AGG TTC AC 3′; Koch et al. 2014), 1.25 unit of Go*Taq* DNA polymerase (Promega), and 1 µl of genomic DNA. The PCR profile began with a hot start denaturation step of 2 min at 95°C, followed by 25 cycles of 45 s at 95°C, 45 s at 50°C, 2 min at 72°C; and a final elongation step of 3 min at 72°C. PCR products were electrophoresed in adjacent lanes in 1.5% agarose gels with Gel Green Nucleic Acid stain (BIOTIUM) and visualized with blue light. The presence of *A. lagunensis* was confirmed when an amplicon was obtained (bands in the agarose gels).

In situ feeding experiments using the devices and methodology explained above were performed on 29 January and 8 February 2016 at the BR site where a dense brown tide was occurring. Only animals that were open for the entire 2 h sampling periods were used in the analysis. Clams were open but did not produce any biodeposits during brown tide experiments and were therefore excluded from further analysis. The feeding physiology of oysters (n = 5) and characteristics of the seston (n = 2) were collected and studied as described in the previous section.

Statistical analyses

Water characteristics (temperature, salinity, dissolved oxygen, and chl *a*) were compared using a 2-way ANOVA with site and sampling period as factors. Seston (TPM, POM, PIM, and the proportion of organic content) values obtained from each 15 min time interval collection (n = 311) were compared using a 2-way ANOVA with site and sampling period as factors. Correlations were established between TPM and PIM using Pearson correlation coefficients. Bivalve feeding behaviors were compared between sites using a blocked 2-way ANOVA with sampling period as the block, and site and species as fixed factors. Sampling period was used as a blocking factor because seasonality was not a main factor of interest.

Nonlinear exponential regression analyses were used to relate the environmental (temperature, salinity, dissolved oxygen, chl *a*) and seston (TPM, POM, PIM, and proportion of organic matter) water characteristics, with each of the physiological variables (clearance rate, filtration rate, rejection, organic ingestion rate, absorption rate, and absorption efficiency) for each species. All environmental and seston water characteristics were first checked for multicollinearity. TPM and PIM were highly correlated (r > 0.9), so only TPM was used in regression analysis. The correlation between the rest of the variables was below the threshold of r = 0.9, so they were retained for the nonlinear exponential regression analysis. Rejection and absorption efficiency were logit transformed prior to analysis. Pearson correlations were tested between the rejection and the feeding variables organic ingestion rate and absorption efficiency for each species to further understand the influence of rejection on the absorptive physiological variables.

Seston characteristics for the brown tide (TPM, POM, PIM, and the proportion of organic content) were compared using a 1-way ANOVA with site as the factor. In this case, sites included data from 2015 sampling in ML, BR, and IR, and brown tide data from 2016. Values from each 15 min interval collection were used in the analysis (n = 335).

Oyster feeding behavior from 2015 was compared with the feeding behavior data from the 2016 brown tide event using an ANOVA (n = 46 in ML; n = 35 in IR; n = 20 in BR; n = 5 brown tide). March and August data from 2015 was combined for each site to be more conservative, as brown tide can potentially occur any time of the year.

All proportion data were logit transformed prior to analysis according to Warton & Hui (2011). For all ANOVAs, data met assumptions for analysis; normality and homogeneity of variances were checked using the Shapiro-Wilk and Levene's tests, respectively. Tukey post hoc tests determined differences within factors. Simple main effects tests were used to determine patterns masked by interactions. The statistical software used was SPSS Statistics 23.0 (IBM).

RESULTS

Water characteristics

Environmental water characteristics varied by sampling period, but only salinity consistently varied by site (Table 2). Water temperature was higher in August than in March at all sites ($F_{1,13}$ = 59.10; p < 0.001) but did not vary among sites ($F_{2,13}$ = 1.82; p = 0.200). Salinity was always higher in ML, regardless

Table 2. Mean (±SE) environmental water characteristics measured at each site in the Indian River Lagoon, Florida (USA), for both sampling periods (n = 8 for March and n = 11 for August). Capital and lowercase superscript letters denote significant differences by site (p < 0.01) and sampling period (p < 0.05), respectively, for each water characteristic based on 2-way ANOVA followed by Tukey post hoc tests. ML: Mosquito Lagoon; BR: Banana River; IR: Indian River

Site	Sampling period	Temperature (°C)	Salinity (ppt)	Dissolved oxygen (mg l^{-1})	Chlorophyll a (µg l^{-1})
ML	March	23.28 ± 1.40[A,a]	31.55 ± 0.27[A,a]	5.68 ± 0.44[A,a]	6.89 ± 0.81[A,a]
	August	29.62 ± 0.98[A,b]	33.12 ± 0.77[A,a]	4.69 ± 0.15[A,b]	20.04 ± 3.72[A,b]
BR	March	24.05 ± 0.15[A,a]	25.75 ± 0.25[B,a]	5.68 ± 0.16[A,a]	5.59 ± 1.03[A,a]
	August	29.17 ± 0.48[A,b]	21.90 ± 0.24[B,a]	4.95 ± 0.03[A,b]	11.64 ± 0.74[A,b]
IR	March	25.34 ± 0.46[A,a]	23.77 ± 0.57[B,a]	6.22 ± 0.47[A,a]	9.19 ± 0.73[A,a]
	August	30.49 ± 0.59[A,b]	23.45 ± 0.19[B,a]	5.24 ± 0.29[A,b]	8.71 ± 2.05[A,b]

of the sampling period ($F_{2,13}$ = 230.28; p < 0.001), although site and sampling period interacted ($F_{2,13}$ = 14.89; p < 0.001). Salinity was similar in IR and BR, although salinities in BR differed by season. Dissolved oxygen was higher in March at all sites ($F_{1,13}$ = 12.27; p < 0.01), but sites did not differ ($F_{2,13}$ = 1.84; p = 0.198). Chl a in ML and BR was higher in August, whereas IR had more chl a in March (sampling period $F_{1,13}$ = 9.92; p = 0.008; interaction site × sampling period; $F_{2,13}$ = 4.51; p = 0.032).

Seston characteristics differed by site (TPM: $F_{2,305}$ = 148.66, p < 0.001; POM: $F_{2,305}$ = 57.27, p < 0.001; PIM: $F_{2,305}$ = 190.49, p < 0.001; proportion organic: $F_{2,305}$ = 185.79, p < 0.001) and sampling period (TPM: $F_{1,305}$ = 4.71, p < 0.031; POM: $F_{1,305}$ = 47.65, p < 0.001;

PIM: $F_{1,305}$ = 50.03, p < 0.001; proportion organic: $F_{1,305}$ = 267.12, p < 0.001; Fig. 3; Table S2 in the Supplement). TPM and PIM were higher in March than in August, and POM and proportion of organic matter in the water were higher in August. TPM, POM, PIM, and proportion of organic matter were highest in ML, lowest in BR, and intermediate in IR. There was also an interaction between site and sampling period (TPM: $F_{2,305}$ = 116.03, p < 0.001; POM: $F_{2,305}$ = 113.23, p < 0.001; PIM: $F_{2,305}$ = 101.64, p < 0.001; proportion organic: $F_{2,305}$ = 12.56, p < 0.001). In August, ML had the highest TPM, POM, and PIM, whereas the lowest TPM, POM, and PIM were observed in BR in March and IR in August. However, the highest proportion of organic matter in the water was recorded in BR in August and the lowest in ML in March.

TPM was positively correlated to the PIM at the 3 studied sites (ML, r = 0.92, p < 0.001; BR, r = 0.93, p < 0.001; IR, r = 0.99, p < 0.001). This relationship indicates that more particulates in the water (TPM) mean more inorganic matter, requiring the bivalves to sort the organic matter available from the seston.
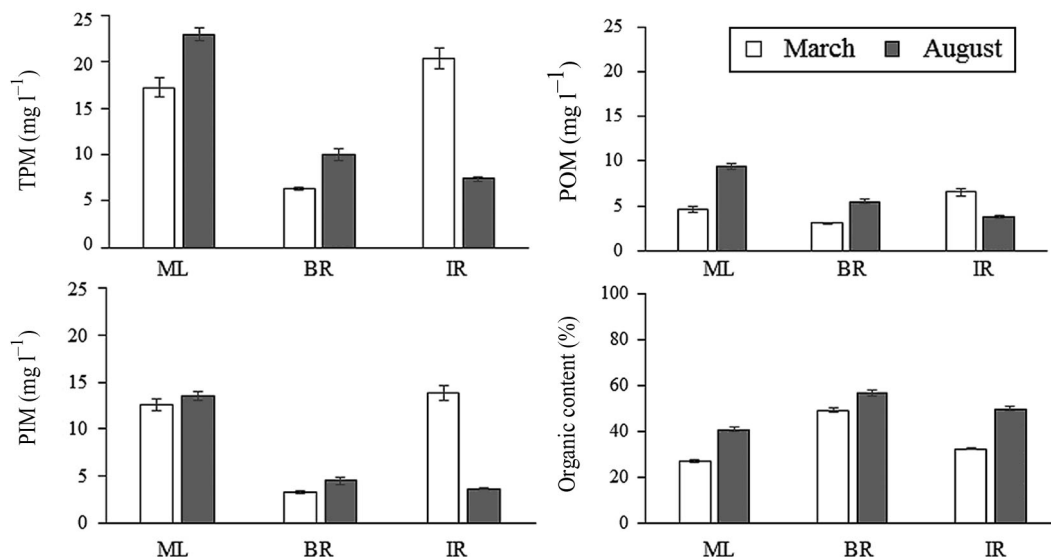

Fig. 3. Mean (± SE) values for the different components of the seston at each site during the 2 sampling periods: ML: Mosquito Lagoon; BR: Banana River; IR: Indian River. The water had different seston characteristics at all sites (p < 0.01) and sampling periods (p < 0.01). Total particulate matter (TPM) and particulate inorganic matter (PIM) were higher in March than in August (p < 0.01), and particulate organic matter (POM) and % organic matter content were higher in August (p < 0.01)
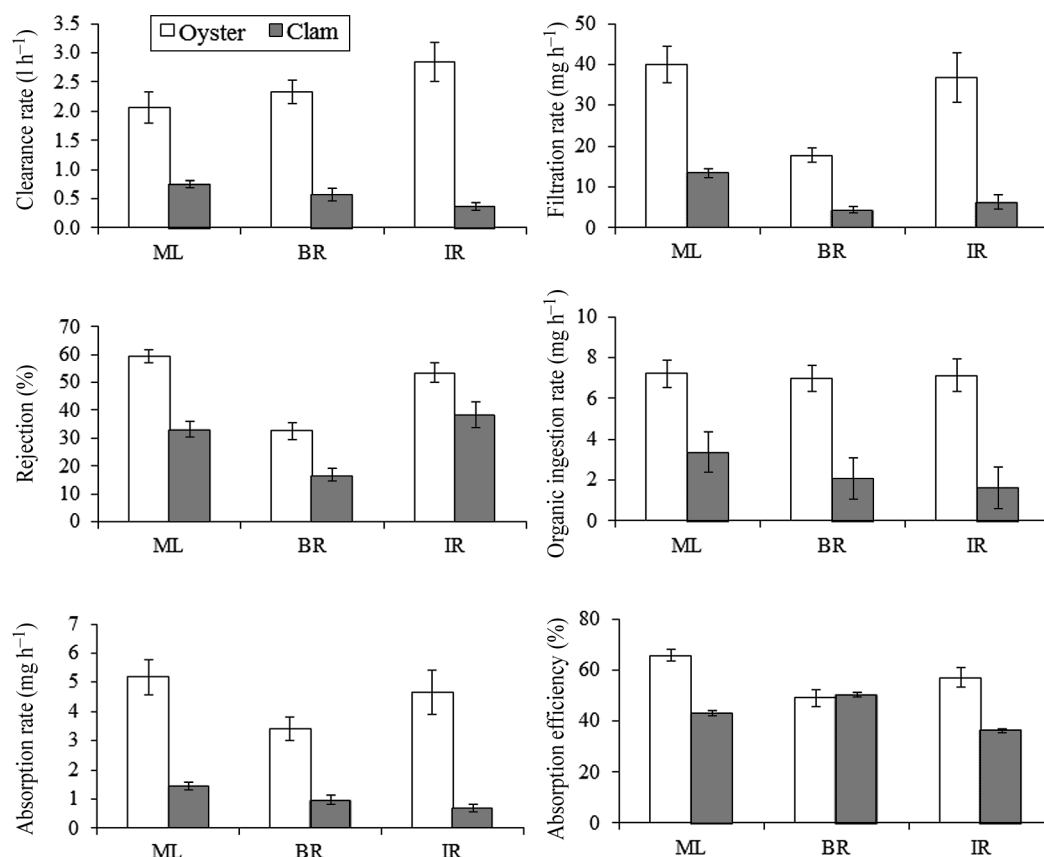
Fig. 4. Mean (±SE) physiological feeding variables measured for both bivalve species (eastern oyster *Crassostrea virginica* and hard clam *Mercenaria mercenaria*) during the *in situ* experiments at the 3 sites for both sampling periods together. ML: Mosquito Lagoon; BR: Banana River; IR: Indian River. Oysters had significantly higher feeding parameters than clams (p < 0.001). BR was different from the other sites only for filtration rate and rejection (p < 0.01)

Physiological feeding parameters

All of the feeding parameters analyzed were significantly higher for oysters than for clams (clearance rate: $F_{1,176} = 87.49$, p < 0.001; filtration rate: $F_{1,176} = 52.35$, p < 0.001; rejection: $F_{1,176} = 26.19$, p < 0.001; organic ingestion rate: $F_{1,176} = 80.24$, p < 0.001; absorption rate: $F_{1,176} = 57.03$, p < 0.001; and absorption efficiency: $F_{1,176} = 40.52$, p < 0.001; Fig. 4; Table S3). Filtration rate ($F_{2,176} = 7.18$, p = 0.001) and rejection ($F_{2,176} = 8.36$, p < 0.001) were lower in BR than in IR or ML. Absorption efficiency was higher in ML than in IR, but BR bivalves were not different from the other sites ($F_{2,176} = 4.79$, p = 0.009). There was an interaction between site and species for clearance rate ($F_{2,176} = 3.27$, p = 0.040), rejection ($F_{2,176} = 4.46$, p = 0.013), and absorption efficiency ($F_{2,176} = 8.10$, p < 0.001). Clearance rate was lower for clams at all sites (simple main effects test, Fig. 5). Rejection in BR was lower for both species than oysters in ML and IR. Clams had lower absorption efficiencies in ML and IR than oysters did. Oysters and clams had similar absorption efficiencies in BR (Fig. 5). Clearance rate ($F_{1,176} = 8.75$, p = 0.004), filtration rate ($F_{1,176} = 12.66$, p < 0.001), and absorption efficiency ($F_{1,176} = 27.83$, p < 0.001) were higher in March than August (Table S3).

Feeding behavior

The feeding behavior of oysters was mainly influenced by chl *a*, organic matter proportion, and salinity (nonlinear exponential regression, Table 3). Clams were mainly influenced by salinity, but filtration rate was also influenced by the organic matter proportion (Table 3).

When correlating the rejection with organic ingestion rate and absorption efficiency, the 2 species were very different. Oysters increased their organic ingestion rate by increasing rejection (r = 0.325, p = 0.001), suggesting they actively increase the amount of organic matter in their digestive system. This increase in organic ingestion rate increased the ab-
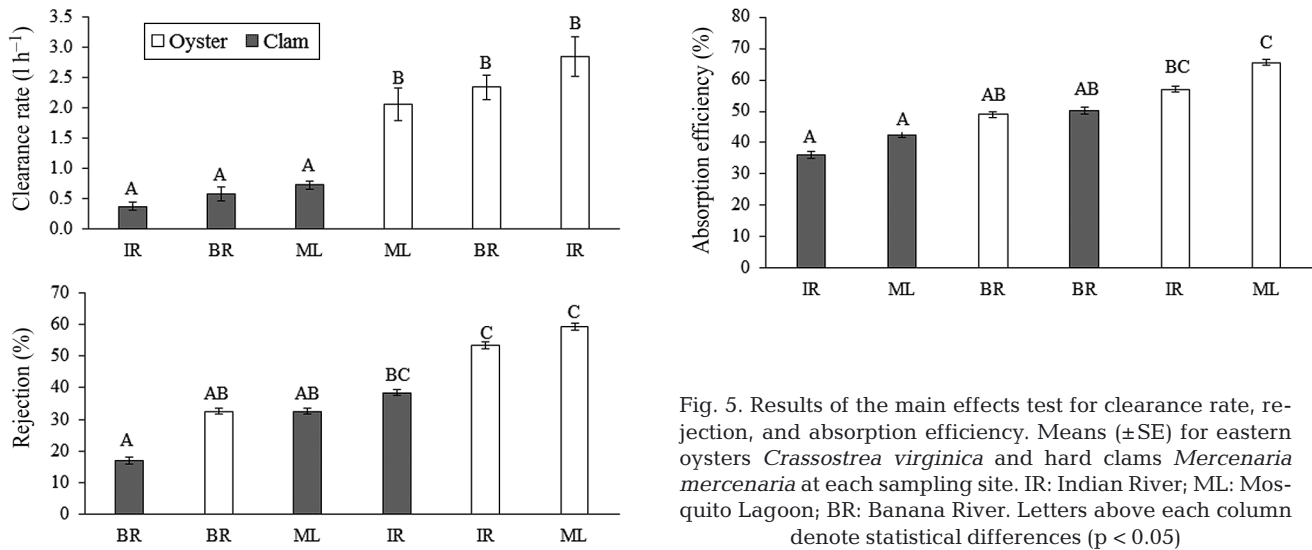
Fig. 5. Results of the main effects test for clearance rate, rejection, and absorption efficiency. Means (±SE) for eastern oysters *Crassostrea virginica* and hard clams *Mercenaria mercenaria* at each sampling site. IR: Indian River; ML: Mosquito Lagoon; BR: Banana River. Letters above each column denote statistical differences (p < 0.05)

Table 3. Nonlinear exponential regressions between the different feeding variables studied and the environmental and seston water characteristics; chl: chlorophyll *a* (µg l^{-1}); organic: proportion of organic matter (%); salinity (ppt). The sign after the e indicates if the regression is positive or negative. p indicates significance; the adjusted regression coefficient is indicated by R^2. NC: no significant relations resulted from the nonlinear exponential regression

| Feeding variable		Oysters	Clams
Clearance rate (CR)	Calculation	CR = 4.47 (±0.66)e$^{-0.07\ (\pm0.01)\ \times\ chl\ a}$	CR = 0.07 (±0.06)e$^{0.07\ (\pm0.03)\ \times\ salinity}$
	F	32.14	5.90
	p	<0.001	0.027
	R^2	0.65	0.22
Filtration rate (FR)	Calculation	FR = 20.09 (±2.26)e$^{-2.12\ (\pm0.44)\ \times\ organic}$	FR = 3.90 (±0.77)e$^{-2.53\ (\pm0.76)\ \times\ organic}$
	F	23.40	11.28
	p	<0.001	0.004
	R^2	0.55	0.36
	Calculation	NC	FR = 0.11 (±0.10)e$^{0.14\ (\pm0.03)\ \times\ salinity}$
	F		17.90
	p		0.001
	R^2		0.50
Rejection (Rej)	Calculation	Rej = 0.68 (±0.03)e$^{-0.71\ (\pm0.17)\ \times\ organic}$	NC
	F	22.44	
	p	<0.001	
	R^2	0.54	
Organic ingestion rate (OIR)	Calculation	NC	OIR = 0.10 (±0.09)e$^{0.11\ (\pm\ 0.04)\ \times\ salinity}$
	F		8.83
	p		0.009
	R^2		0.32
Absorption rate (AR)	Calculation	NC	AR = 0.02 (± 0.03)e$^{0.13\ (\pm\ 0.05)\ \times\ salinity}$
	F		6.68
	p		0.020
	R^2		0.25
Absorption efficiency (AE)	Calculation	AE = 0.79 (± 0.05)e$^{0.48\ (\pm\ 0.22)\ \times\ organic}$	NC
	F	4.65	
	p	0.046	
	R^2	0.17	
	Calculation	AE = 0.43 (± 0.13)e$^{0.02\ (\pm\ 0.01)\ \times\ salinity}$	
	F	4.87	
	p	0.042	
	R^2	0.19	
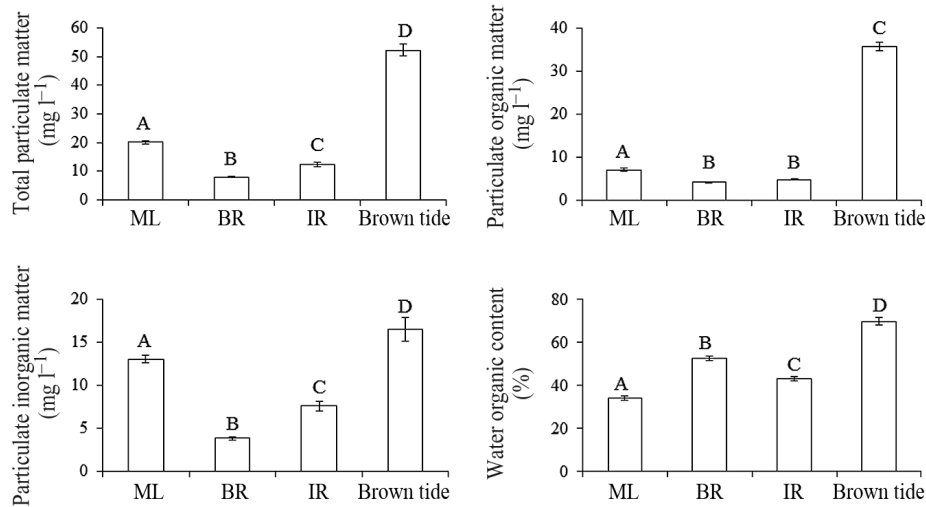
Fig. 6. Mean (±SE) values for the different components of the seston at each site and during the brown tide event. ML: Mosquito Lagoon; BR: Banana River; IR: Indian River; TPM: total particulate matter; PIM (POM): particulate inorganic (organic) matter. Letters above each column denote statistical differences (ANOVA, p < 0.05)

sorption efficiency in oysters (r = 0.673, p < 0.001). Clams, however, did not alter their rejection to modify their organic ingestion rate or absorption efficiency (r = −0.156, p = 0.168; r = −0.128, p = 0.260, respectively).

Brown tide

The algal bloom had an average concentration of $3.26 \times 10^6 \pm 0.20 \times 10^6$ (SE) cells ml^{-1} and accounted for $87.15 \pm 2.69\%$ of the total particulates in the
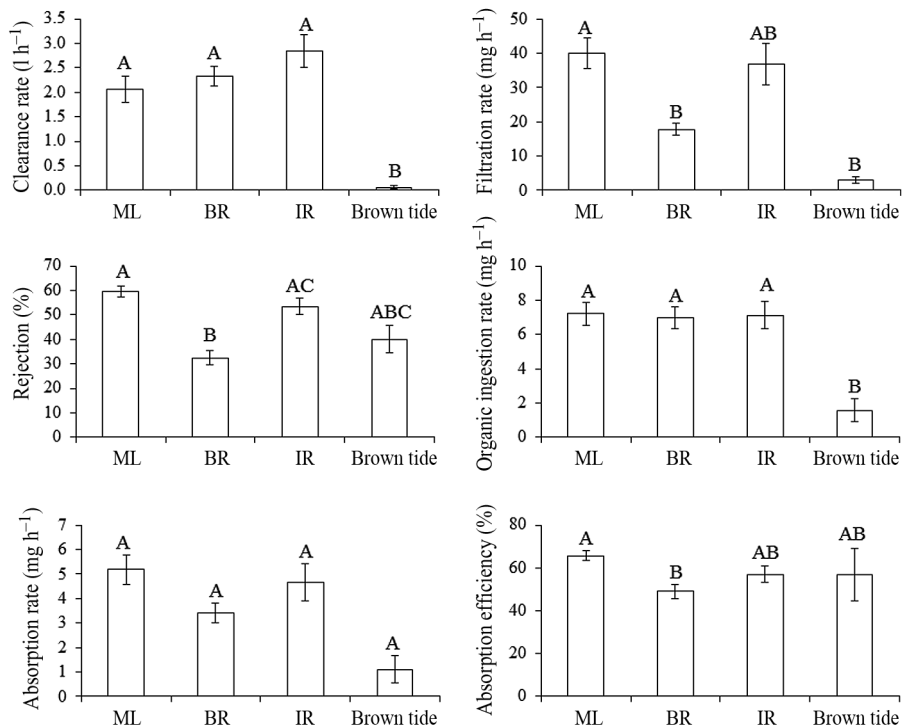

Fig. 7. Mean (±SE) physiological feeding variables measured for eastern oysters *Crassostrea virginica* during the *in situ* experiments at the 3 sites for both sampling periods together. ML: Mosquito Lagoon; BR: Banana River; IR: Indian River; brown tide: experiments at the same site as BR conducted during the brown tide in 2016. Letters above each column denote statistical differences (ANOVA, p < 0.05, followed by Tukey post hoc tests)

water. The population identified as *Aureoumbra lagunensis* in the flow cytometer matched with the algal culture from Texas (Fig. S1 in the Supplement). Water samples collected on 19, 27, and 29 February 2016 were confirmed to contain *A. lagunensis* based on the presence of the correct *Aureoumbra*-specific amplicon of 18S rRNA.

All seston characteristics differed between bloom and non-bloom conditions (Fig. 6; Table S4). TPM, POM, PIM, and proportion of organic matter in the water were highest when the brown tide was present (TPM: $F_{3,335}$ = 318.32, p < 0.001; POM: $F_{3,335}$ = 1005.27, p < 0.001; PIM: $F_{3,335}$ = 90.56, p < 0.001; proportion organic: $F_{3,335}$ = 120.66, p < 0.001).

Oyster clearance rates when feeding on the brown tide were much lower than those obtained in 2015, with an average of 0.06 ± 0.02 l h^{-1} ($F_{3,104}$ = 4.27, p = 0.007; Fig. 7; Table S5). Filtration rate, the amount of particles retained on the gills, was significantly different ($F_{3,104}$ = 4.79, p = 0.004), but only between brown tide and ML. Rejection by the oysters differed in the 4 different water conditions ($F_{3,104}$ = 13.03, p < 0.001), though only among the water bodies and not with brown tide. The organic ingestion rate was lower when oysters were feeding on the brown tide ($F_{3,104}$ = 2.75, p = 0.046). The absorption rates did not differ ($F_{3,104}$ = 2.40, p = 0.071), but absorption efficiency was different ($F_{3,104}$ = 4.12, p = 0.008) between ML and BR but not for the brown tide.

DISCUSSION

We investigated the interaction between bivalve feeding behavior and environmental conditions in a shallow estuarine ecosystem, the IRL. Oysters were more efficient in feeding on the available seston and were able to reject the excess inorganic matter in the water, leading to increased ingestion of organic matter, despite high levels of inorganic particles in the water. In contrast, clams were overloaded by high amounts of inorganic matter with a low ability to reject it; therefore, they were unable to increase the ingested organic matter. Changes in the feeding behavior of the clams were mainly influenced by the salinity of the water. Acute environmental change brought on by a brown tide reduced the ingestion and rejection rates of oysters, but these bivalves were able to maintain the absorption efficiency of the feeding process, even under increased seston concentrations.

The 3 basins of the IRL differed in TPM and POM. Total seston loads were directly related to inorganic matter in all basins, a characteristic of shallow estuaries (Hawkins et al. 1996, Galimany et al. 2011). Therefore, the IRL is used as an example of a shallow estuary, where wind, rainfall, or freshwater releases into the lagoon can easily resuspend the sediment and increase the amounts of total and inorganic matter.

The feeding behavior of bivalves is influenced by food quantity and quality, which is traditionally measured in terms of amount of organic matter or chl *a* in the seston. Bivalves may respond to these seston organic loads by modulating ingestion through regulation of clearance and/or filtration rates and pseudofeces production (Ward & Shumway 2004). In our study, oysters were able to use both strategies. First, oysters decreased their clearance rate with increasing amounts of chl *a*. They then decreased filtration rate and rejection when the organic proportion of the seston increased. These behaviors allowed the oysters to increase the organic portion of the ingested matter, enhancing their absorption efficiencies (Bayne et al. 1993) and increasing their overall feeding performance. In contrast, clams appear to regulate their ingestion rate at high seston concentrations primarily via reduction in clearance rates rather than through pseudofeces production (Bricelj & Malouf 1984). Similarly, clams in the IRL decreased filtration as water organic fraction increased, but no other feeding parameter was affected by water seston loads. Differences in the absorption efficiency between oysters and clams illustrate the reduced ability of clams to incorporate the organic matter from the lagoon water in their digestive system, and may be a reason why clams do not seem to thrive in the IRL. Nevertheless, clam restoration followed by stock protection might be useful in certain areas to enhance the fragile natural clam population, as suggested by Arnold (2001).

The environmental water characteristics recorded in the present study (temperature, dissolved oxygen, and salinity) were mainly within the environmental thresholds described for both oysters and clams. Nevertheless, some of these influenced the feeding behavior of the bivalves. Salinity had the largest effect on bivalve physiology, particularly for clams. Adult oysters have been traditionally described as a euryhaline species, but they can also thrive in oceanic salinities (35 ppt), and can even tolerate levels up to 42 ppt (Arnold & Berrigan 2002, EOBRT 2007). Clams live in saline (>25 ppt) waters and cannot tolerate low salinities or fresh water for an extended period (Whetstone et al. 2005). The salinity values recorded ranged from 21 to 33 ppt, and salinity was positively correlated to absorption efficiency

in oysters and to clearance rate, filtration rate, organic ingestion rate, and absorption rate in clams. In our study, clams were at or below their lower salinity threshold for many of the experimental days. As a result, low salinity is the water characteristic that most affected the feeding behavior of the clams, and likely impaired their feeding performance. Oysters were within their described salinity range, but performed better at higher salinities. However, this idea must be tempered by our knowledge of how geography can affect the physiology of the species.

Bivalves that are geographically separated have been demonstrated to display different physiological adaptations (Shumway 1996). Therefore, potential environmental stressors, such as salinity, can differentially affect oyster populations collected from geographically distant locations. For example, oysters from Port Isabel, Texas, have different physiological thresholds because of their lower salinity tolerances than those from the northeast, with spat surviving in salinities as low as 1.4 ppt (Shumway 1996). The oysters used for these feeding assays were from Sebastian, Florida, where salinity during the previous year averaged 29.19 ± 0.18 ppt, higher than the salinities at our sites on many of the experimental days. Despite being acclimatized to study site water conditions for several days before each experiment, oyster absorption efficiency was higher at salinities closer to their native conditions. These data illustrate the importance of understanding the interaction between bivalve physiology and environmental conditions for both restoration and experimental work.

Harmful algal blooms (HABs) are becoming more prevalent and widespread worldwide (Hallegraeff 1993), acting as acute, and sometimes chronic, disturbances in coastal ecosystems. The IRL first recorded fish kills associated with toxic phytoplankton in the early 1950s (Howell 1953). Since then, a wide diversity of HABs have been described, with some blooms being very intense (Phlips et al. 2011). *Aureoumbra lagunensis* is a marine microalga that can create non-toxic HABs known as brown tides (Stockwell et al. 1993), which can be very persistent; for example, an *A. lagunensis* bloom lasted without interruption in Laguna Madre, Texas, for about 8 yr (Buskey et al. 2001). In 2012, a brown tide (~10^6 cells ml^{-1}) was reported in the IRL, the first time outside Texas (Gobler et al. 2013). Despite the nontoxicity of *A. lagunensis*, the cells are surrounded by a mucous layer of exopolymer secretions which impairs protozoan grazing efficiencies (Liu & Buskey 2000). Similarly, clams and oysters decreased their clearance rates when fed bloom densities of *A. lagunensis* compared

to *Isochrysis galbana*, a nutritious microalga (Gobler et al. 2013). Our study is the first report of oysters and clams feeding *in situ* on a brown tide alga. As previously stated, clams are not efficient when feeding on high seston loads, and the brown tide probably overloaded their gills. Oysters also reduce their clearance rates when feeding on *A. lagunensis* compared to *I. galbana*, a nutritious alga, from 2.19 to ≤ 0.12 l h^{-1} g^{-1}, respectively, probably because pseudofeces production was not enough to compensate for the high seston loads from the brown tide (Gobler et al. 2013). The exopolymer secretions from the microalgal cells might have also affected clearance and filtration rates in bivalves, as the stickiness may inhibit the cilia in transporting particles through the gills to the mouth. Nevertheless, oysters had similar absorption rates and efficiency when feeding from the natural seston and the brown tide, suggesting that they are able to sustain their feeding requirements on the brown tide.

Oysters and clams have different physiological capabilities that drive their ability to survive and grow in shallow estuaries where environmental conditions are highly dynamic. Oysters are able to function across a wider range of sites and seston characteristics due to their ability to adjust their feeding physiology; oyster absorption efficiency was highest at high salinities and water organic proportion. In contrast, clams required more specific conditions (or thrived under a much narrower range of environmental conditions), with the inability to sort out and reject inorganic matter, relegating them to sites where inorganic matter content is low. This study suggests that the ecosystem functions performed by bivalves are highly dependent on local environmental conditions and highlights the importance of understanding bivalve physiology when undertaking restoration efforts and management plans.

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