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

2 *charruana*

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4 **Running page head:** oyster and mussel competition

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13 **ABSTRACT**

14 The sub-tropical mussel *Mytella charruana* has been reported as invasive along the southeast

15 coast of the USA since 1986. This mussel has been found to negatively impact the keystone species in its

16 invaded range, the eastern oyster *Crassostrea virginica*. To date, however, no mechanism for this

17 negative impact has been determined. To elucidate the role of the invasive mussel on economically

18 important oyster reefs, we compared the feeding physiology of both species in a lagoon along the east

19 coast of Florida (USA). Three different methodologies were used: 1) *in situ* filter-feeding experiments

20 using the biodeposition method to estimate feeding behavior; 2) laboratory assays to estimate the

21 depletion of bacterial particles using a flow cytometer; and 3) stable isotope analysis in conjunction with

22 ellipse-based metrics to investigate the niche size and overlap of these two species. The *in situ* filter-

23 feeding experiments revealed that *M. charruana* had significantly higher clearance, filtration, rejection,

24 organic ingestion and absorption rates, as well as higher rejection percentage and absorption efficiency,

25 but rejected the same amount of inorganic particles. Flow cytometry data suggested that bacteria were a

26 food source for both bivalve species. Stable isotope values confirmed that *M. charruana* and *C. virginica*

27 filled similar functional niches in this ecosystem. These results suggest that *M. charruana* can out-

28 compete native oysters, the findings also demonstrate that an invasion of *M. charruana* might

29 significantly alter plankton abundance, potentially limiting food sources available to other less efficient

30 native filter-feeders such as clams.

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32 **Key words:** bivalve, oyster reef, feeding behavior, stable isotope, Indian River Lagoon.

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INTRODUCTION

Coastal systems are some of the most highly invaded ecosystems on earth, often as a result of human activities (Grosholz 2002). These non-native and invasive species may have a negative effect on local biodiversity, by out-competing local species and thus eradicating native fauna (Ricciardi et al. 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 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 et al. 1998). Zebra mussels, along with the quagga mussel, *Dreissena 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).

Along the Atlantic and Gulf Coasts of the United States, 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 recreational 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 mussel, *Mytella charruana*, known as the charru mussel, 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 United States from Titusville, Florida to Charleston, South Carolina (Spinuzzi et al. 2013). Densities of this non-native species in the United States 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

68   native (11,036 mussels m^{-2}, Brazil) and invasive (13,400 mussels m^{-2}, Colombia) habitats (Pereira et al.

69   2003, Puyana et al. 2012).

70   With the potential for *M. charruana* to compete with resident filter-feeding organisms, it is

71   important to assess the invasive potential or competitive ability of *M. charruana* against other species

72   with a similar role in the ecosystem, i.e., native bivalves. Potential food sources for bivalves include

73   bacteria, phytoplankton, zooplankton, and dissolved organic matter (Hartland & Timoney 1979, Lehane

74   & Davenport 2002, Gosling 2003). Nevertheless, the retention efficiency is species-specific and

75   dependent on different particle characteristics. For example, *C. virginica* and blue mussels (*Mytilus*

76   *edulis*) can retain particles larger than 6 μm with 100% efficiency, whereas the efficiency for particles

77   smaller than 2μm decreases to 50% (Jørgensen 1975, Møhlenberg & Riisgård 1978, Riisgård 1988).

78   However, the bay scallop *Argopecten irradians* has an even lower retention efficiency of 2 μm particles

79   retaining only 15% (Riisgård 1988). Small particles, such as bacteria, are extremely abundant in aquatic

80   ecosystems but they are not readily available to bivalves, as seen by their generally low retention

81   efficiency. However, Kach and Ward (2008) demonstrated that suspension feeders can ingest

82   picoplankton-size (0.2-2.0 μm) particles when in aggregates, suggesting that small particles can be an

83   important food source as they become available to bivalves as larger particle masses. Despite the wide

84   range of particle sizes that bivalves are capable of ingesting, they do not ingest everything that is retained

85   by the gills. Pre-ingestive selection may occur on the gills and labial palps, which results in the release of

86   rejected particles as pseudofeces (Kiørboe & Møhlenberg 1981, Shumway et al. 1985, Ward et al. 1998).

87   Pre-ingestive selection is dependent upon the characteristics of the available food particles, and

88   production of pseudofeces occurs when the ingestive capacity of a bivalve is overloaded or the particles

89   are unsuitable, such as silt and other inorganic matter (Beninger & St-Jean 1997).

90   Invasion biology is founded on understanding what happens to a system when an invasion occurs

91   (Carlton 2001). Yuan et al. (2016a) found that survival and growth of *C. virginica* was negatively affected

92   by *M. charruana* when the two species were grown in contact in 6-week long field studies in Mosquito

93   Lagoon, while native mussels (*Geukensia demissa*) had no effect on the oysters, proposing that feeding

94   competition was the mechanism underlying this result. Our study now directly tests this idea by

95   investigating the feeding behavior and removal of bacteria by each of these species. In addition, because

96   stable isotope analysis provides a time-integrated assessment of an organisms' diet, we used the stable

97   isotope ratios of C and N (δ^{13}C and δ^{15}N) to determine if these two species had overlapping diets (Fry

98   2006, Jackson et al. 2012) and fill the same functional "niche" (Newsome et al. 2007, Layman et al. 2012)

99   in this system. We are testing the null hypothesis that both bivalves are equally effective at feeding and

100   have distinct diets in the waters of the Mosquito Lagoon. The alternative hypothesis is that the invasive

101   *M. charruana* out-competes *C. virginica* by more efficient filter-feeding and an overlapping diet. These

102 types of ecophysiological studies can help provide a mechanistic understanding of invasive potential and

103 potential impacts arising from introduced species.

104

105 **MATERIALS AND METHODS**

106 **Study site and bivalve collection**

107 Experiments were conducted in May 2015 in the waters of the Mosquito Lagoon within

108 Canaveral National Seashore, New Smyrna, FL at the Fellers House Field Station operated by the

109 University of Central Florida (28 54'23.96"N; 80 49'11.54"W). All organisms were collected by hand

110 from an intertidal oyster reef within 0.5 km of the station. Oysters (*Crassostrea virginica*) and invasive

111 mussels (*Mytella charruana*), 50 of each, were collected the day before the beginning of the experiments,

112 cleaned of epiphytes and other encrusting organisms, and suspended from the dock of the field station in

113 plastic mesh bags. Animals were maintained in bags for the duration of the experiments. Physical water

114 characteristics (temperature, salinity, oxygen, and chlorophyll *a*) were recorded daily.

115 **Filter-feeding experiments**

116 To assess the feeding behavior of both bivalve species, we conducted *in situ* filter-feeding

117 experiments from the same dock where animals were kept using two portable, flow-through devices (Fig.

118 1), which include a PVC "reservoir" tank (20 L capacity) that received lagoon water from an underwater

119 bilge pump suspended at 1 m depth. This reservoir tank was aerated to maintain suspension of particles in

120 the water. Water from the reservoir tank flowed through tubes into individual chambers that each held a

121 single bivalve. With 10 individual chambers in each device, this design allowed for 18 replicate bivalves

122 and 2 controls (empty shell) to be sampled simultaneously. A thorough description of design and

123 operation is available in Galimany et al. (2011). Experiments were conducted over three consecutive

124 days.

125 Each day, nine adults of each species (mean shell length 50.43±1.15 mm and 33.43±0.86 mm for

126 oysters and mussels, respectively, and mean dry weight 0.68±0.05 g and 0.24±0.02 g for oysters and

127 mussels, respectively) were chosen haphazardly from the previously collected bivalves and placed within

128 the two flow-through devices. Each device held only one species to simplify the collection of biodeposits.

129 To affix mussels within each chamber a small plastic, velcro strip was glued to each mussel. Oysters were

130 not affixed as they do not move once placed within the chamber. Each bivalve was exposed to a constant

131 flow rate of 12 L h^{-1} of ambient water. Animals were allowed to acclimate within their flow-through

132 chambers for 2 h to recover from any stress associated with handling. Shells without live C. *virginica* and

133 *M. charruana* were added into the final chambers to act as controls (Galimany et al. 2011).

134 Water (110 ml) was collected from the overflow of each control chamber to characterize the

135 ambient particulate matter. Samples were taken every 15 minutes for two hours. Prior to the start of the

136 experiment, individual chambers were cleaned to remove any fecal material. Then, feces and pseudofeces

137 from each live bivalve were collected separately throughout the experiment with a pipette for 2h. Water

138 samples and, at the end of the experiment, feces and pseudofeces from each chamber were filtered

139 through washed, pre-combusted (450 °C at 4 h), pre-weighed Whatman GF/C filters (25 mm) and rinsed

140 with isotonic ammonium formate to remove residual salt on the filter. Filters were kept on ice and

141 transferred to the Smithsonian Marine Station where they were dried at 60 °C for 48 h and weighed. For

142 the filters used for water, this weight represents the total particulate matter (TPM). Filters were then ashed

143 at 450 °C for 4 h and weighed again to obtain the particulate inorganic matter (PIM). The particulate

144 organic matter (POM) was calculated as the difference between TPM and PIM. The organic content of the

145 water (f) was calculated as the mean organic fraction of total particulates (f = POM/TPM). The different

146 seston values obtained at each experiment were compared using a one-way ANOVA.

147   Three water samples were filtered through Whatman GF/C filters (25 mm Ø) until clogged to

148 collect biomass for chlorophyll a (chl a) analyses. Filters for chl a analysis were frozen, lyophilized

149 overnight, and extracted with 5 ml of 90 % acetone at 4 °C overnight. The concentration of chl a was

150 quantified by measuring extract absorbance at 750, 664, 647, and 630 nm and with the equations of

151 Parsons et al. (1984). Final values were corrected for the volume of water filtered through each filter and

152 are reported in μg L^{-1}.

153   Filters with feces and pseudofeces were used to estimate physiological feeding variables (e.g.

154 clearance rate, filtration rate, etc.; Table 1). Data from animals that produced no feces or pseudofeces

155 (i.e., did not open) during the measurement period were not included in subsequent analyses. The feeding

156 behavior of both species were calculated according to the biodeposition method (Iglesias et al. 1998).

157 This method is based on using the inorganic matter as a tracer for the feeding processes.

158   To synchronize the seston available with the corresponding biodeposits produced by the bivalves,

159 it was necessary to estimate the gut transit time (GTT). Gut transit time is defined as the minimum time

160 for an organic particle to pass through the digestive tract of a bivalve after ingestion. This variable was

161 calculated before each measurement period by providing replicate individuals of each bivalve species

162 with a mixture of local ambient water and cultured *Tetraselmis* sp. (Oahu, HI) (adapted from Hawkins et

163 al. 1996). The time in minutes that elapsed between the addition of cultured *Tetraselmis* sp. and the first

164 deposition of green-colored feces by one of each of the bivalves was considered to be the gut transit time.

165   At the end of each experiment, animals were then frozen and transported to the Smithsonian

166 Marine Station where shell length and dry tissue weight (48 h at 60 °C) were measured.

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

168 equation:

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

170   where, Y_e is the experimentally-determined rate, and W_e is the dry body mass measured for each

171   bivalve. For the predetermined feeding rate constant *b* values, 0.73 was used for *C. virginica* (Riisgård

172   1988) and 0.67 for *M. charruana,* as is commonly used in mussel feeding studies with the mussel *Mytilus*

173   *edulis* (Bayne et al. 1989, Hawkins et al. 1997).

174   The different feeding physiological variables estimated were compared using a blocked ANOVA

175   with day as the block and species as a fixed factor. Only bivalves that were open and actively feeding

176   were used for the statistical analyses, which included 17 *C. virginica* and 23 *M. charruana.*

177   **Bacterial assay**

178   To quantify the removal of bacterial populations and examine feeding by these two bivalves we

179   conducted a laboratory *in situ* bacterial depletion experiment on each of the three experimental days.

180   Lagoon water was collected from the dock next to the laboratory and used to fill eleven 1L-plastic

181   beakers, which were continuously aerated throughout each experiment. Five oysters and five mussels

182   were haphazardly selected from the mesh bag containing previously collected bivalves and placed

183   individually in the 1L beakers. One extra beaker was left as a control to detect changes in the natural

184   bacterial population. Samples were taken every 5 min, starting at time 0 and continuing for 30 min, after

185   which samples were taken every 10 min until 1 h had passed. Each of these 10 samples was collected with

186   a pipette and preserved with 1% formalin (final concentration) until analysis. At the end of the

187   experiment, bivalves were dissected and dried at 60 °C for 48 h to determine individual dry tissue weight.

188   To calculate the ambient planktonic bacteria abundance, water samples were processed according

189   to Gasol et al. (1999); samples were stained with 2.5 µM Syto 13, a green fluorescing nucleic acid stain,

190   (10:1 stock dilution; Molecular Probes) and left for 10 min in the dark. Then 10 µl of Fluoresbrite yellow-

191   green 1µm microspheres (Polysciences) were added to each sample as a size standard. Each sample was

192   run on a C6 flow cytometer (BD Biosciences, San Jose, CA) for 2 minutes on the medium flow rate

193   setting (35 µL min^{-1}). Bacteria in the samples were identified and quantified using the plots contrasting

194   side scatter (SSC) vs green fluorescence (FL1) produced by the flow cytometer.

195   Bacterial concentrations from both species and the control were compared with a blocked

196   ANOVA with day as the block and species as a fixed factor. Moreover, an ANCOVA was run with

197   bacterial depletion using species as a fixed factor and bivalve dry weight as covariate. All bivalves were

198   open and actively feeding and therefore used in the statistical analyses (N = 15 for each species). The

199   three controls, one from each experimental day, were also added to the analyses for comparison purposes.

200   **Stable isotopes**

201   A subset of the *C. virginica* and *M. charruana* individuals collected from the oyster reef were

202   held overnight in the laboratory in filtered lagoon water to ensure that their guts were empty prior to

203   stable isotope analysis. Replicate water samples from the oyster reef were pre-filtered through a 105 µm

204 mesh to remove larger particles and then filtered through a pre-combusted (450 °C for 4 h) 47 mm quartz

205 fiber filter (~2 μm retention) to collect particulate organic matter (POM), a potential source of C and N

206 for filter-feeding organisms. Filters and bivalves were frozen until further analysis. Back at the

207 Smithsonian Marine Station, shell length and dry weight (following 24 h at 60 °C) of all bivalves were

208 measured and, in preparation for stable isotope analysis, dried whole bivalve tissue was ground to a fine

209 powder using a mortar and pestle. Bivalve tissue for 14 and 17 *C. virginica* and *M. charruana*,

210 respectively, and four filters containing POM, were acidified by exposure to 12N HCl fumes in a closed

211 environment for 12 h. After drying at 60 °C to remove residual acid, samples were weighed to the nearest

212 0.001 mg into tared tin capsules. Samples were analyzed at the OUSS/MCI Stable Isotope Mass

213 Spectrometry Laboratory at the Smithsonian Museum Conservation Institute in Suitland, Maryland.

214 Analysis was carried out on a Thermo Delta V Advantage mass spectrometer in continuous flow mode

215 coupled to a Costech 4010 Elemental Analyzer (EA) via a Thermo Conflo IV. Isotope values are reported

216 in δ notation in units of per mil (‰) following equations outlined in Fry (2006). Individual samples were

217 run in duplicate and then averaged prior to analysis. Precision across samples was ± 0.2 ‰ for both $\delta^{13}C$

218 and $\delta^{15}N$.

219   Because the location of an organism within bivariate ($\delta^{13}C$ and $\delta^{15}N$) isotopic space is based on

220 the sources of C and N it assimilates, internal processing of these nutrients, and the surrounding

221 environment, the placement of an organism in isotopic space may be presented as the functional "niche" it

222 fills in a system (Newsome et al. 2007, Layman et al. 2012). To visualize and quantify the isotopic niche

223 area for each bivalve species, we calculated the Standard Ellipse Area (SEA_c) using Bayesian inference

224 based on the ellipse-based metrics within the SIBER (Stable Isotope Bayesian Ellipses in R) program

225 (Jackson et al. 2011). These analyses allowed us to quantitatively compare the niche size of each species

226 and estimate the percent overlap of these niches within isotopic space.

227

228 **RESULTS**

229 **Water and seston characteristics**

230   Average values for Mosquito Lagoon water characteristics (temperature, salinity, oxygen, chl *a*)

231 are shown in Table 2. All characteristics are within published ranges for both species studied. Total

232 particulate matter ($F_{2,39} = 3.42$; $p = 0.043$) and particulate inorganic matter ($F_{2,39} = 3.45$; $p = 0.042$)

233 differed between days. Particulate organic matter and the organic content of the water (*f*) showed no

234 significant differences between days ($F_{2,39} = 2.22$; $p = 0.122$, and $F_{2,39} = 1.26$; $p = 0.296$, respectively).

235 **Filter feeding experiments**

236   *M. charruana* had higher values for all physiological parameters measured than *C. virginica* (Fig.

237 2; Electronic supplement Table1). The clearance rate (Fig. 2a; $F_{1,36} = 14.73$, $p < 0.001$), filtration rate

238 (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

239 high in *M. charruana* compared to *C. virginica*. Mean rejection percentage was approximately 20%

240 higher in the invasive species (Fig. 2d; $F_{1, 36}$ = 17.96, p = < 0.001). Nevertheless, the amount of inorganic

241 matter rejected through pseudofeces was exactly the same for both species of bivalves ($F_{1, 36}$ = 0.96, p =

242 0.335). Both organic ingestion rate and absorption rate for *M. charruana* were significantly higher than *C.*

243 *virginica* (Fig. 2e & f; $F_{1, 36}$ = 8.94, p = 0.005; $F_{1,36}$ = 9.26, p = 0.004, respectively). Absorption efficiency

244 was higher for *M. charruana*, with an efficiency of 0.65 and 0.76 for the native bivalves (*C. virginica*)

245 and the invasive species (*M. charruana*), respectively (Fig. 2g; $F_{1, 36}$ = 6.92, p = 0.012). Physiological

246 rates were, in general, not affected by date (p > 0.05), though rejection rate was different among

247 experimental dates ($F_{2, 36}$ = 3.38, p = 0.045). The effect of date on rejection rate may be attributable to

248 differences in water conditions.

249 **Bacterial assay**

250   The bivalves did deplete bacteria ($F_{2, 28}$ = 7.68, p = 0.002), though the two studied species were

251 not different from each other (Fig. 3). After taking into account differences in body size, both bivalves

252 removed the same amount of bacteria (ANCOVA $F_{1, 27}$ = 0.72, p = 0.405). After one hour, the number of

253 bacteria remaining in the experimental beakers ranged from 1.04 10^5 to 4.01 10^5 bacteria ml^{-1}.

254 **Stable isotopes**

255   The mean δ^{13}C and δ^{15}N values of *C. virginica* and *M. charruana* were -22.31 ‰ and 4.60 ‰,

256 and -21.84 ‰ and 4.65 ‰, respectively. Both bivalve species had relatively narrow Standard Ellipse

257 Areas (SEA$_c$) that were of similar size (SEA$_c$ of 0.477 and 0.389 for *C. virginica* and *M. charruana*,

258 respectively SIBER; p > 0.05; Fig. 4) and the SEA$_c$ of these two species overlapped by approximately 20

259 %. Mean (+/-) δ^{13}C and δ^{15}N values of POM were -22.83 ‰ (0.33) and 4.09 ‰ (0.23), respectively.

260

261 **DISCUSSION**

262   The results of this study suggest that the invasive mussel *M. charruana* shares the ecological

263 niche of the native, eastern oyster *C. virginica* and thus competes for the same resources. When non-

264 native species colonize a new habitat, the effects of the feeding activity can be very negative for the

265 ecosystem. For example, the ability of the Asian clam, *Corbicula fluminea,* to couple benthic and pelagic

266 environments with terrestrial ecosystems has been demonstrated to have a strong potential to alter food

267 web flows in aquatic ecosystems (Dias et al. 2014). In Mosquito Lagoon, the invasive mussel *M.*

268 *charruana* significantly reduced the survival and growth of the native eastern oyster *C. virginica* and it

269 was suggested that this was the result of competition for food (Yuan et al. 2016a). The results of our

270 present study confirm this idea.

8

271   Bivalves are filter-feeding organisms that force water into their bodies, where gills allow the

272   filtration of particles from the water (Gosling 2003). The feeding behavior of bivalves depends on water

273   characteristics such as temperature, salinity and dissolved oxygen. With the values recorded in our study

274   falling within the range tolerance of both species, we do not expect their feeding behavior to be limited by

275   these water characteristics (Kennedy et al. 1996, Yuan et al. 2016b). The flow of water (clearance rate)

276   and the particulate matter retained in the gills (filtration rate) are physiological parameters that depend on

277   the water characteristics (Hawkins et al. 1996). For example, *in situ* measurements of the feeding

278   behavior of *C. virginica* in a New Hampshire (USA) estuary found clearance rates of 3.51 L h^{-1} but

279   increases in inorganic material increased clearance rates to 7.31 L h^{-1} though rejection remained constant

280   (Hoellein et al. 2015). Mosquito Lagoon is a shallow embayment with high loads of inorganic matter

281   probably caused by silt resuspension where mixing events would tend to diminish the organic content of

282   particles in the water column (Hawkins et al. 1996, Galimany et al. 2011). Despite organic matter only

283   comprising 27% of the total particulates in the water, the clearance and filtration rates estimated for *C.*

284   *virginica* were about 50% lower than those reported by Hoellein et al. (2015). The lower feeding response

285   found in our study for *C. virginica* is likely related to pre-ingestive selection as rejection was higher in

286   our study site (up to 60%). Pre-ingestive selection allows bivalves to handle excess inorganic matter in

287   the water column by rejecting it as pseudofeces, thus increasing the organic fraction of the ingested matter

288   (Hawkins et al. 1996). The high rejection rate estimated in this study maximized energy gain since the

289   animals in these sites were feeding upon natural suspensions with low organic content (Iglesias et al.

290   1992, Bayne et al. 1993).

291   Invasive mussels, even taxonomically unrelated, have similar ecosystem impacts because of the

292   ecological niche they share (Karatayev et al. 2007). The presence of invasive species with such efficient

293   feeding physiology may influence trophic interactions and food availability for both pelagic and benthic

294   species (Karatayev et al. 1997, Boltovskoy et al. 2006) (Karatayev et al. 1997, Boltovskoy et al. 2006)

295   and impact nutrient mineralization, oxygen availability and sedimentation rates (Karatayev et al. 1997,

296   Boltovskoy et al. 2006). In addition, competition with native species may be exacerbated by the ability of

297   invasive mussels to withstand stressful conditions (Lorenz & Pusch 2013).This is the first study

298   describing the feeding behavior of *M. charruana* and, overall, *M. charruana* absorbed more organic

299   matter and was more efficient in the feeding process that oysters. Therefore, we suggest that the invasive

300   mussel *M. charruana* is out-competing the native oyster *C. virginica*, and likely other native filter-feeding

301   species as well, such as the clam *Mercenaria mercenaria*. The effects of *M. charruana* on the ecosystem

302   might be as severe as those reported for other invasive mussels (i.e. the zebra mussel *Dreissena*

303   *polymorpha*).

304   Both studied bivalves cleared bacteria from the water, using this food item to supplement the low
305   organic matter available for their diets. Although bacterial depletion has been shown in bivalves
306   previously, not all species are equally efficient at clearing bacteria from the water. Wright et al. (1982)
307   observed that the ribbed mussel *Geukensia demissa* cleared bacteria more effectively than the blue mussel
308   *Mytilus edulis* and soft-shell clams (*Mya arenaria*). Nevertheless, bacteria can be an important component
309   of the seston in two different forms, as free-living organisms, or in association with phytoplankton cells
310   and/or organic matter (Bell & Mitchell 1972). In estuaries along the east coast of the USA, free bacterial
311   abundance ranged from 10^5 to 10^9 cells ml^{-1} (Findlay et al. 1991, Ducklow et al. 1999). Our results
312   indicate that Mosquito Lagoon had a low abundance of free bacteria, although the total bacterial
313   population might be higher than measured if the bacteria were associated with organic components of the
314   seston. The ability of bivalves to filter the water allows the bacteria to act as a food source in association
315   with organic matter by creating larger aggregates. In fact, such small organisms have become a health
316   concern as bivalves can ingest human-harmful bacteria (Su & Liu 2007) leading to the development of
317   shellfish monitoring programs worldwide (Shumway 2001).

318   Stable isotope analysis revealed that the invasive mussel *M. charruana* shared isotopic niche
319   space with the native oyster *C. virginica*, supporting the contention that these sympatric species have
320   dietary overlap that could contribute to interspecific competition (Dubois & Colombo 2014). Because
321   consumers are generally enriched in δ^{15}N and δ^{13}C relative to their diet (Fry 2006), enriched (~0.5 ‰ to
322   0.9 ‰) δ^{15}N and δ^{13}C values of both species relative to particulate organic matter (POM) implies that
323   these bivalve species were assimilating C and N from bulk particulate sources in the water column.
324   Minimal isotopic variation across most individuals, and the resulting narrow niche widths of both species,
325   however, are indicative of specialized feeding on a specific fraction of the overall POM pool. Classic
326   theory and recent empirical evidence suggest that there is an inverse relationship between the level of
327   interspecific competition exacted on a species and its realized niche size (estimated by SEA$_c$ or other
328   quantitative metrics as in Layman et al. (2007)) and placement within isotopic space (Jackson et al. 2012,
329   Dubois & Colombo 2014, Jackson et al. 2014, Karlson et al. 2015). For instance, Jackson et al. (2012)
330   found a strong reduction in the isotopic niche size of an invasive species following the establishment of
331   another invasive species belonging to the same functional group. While the small SEA$_c$ values in this
332   study may reflect competition of these bivalves for local resources, additional studies investigating the
333   dynamics between *C. virginica* SEA$_c$ size and *M. charruana* abundance are needed to fully elucidate this
334   (Jackson et al. 2012).

335   In conclusion, our study suggests that the invasive mussel *M. charruana* may have a competitive
336   advantage among other filter-feeders due to its efficiency at removing and sorting available nutrients from
337   the water column. Instead of filling a vacant niche in this system through the acquisition of unexploited

338 resources, *M. charruana* competes directly with sympatric resident bivalves including the oyster, *C.*
339 *virginica*. Interestingly, with overlapping niches, these two species may fill similar roles in these local
340 ecosystems, raising important questions about how ecosystem processes and overall ecosystem function is
341 affected by *M. charruana* invasion on these reefs. We demonstrate that *M. charruana* poses a threat to
342 native oysters in central Florida, and provide evidence that this species may impact ecosystem services
343 and nutrient cycling within the Southeast US and in other locations within its invasive range.
344

Fig. 1: Image of the two 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.

362　Fig. 2: Mean (+/- SE) physiological feeding variables measured for both species during the in-situ

363　experiment: Clearance Rate in L h^{-1} (a); Filtration Rate in mg h^{-1} (b); Rejection Rate in mg h^{-1} (c);

364　Rejection Proportion in % (d); Inorganic Rejection Proportion in % (e); organic ingestion rate in mg h^{-1}

365　(f); Absorption Rate in mg h^{-1} (g); Absorption Efficiency (h). Stars denote significance (ANOVA: p <

366　0.05).
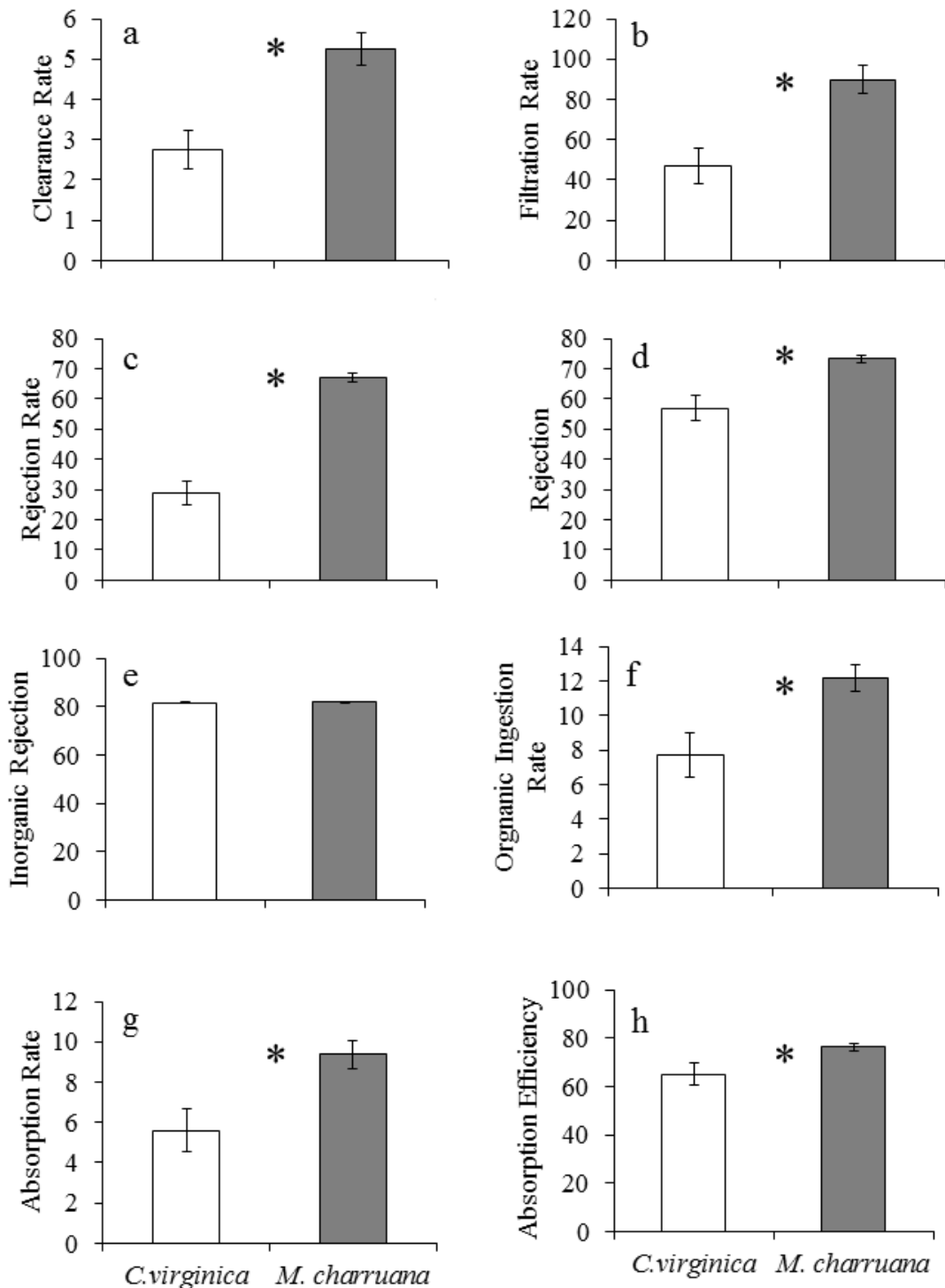
367 Fig. 3: Bacterial concentration in the water (±SE) for both studied bivalve species and controls. Letters

368 denote significant differences (ANOVA: $p < 0.05$) (i.e., controls are different than the two bivalve

369 species). ANCOVA results using dry weight as covariate also showed no significant differences between

370 species ($p > 0.05$).
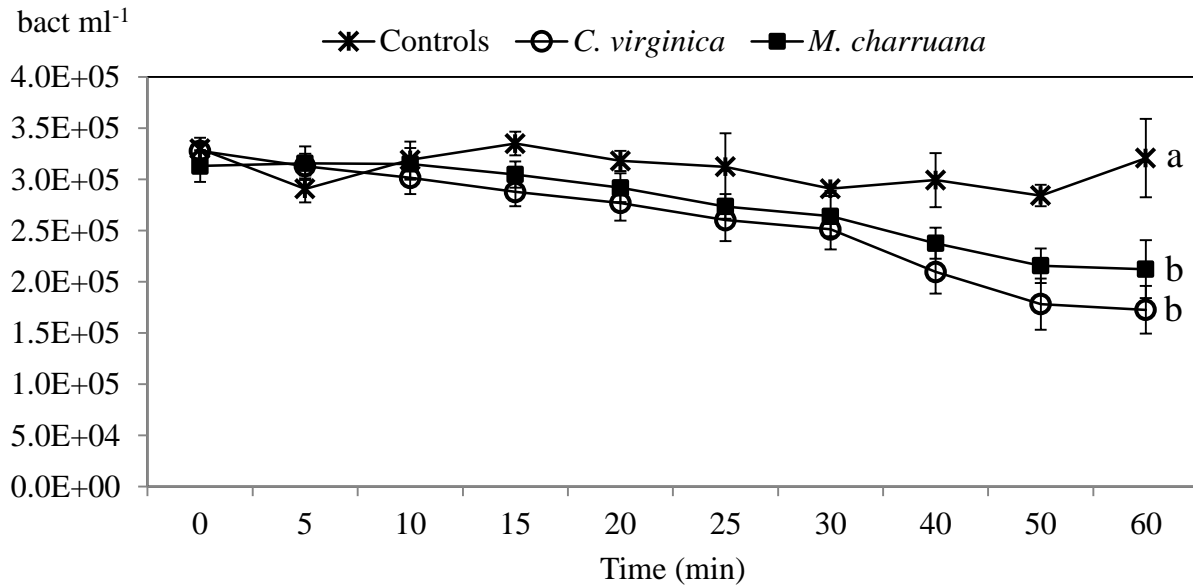
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373   Fig. 4: Bivariate (δ^{13}C and δ^{15}N) plot depicting the placement of C. *virginica* and *M. charruana*

374   individuals within the isotopic niche space of the studied oyster bed. Standard Ellipse Areas (SEA$_c$)

375   depicted by solid lines provide estimates of the niche area of each species. Mean δ^{13}C and δ^{15}N values of

376   particulate organic matter (POM) are shown for reference. N=14 for C. *virginica* and N=17 for *M.*

377   *charruana*.

378
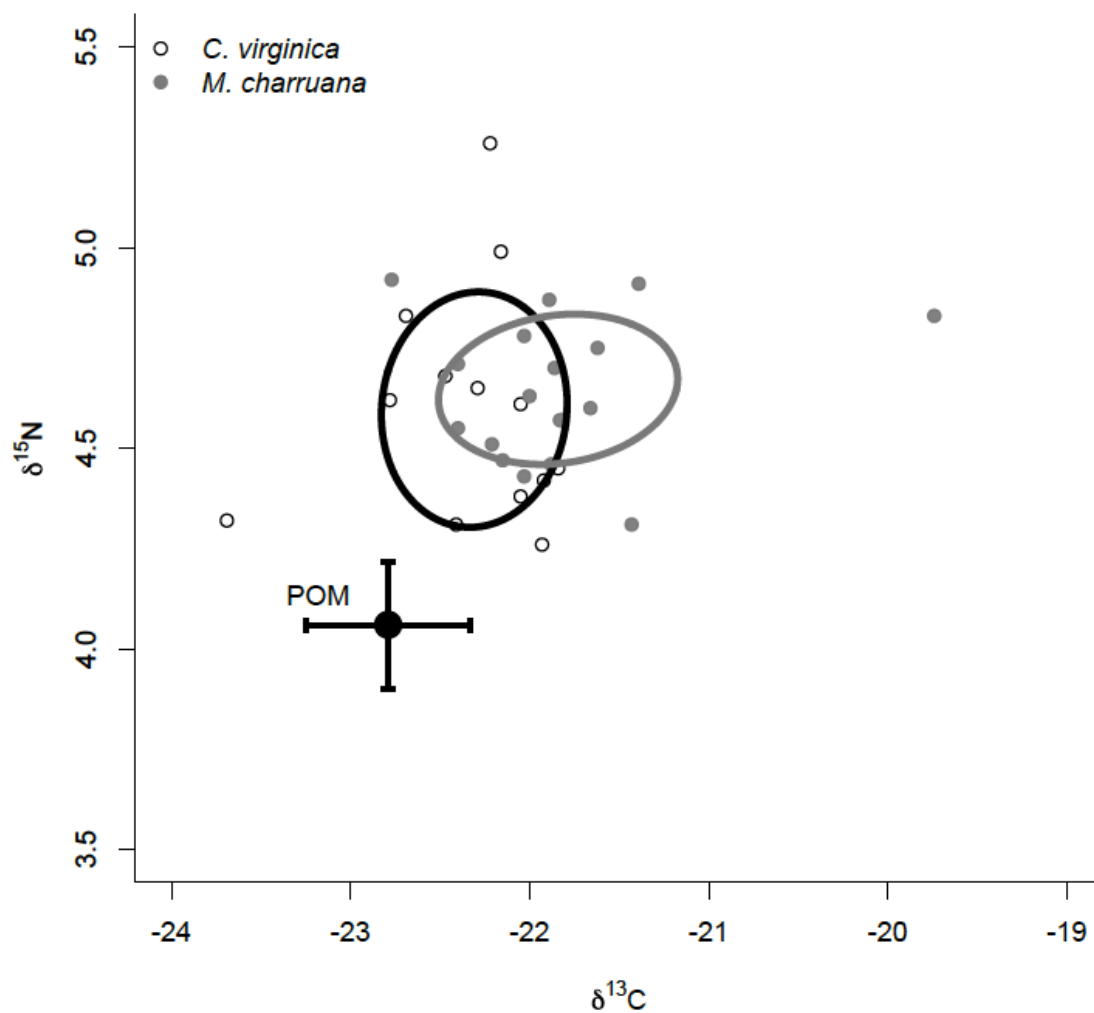
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380

381 Table 1: Description of the physiological components of absorptive balance for bivalves. TPM: total particulate matter from the water (mg L^{-1});

382 PIM: particulate inorganic matter from the water (mg L^{-1}); POM: particulate 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}	Total particulate matter from the seawater retained in the gills per unit of time	CR x TPM
Rejection rate	RR	mg h^{-1}	Total particulate matter that has been retained in the gills but rejected prior 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 ingestion	(RR / FR) x 100
Inorganic rejection proportion	IRP	%	Proportion of particulate inorganic matter that has been retained in the gills but rejected prior 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})) x 100
Organic ingestion rate	OIR	mg h^{-1}	Particulate organic matter retained in the gills and ingested by the bivalve per unit of time	(CR x POM) – (mg organic matter from pseudofeces (mg h^{-1}))
Absorption rate	AR	mg h^{-1}	Particulate organic matter 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

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385   Table 2: Mean values for Mosquito Lagoon water characteristics (±SE) recorded during the experimental

386   days. T: Temperature; Sal: salinity; Oxy: Oxygen; Chl a: Chlorophyll a; TPM: Total Particulate Matter;

387   POM: Particulate Organic Matter; PIM: Particulate Inorganic Matter; f: Organic matter in the water

388   ((TPM/POM) x 100).

389

T (°C)	Sal (ppt)	Oxy (mg L^{-1})	Chl a (µg L^{-1})	TPM (mg L^{-1})	POM (mg L^{-1})	PIM (mg L^{-1})	f (%)
27.80±0.57	36.27±0.20	4.58±0.69	7.39±0.85	17.80±0.90	4.93±0.27	12.87±0.70	28.01±0.90

390

391

392   Electronic supplement Table 1: blocked one-way ANOVA table for the feeding behavior of bivalve

393   species, *C. virginica* and *M. charruana*, with day as the block and species as a fixed factor. CR: Clearance

394   rate; FR: Filtration rate; RR: Rejection rate; RP: rejection proportion arcsine square root transformed for

395   analysis; IRP: inorganic rejection proportion arcsine square root transformed for analysis; OIR: Organic

396   ingestion rate; AR: Absorption rate; AE: Absorption efficiency arcsine square root transformed for

397   analysis.

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399

Tests of Between-Subjects Effects

Source	Dependent Variable	df	Mean Square	F	Sig.
Corrected Model	CR	3	24.357	6.437	0.001
	FR	3	7844.040	6.764	0.001
	RR	3	6256.593	9.360	<0.000
	RP	3	0.133	8.286	<0.000
	IRP	3	0.000	1.563	0.215
	OIR	3	65.643	3.090	0.039
	AR	3	46.982	3.119	0.038
	AE	3	0.056	2.717	0.059
Intercept	CR	1	633.528	167.419	0.000
	FR	1	184765.119	159.328	0.000
	RR	1	91095.862	136.280	0.000
	RP	1	34.449	2145.245	0.000
	IRP	1	25.749	177279.743	0.000
	OIR	1	3837.775	180.636	0.000
	AR	1	2162.999	143.578	0.000
	AE	1	38.924	1889.469	0.000
Spp	CR	1	55.729	14.727	0.000
	FR	1	17620.697	15.195	0.000
	RR	1	14102.771	21.098	0.000
	RP	1	0.288	17.959	0.000
	IRP	1	0.000	0.956	0.335
	OIR	1	189.913	8.939	0.005
	AR	1	139.448	9.256	0.004
	AE	1	0.143	6.919	0.012
Day	CR	2	5.809	1.535	0.229
	FR	2	2893.637	2.495	0.097

		df	Mean Square	F	Sig.
	RR	2	2261.460	3.383	0.045
	RP	2	0.047	2.909	0.067
	IRP	2	0.000	2.018	0.148
	OIR	2	2.194	0.103	0.902
	AR	2	1.112	0.074	0.929
	AE	2	0.013	0.628	0.539
Error	CR	36	3.784		
	FR	36	1159.651		
	RR	36	668.444		
	RP	36	0.016		
	IRP	36	0.000		
	OIR	36	21.246		
	AR	36	15.065		
	AE	36	0.021		
Total	CR	40			
	FR	40			
	RR	40			
	RP	40			
	IRP	40			
	OIR	40			
	AR	40			
	AE	40			
Corrected Total	CR	39			
	FR	39			
	RR	39			
	RP	39			
	IRP	39			
	OIR	39			
	AR	39			
	AE	39			

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