# Estuaries and Coasts

# Feeding behavior of the native mussel Ischadium recurvum and the invasive mussels Mytella charruana and Perna viridis in Florida, USA, across a salinity gradient

--Manuscript Draft--

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| **Abstract:** | The feeding behavior of three species of mussels, the native Ischadium recurvum and the invasives Mytella charruana and Perna viridis, was studied in an invaded ecosystem in Florida (USA). In situ feeding experiments using the biodeposition method were performed along a salinity gradient in the St. Johns River. Additionally, water characteristics (salinity, temperature, dissolved oxygen, and seston loads) were related to the feeding behavior. Clearance, filtration, organic ingestion, and absorption rates of I. recurvum were negatively affected by salinity. For M. charruana, rejection proportion was positively related to salinity while total ingestion, organic ingestion, and absorption rates were positively related to the percentage of organic matter in the seston. Total and organic ingestion rates of P. viridis were negatively affected by salinity but positively affected by total particulate matter. Condition indices for P. viridis and M. charruana were 13.16 ± 0.64 and 6.63 ± 0.43, respectively, compared to 4.82 ± 0.41 for the native species I. recurvum, indicating that these mussels are well adapted to the environmental conditions in the area. This study indicates that the three species thrive in different water characteristics. Thus, the invasive mussels will not totally occupy the niche of the native mussel in Florida despite overlapping distributions. |

Smithsonian Marine Station at Fort Pierce

May 14, 2018

Dr. Paul E. Montagna
Co-Editor-in-Chief
Estuaries and Coasts

Dear Dr. Montagna,

We would like to re-submit the Research Article entitled “Feeding behavior of the native mussel *Ischadium recurvum* and the invasive mussels *Mytella charruana* and *Perna viridis* in Florida, USA, across a salinity gradient” to the journal Estuaries and Coasts.

We submitted a tracked and non-tracked versions of the manuscript yesterday because we thought they both would help understand the changes made in the manuscript. However, as requested, we are now resubmitting just the clean version with no tracked changes.

My coauthors and I have considered all of the comments and have been able to address them. We apologize for any confusion caused by the first revision and the very recent submission and hope that this version of the manuscript will satisfy the Co-Editor-in-Chief and Reviewer 2 hesitations.

We are very thankful for all the comments and suggestions; the article has greatly improved after addressing them. Please find attached a non-tracked version of the manuscript and a document with detailed information provided on how we addressed each suggestion in the revised manuscript.

Best regards,

Dr. Eve Galimany

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**Co-Editor in Chief**

One problem is that the letter with the revision is inadequate. A letter in response to a review should contain an explicit response, i.e., how the text was changed, and where the text was changed (i.e., line numbers). Your responses are more of a narrative explanation that no reader will ever see, and it is not clear that changes were made to the manuscript. You have to tell the editor how you changed the manuscript. Using continuous line numbering makes this easier.

We apologize for the confusion over the changes made to the manuscript; we misunderstood the guide for authors about numbering. We have changed our line numbers to continuous as suggested. We have endeavored to explain the changes made in response to Reviewer 2's comments and more clearly denote the changes made in the manuscript.

**Reviewer # 2**

Although the revised ms has improved in the sense that essential errors in the previous version have been resolved, the paper still lacks scientific depth. It is interesting to measure physiological parameters of 3 species in a salinity gradient but I do not see how this contributes to explaining the observations, on habitat preferences. Also many more factors determine habitat, and the authors did change the title but are still addressing the habitat preferences. Moreover, the research question as formulated by the authors: "We hypothesized that invasive mussels had higher feeding rates because successful invasive bivalves seem to be very efficient at using the natural resources, often even more efficient than native species" is not testable as it is a circular argument: they have higher feeding rates because they are efficient.

It seems that the salinity gradient determines the distribution of the species whether native or invasive is another issue. This spatial differentiation prevents competition and possible loss of natives, so there is hardly any issue linked to invasion in this case.

We appreciate the Reviewer's concern that we are overstating the results of our study in regards to habitat preferences. We agree that many factors in addition to salinity affect habitat preference, and have endeavored to shift the focus of the paper to the effects of salinity and seston loads on feeding behaviors (Lines 27, 81-85, 292-302, 315-318). Additionally, we have expanded the last paragraph to include discussions of how our results could be influenced by other factors (Lines 331-343).

We disagree with the Reviewer that our hypothesis is a circular argument. We are hypothesizing that invasive species being tested have higher feeding rates than our native species because previous research found that invasive species tend to be more efficient than their native counterparts are. These parameters had not been previously tested and we did not know if *Perna* and

*Mytella* were more efficient than *Ischadium.* We have reworded the sentence to clarify our intent (Lines 84-89).

The literature reports all three species do have overlaps in salinity range, a fact we have expanded upon in this version (Lines 66-68, 70-74, 79-81). However, our results suggest that there are salinity preferences, which may allow these species to coexist. We have stated this relationship more clearly (Lines 316-318).

# Feeding behavior of the native mussel *Ischadium recurvum* and the invasive mussels *Mytella charruana* and *Perna viridis* in Florida, USA, across a salinity gradient

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## Abstract

The feeding behavior of three species of mussels, the native *Ischadium recurvum* and the invasives *Mytella charruana* and *Perna viridis*, was studied in an invaded ecosystem in Florida (USA). *In situ* feeding experiments using the biodeposition method were performed along a salinity gradient in the St. Johns River. Additionally, water characteristics (salinity, temperature, dissolved oxygen, and seston loads) were related to the feeding behavior. Clearance, filtration, organic ingestion, and absorption rates of *I. recurvum* were negatively affected by salinity. For *M. charruana*, rejection proportion was positively related to salinity while total ingestion, organic ingestion, and absorption rates were positively related to the percentage of organic matter in the seston. Total and organic ingestion rates of *P. viridis* were negatively affected by salinity but positively affected by total particulate matter. Condition indices for *P. viridis* and *M. charruana* were 13.16 ± 0.64 and 6.63 ± 0.43, respectively, compared to 4.82 ± 0.41 for the native species *I. recurvum*, indicating that these mussels are well adapted to the environmental conditions in the area. This study indicates that the three species thrive in different water characteristics. Thus, the invasive mussels will not totally occupy the niche of the native mussel in Florida despite overlapping distributions.

**Key words**: feeding behavior, bivalve, seston, salinity, St. Johns River.

## Introduction

Mussels are considered keystone species in ecosystems, contributing to the stabilization of benthic or intertidal habitats by forming large beds (Meadows et al. 1998). They also have important ecological roles such as maintaining water quality and participating in the cycling of nutrients from the water column to the benthos (Prins and Smaal 1994; Ostroumov 2005). These benefits are primarily a result of their filter feeding capacity. Mussels filter water through their gills in order to obtain their food source, suspended organic matter mainly in the form of phytoplankton (Gosling 1992). The amount of water filtered may vary according to the amount of suspended particles found in the water. For example, mussels may decrease their clearance rate (i.e.: circulate less water through their gills) when particle concentration is high in the water (Widdows et al. 1979; Babarro et al. 2000). Mussels may also deal with high particle concentrations by selecting the types of particles they want to ingest and rejecting the undesired ones in the form of pseudofeces (Shumway et al. 1985; Ward and Shumway 2004). Understanding the feeding preferences of mussels is of ecological importance, as it may determine the success and competitive advantages of non-native species.

As species arrive in new locations they have the potential to affect terrestrial and aquatic ecosystems on a global scale (Simberloff et al. 2013). The success of species in their new habitat will depend on environmental conditions in relation to their characteristics and preferences (Leung and Mandrak 2007). If individuals succeed in reaching a new site, most will perish from a multitude of physical or biotic agents (Mack 1995). If individuals persist in the new habitat, they may increase in abundance and spread, potentially causing detrimental environmental effects (Elton 1958; Mack et al. 2000). Coastal systems are some of the most highly invaded ecosystems on earth, often as a result of human activities (Grosholz 2002); ships (ballast water, hull fouling), aquaculture imports, and live trade are vectors typically responsible for introducing aquatic invertebrate species (Ruiz and Carlton 2003). Moreover, hard artificial structures in the marine environment may be acting as ‘stepping stones’ between regions that facilitate the spread of invasive species (Airoldi et al. 2015).

The east coast of Florida, including the St. Johns River and the Indian River Lagoon, has populations of many non-native species including two mussel species. The charru mussel, *Mytella 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). The charru mussel was first reported in Florida from a population in an intake valve at the Jacksonville power plant (Lee 1987). However, it was considered extirpated by a freeze in 1987. Presently, the charru mussel is found along the southeastern coast of the US from central Florida to South Carolina (Spinuzzi et al. 2013). This species has a wide salinity range  ranging from 2 to 31 ppt with 100% mortality at 0 ppt and 45 ppt (Yuan et al. 2010). The green mussel, *Perna viridis*, is an economically important marine bivalve in the Pacific, native to the tropical Indo-Pacific (Siddall 1980; Vakily 1989; Baker et al. 2007), but has been introduced to Asian and Central American countries and to North America (Baker et al. 2007). The preferred salinity of

*P. viridis* is 27 to 33 ppt, though it is found in salinities from 19 - 44 ppt (Siddall 1980; Vakily 1989). However, the species is fairly tolerant of large fluctuations in salinity, surviving in the laboratory at salinities as low as 5 ppt (Yuan et al 2016), though salinities lower than ~20 ppt will affect survival (Siddall 1980; Vakily 1989). Within the United States, the invasive range of *P. viridis* is spreading along the Florida coast and northward towards South Carolina (Spinuzzi et al. 2013). These regions are characterized by sedimentary habitats, and the spread of this species has been facilitated by artificial structures (Firth et al. 2016).

*Ischadium recurvum* is an abundant, intertidal estuarine species found on oyster reefs and other hard substrates in the southeastern United States (Bahr 1981). The hooked mussel is tolerant of a wide salinity range, and has been reported in the field at salinities of 2.5-40 ppt; however, increased mortality is found at salinities lower than 5ppt (Parker 1959, Allen 1960, Castagna and Chanley 1973). Using *in situ* feeding assays, we assessed the feeding behavior of *I. recurvum* and the two invasive mussel species (*Mytella charruana* and *Perna viridis*) under natural conditions across invaded sites within the St. Johns River. We sought to determine the potential competition among these species by comparing feeding behavior throughout their salinity ranges. We hypothesized that the invasive mussels would have higher feeding rates because successful invaders often have more general and/or efficient feeding habits than native species. Moreover, we related the feeding behavior with water characteristics (e.g. salinity and seston content) in multiple sites along a salinity gradient to elucidate potential distribution of the invasive and native mussels and determine any overlapping distributions. This field study is of importance for predicting future potentially invaded areas and warning of possible ecosystem disruption by the invasive mussels.

## Materials and Methods

### Location and animal collection

Three mussel species were studied: two invasive species, the charru mussel (*Mytella charruana* (d'Orbigny, 1842)) and the green mussel (*Perna viridis* (Linnaeus, 1758)), and the native hooked mussel (*Ischadium recurvum* (Rafinesque, 1820)) (Online Resource 1). Filter feeding experiments were performed during four consecutive days in a different location each day along the St. Johns River in Jacksonville, Florida, during May 2016 (Fig. 1). The sites, Metropolitan Park Marina (30.319, -81.642); Lonnie Wurn Boat Ramp (30.375, -81.585); Browns Creek fish camp (30.417, -81.533); and Mayport Boat Ramp (30.396, -81.428), were chosen to relate the feeding behavior of the three species to different environmental parameters and water characteristics, e.g. salinity, and total and organic matter in the water column. Each experiment contained six adult mussels of each species (average shell lengths ± SE: 34.8 ± 1.4 mm for *I. recurvum*, 32.5 ± 1.0 mm for *M. charruana*, and 44.4 ± 1.8 mm for *P. viridis)*.

All mussels were collected in Jacksonville, Florida, at two locations. *I. recurvum* and *M. charruana* were collected from the floating dock at Lonnie Wurn Boat Ramp, whereas *P. viridis* individuals were collected from the jetty at Huguenot Memorial Park (Fig. 1). All mussels were cleaned of epiphytes and other encrusting organisms and hung in mesh bags at each site at least two days before experiments to allow them to acclimate to environmental conditions. Controls were made by removing the mussels' meat and gluing their respective shells together (two controls per experiment).

## Water characteristics

Physical water characteristics (temperature (°C), salinity (ppt), and dissolved oxygen (mg $L^{-1}$)) were measured with a YSI meter at the beginning and end of each experiment.

To determine characteristics of the seston, between 80 and 140 ml (enough to clog the filters) of St. Johns River water were collected from the control filter-feeding chambers (explained below) every 15 minutes for 2 hours (n = 7 for each experiment). In the laboratory, all filters were dried at 60 ºC for 48 hours and weighed to obtain the total particulate matter (TPM). The filters were then ashed at 450 ºC for 4 hours to obtain the particulate inorganic matter (PIM). The particulate organic matter (POM) was calculated as the weight loss between TPM and PIM. Percentage of organic matter in the water was calculated as (POM / TPM) x 100.

## Physiological feeding parameters

Two portable, filter feeding, flow-through devices were designed to simulate *in situ* conditions of bivalve feeding (Fig. 2). The devices were slightly modified from Galimany et al. (2011). Each device consisted of a common PVC tank (66 cm length x 30.5 cm width x 14.5 cm height) that received water from an underwater pump. Aeration in the common tank prevented particle settlement. The common tank connected to the 10 PVC chambers (18.5 cm length x 5.7 cm width x 5.7 cm height) through individual rubber tubes. Each chamber contained a single live mussel, except for the control. Individual mussels were positioned near the flow exit tube of the chambers and 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 $h^{-1}$.

Six mussels (each one considered a replicate) of each species and two controls were placed in the individual chambers of the feeding devices (three individuals of each species and one control in each feeding device). All experimental mussels were allowed to recover from any stress associated with handling for at least one hour. 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 devices, feces and pseudofeces were collected with a pipette as soon as they were produced, and kept separately throughout the 2h experimental collection time. All samples of feces and pseudofeces were filtered separately by chamber through preweighed Whatman GF/C filters (25 mm Ø) and rinsed with ammonium formate to dissolve salt from the samples on the filters. In the laboratory, all filters were dried at 60 ºC for 48 hours and weighed to determine dry weights of feces and pseudofeces. Then, they were ashed at 450 ºC for 4 hours to obtain the ash weight (inorganic matter). The organic matter (ash free dry weight) contained in the feces and pseudofeces was calculated as the difference between dry weight and ash weight.

The physiological parameters of the feeding behavior of the mussels (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, rejection, and egestion feeding processes. The ingestion processes are those related to the intake of food. Rejection processes are related to the elimination of material captured by the gills but not ingested, whereas the egestion processes are those related to the elimination of undigested matter. All the feeding parameters were then standardized to 1 g of dried bivalve flesh using the following equation:

$$Y_s = Y_e \text{ x } (1/W_e)^{b}$$

$Y_s$ 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 0.67, as commonly used in mussel feeding studies (Bayne et al. 1989; Bayne et al. 1993; Hawkins et al. 1997).

To determine when to start feces and pseudofeces collection we calculated the gut transit time (GTT) of all mussel species before each experiment. GTT was calculated using a method adapted from Hawkins et al. (1996). Two mussels of each species were placed individually in beakers in a mixture of water and nutritious phytoplankton (*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).

## Condition index

After the feeding behavior determinations, the length of each mussel was measured by an electronic caliper. The dry weight of mussel tissue calculated to standardize the feeding behavior was also used to estimate the condition index (CI) following the equation from Martin et al. (1984):

CI = (tissue dry weight (g) x 100) / shell length (cm)

## Statistical analyses

Seston and St. Johns River water characteristics at each site were compared using a one-way ANOVA. Characteristics compared were temperature, salinity, dissolved oxygen, TPM, POM, PIM and proportion of organic matter of the river water. Proportion of water organic content was arcsine square root transformed prior to analysis. A Tukey's post hoc test determined statistical differences between the different sites.

The feeding behavior parameters studied in the four locations were analyzed using a two-way ANOVA with species and site as factors. Rejection percentage and selection efficiency were arcsine square root transformed prior to analysis. Two-way ANOVA showed significant species x site interactions for most feeding parameters; therefore, to elucidate differences in the feeding behavior among the mussels, the feeding rates were analyzed within each site with a one-way ANOVA comparing the different species. A Tukey's post hoc test determined statistical differences between the species. Non-linear exponential regression analyses were used to relate the water characteristics (salinity, TPM, and proportion of organic matter), with the physiological feeding variables, including clearance rate, filtration rate, rejection percentage, inorganic rejection rate, total ingestion rate, organic ingestion rate, and absorption rate, for each species. Salinity, TPM, and proportion of organic matter were previously checked for multicollinearity and found that they were not correlated ($R^2 < 0.9$). Correlations were examined between rejection parameters (rejection percentage and inorganic rejection rate), and ingestion rates (clearance rate, filtration rate, total ingestion rate, organic ingestion rate, and absorption rate) for each species to further understand the interaction between ingestion and rejection processes.

Condition index was compared between sites and species using a two-way ANOVA. A Tukey's post hoc test determined statistical differences between the species.

The statistical software used was SPSS Statistics 23.0 (Armonk, NY: IBM Corp.).

# **Results**

## Water characteristics

Seston characteristics were significantly different among sites (TPM: $F_{3,40} = 49.00$, $p < 0.001$; POM: $F_{3,40} = 51.37$, $p < 0.001$; PIM: $F_{3,40} = 48.11$, $p < 0.001$; Proportion of organic matter: $F_{3,40} = 53.52$, $p < 0.001$) (Fig. 3a; Online Resource 2). A Tukey's post hoc test showed that Lonnie Wurn had the most TPM, POM and PIM, whereas Metropolitan Park Marina had the lowest TPM and PIM (Fig. 3a).

Temperature and dissolved oxygen were not different among sites ($F_{3,4} = 5.17$, $p = 0.073$; $F_{3,4} = 0.26$, $p = 0.854$, respectively) (Online Resource 2). On average (± SE), temperatures were 24.58 ± 0.40°C and dissolved oxygen was 7.42 ± 0.18 mg $L^{-1}$. In contrast, salinity and percentage of organic matter in the water were different among sites ($F_{3,4} = 96.61$, $p < 0.001$ and $F_{3,40} = 53.52$, $p < 0.001$, respectively). Salinity was the highest at the mouth of the river (Browns Creek and Mayport) and the lowest in Metropolitan Park Marina (Fig. 3b; Online Resource 2). Inversely, the percentage of organic matter was highest in Metropolitan Park Marina, whereas Mayport, the river mouth, showed the lowest values.

## Physiological feeding parameters

There were significant effects of species and site on feeding as well as significant interactions between species and site. The species x site interaction was significant for all feeding parameters except selection efficiency (clearance rate, $F_{5,44} = 7.31$, $p < 0.001$; filtration rate, $F_{5,44} = 7.27$, $p < 0.001$; rejection percentage, $F_{5,44} = 7.02$, $p < 0.001$; inorganic rejection rate, $F_{5,44} = 7.15$, $p < 0.001$; total ingestion rate, $F_{5,44} = 2.81$, $p = 0.028$; organic ingestion rate, $F_{5,44} = 5.72$, $p < 0.001$; absorption rate, $F_{5,44} = 7.22$, $p < 0.001$) (Online Resource 3). Selection efficiency differed among sites but not among species (Online Resource 3). Results plotting the one-way ANOVA tests are shown in two separated graphs (Fig. 4 and Fig. 5) to better read the values on the y-axis. One-way ANOVA showed no significant differences among the mussels for any feeding parameters at Metropolitan Park Marina (Fig. 4 and Fig. 5, Online Resource 4). At Lonnie Wurn Boat Ramp, the rejection percentage was the only parameter that showed statistical differences ($F_{2,12} = 6.50$, $p = 0.012$) (Online Resource 4); *M. charruana* had the lowest value whereas *P. viridis* had the highest. At Browns Creek, all feeding parameters showed significant differences except for rejection percentage (Online Resource 4). *M. charruana* had the highest clearance rate ($F_{2,13} = 17.72$, $p < 0.001$), filtration rate ($F_{2,13} = 15.10$, $p < 0.001$), inorganic rejection rate ($F_{2,13} = 9.84$, $p = 0.002$), and absorption rate ($F_{2,13} = 13.42$, $p = 0.001$). The total ingestion rate was the lowest for *I. recurvum* ($F_{2,13} = 13.24$, $p = 0.001$), whereas the organic ingestion rate was significantly different for the three species ($F_{2,13} = 17.61$, $p < 0.001$) with *I. recurvum* having the lowest value and *P. viridis* the highest. At Mayport, the three species differed in clearance rate ($F_{2,10} = 5.87$, $p = 0.021$), filtration rate ($F_{2,10} = 5.90$, $p = 0.020$), total ingestion rate ($F_{2,10} = 12.24$, $p = 0.002$), and organic ingestion rate ($F_{2,10} = 9.24$, $p = 0.005$) (Online Resource 4). In all cases, *P. viridis* had the highest values.

## Relationship between feeding parameters and water characteristics

The relationship between feeding parameters and water characteristics (e.g. salinity, TPM, and proportion of organic matter; Table 2) was different for each of the three mussels. *I. recurvum* had a negative relationship between salinity and clearance rate (F = 14.06, p = 0.002), filtration rate (F = 6.02, p = 0.028), organic ingestion rate (F = 6.98, p = 0.019), and absorption rate (F = 5.12, p = 0.040). For the invasive mussel *M. charruana*, rejection percentage (F = 7.58, p = 0.013) and inorganic rejection rate (F = 5.62, p = 0.029) were positively related to salinity. Moreover, a positive relationship was observed for this same species between total ingestion rate (F = 5.82, p = 0.026), organic ingestion rate (F = 10.15, p = 0.005) and absorption rate (F = 13.13, p = 0.002) and the proportion of organic matter in the water. The invasive mussel *P. viridis* had two different water characteristics related to feeding parameters: total and organic ingestion rates were negatively related to salinity (F = 10.76, p = 0.005; F = 13.23, p = 0.002, respectively) and positively related to total particulate matter (F = 22.70, $p < 0.001$; F = 11.67, p = 0.004, respectively).

## Correlations between feeding parameters

The statistically significant values for the correlations established between rejection percentage and inorganic rejection rate, and the feeding variables (clearance rate, filtration rate, total ingestion rate, organic ingestion rate, and absorption rate) for each species are shown in Table 3. There was a positive relationship between all feeding variables and inorganic rejection rate ($p \leq 0.005$) for the native mussel *I. recurvum*; however, none of the feeding variables was related to rejection percentage. For *M. charruana*, total ingestion rate was negatively correlated with rejection percentage (p = 0.032). Additionally, the inorganic rejection rate of *M. charruana* was correlated ($p < 0.001$) to all ingestion-related variables except for total ingestion rate. The rejection percentage of the invasive *P. viridis* was positively correlated with clearance, filtration, and absorption rates ($p < 0.05$). All feeding parameters were positively correlated to inorganic rejection rate for *P. viridis* ($p \leq 0.01$) (Table 3).

## Condition Index

Condition index was different among species ($F_{2,49} = 56.47$, $p < 0.001$) (Fig. 6). A Tukey's post hoc test indicated that the native mussel had the lowest condition index and the invasive *P. viridis* had the highest. The condition index was not different among sites ($F_{3,49} = 2.32$, p = 0.087), and there was no significant species x site interaction ($F_{5,49} = 0.11$, p = 0.99) (Online Resource 5).

# Discussion

The invasive mussel species (*M. charruana* and *P. viridis*) found on the east coast of Florida have a similar feeding performance as the native mussels (*I. recurvum*) and salinity was the main driver of the feeding behavior. *I. recurvum* preferred low salinity, and salinity related to most of the measured feeding parameters. The invasive species preferred high loads of total particulate matter and proportion of organic matter, while salinity was only related to some of their feeding parameters. Understanding feeding behavior and preferred environmental conditions can help to predict invasion potential. Moreover, this understanding may elucidate the role and effects of filter feeding in the ecosystem. The different preferences observed in this study indicate feeding overlap among the species but also limits on their distributions. Thus, *M. charruana* and *P. viridis* represent a potential threat for native mussels in some areas but not others.

Water characteristics may affect the feeding physiology of bivalves by decreasing their feeding under stressful environmental conditions (Gosling 2003). As the invasive mussels were found within the native habitat of *I. recurvum*, it is evident that the water characteristics, i.e. temperature, salinity and dissolved oxygen, were well within the tolerance limits of the three species. However, salinity was one of the most important environmental factors influencing the feeding behavior of the three studied species. When salinity decreased to 8.7 ppt, *P. viridis* stopped feeding and did not produce any biodeposits. Salinities under 19 ppt are stressful for this species, though survival has been observed at salinities as low as 5 ppt in the laboratory (Siddall 1980; Vakily 1989). Similarly, the invasive Indo-Pacific mussel *Brachidontes pharaonic* in the Mediterranean Sea decreased its clearance rate when salinity and temperature reached their lower thresholds (Sarà et al. 2008). However, salinity was negatively related to total and organic ingestion rates indicating that the highest salinities are not ideal for *P. viridis* either. Salinity was also inversely related to the feeding behavior of *I. recurvum*. Under laboratory conditions and salinity at 10ppt, *I. recurvum* has been reported to have maximum clearance rates of 4.6 L $h^{-1}$ at 26°C and minimum clearance rates, close to 0, below 10°C (Gedan et al. 2014). Conversely, higher salinities enhanced *M. charruana* feeding. These data indicate that while these species can exploit a wide range of salinities their preferred ranges may allow for niche partitioning that precludes the native species from being eliminated.

Bivalve feeding behavior is also affected by the seston in the water as bivalves regulate their feeding according to seston concentrations (Hawkins et al. 1998a; Ward and Shumway 2004). In our study, *I. recurvum* was not affected by the seston levels, while *P. viridis* and *M. charruana* were. Clearance rate for *P. viridis* was the highest compared to the other species at Mayport, where the percentage of organic matter in the water was the lowest. This is in keeping with previous research demonstrating that *P. viridis* had clearance rates above average when the organic particulate matter was <

1 mg $L^{-1}$ (Hawkins et al. 1998b). For *M. charruana*, higher organic matter led to higher total, organic ingestion, and absorption rates, suggesting that this species thrives in high organic environments. These strategies maximize the food resources and suggest that both invasive species can tolerate high seston loads. Another way for bivalves to enhance food resources is through pre-ingestive organic selection. Mussels may preferentially reject inorganic particles in the form of pseudofeces to increase the amount of organic matter ingested and promote higher absorption (Bayne et al. 1993; Newell and Shumway 1993; Hawkins et al. 1996). The invasive mussels were also more efficient at pseudofeces production, leading to *I. recurvum* having lower organic ingestion. The overlap in feeding behaviors and increased efficiency of the invasive mussels suggest interspecific competition for food resources is possible; however, the three species have different preferences, which may allow them to coexist within separate microhabitats.

Condition indices (CI) among mussel species can be compared at similar gonadal stages, as the gonad develops within the mantle tissue and increases tissue weight (Gosling 1992). Though we did not study their gonadal cycles, the three mussel species had very well developed mantles when dissected, indicating gonadal maturation. In fact, both invasive mussels have been reported to reproduce the whole year round in Florida (Stenyakina et al. 2010; McFarland et al. 2016). Therefore, the differences found in the CI of the mussels are most likely an indication that the invasive mussels seem to be well adapted to the environmental conditions in the area. Moreover, invasive bivalves have been reported to have a wider tolerance range and higher survival rates under stressful environmental situations (Lenz et al. 2011), which is one reason why invasive bivalves succeed in new environments.

Understanding the distribution of invasive species and identifying potential invaded areas are essential for conservation and management purposes. The invasive mussels in Florida do not seem to be predominant in the ecosystems, but there is no official record of their abundance nor predictions on their invasive potential. Our study indicates that in the St. Johns River the invasive mussels do not share the same environmental preferences with the native *I. recurvum*; thus, the species do not fully overlap. Within the St. Johns River, salinity seems to be the main water characteristic influencing the feeding responses. This is surprising since they are all found in similar salinity ranges. However, salinity is not the only important factor in determining a species range. For example, temperature can alter salinity tolerance. *M. charruana* can survive salinities 5-44 ppt at 20°C, but their salinity range narrows considerably when the temperature fluctuates (Yuan et al 2016). In this study, we focused on comparing behaviors along a salinity gradient at similar temperatures. This was to investigate how these species might compete within local systems and determine if *I. recurvum* could potentially be outcompeted by these recent invaders. While the species do overlap, different preferences in salinity and water characteristics indicate that the three species may coexist by habitat partitioning within ecosystems. However, the efficiency, and wide ranges tolerated by *M. charruana* and *P. viridis* represent a potential threat to other, untested native filter feeders.

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Fig. 1: Detailed map of the mussel collection and sampling sites. Black arrows indicate mussel collection sites, from left to right: Lonnie Wurn Boat Ramp (collection site for *I. recurvum* and *M. charruana*); Huguenot Memorial Park (collection site for *P. viridis*). White stars indicate sites of feeding experiments, from left to right: Metropolitan Park Marina; Lonnie Wurn Boat Ramp; Browns Creek fish camp; and Mayport Boat Ramp. The top left square image shows the SE of the USA with a black square locating the Jacksonville area.

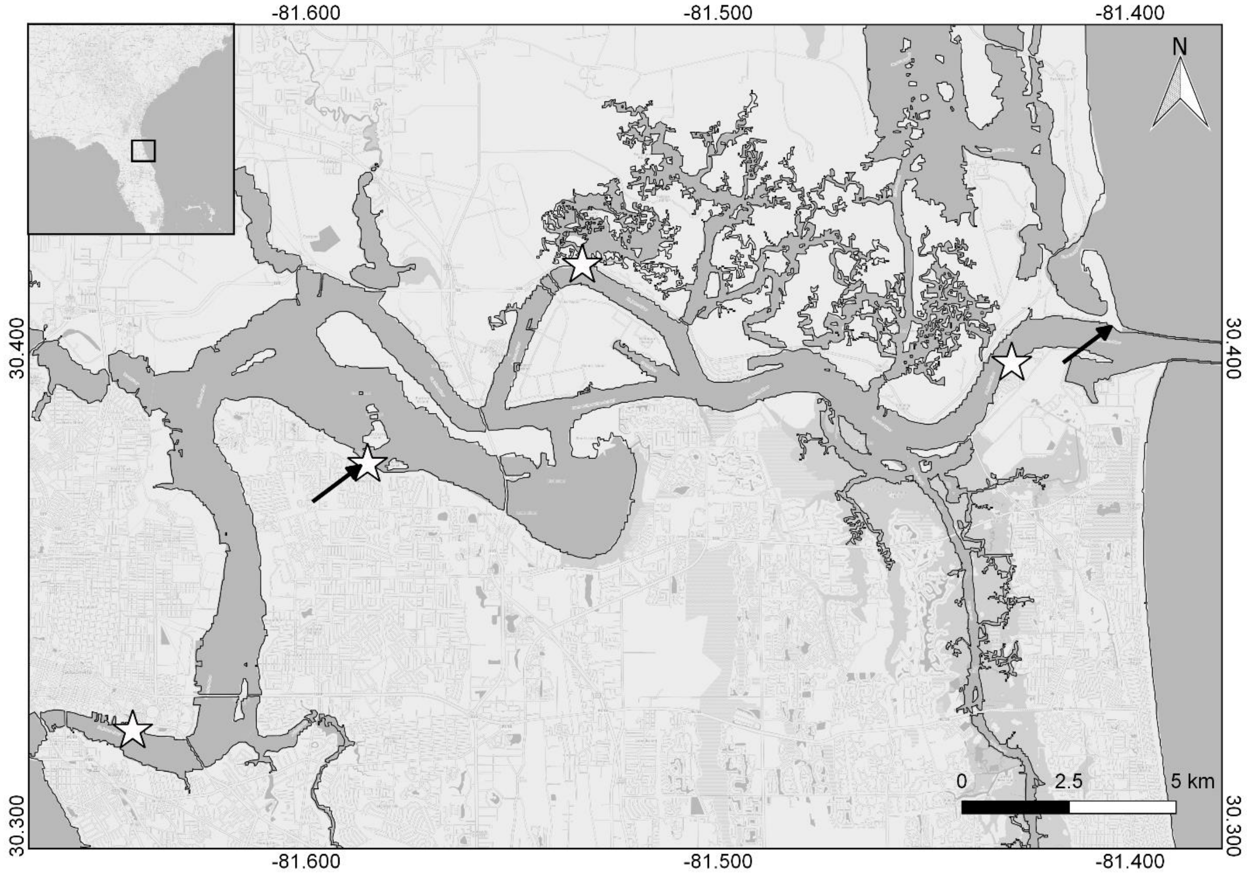

Fig. 2: Image detail of a portable, flow-through device used for the filter feeding experiments. 1: tube transporting water pumped from the environment to the device; 2: PVC “reservoir” tank; 3: plastic tubes with valves to regulate flow connecting the “reservoir” tank with each individual chamber; 4: individual chambers that each hold a single bivalve. White arrows show water flow direction.

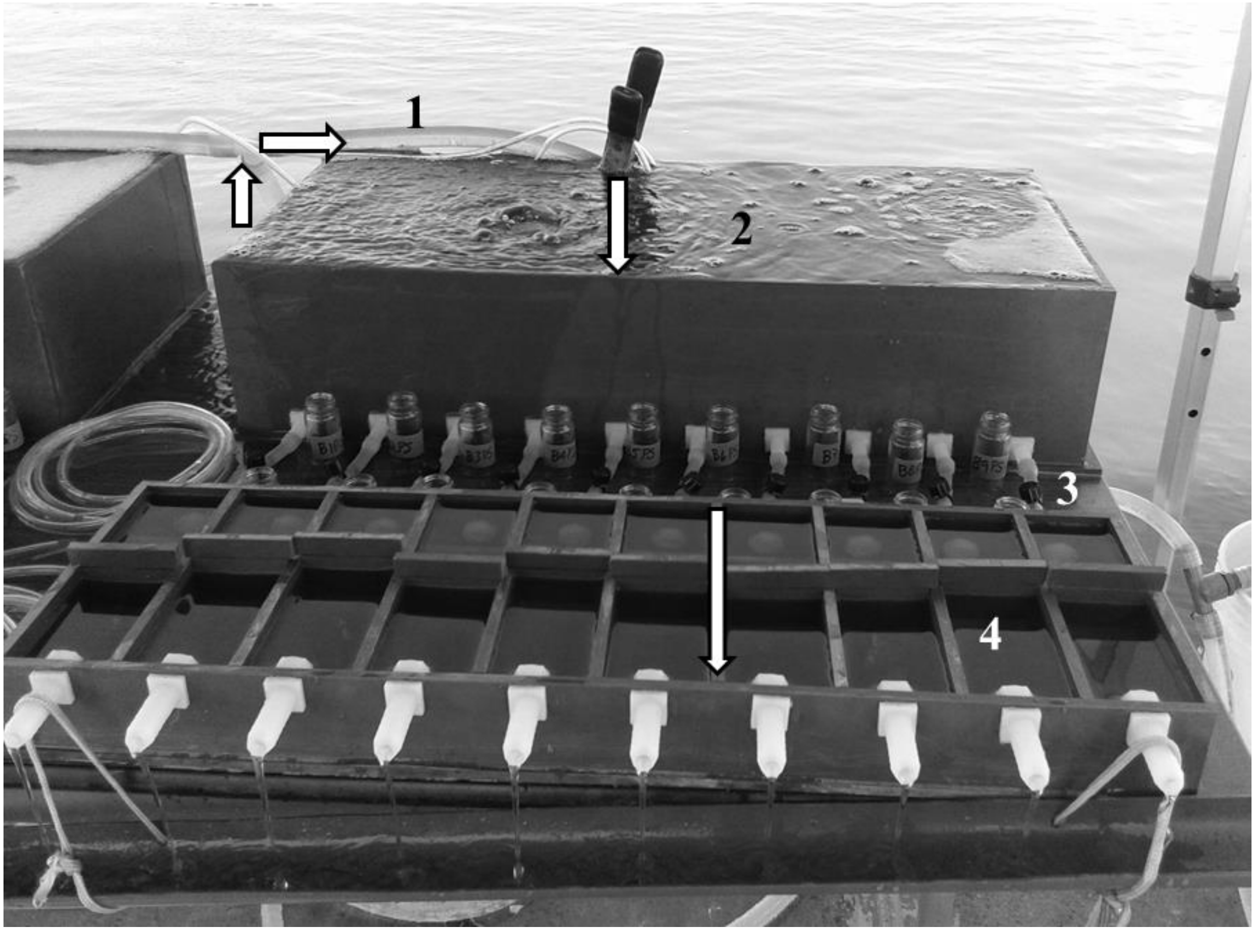

Fig. 3: Average (± SE) values for seston and water characteristics at each site. a: Column bars denote values for the total particulate matter (TPM) which are subdivided into particulate organic matter (POM) and particulate inorganic matter (PIM). Error bars on the top of the column are for POM whereas lower errors bars, on top of the grey bar, are for PIM. Numbers denote significant differences for TPM, letters denote significant differences for POM and letters in italics denote significant differences for PIM. b: Percentage of organic matter in the water (Organic) on the left Y axis (black line) and Salinity on the right Y axis (gray line). Letters denote significant differences for organic and letters in italics denote significant differences for salinity.

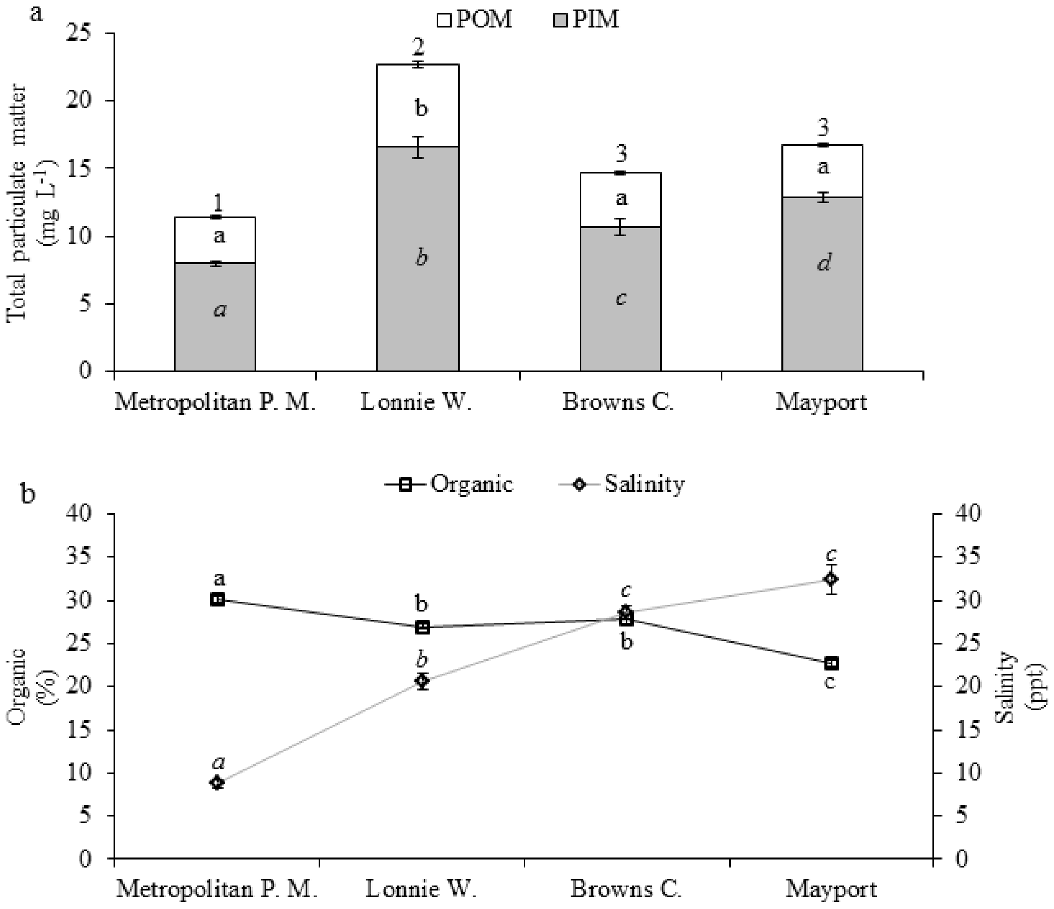

Fig. 4: Results of the one-way ANOVA tests for the feeding parameters clearance rate (CR; L $h^{-1}$), organic ingestion rate (OIR; mg $h^{-1}$), and absorption rate (AR; mg $h^{-1}$). Column bars denote average values (± SE) at each sampling site for the three mussel species: *I. recurvum*, *M. charruana*, and *P. viridis*. *P. viridis* bars are absent from the Metropolitan Park Marina site because none of the animals fed. Letters on top of each column denote statistical differences by Tukey's tests ($p < 0.05$); no letters denote no significant differences.

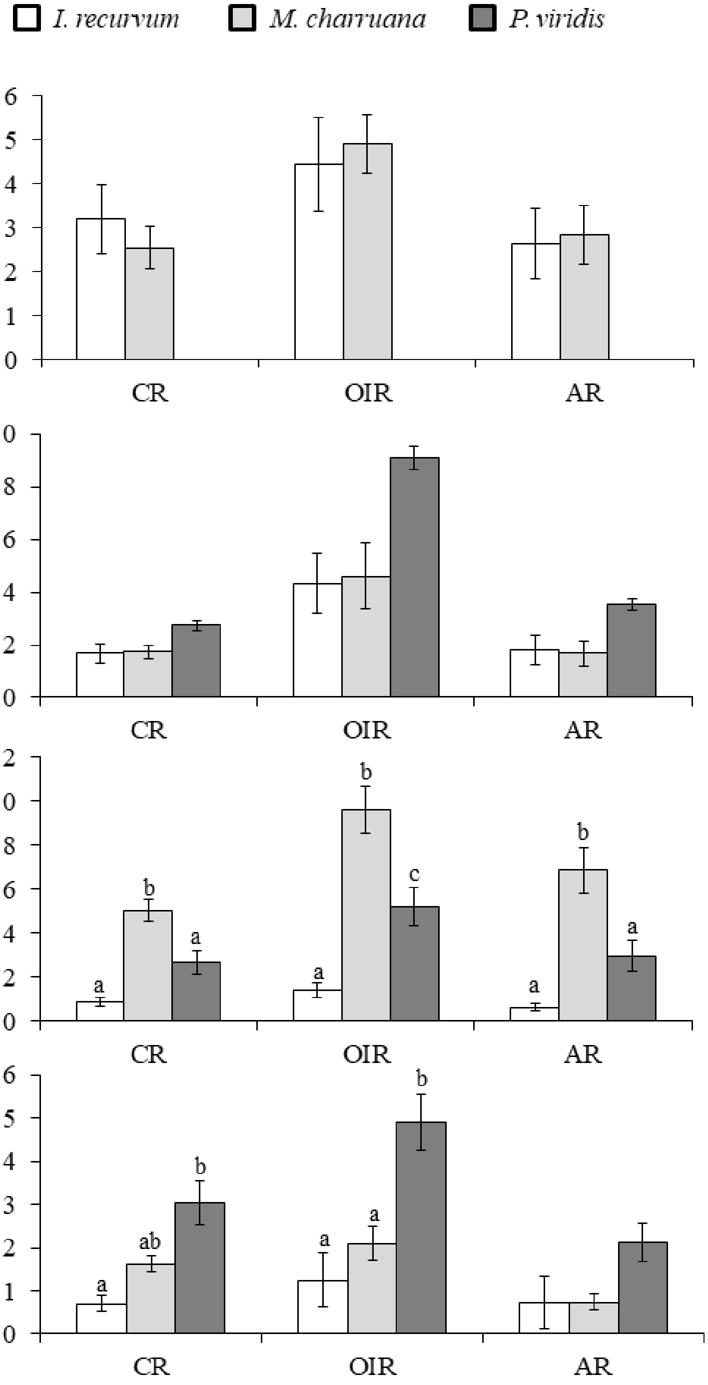

Fig. 5: Results of the one-way ANOVA tests for the feeding parameters filtration rate (FR; mg $h^{-1}$), rejection percent (RP; %), inorganic rejection rate (IRR; mg $h^{-1}$), and total ingestion rate (TIR; mg $h^{-1}$). Column bars denote average values (± SE) at each sampling site for the three mussel species: *I. recurvum*, *M. charruana*, and *P. viridis*. *P. viridis* bars are absent from the Metropolitan Park Marina site because none of the animals fed. Letters on top of each column denote statistical differences by Tukey's tests ($p < 0.05$); no letters denote no significant differences.

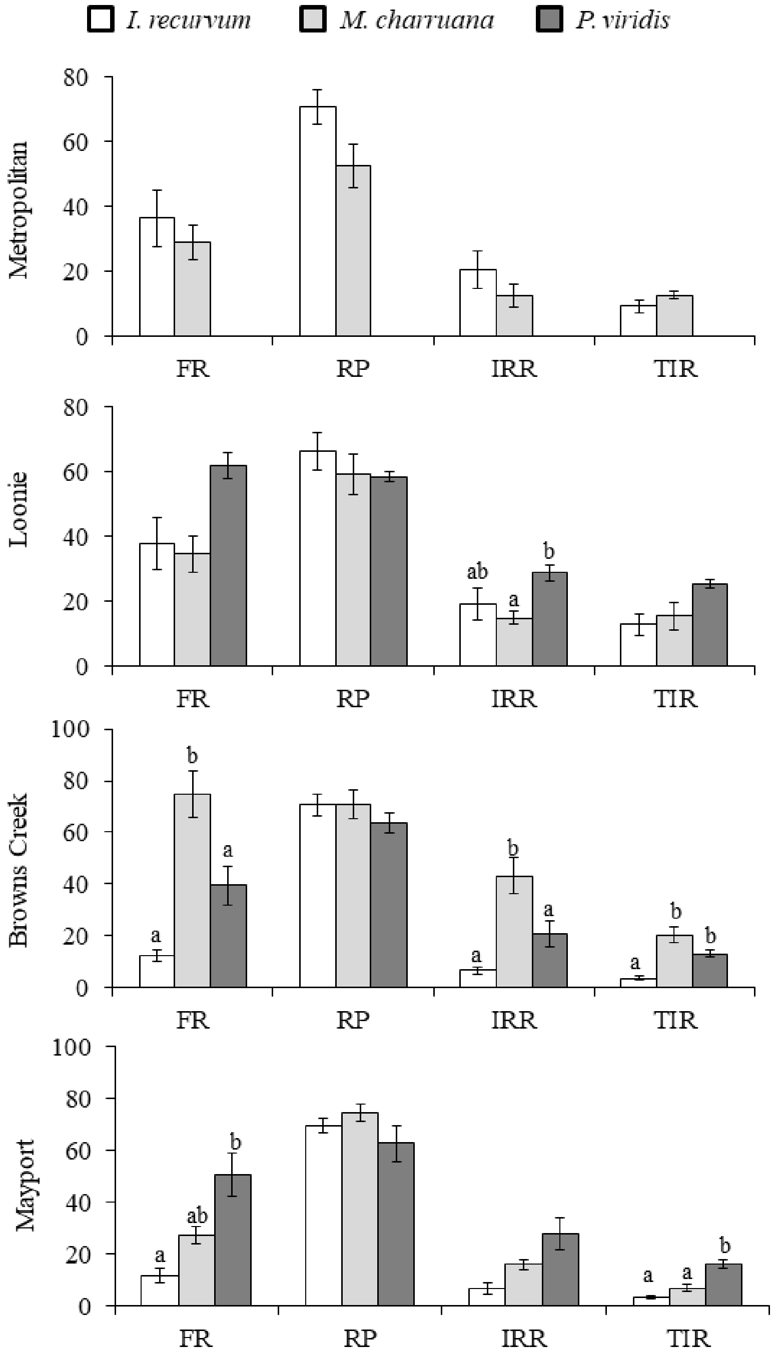

Fig. 6: Average (± SE) of the condition index for the different mussel species. Letters denote significant differences by Tukey's test.

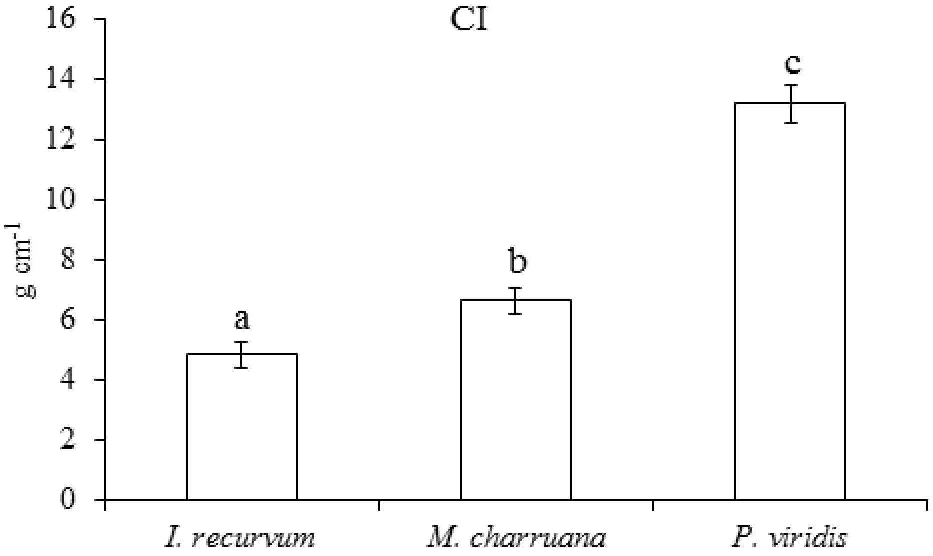

Table 1: Description of the different variables of the feeding behavior of the mussels. TPM: total particulate matter from the water (mg $L^{-1}$); PIM: particulate inorganic matter from the water (mg $L^{-1}$); POM: particulate organic matter from the water (mg $L^{-1}$).

| Parameter (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 $h^{-1}$) | Total particulate matter from the seawater retained in the gills per unit of time | Clearance rate x TPM water |
| Rejection percentage (%) | Total particulate matter 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) x 100 |
| Inorganic rejection rate (mg $h^{-1}$) | Particulate inorganic matter that has been retained in the gills but rejected prior to ingestion | mg inorganic matter from pseudofeces per unit of time |
| Selection efficiency (%) | Pre-ingestive selection of organic matter through pseudofeces production | [1 – ((mg organic matter from pseudofeces per unit of time / mg organic and inorganic matter from pseudofeces per unit of time) / (organic matter in the water))] x 100 |
| Total ingestion rate (mg $h^{-1}$) | Total particulate matter retained in the gills and ingested by the bivalve per unit of time | Filtration rate – mg organic and inorganic matter from pseudofeces per unit of time |
| Organic ingestion rate (mg $h^{-1}$) | Particulate organic matter retained in the gills and ingested by the bivalve per unit of time | (Clearance rate x POM water) – (mg organic matter from pseudofeces per unit of time) |
| Absorption rate (mg $h^{-1}$) | Particulate organic matter 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) |

Table 2: Exponential regression analyses results between the feeding physiological parameters and the water characteristics, i.e. salinity, total particulate matter (TPM), and proportion of organic matter (organic). NC: no correlations.

| Feeding variable (acronym) | *I. recurvum* | *M. charruana* | *P. viridis* |
|---|---|---|---|
| Clearance rate (CR) | $CR = 4.47\ (\pm 1.50)\ e^{(-0.06 \pm 0.02)\text{ x salinity}}$<br>F = 14.06; p = 0.002; $R^2 = 0.47$ | NC | NC |
| Filtration rate (FR) | $FR = 49.71\ (\pm 18.51)\ e^{(-0.04 \pm 0.02)\text{ x salinity}}$<br>F = 6.02; p = 0.028; $R^2 = 0.25$ | NC | NC |
| Rejection percentage (RP) | NC | $RP = 0.67\ (\pm 0.07)\ e^{(0.01 \pm 0.01)\text{ x salinity}}$<br>F = 7.58; p = 0.013; $R^2 = 0.25$ | NC |
| Inorganic rejection rate (IRR) | NC | $IRR = 7.33\ (\pm 2.89)\ e^{(0.04 \pm 0.02)\text{ x salinity}}$<br>F = 5.62; p = 0.029; $R^2 = 0.19$ | NC |
| Total ingestion rate (TIR) | NC | $TIR = 0.04\ (\pm 0.10)\ e^{(10.54 \pm 4.37)\text{ x organic}}$<br>F = 5.82; p = 0.026; $R^2 = 0.19$ | $TIR = 62.55\ (\pm 25.08)\ e^{(-0.05 \pm 0.01)\text{ x salinity}}$<br>F = 10.76; p = 0.005; $R^2 = 0.37$<br>$TIR = 3.95\ (\pm 1.24)\ e^{(0.08 \pm 0.02)\text{ x TPM}}$<br>F = 22.70; $p < 0.001$; $R^2 = 0.56$ |
| Organic ingestion rate (OIR) | $OIR = 6.53\ (\pm 2.71)\ e^{(-0.05 \pm 0.02)\text{ x salinity}}$<br>F = 6.98; p = 0.019; $R^2 = 0.28$ | $OIR = 0.01\ (\pm 0.01)\ e^{(14.66 \pm 4.60)\text{ x organic}}$<br>F = 10.15; p = 0.005; $R^2 = 0.31$ | $OIR = 30.29\ (\pm 13.88)\ e^{(-0.06 \pm 0.02)\text{ x salinity}}$<br>F = 13.23; p = 0.002; $R^2 = 0.42$<br>$OIR = 1.33\ (\pm 0.59)\ e^{(0.08 \pm 0.02)\text{ x TPM}}$<br>F = 11.67; p = 0.004; $R^2 = 0.39$ |
| Absorption rate (AR) | $AR = 3.47\ (\pm 2.08)\ e^{(-0.06 \pm 0.03)\text{ x salinity}}$<br>F = 5.12; p = 0.040; $R^2 = 0.22$ | $AR = 9.97 \text{ x } 10^{-6}\ (\pm 0.00)\ e^{(22.57 \pm 6.23)\text{ x organic}}$<br>F = 13.13; p = 0.002; $R^2 = 0.38$ | NC |

Table 3: Pearson correlation (r) values and statistical significance (p) for the relationships between feeding variables for the three mussel species. Pearson r value indicates if the correlation is positive or negative. RP: Rejection percentage arcsine square root transformed for analysis; IRR: Inorganic rejection rate; CR: Clearance rate; FR: Filtration rate; TIR: Total ingestion rate; OIR: Organic ingestion rate; AR: Absorption rate. Asterisks denote significant differences.

| | | RP | | | IRR | | |
|---|---|---|---|---|---|---|---|
| | | *I. recurvum* | *M. charruana* | *P. viridis* | *I. recurvum* | *M. charruana* | *P. viridis* |
| CR | r | 0.334 | 0.340 | 0.837* | 0.911* | 0.926* | 0.937* |
| | p | 0.207 | 0.131 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| FR | r | 0.234 | 0.267 | 0.632* | 0.977* | 0.929* | 0.960* |
| | p | 0.383 | 0.241 | 0.005 | < 0.001 | < 0.001 | < 0.001 |
| TIR | r | -0.308 | -0.468* | 0.099 | 0.668* | 0.307 | 0.590* |
| | p | 0.246 | 0.032 | 0.697 | 0.005 | 0.176 | 0.010 |
| OIR | r | -0.040 | -0.127 | 0.229 | 0.848* | 0.700* | 0.643* |
| | p | 0.882 | 0.583 | 0.360 | < 0.001 | < 0.001 | 0.004 |
| AR | r | 0.189 | 0.205 | 0.501* | 0.898* | 0.893* | 0.712* |
| | p | 0.484 | 0.373 | 0.034 | < 0.001 | < 0.001 | 0.001 |

Online Resource 1: Example of each species of mussel used for the feeding study. 1. *Perna viridis*; 2. *Ischadium recurvum*; 3. *Mytella charruana*.

Online Resource 2: One-way ANOVA comparing seston and river water characteristics at each site. TPM: total particulate matter; POM: particulate organic matter; PIM: particulate inorganic matter; Organic: proportion of organic matter arcsine square root transformed for analysis; DO: dissolved oxygen.

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| TPM | Between Groups | 674.313 | 3 | 224.771 | 49.005 | < 0.001 |
| | Within Groups | 183.466 | 40 | 4.587 | | |
| | Total | 857.779 | 43 | | | |
| POM | Between Groups | 43.981 | 3 | 14.660 | 51.367 | < 0.001 |
| | Within Groups | 11.416 | 40 | 0.285 | | |
| | Total | 55.397 | 43 | | | |
| PIM | Between Groups | 401.710 | 3 | 133.903 | 48.115 | < 0.001 |
| | Within Groups | 111.321 | 40 | 2.783 | | |
| | Total | 513.031 | 43 | | | |
| Organic | Between Groups | 0.045 | 3 | 0.015 | 53.524 | < 0.001 |
| | Within Groups | 0.011 | 40 | 0.000 | | |
| | Total | 0.057 | 43 | | | |
| Temperature | Between Groups | 7.245 | 3 | 2.415 | 5.166 | 0.073 |
| | Within Groups | 1.870 | 4 | 0.468 | | |
| | Total | 9.115 | 7 | | | |
| Salinity | Between Groups | 654.355 | 3 | 218.118 | 96.608 | < 0.001 |
| | Within Groups | 9.031 | 4 | 2.258 | | |
| | Total | 663.386 | 7 | | | |
| DO | Between Groups | 0.307 | 3 | 0.102 | 0.256 | 0.854 |
| | Within Groups | 1.600 | 4 | 0.400 | | |
| | Total | 1.907 | 7 | | | |

Online Resource 3: Two-way ANOVA table for the feeding behavior with site and species as factors. CR: Clearance rate; FR: Filtration rate; RP: Rejection percentage arcsine square root transformed for analysis; IRR: Inorganic rejection rate; SE: Selection efficiency arcsine square root transformed for analysis; TIR: Total ingestion rate; OIR: Organic ingestion rate; AR: Absorption rate.

| Source | Dependent Variable | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Corrected Model | CR | 70.127[a] | 10 | 7.013 | 5.718 | < 0.001 |
| | FR | 16813.399[b] | 10 | 1681.340 | 5.905 | < 0.001 |
| | RP | 8616.422[c] | 10 | 861.642 | 4.576 | < 0.001 |
| | IRR | 5616.841[d] | 10 | 561.684 | 4.734 | < 0.001 |
| | SE | 0.323[e] | 10 | 0.032 | 2.442 | 0.020 |
| | TIR | 2400.766[f] | 10 | 240.077 | 6.389 | < 0.001 |
| | OIR | 359.013[g] | 10 | 35.901 | 7.302 | < 0.001 |
| | AR | 158.538[h] | 10 | 15.854 | 6.989 | < 0.001 |
| Intercept | CR | 303.796 | 1 | 303.796 | 247.719 | < 0.001 |
| | FR | 78157.777 | 1 | 78157.777 | 274.494 | < 0.001 |
| | RP | 33242.380 | 1 | 33242.380 | 176.558 | < 0.001 |
| | IRR | 20549.889 | 1 | 20549.889 | 173.184 | < 0.001 |
| | SE | 8.940 | 1 | 8.940 | 676.863 | < 0.001 |
| | TIR | 9456.044 | 1 | 9456.044 | 251.637 | < 0.001 |
| | OIR | 1287.988 | 1 | 1287.988 | 261.977 | < 0.001 |
| | AR | 328.399 | 1 | 328.399 | 144.763 | < 0.001 |
| Site | CR | 13.747 | 3 | 4.582 | 3.736 | 0.018 |
| | FR | 1788.232 | 3 | 596.077 | 2.093 | 0.115 |
| | RP | 498.418 | 3 | 166.139 | 0.882 | 0.458 |
| | IRR | 296.808 | 3 | 98.936 | 0.834 | 0.483 |
| | SE | 0.145 | 3 | 0.048 | 3.653 | 0.019 |
| | TIR | 842.159 | 3 | 280.720 | 7.470 | < 0.001 |
| | OIR | 92.474 | 3 | 30.825 | 6.270 | 0.001 |
| | AR | 33.473 | 3 | 11.158 | 4.918 | 0.005 |
| Species | CR | 15.708 | 2 | 7.854 | 6.404 | 0.004 |
| | FR | 4722.271 | 2 | 2361.135 | 8.292 | 0.001 |
| | RP | 1559.971 | 2 | 779.986 | 4.143 | 0.022 |
| | IRR | 1036.605 | 2 | 518.302 | 4.368 | 0.019 |
| | SE | 0.055 | 2 | 0.028 | 2.096 | 0.135 |
| | TIR | 862.969 | 2 | 431.485 | 11.482 | < 0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | OIR | 102.593 | 2 | 51.297 | 10.434 | < 0.001 |
| | AR | 27.221 | 2 | 13.610 | 6.000 | 0.005 |
| Site x Species | CR | 44.818 | 5 | 8.964 | 7.309 | < 0.001 |
| | FR | 10347.166 | 5 | 2069.433 | 7.268 | < 0.001 |
| | RP | 6606.750 | 5 | 1321.350 | 7.018 | < 0.001 |
| | IRR | 4242.652 | 5 | 848.530 | 7.151 | < 0.001 |
| | SE | 0.100 | 5 | 0.020 | 1.508 | 0.207 |
| | TIR | 527.566 | 5 | 105.513 | 2.808 | 0.028 |
| | OIR | 140.730 | 5 | 28.146 | 5.725 | < 0.001 |
| | AR | 81.930 | 5 | 16.386 | 7.223 | < 0.001 |
| Error | CR | 53.960 | 44 | 1.226 | | |
| | FR | 12528.294 | 44 | 284.734 | | |
| | RP | 8284.309 | 44 | 188.280 | | |
| | IRR | 5221.004 | 44 | 118.659 | | |
| | SE | 0.581 | 44 | 0.013 | | |
| | TIR | 1653.435 | 44 | 37.578 | | |
| | OIR | 216.322 | 44 | 4.916 | | |
| | AR | 99.815 | 44 | 2.269 | | |
| Total | CR | 486.854 | 55 | | | |
| | FR | 123946.859 | 55 | | | |
| | RP | 57452.620 | 55 | | | |
| | IRR | 35952.116 | 55 | | | |
| | SE | 10.614 | 55 | | | |
| | TIR | 15333.603 | 55 | | | |
| | OIR | 2128.132 | 55 | | | |
| | AR | 663.964 | 55 | | | |
| Corrected Total | CR | 124.088 | 54 | | | |
| | FR | 29341.692 | 54 | | | |
| | RP | 16900.730 | 54 | | | |
| | IRR | 10837.845 | 54 | | | |
| | SE | 0.904 | 54 | | | |
| | TIR | 4054.201 | 54 | | | |
| | OIR | 575.335 | 54 | | | |
| | AR | 258.354 | 54 | | | |

a. $R^2 = 0.565$ (Adjusted $R^2 = 0.466$)

b. $R^2 = 0.573$ (Adjusted $R^2 = 0.476$)

c. $R^2 = 0.510$ (Adjusted $R^2 = 0.398$)

d. $R^2 = 0.518$ (Adjusted $R^2 = 0.409$)
e. $R^2 = 0.357$ (Adjusted $R^2 = 0.211$)
f. $R^2 = 0.592$ (Adjusted $R^2 = 0.499$)
g. $R^2 = 0.624$ (Adjusted $R^2 = 0.539$)
h. $R^2 = 0.614$ (Adjusted $R^2 = 0.526$)

Online Resource 4: One-way ANOVA table for the feeding behavior of the three species at the four different studied locations. CR: Clearance rate; FR: Filtration rate; RP: Rejection percentage arcsine square root transformed for analysis; IRR: Inorganic rejection rate; SE: Selection efficiency arcsine square root transformed for analysis; TIR: Total ingestion rate; OIR: Organic ingestion rate; AR: Absorption rate.

| Metropolitan Park Marina | | | | | | |
|---|---|---|---|---|---|---|
| Dependent variable | | Sum of Squares | df | Mean Square | F | Sig. |
| CR | Between Groups | 1.147 | 1 | 1.147 | 0.444 | 0.522 |
| | Within Groups | 23.264 | 9 | 2.585 | | |
| | Total | 24.411 | 10 | | | |
| FR | Between Groups | 149.224 | 1 | 149.224 | 0.461 | 0.514 |
| | Within Groups | 2912.673 | 9 | 323.630 | | |
| | Total | 3061.897 | 10 | | | |
| RP | Between Groups | 0.103 | 1 | 0.103 | 4.760 | 0.057 |
| | Within Groups | 0.195 | 9 | 0.022 | | |
| | Total | 0.299 | 10 | | | |
| IRR | Between Groups | 175.082 | 1 | 175.082 | 1.311 | 0.282 |
| | Within Groups | 1202.221 | 9 | 133.580 | | |
| | Total | 1377.303 | 10 | | | |
| TIR | Between Groups | 29.781 | 1 | 29.781 | 1.938 | 0.197 |
| | Within Groups | 138.326 | 9 | 15.370 | | |
| | Total | 168.106 | 10 | | | |
| OIR | Between Groups | 0.581 | 1 | 0.581 | 0.123 | 0.733 |
| | Within Groups | 42.393 | 9 | 4.710 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total | 42.974 | 10 | | | |
| AR | Between Groups | 0.119 | 1 | 0.119 | 0.038 | 0.850 |
| | Within Groups | 28.051 | 9 | 3.117 | | |
| | Total | 28.170 | 10 | | | |

| Lonnie Wurn Boat Ramp | | | | | | |
|---|---|---|---|---|---|---|
| Dependent variable | | Sum of Squares | df | Mean Square | F | Sig. |
| CR | Between Groups | 3.761 | 2 | 1.880 | 3.817 | 0.052 |
| | Within Groups | 5.912 | 12 | 0.493 | | |
| | Total | 9.672 | 14 | | | |
| FR | Between Groups | 1936.367 | 2 | 968.183 | 3.849 | 0.051 |
| | Within Groups | 3018.602 | 12 | 251.550 | | |
| | Total | 4954.969 | 14 | | | |
| RP | Between Groups | 0.089 | 2 | 0.044 | 3.263 | 0.074 |
| | Within Groups | 0.163 | 12 | 0.014 | | |
| | Total | 0.252 | 14 | | | |
| IRR | Between Groups | 679.191 | 2 | 339.596 | 6.500 | 0.012 |
| | Within Groups | 626.957 | 12 | 52.246 | | |
| | Total | 1306.148 | 14 | | | |
| TIR | Between Groups | 390.097 | 2 | 195.049 | 2.251 | 0.148 |
| | Within Groups | 1039.656 | 12 | 86.638 | | |
| | Total | 1429.753 | 14 | | | |
| OIR | Between Groups | 55.894 | 2 | 27.947 | 3.503 | 0.063 |
| | Within Groups | 95.746 | 12 | 7.979 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total | 151.640 | 14 | | | |
| AR | Between Groups | 7.806 | 2 | 3.903 | 2.977 | 0.089 |
| | Within Groups | 15.732 | 12 | 1.311 | | |
| | Total | 23.538 | 14 | | | |

| Browns Creek | | | | | | |
|---|---|---|---|---|---|---|
| Dependent variable | | Sum of Squares | df | Mean Square | F | Sig. |
| CR | Between Groups | 43.517 | 2 | 21.759 | 17.723 | <0.001 |
| | Within Groups | 15.961 | 13 | 1.228 | | |
| | Total | 59.478 | 15 | | | |
| FR | Between Groups | 9672.955 | 2 | 4836.478 | 15.097 | <0.001 |
| | Within Groups | 4164.747 | 13 | 320.365 | | |
| | Total | 13837.702 | 15 | | | |
| RP | Between Groups | 0.024 | 2 | 0.012 | 0.853 | 0.449 |
| | Within Groups | 0.184 | 13 | 0.014 | | |
| | Total | 0.208 | 15 | | | |
| IRR | Between Groups | 3432.076 | 2 | 1716.038 | 9.845 | 0.002 |
| | Within Groups | 2266.013 | 13 | 174.309 | | |
| | Total | 5698.089 | 15 | | | |
| TIR | Between Groups | 672.110 | 2 | 336.055 | 13.238 | 0.001 |
| | Within Groups | 330.017 | 13 | 25.386 | | |
| | Total | 1002.127 | 15 | | | |
| OIR | Between Groups | 166.388 | 2 | 83.194 | 17.615 | <0.001 |
| | Within Groups | 61.398 | 13 | 4.723 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total | 227.786 | 15 | | | |
| AR | Between Groups | 100.458 | 2 | 50.229 | 13.424 | 0.001 |
| | Within Groups | 48.641 | 13 | 3.742 | | |
| | Total | 149.100 | 15 | | | |

| Mayport | | | | | | |
|---|---|---|---|---|---|---|
| Dependent variable | | Sum of Squares | df | Mean Square | F | Sig. |
| CR | Between Groups | 10.352 | 2 | 5.176 | 5.866 | 0.021 |
| | Within Groups | 8.824 | 10 | 0.882 | | |
| | Total | 19.176 | 12 | | | |
| FR | Between Groups | 2868.411 | 2 | 1434.206 | 5.897 | 0.020 |
| | Within Groups | 2432.272 | 10 | 243.227 | | |
| | Total | 5300.683 | 12 | | | |
| RP | Between Groups | 0.046 | 2 | 0.023 | 1.236 | 0.331 |
| | Within Groups | 0.185 | 10 | 0.019 | | |
| | Total | 0.231 | 12 | | | |
| IRR | Between Groups | 794.476 | 2 | 397.238 | 3.528 | 0.069 |
| | Within Groups | 1125.813 | 10 | 112.581 | | |
| | Total | 1920.289 | 12 | | | |
| TIR | Between Groups | 356.151 | 2 | 178.075 | 12.244 | 0.002 |
| | Within Groups | 145.436 | 10 | 14.544 | | |
| | Total | 501.587 | 12 | | | |
| OIR | Between Groups | 31.017 | 2 | 15.509 | 9.239 | 0.005 |
| | Within Groups | 16.786 | 10 | 1.679 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total | 47.803 | 12 | | | |
| AR | Between Groups | 6.330 | 2 | 3.165 | 4.282 | 0.051 |
| | Within Groups | 7.391 | 10 | 0.739 | | |
| | Total | 13.721 | 12 | | | |

Online Resource 5: Two-way ANOVA table for the condition index with site and species as factors.

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 750.853[a] | 10 | 75.085 | 15.194 | < 0.001 |
| Intercept | 3738.439 | 1 | 3738.439 | 756.522 | < 0.001 |
| Site | 34.339 | 3 | 11.446 | 2.316 | 0.087 |
| Species | 558.121 | 2 | 279.060 | 56.472 | < 0.001 |
| Site x Species | 2.715 | 5 | 0.543 | 0.110 | 0.990 |
| Error | 242.139 | 49 | 4.942 | | |
| Total | 4845.009 | 60 | | | |
| Corrected Total | 992.992 | 59 | | | |

a. $R^2 = 0.756$ (Adjusted $R^2 = 0.706$)