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Feeding competition between the native oyster *Crassostrea virginica* and the invasive mussel *Mytella charruana*

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ABSTRACT: The sub-tropical mussel *Mytella charruana* has been reported as invasive along the southeast coast of the USA since 1986. This mussel has been found to negatively impact the keystone species in its invaded range, the eastern oyster *Crassostrea virginica*. To date, however, no mechanism for this negative impact has been determined. To elucidate the role of the invasive mussel on economically important oyster reefs, we compared the feeding of both species in a lagoon along the east coast of Florida (USA). Three different methodologies were used: (1) *in situ* filter-feeding experiments using the biodeposition method to estimate feeding behavior; (2) laboratory assays to estimate the depletion of bacterial particles using a flow cytometer; and (3) stable isotope analysis in conjunction with ellipse-based metrics to investigate the niche size and overlap of these 2 species. The *in situ* filter-feeding experiments revealed that, compared to *C. virginica*, *M. charruana* had significantly higher clearance, filtration, rejection, organic ingestion, and absorption rates, as well as higher rejection percentage and absorption efficiency, but rejected the same amount of inorganic particles. Flow cytometry data suggested that bacteria were a food source for both bivalve species. Stable isotope values confirmed that *M. charruana* and *C. virginica* filled similar functional niches in this ecosystem. These results suggest that *M. charruana* can out-compete native oysters; the findings also demonstrate that an invasion of *M. charruana* might significantly alter plankton abundance, potentially limiting food sources available to other less efficient native filter-feeders such as clams.

KEY WORDS: Bivalve · Oyster reef · Feeding behavior · Stable isotope · Indian River Lagoon · Florida

INTRODUCTION

Coastal systems are some of the most highly invaded ecosystems on earth, often as a result of human activities (Grosholz 2002). Non-native and invasive species may have a negative effect on local biodiversity, by out-competing local species and thus eradicating native fauna (Ricciardi & Rasmussen 1998, Whyte et al. 2008, Thomsen et al. 2014). Non-native, invasive bivalves may significantly alter ecosystem structure and function and, as a consequence, such bivalves often have very large economic impacts (Sousa et al. 2009). Within bivalves, mussels are notorious invasive species in both fresh and marine ecosystems (Ricciardi & Rasmussen 1998, Yuan et al. 2016a). Zebra mussels *Dreissena polymorpha* were introduced to the Great Lakes region in the late 1980s and have drastically altered benthic communities and water quality, pushing native mussels to the brink of extinction (Fahnenstiel et al. 1995, Ricciardi & Rasmussen 1998). Zebra mussels, along with quagga mussels *D. bugensis*, introduced from the Ukraine (Mills et al. 1993), have changed the nutrient and carbon distribution and cycling patterns in the invaded ecosystems (Ozersky et al. 2015).

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Along the Atlantic and Gulf of Mexico coasts of the US, reefs of the eastern oyster *Crassostrea virginica* are a crucial part of the coastal landscape. Oysters, whether subtidal or intertidal, are a foundation species, providing essential habitats for recreationally and commercially important organisms, as well as providing a fishery themselves (Grabowski et al. 2005, Grabowski & Peterson 2007). Although oyster reefs are ecologically and economically important, they are declining because of destructive fishing practices, increased diseases, and human activities, which negatively affect coastal watersheds (Beck et al. 2009). As a result of this decline, restoration efforts are widespread for both fisheries enhancement and ecosystem functioning (Coen & Luckenbach 2000, Luckenbach et al. 2005, Coen et al. 2007). However, as range expansions and species introductions continue, oyster reefs may also be threatened by invasive filter-feeders that could out-compete native oysters.

The invasive charru mussel *Mytella charruana* has been found on intertidal oyster reefs along the east coast of Florida (Boudreaux et al. 2006, Spinuzzi et al. 2013). The charru mussel is a non-native species thought to be introduced through ballast water releases in Jacksonville, Florida (Lee 1987). *M. charruana* is native to Central and South America, with a distribution on the Pacific coast from Mexico to Ecuador, including the Galapagos Islands (Keen 1971, Carlton 1992, Szefer et al. 1998, Boehs et al. 2004), and on the south Atlantic coast from Argentina to Uruguay (Lee 1987). Within its invasive range, *M. charruana* has been found along the southeastern coast of the USA from Titusville, Florida, to Charleston, South Carolina (Spinuzzi et al. 2013). Densities of this non-native species in the USA have been measured at their highest to be approximately 12 mussels m^{-2} in Jacksonville, Florida (Spinuzzi et al. 2013), which is much lower than the densities found in both native (11 036 mussels m^{-2}, Brazil) and invasive (13 400 mussels m^{-2}, Colombia) habitats (Pereira et al. 2003, Puyana et al. 2012).

With the potential for *M. charruana* to compete with resident filter-feeding organisms, it is important to assess the invasive potential or competitive ability of *M. charruana* against other species with a similar role in the ecosystem, i.e. native bivalves. Potential food sources for bivalves include bacteria, phytoplankton, zooplankton, and dissolved organic matter (Hartland & Timoney 1979, Lehane & Davenport 2002, Gosling 2003). Nevertheless, the retention efficiency is species-specific and dependent on different particle characteristics. For example, *C. virginica* and blue mussels *Mytilus edulis* can retain particles > 6 µm with 100% efficiency, whereas the efficiency for particles < 2 µm decreases to 50% (Jørgensen 1974, Møhlenberg & Riisgård 1978, Riisgård 1988). However, the bay scallop *Argopecten irradians* has an even lower retention efficiency of 2 µm particles, retaining only 15% (Riisgård 1988). Small particles, such as bacteria, are extremely abundant in aquatic ecosystems but they are not readily available to bivalves, as seen by their generally low retention efficiency. However, Kach & Ward (2008) demonstrated that suspension feeders can ingest picoplankton-size (0.2–2.0 µm) particles when in aggregates, suggesting that small particles can be an important food source as they become available to bivalves as larger particle masses. Despite the wide range of particle sizes that bivalves are capable of ingesting, they do not ingest everything that is retained by the gills. Pre-ingestive selection may occur on the gills and labial palps, which results in the release of rejected particles as pseudofeces (Kiørboe & Møhlenberg 1981, Shumway et al. 1985, Ward et al. 1998). Pre-ingestive selection is dependent upon the characteristics of the available food particles, and production of pseudofeces occurs when the ingestive capacity of a bivalve is overloaded or the particles are unsuitable, such as silt and other inorganic matter (Beninger & St-Jean 1997).

Invasion biology is founded on understanding what happens to a system when an invasion occurs (Carlton 2001). Yuan et al. (2016a) found that survival and growth of *C. virginica* was negatively affected by *M. charruana* when the 2 species were grown in contact in 6 wk long field studies in Mosquito Lagoon, while native mussels *Geukensia demissa* had no effect on the oysters, suggesting that feeding competition was the mechanism underlying this result. Our study directly tested this idea by investigating the feeding behavior and removal of bacteria by each of these species. In addition, because stable isotope analysis provides a time-integrated assessment of an organism's diet, we used the stable isotope ratios of C and N ($\delta^{13}C$ and $\delta^{15}N$) to determine whether these 2 species have overlapping diets (Fry 2006, Jackson et al. 2012) and fill the same functional 'niche' (Newsome et al. 2007, Layman et al. 2012) in this system. We tested the null hypothesis that both bivalves are equally effective at feeding and have distinct diets in the waters of the Mosquito Lagoon. The alternative hypothesis was that the invasive *M. charruana* outcompetes *C. virginica* by more efficient filter-feeding and an overlapping diet. These types of ecophysiological studies can help provide a mechanistic understanding of invasive potential and potential impacts arising from introduced species.

MATERIALS AND METHODS

Study site and bivalve collection

Experiments were conducted in May 2015 in the waters of the Mosquito Lagoon, a sub-lagoon of the Indian River Lagoon, within Canaveral National Seashore, New Smyrna, Florida. Research was carried out at the Fellers House Field Station operated by the University of Central Florida (28° 54′ 23.96″ N, 80° 49′ 11.54″ W). All organisms were collected by hand from an intertidal oyster reef within 0.5 km of the station. Oysters *Crassostrea virginica* and invasive mussels *Mytella charruana*, 50 of each, were collected the day before the beginning of the experiments, cleaned of epiphytes and other encrusting organisms, and suspended from the dock of the field station in plastic mesh bags. Animals were maintained in bags for the duration of the experiments. Physical water characteristics (temperature, salinity, dissolved oxygen, and chlorophyll *a* [chl *a*]) were recorded daily.

Filter-feeding experiments

To assess the feeding behavior of both bivalve species, we conducted *in situ* filter-feeding experiments from the same dock where animals were kept using 2 portable, flow-through devices (Fig. 1), which included a PVC 'reservoir' tank (20 l capacity) that received lagoon water from an underwater bilge pump suspended at 1 m depth. This reservoir tank was aerated to maintain suspension of particles in the water. Water from the reservoir tank flowed through tubes into individual chambers that each held a single bivalve. With 10 individual chambers in each device, this design allowed for 18 replicate bivalves and 2 controls (empty shell) to be sampled simultaneously. A thorough description of design and operation is available in Galimany et al. (2011). Experiments were conducted over 3 consecutive days.

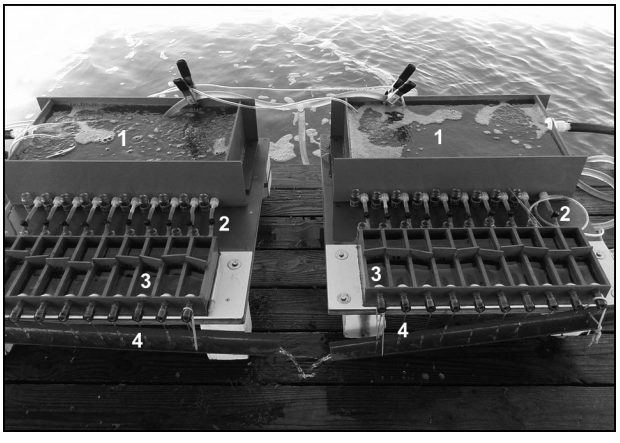

Fig. 1. Portable, flow-through devices used for the filter-feeding experiments. 1: PVC reservoir tank; 2: plastic tubes with valves to regulate flow connecting the reservoir tank with each individual chamber; 3: individual chambers that each hold a single bivalve; 4: overflow of the chamber

Each day, 9 adults of each species (mean ± SE shell length 50.43 ± 1.15 and 33.43 ± 0.86 mm, and mean ± SE dry weight 0.68 ± 0.05 and 0.24 ± 0.02 g for oysters and mussels, respectively) were chosen haphazardly from the previously collected bivalves and placed within the 2 flow-through devices. Each device held only 1 species to simplify the collection of biodeposits. To affix mussels within each chamber, a small plastic velcro strip was glued to each mussel. Oysters were not affixed, as they do not move once placed within the chamber. Each bivalve was exposed to a constant flow rate of 12 l h^{-1} of ambient water. Animals were allowed to acclimate within their flow-through chambers for 2 h to recover from any stress associated with handling. Shells without live *C. virginica* and *M. charruana* were added into the final chambers to act as controls (Galimany et al. 2011).

Water (110 ml) was collected from the overflow of each control chamber to characterize the ambient particulate matter. Samples were taken every 15 min for 2 h. Prior to the start of the experiment, individual chambers were cleaned to remove any fecal material. Feces and pseudofeces from each live bivalve were then collected separately throughout the experiment with a pipette for 2 h. Water samples and, at the end of the experiment, feces and pseudofeces from each chamber were filtered through washed, pre-combusted (450°C at 4 h), pre-weighed Whatman GF/C filters (25 mm) and rinsed with isotonic ammonium formate to remove residual salt on the filter. Filters were kept on ice and transferred to the Smithsonian Marine Station where they were dried at 60°C for 48 h and weighed. For the filters used for water, this weight represents the total particulate matter (TPM). Filters were then ashed at 450°C for 4 h and weighed again to obtain the particulate inorganic matter (PIM). The particulate organic matter (POM) was calculated as the difference between TPM and PIM. The organic content of the water (f) was calculated as the mean organic fraction of total particulates (f = POM/TPM). The different seston values obtained in each experiment were compared using a 1-way ANOVA.

Three water samples were filtered through Whatman GF/C filters (25 mm ∅) until clogged to collect biomass for 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}.

Filters with feces and pseudofeces were used to estimate feeding variables (clearance, filtration, rejection, organic ingestion, and absorption rates; proportions of rejection of particulate matter and PIM; and absorption efficiency. See Table 1 for units, detailed descriptions, and methods of calculation). Data from animals that produced no feces or pseudofeces (i.e. did not open) during the measurement period were not included in subsequent analyses. The feeding parameters of both species were calculated according to the biodeposition method (Iglesias et al. 1998). This method is based on using the inorganic matter as a tracer for the feeding processes.

To synchronize the seston available with the corresponding biodeposits produced by the bivalves, it was necessary to estimate the gut transit time, which is defined as the minimum time for an organic particle to pass through the digestive tract of a bivalve after ingestion. This variable was calculated before each measurement period by providing replicate individuals of each bivalve species with a mixture of local ambient water and cultured *Tetraselmis* sp. (Oahu, AlgaGen; adapted from Hawkins et al. 1996). The time in minutes that elapsed between the addition of cultured *Tetraselmis* sp. and the first deposition of green-colored feces by one of each of the bivalves was considered to be the gut transit time. At the end of each experiment, animals were frozen and transported to the Smithsonian Marine Station, where shell length and dry tissue weight (48 h at 60°C) were measured.

All feeding variables were standardized (Y_s) to 1 g of dried bivalve flesh using the following equation:

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

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, we used 0.73 for *C. virginica* (Riisgård 1988) and 0.67 for *M. charruana,* as is commonly used in mussel feeding studies with the mussel *Mytilus edulis* (Bayne et al. 1989, Hawkins et al. 1997).

The different feeding variables estimated were compared using a blocked ANOVA with day as the block and species as a fixed factor. Only bivalves that were open and actively feeding were used for the statistical analyses, which included 17 *C. virginica* and 23 *M. charruana*.

Bacterial assay

To quantify the removal of bacterial populations and examine feeding by the 2 bivalves, we conducted a laboratory *in situ* bacterial depletion exper-

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

Parameter	Acronym	Units	Description	Calculation
Clearance rate	CR	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 [mg h^{-1}]) / PIM
Filtration rate	FR	mg h^{-1}	TPM from the seawater retained in the gills per unit of time	CR × TPM
Rejection rate	RR	mg h^{-1}	TPM that has been retained in the gills but rejected prior to ingestion	mg inorganic and organic matter from pseudofeces per unit of time (mg h^{-1})
Rejection proportion	RP	%	Proportion of particulate matter that has been retained in the gills but rejected prior to ingestion	(RR / FR) × 100
Inorganic rejection proportion	IRP	%	Proportion of PIM that has been retained in the gills but rejected prior to ingestion	(mg inorganic matter from pseudofeces per unit of time [mg h^{-1}]) / (mg inorganic and organic matter from pseudofeces per unit of time [mg h^{-1}]) × 100
Organic ingestion rate	OIR	mg h^{-1}	POM retained in the gills and ingested by the bivalve per unit of time	(CR × POM) – (mg organic matter from pseudofeces [mg h^{-1}])
Absorption rate	AR	mg h^{-1}	POM ingested by the bivalve and not egested as feces per unit of time	OIR – (mg organic matter from feces [mg h^{-1}])
Absorption efficiency	AE	Fraction	Efficiency of the feeding process	AR / OIR

iment on each of the 3 experimental days. Lagoon water was collected from the dock next to the laboratory and used to fill 1 l plastic beakers (n = 11), which were continuously aerated throughout each experiment. Five oysters and 5 mussels were haphazardly selected from the mesh bag containing previously collected bivalves and placed individually in the 1 l beakers. One extra beaker was left as a control to detect changes in the natural bacterial population. Samples 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. At the end of the experiment, bivalves were dissected and dried at 60°C for 48 h to determine individual dry tissue weight.

To calculate the ambient planktonic bacterial abundance, water samples were processed according to Gasol et al. (1999); samples were stained with 2.5 µM Syto 13, a green fluorescing nucleic acid stain (10:1 stock dilution; Molecular Probes) and left for 10 min in the dark. Then 10 µl of Fluoresbrite yellow-green 1 µm microspheres (Polysciences) were added to each sample as a size standard. Each sample was run on a C6 flow cytometer (BD Biosciences) for 2 min on the medium flow rate setting (35 µl min^{-1}). Bacteria in the samples were identified and quantified using the plots contrasting side scatter vs. green fluorescence produced by the flow cytometer.

Bacterial concentrations from both species and the control were compared with a blocked ANOVA with day as the block and species as a fixed factor. Moreover, an ANCOVA was run with bacterial depletion using species as a fixed factor and bivalve dry weight as a covariate. All bivalves were open and actively feeding and were therefore used in the statistical analyses (N = 15 for each species). The 3 controls, 1 from each experimental day, were also added to the analyses for comparison purposes.

Stable isotopes

A subset of the *C. virginica* and *M. charruana* individuals collected from the oyster reef were held overnight in the laboratory in filtered lagoon water to ensure that their guts were empty prior to stable isotope analysis. Replicate water samples from the oyster reef were pre-filtered through a 105 µm mesh to remove larger particles and then filtered through a pre-combusted (450°C for 4 h) 47 mm quartz fiber filter (~2 µm retention) to collect POM, a potential source of C and N for filter-feeding organisms. Filters and bivalves were frozen until further analysis. At the Smithsonian Marine Station, shell length and dry weight (following 24 h at 60°C) of all bivalves were measured and, in preparation for stable isotope analysis, dried whole bivalve tissue was ground to a fine powder using a mortar and pestle. Bivalve tissue for 14 and 17 *C. virginica* and *M. charruana*, respectively, and 4 filters containing POM, were acidified by exposure to 12N HCl fumes in a closed environment for 12 h. After drying at 60°C to remove residual acid, samples were weighed to the nearest 0.001 mg into tared tin capsules. Samples were analyzed at the OUSS/MCI Stable Isotope Mass Spectrometry Laboratory at the Smithsonian Museum Conservation Institute in Suitland, Maryland. Analysis was carried out on a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to a Costech 4010 Elemental Analyzer (EA) via a Thermo Conflo IV. Isotope values are reported in δ notation in units of per mil (‰) following equations outlined in Fry (2006). Individual samples were run in duplicate and then averaged prior to analysis. Precision across samples was ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Because the location of an organism within bivariate ($\delta^{13}C$ and $\delta^{15}N$) isotopic space is based on the sources of C and N it assimilates, internal processing of these nutrients, and the surrounding environment, the placement of an organism in isotopic space may be presented as the functional 'niche' that it fills in a system (Newsome et al. 2007, Layman et al. 2012). To visualize and quantify the isotopic niche area for each bivalve species, we calculated the standard ellipse area (SEA_c) using Bayesian inference based on the ellipse-based metrics within the Stable Isotope Bayesian Ellipses in R (SIBER) program (Jackson et al. 2011). These analyses allowed us to quantitatively compare the niche size of each species and estimate the percent overlap of these niches within isotopic space.

RESULTS

Water and seston characteristics

Average values for Mosquito Lagoon water characteristics (temperature, salinity, oxygen, chl *a*) are shown in Table 2. All characteristics are within published ranges for both species studied. TPM ($F_{2,39}$ = 3.42; p = 0.043) and PIM ($F_{2,39}$ = 3.45; p = 0.042) differed between days. POM and *f* showed no significant differences between days ($F_{2,39}$ = 2.22; p = 0.122, and $F_{2,39}$ = 1.26; p = 0.296, respectively).

Table 2. Mean values for Mosquito Lagoon (Florida, USA) water characteristics (± SE) recorded during the experimental days. TPM: total particulate matter, POM (PIM): particulate organic (inorganic) matter, *f*: organic matter in the water ([TPM/POM] × 100)

Parameter	Value
Temperature (°C)	27.80 ± 0.57
Salinity (ppt)	36.27 ± 0.20
Oxygen (mg l^{-1})	4.58 ± 0.69
Chl *a* (µg l^{-1})	7.39 ± 0.85
TPM (mg l^{-1})	17.80 ± 0.90
POM (mg l^{-1})	4.93 ± 0.27
PIM (mg l^{-1})	12.87 ± 0.70
f (%)	28.01 ± 0.90

Filter-feeding experiments

Mytella charruana had higher values for all feeding parameters measured than *Crassostrea virginica* (Fig. 2, and see Table S1 in the Supplement at www.int-res.com/articles/suppl/m564p057_supp.pdf). The clearance rate (Fig. 2a; $F_{1,36}$ = 14.73, p < 0.001), filtration rate (Fig. 2b; $F_{1,36}$ = 15.20, p < 0.001), and rejection rate (Fig. 2c; $F_{1,36}$ = 21.10, p < 0.001) were all twice as high in *M. charruana* compared to *C. virginica*. Mean rejection percentage was approximately 20% higher in the invasive species (Fig. 2d; $F_{1,36}$ = 17.96, p < 0.001). Nevertheless, the amount of inorganic matter rejected through pseudofeces was exactly the same for both species of bivalves (Fig. 2e; $F_{1,36}$ = 0.96, p = 0.335). Both organic ingestion rate and absorption rate for *M. charruana* were significantly higher than *C. virginica* (Fig. 2f,g; $F_{1,36}$ = 8.94, p = 0.005; $F_{1,36}$ = 9.26, p = 0.004, respectively). Absorption efficiency was higher for *M. charruana*, with an efficiency of 0.65 and 0.76 for the native bivalves (*C. virginica*) and the invasive species (*M. charruana*), respectively (Fig. 2h; $F_{1,36}$ = 6.92, p = 0.012). Physiological rates were, in general, not affected by date (p > 0.05), although rejection rate was different among experimental dates ($F_{2,36}$ = 3.38, p = 0.045). The effect of date on rejection rate may be attributable to differences in water conditions.

Bacterial assay

Both bivalve species depleted bacteria compared to controls ($F_{2,28}$ = 7.68, p = 0.002), (Fig. 3). After taking into account differences in body size, both bivalves removed the same amount of bacteria (ANCOVA, $F_{1,27}$ = 0.72, p = 0.405). After 1 h, the number of bacteria remaining in the experimental beakers ranged from 1.04 × 10^5 to 4.01 × 10^5 bacteria ml^{-1}.

Stable isotopes

The mean $\delta^{13}C$ and $\delta^{15}N$ values of *C. virginica* and *M. charruana* were −22.31‰ and 4.60‰, and −21.84‰ and 4.65‰, respectively. Both bivalve species had relatively narrow SEA_c of similar size (0.477 and 0.389 for *C. virginica* and *M. charruana*, respectively; SIBER analysis: p > 0.05; Fig. 4), and the SEA_c of these 2 species overlapped by approximately 20%. Mean (±) $\delta^{13}C$ and $\delta^{15}N$ values of POM were −22.83 ‰ (0.33) and 4.09 ‰ (0.23), respectively.

DISCUSSION

The results of this study suggest that the invasive mussel *Mytella charruana* shares the ecological niche of the native, eastern oyster *Crassostrea virginica* and thus competes for the same resources. When non-native species colonize a new habitat, the effects of their feeding activity can be very negative for the ecosystem. For example, the ability of the Asian clam *Corbicula fluminea* to couple benthic and pelagic environments with terrestrial ecosystems has been demonstrated to have a strong potential to alter food web flows in aquatic ecosystems (Dias et al. 2014). In Mosquito Lagoon, the invasive mussel *M. charruana* significantly reduced the survival and growth of the native eastern oyster *C. virginica*, possibly as the result of competition for food (Yuan et al. 2016a). The results of our present study confirm this idea.

Bivalves are filter-feeding organisms that force water into their bodies, where gills allow the filtration of particles from the water (Gosling 2003). The feeding behavior of bivalves depends on water characteristics such as temperature, salinity, and dissolved oxygen. With the values recorded in our study falling within the range tolerance of both species, we do not expect their feeding behavior to be limited by these water characteristics (Kennedy et al. 1996, Yuan et al. 2016b). The flow of water (clearance rate) and the particulate matter retained in the gills (filtration rate) are feeding parameters that depend on the water characteristics (Hawkins et al. 1996). For example, *in situ* measurements of the feeding behavior of *C. virginica* in a New Hampshire (USA) estuary found clearance rates of 3.51 l h^{-1}, but increases in

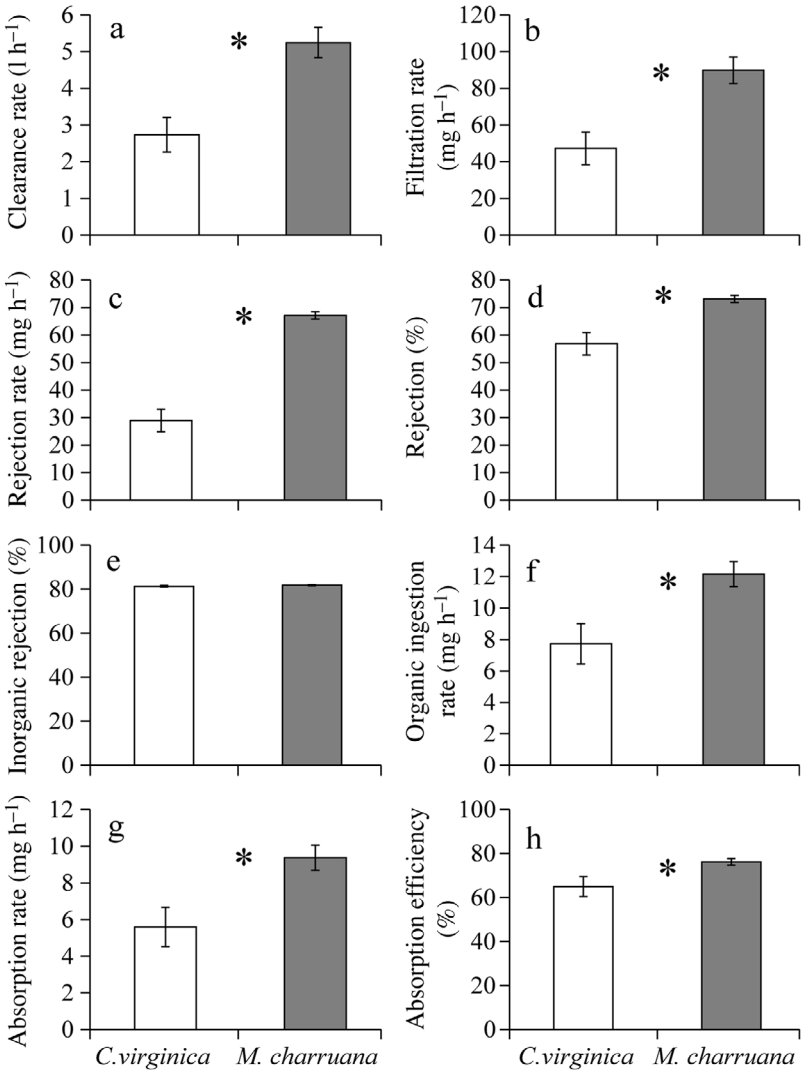

Fig. 2. Mean (±SE) feeding variables measured for *Crassostrea virginica* and *Mytella charruana* during the *in situ* experiment: (a) clearance rate, (b) filtration rate, (c) rejection rate, (d) rejection proportion, (e) inorganic rejection proportion, (f) organic ingestion rate, (g) absorption rate, (h) absorption efficiency. Asterisks denote significant differences between species (ANOVA: $p < 0.05$)

inorganic material increased clearance rates to 7.31 l h^{-1} even though rejection rate remained constant (Hoellein et al. 2015). Mosquito Lagoon is a shallow embayment with high loads of inorganic matter, probably caused by silt resuspension where mixing events would tend to diminish the organic content of particles in the water column (Hawkins et al. 1996, Galimany et al. 2011). Despite organic matter only comprising 27% of the total particulates in the water, the clearance and filtration rates estimated for *C. virginica* were about 50% lower than those reported by Hoellein et al. (2015). The lower feeding response found in our study for *C. virginica* is likely related to pre-ingestive selection, as rejection was higher in our study site (up to 60%). Pre-ingestive selection allows bivalves to handle excess inorganic matter in the water column by rejecting it as pseudofeces, thus increasing the organic fraction of the ingested matter (Hawkins et al. 1996). The high rejection rate estimated in this study maximized energy gain since the animals at these sites were feeding upon natural suspensions with low organic content (Iglesias et al. 1992, Bayne et al. 1993).

Invasive mussels, even taxonomically unrelated species, have similar ecosystem impacts because of the ecological niche they share (Karatayev et al. 2007). The presence of invasive species with such efficient feeding may influence trophic interactions and food availability for both pelagic and benthic species (Karatayev et al. 1997, Boltovskoy et al. 2006) and impact nutrient mineralization, oxygen availability, and sedimentation rates (Karatayev et al. 1997, Boltovskoy et al. 2006). In addition, competition with native species may be exacerbated by the ability of invasive mussels to withstand stressful conditions (Lorenz & Pusch 2013). This is the first study describing the feeding behavior of *M. charruana* and, overall, *M. charruana* absorbed more organic matter and was more efficient in the feeding process than oysters. Therefore, we suggest that the invasive mussel *M. charruana* is out-competing the native oyster *C. virginica* and likely other native filter-feeding species as well, such as the clam *Mercenaria mercenaria*. The effects of *M. charruana* on the ecosystem might be as severe as those reported for other invasive mussels (e.g. the zebra mussel *Dreissena polymorpha*).

Both studied bivalves cleared bacteria from the water, using this food item to supplement the low organic matter available for their diets. Although bacterial depletion has been shown in bivalves previously, not all species are equally efficient at clearing bacteria from the water. Wright et al. (1982) observed that the ribbed mussel *Geukensia demissa* cleared bacteria more effectively than the blue mussel *Mytilus edulis* and soft-shell clams *Mya arenaria*. Nevertheless, bacteria can be an important component of the seston in 2 different forms, either as free-

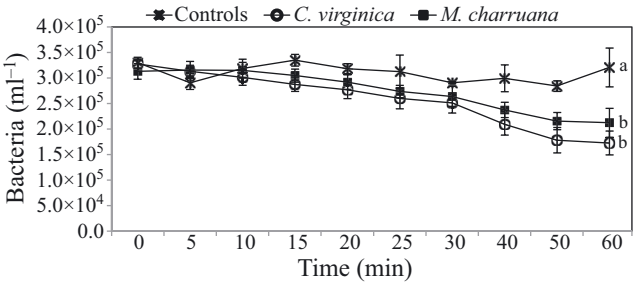

Fig. 3. Bacterial concentration in the water (±SE) for *Crassostrea virginica* and *Mytella charruana* and controls. Letters denote significant differences (ANOVA: $p < 0.05$; i.e. controls are different than the 2 bivalve species). ANCOVA results using dry weight as a covariate also showed no significant differences between species ($p > 0.05$)

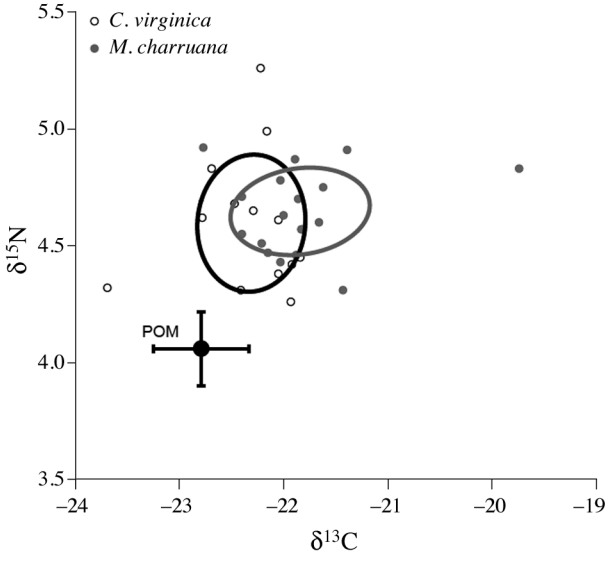

Fig. 4. Bivariate ($\delta^{13}C$ and $\delta^{15}N$) plot depicting the placement of *Crassostrea virginica* (N = 14) and *Mytella charruana* (N = 17) individuals within the isotopic niche space of the studied oyster bed. Standard ellipse areas depicted by solid lines provide estimates of the niche area of each species. Mean $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter (POM) are shown for reference

living organisms or in association with phytoplankton cells and/or organic matter (Bell & Mitchell 1972). In estuaries along the east coast of the USA, free bacterial abundance ranged from 10^5 to 10^9 cells ml^{-1} (Findlay et al. 1991, Ducklow et al. 1999). Our results indicate that Mosquito Lagoon had a low abundance of free bacteria, although the total bacterial population might be higher than measured if the bacteria were associated with organic components of the seston. The ability of bivalves to filter the water allows the bacteria to act as a food source in association with organic matter by creating larger aggregates. In fact, such small organisms have become a health concern, as bivalves can ingest human-harmful bacteria (Su & Liu 2007) leading to the development of shellfish monitoring programs worldwide (Shumway 2001).

Stable isotope analysis revealed that the invasive mussel *M. charruana* shared isotopic niche space with the native oyster *C. virginica*, supporting the contention that these sympatric species have dietary overlap that could contribute to interspecific competition (Dubois & Colombo 2014). Because consumers are generally enriched in $\delta^{15}N$ and $\delta^{13}C$ relative to their diet (Fry 2006), enriched (~0.5 to 0.9 ‰) $\delta^{15}N$ and $\delta^{13}C$ values of both species relative to POM implies that these bivalve species were assimilating C and N from bulk particulate sources in the water column. Minimal isotopic variation across most individuals, and the resulting narrow niche widths of both species, however, are indicative of specialized feeding on a specific fraction of the overall POM pool. Classic theory and recent empirical evidence suggest an inverse relationship between the level of interspecific competition exacted on a species and its realized niche size (estimated by SEA_c or other quantitative metrics as in Layman et al. 2007) and placement within isotopic space (Jackson et al. 2012, 2014, Dubois & Colombo 2014, Karlson et al. 2015). For instance, Jackson et al. (2012) found a strong reduction in the isotopic niche size of an invasive species following the establishment of another invasive species belonging to the same functional group. While the small SEA_c values in this study may reflect competition of these bivalves for local resources, additional studies investigating the dynamics between *C. virginica* SEA_c size and *M. charruana* abundance are needed to fully elucidate this (Jackson et al. 2012).

In conclusion, our study suggests that the invasive mussel *M. charruana* may have a competitive advantage among other filter-feeders due to its efficiency at removing and sorting available nutrients from the water column. Instead of filling a vacant niche in this system through the acquisition of unexploited resources, *M. charruana* competes directly with sympatric resident bivalves including the oyster *C. virginica*. Interestingly, with overlapping niches, these 2 species may fill similar roles in these local ecosystems, raising important questions about how ecosystem processes and overall ecosystem function is affected by *M. charruana* invasion on these reefs. We demonstrate that *M. charruana* poses a threat to native oysters in central Florida and provide evidence that this species may impact ecosystem services and nutrient cycling within southeast USA and in other locations within its invasive range.

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LITERATURE CITED

Bayne BL, Hawkins AJS, Navarro E, Iglesias JIP (1989) Effects of seston concentration on feeding, digestion and growth in the mussel *Mytilus edulis.* Mar Ecol Prog Ser 55:47–54

Bayne BL, Iglesias JIP, Hawkins AJS, Navarro E, Heral M, Deslous-Paoli JM (1993) Feeding behavior of the mussel, *Mytilus edulis*: responses to variations in quantity and organic content of the seston. J Mar Biol Assoc UK 73: 813–829

Beck MW, Brumbaugh RD, Airoldi L, Carranza A and others (2009) Shellfish reefs at risk: a global analysis of problems and solutions. The nature conservancy, Arlington, VA

Bell W, Mitchell R (1972) Chemotactic and growth responses of marine bacteria to algal extracellular products. Biol Bull 143:265–277

Beninger PG, St-Jean SD (1997) Particle processing on the labial palps of *Mytilus edulis* and *Placopecten magellanicus* (Mollusca: Bivalvia). Mar Ecol Prog Ser 147:117–127

Boehs G, Absher T, Da Cruz-Kaled A (2004) Composition and distribution of benthic mollusks on intertidal flats of Paranagua Bay (Parana, Brazil). Sci Mar 68:537–543

Boltovskoy D, Correa N, Cataldo D, Sylvester F (2006) Dispersion and ecological impact of the invasive freshwater bivalve *Limnoperna fortunei* in the Río de la Plata watershed and beyond. Biol Invasions 8:947–963

Boudreaux ML, Stiner JL, Walters LJ (2006) Biodiversity of sessile and motile macrofauna on intertidal oyster reefs in Mosquito Lagoon, Florida. J Shellfish Res 25: 1079–1089

Carlton JT (1992) Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. J Shellfish Res 11:489–505

Carlton J (2001) Introduced species in US coastal waters: environmental impacts and management priorities. Pew Oceans Commission, Arlington, VA

Coen LD, Luckenbach MW (2000) Developing success criteria and goals for evaluating oyster reef restoration: ecological function or resource exploitation? Ecol Eng 15: 323–343

Coen LD, Brumbaugh RD, Bushek D, Grizzle R and others (2007) Ecosystem services related to oyster restoration. Mar Ecol Prog Ser 341:303–307

Dias E, Morais P, Antunes C, Hoffman JC (2014) Linking terrestrial and benthic estuarine ecosystems: organic matter sources supporting the high secondary production of a non-indigenous bivalve. Biol Invasions 16:2163–2179

Dubois SA, Colombo F (2014) How picky can you be? Temporal variations in trophic niches of co-occurring suspension-feeding species. Food Webs 1:1–9

Ducklow H, Schultz G, Raymond P, Bauer J, Shiah F (1999) Bacterial dynamics in large and small estuaries. In: Bell CR, Brylinsky M, Johnson-Green P (eds) Microbial biosystems: new frontiers. Proc 8th Int Symp Microb Ecol, Halifax, Canada, August 9–14, 1998. Atlantic Canada Society for Microbial Ecology, Halifax, NS, p 105–112

Fahnenstiel GL, Lang GA, Nalepa TF, Johengen TH (1995) Effects of zebra mussel (*Dreissena polymorpha*) colonization on water quality parameters in Saginaw Bay, Lake Huron. J Great Lakes Res 21:435–448

Findlay S, Pace ML, Lints D, Cole JJ, Caraco NF, Peierls B (1991) Weak coupling of bacteria and algal production in a heterotrophic ecosystem: the Hudson River estuary. Limnol Oceanogr 36:268–278

Fry B (2006) Stable isotope ecology. Springer, New York, NY

Galimany E, Ramón M, Ibarrola I (2011) Feeding behavior of the mussel *Mytilus galloprovincialis* (L.) in a Mediterranean estuary: a field study. Aquaculture 314:236–243

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström Å (1999) Significance of size and nucleid acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65: 4475–4483

Gosling E (2003) Bivalve molluscs: biology, ecology, and culture. Blackwell Publishing, Oxford

Grabowski JH, Peterson CH (2007) Restoring oyster reefs to recover ecosystem services. In: Cuddington K, Byers JE, Wilson WG, Hastings A (eds) Ecosystem engineers: plants to protists. Academic Press, Burlington, MA, p 281–298

Grabowski JH, Hughes AR, Kimbro DL, Dolan MA (2005) How habitat setting influences restored oyster reef communities. Ecology 86:1926–1935

Grosholz ED (2002) Ecological consequences of coastal invasions. Trends Ecol Evol 17:22–27

Hartland BJ, Timoney JF (1979) In vivo clearance of enteric bacteria from the hemolymph of the hard clam and the American oyster. Appl Environ Microbiol 37: 517–520

Hawkins AJS, Smith RFM, Bayne BL, Héral M (1996) Novel observations underlying the fast growth of suspension-feeding shellfish in turbid environments: *Mytilus edulis.* Mar Ecol Prog Ser 131:179–190

Hawkins AJS, Smith RFM, Bougrier S, Bayne BL, Héral M (1997) Manipulation of dietary conditions for maximal growth in mussels, *Mytilus edulis* L., from the Marennes-Oléron, France. Aquat Living Resour 10:13–22

Hoellein TJ, Zarnoch CB, Grizzle RE (2015) Eastern oyster (*Crassostrea virginica*) filtration, biodeposition, and sediment nitrogen cycling at two oyster reefs with contrasting water quality in Great Bay Estuary (New Hampshire, USA). Biogeochemistry 122:113–129

Iglesias JIP, Navarro E, Alvarez Jorna P, Armentia I (1992) Feeding, particle selection and absorption in cockles *Cerastoderma edule* (L.) exposed to variable conditions of food concentration and quality. J Exp Mar Biol Ecol 162:177–198

Iglesias JIP, Urrutia MB, Navarro E, Ibarrola I (1998) Measuring feeding and absorption in suspension-feeding bivalves: an appraisal of the biodeposition method. J Exp Mar Biol Ecol 219:71–86

Jackson AL, Inger R, Parnell A, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER — Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80:595–602

Jackson MC, Donohue I, Jackson AL, Britton JR, Harper DM, Grey J (2012) Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. PLOS ONE 7:e31757

Jackson MC, Jones T, Milligan M, Sheath D and others (2014) Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. Freshw Biol 59:1123–1135

Jørgensen CB (1974) On gill function in the mussel *Mytilus edulis* L. Ophelia 13:187–232

Kach DJ, Ward JE (2008) The role of marine aggregates in the ingestion of picoplankton-size particles by suspension-feeding molluscs. Mar Biol 153:797–805

Karatayev AY, Burlakova LE, Padilla DK (1997) The effects of *Dreissena polymorpha* (Pallas) invasion on aquatic communities in Eastern Europe. J Shellfish Res 16: 187–203

Karatayev AY, Boltovskoy D, Padilla DK, Burlakova LE (2007) The invasive bivalves *Dreissena polymorpha* and *Limnoperna fortunei*: parallels, contrasts, potential spread and invasion impacts. J Shellfish Res 26:205–213

Karlson AML, Gorokhova E, Elmgren R (2015) Do deposit-feeders compete? Isotopic niche analysis of an invasion in a species-poor system. Sci Rep 5:9715

Keen AM (1971) Sea shells of tropical West America: marine mollusks from Baja California to Peru. Stanford University Press, Stanford, CA

Kennedy VS, Newell RIE, Eble AF (eds) (1996) The eastern oyster: *Crassostrea virginica*. Maryland Sea Grant College, College Park, MD

Kiørboe T, Møhlenberg F (1981) Particle selection in suspension-feeding bivalves. Mar Ecol Prog Ser 5:291–296

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide quantitative measures of trophic diversity within food webs? Ecology 88:42–48

Layman CA, Araujo MS, Boucek R, Harrison E and others (2012) Applying stable isotopes to examine food web structure: an overview of analytical tools. Biol Rev Camb Philos Soc 87:545–562

Lee HG (1987) Immigrant mussel settles in Northside generator. The Shell-O-Gram 28:7–9

Lehane C, Davenport J (2002) Ingestion of mesozooplankton by three species of bivalve; *Mytilus edulis*, *Cerastoderma edule* and *Aequipecten opercularis*. J Mar Biol Assoc UK 82:615–619

Lorenz S, Pusch MT (2013) Filtration activity of invasive mussel species under wave disturbance conditions. Biol Invasions 15:2681–2690

Luckenbach MW, Coen LD, Ross PG Jr, Stephen JA (2005) Oyster reef habitat restoration: relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. J Coast Res Spec Iss 40:64–78

Mills E, Dermott RM, Roseman EF, Dustin D, Mellina E, Conn DB, Spidle AP (1993) Colonization, ecology, and population structure of the quagga mussel (Bivalvia, Dreissenidae) in the lower Great Lakes. Can J Fish Aquat Sci 50:2305–2314

Møhlenberg F, Riisgård HU (1978) Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17:239–246

Newsome SD, Del Rio CM, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Front Ecol Environ 5: 429–436

Ozersky T, Evans DO, Ginn BK (2015) Invasive mussels modify the cycling, storage and distribution of nutrients and carbon in a large lake. Freshw Biol 60:827–843

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, NY

Pereira OM, Hilberath RC, Ansarah PRAC, Galvao MSN (2003) Production estimate of *Mytella falcata* and *M. guyanensis* in natural beds of Ilha Comprida Estuary (Sao Paulo, Brasil). Bol Inst Pesca Sao Paulo 29:139–149

Puyana M, Prato J, Díaz JM (2012) *Mytella charruana* (D'Orbigny) (Mollusca: Bivalvia: Mytilidae) en la bahía de Cartagena, Colombia. Bol Investig Mar Costeras 41: 213–217

Ricciardi A, Rasmussen JB (1998) Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can J Fish Aquat Sci 55: 1759–1765

Riisgård HU (1988) Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Mar Ecol Prog Ser 45:217–223

Shumway SE (2001) Shellfish aquaculture and the environment. Wiley-Blackwell, Ames, IA

Shumway SE, Cucci TL, Newell RC, Yentsch CM (1985) Particle selection, ingestion, and absorption in filter-feeding bivalves. J Exp Mar Biol Ecol 91:77–92

Sousa R, Gutiérrez JL, Aldridge DC (2009) Non-indigenous invasive bivalves as ecosystem engineers. Biol Invasions 11:2367–2385

Spinuzzi SS, Schneider KR, Walters LJ, Yuan WS, Hoffman EA (2013) Tracking the distribution of non-native marine invertebrates (*Mytella charruana, Perna viridis*, and *Megabalanus coccopoma*) along the southeastern USA. Mar Biodivers Rec 6:55–67

Su YC, Liu C (2007) *Vibrio parahaemolyticus*: a concern of seafood safety. Food Microbiol 24:549–558

Szefer P, Geldon J, Ali A, Páez-Osuna F, Ruiz-Fernandes A, Galvan S (1998) Distribution and association of trace metals in soft tissue and byssus of *Mytella strigata* and other benthal organisms from Mazatlan Harbour, mangrove lagoon of the northwest coast of Mexico. Environ Int 24:359–374

Thomsen MS, Byers JE, Schiel DR, Bruno JF, Olden JD, Wernberg T, Silliman BR (2014) Impacts of marine invaders on biodiversity depend on trophic position and functional similarity. Mar Ecol Prog Ser 495:39–47

Ward JE, Levinton JS, Shumway SE, Cucci TL (1998) Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Mar Biol 131:283–292

Whyte RS, Trexel-Kroll D, Klarer DM, Shields R, Francko DA (2008) The invasion and spread of *Phragmites australis* during a period of low water in a Lake Erie coastal wetland. J Coast Res 55:111–120

Wright RT, Coffin RB, Ersing CP, Pearson D (1982) Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol Oceanogr 27:91–98

Yuan WS, Hoffman EA, Walters LJ (2016a) Effects of nonnative invertebrates on two life stages of the native eastern oyster *Crassostrea virginica.* Biol Invasions 18:689–701

Yuan WS, Walters LJ, Brodsky SA, Schneider KR, Hoffman EA (2016b) Synergistic effects of salinity and temperature on the survival of two nonnative bivalve molluscs, *Perna viridis* (Linnaeus 1758) and *Mytella charruana* (d'Orbigny 1846). J Mar Biol 2016:9261309

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