

Facilitation in the low intertidal: effects of an invasive species on the structure of an estuarine macrozoobenthic assemblage

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ABSTRACT: The Asian clam *Corbicula fluminea* (Müller, 1774) has been recognized as one of the most important invasive alien species in aquatic ecosystems and may have significant ecological and economic impacts. Recently, the presence of *C. fluminea* was associated with changes in benthic and epibenthic fauna. In this study, we aimed to understand the mechanisms underlying the effects of *C. fluminea* on an estuarine macrozoobenthic assemblage using a manipulative experiment. We used 5 different treatments (control, rock, closed, live, open), which were placed in a low sandy intertidal soft bottom area in the Minho estuary (NW Iberian Peninsula) for 2 months. We found that the presence of live and open empty shells of *C. fluminea* had positive effects on the density, biomass and species richness of macrozoobenthos, specifically on species belonging to Annelida, Mollusca and Crustacea. Our results may be explained by 2 main mechanisms: (1) the production of feces and pseudofeces by *C. fluminea*, which increases organic matter content and food resources for some macrozoobenthic species; and (2) ecosystem engineering activities by *C. fluminea*, which can create conditions for the establishment of other species via shell production and bioturbation in the sediments.

KEY WORDS: *Corbicula fluminea* · Alien species · Ecosystem engineering · Invertebrates · Minho estuary

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INTRODUCTION

The introduction of invasive alien species (IAS) is one of the main threats to global biodiversity, causing significant changes in ecosystem structure and functioning (Grosholz 2002, Cox 2004, Davis 2009, Simberloff et al. 2013). Impacts generated by these introductions have contributed to biotic homogenization, reduction in global biodiversity and extinctions of native species (Olden 2006, Strayer 2010).

Many studies have addressed the negative impacts of IAS on native species biodiversity in terrestrial and aquatic ecosystems (Mack et al. 2000, Pimentel et al.

2000, Byers 2009). In most cases, the negative influences were due to new biotic interactions (predation, competition, allelopathy, introduction of diseases and parasitism) or by changes in biogeochemical cycles or physical structures (Ehrenfeld 2010, Sousa et al. 2011). However, some IAS can interact positively with native species by providing food resources and habitat to rare species (see for example Schlaepfer et al. 2011). In some circumstances, IAS may behave as a foundation species because they have the ability to create habitats and/or modify environmental conditions, as well as change species interactions and resource availability in invaded ecosystems (Bruno &

Bertness 2001, Crooks 2002, Altieri & van de Koppel 2014). There has been growing evidence in recent years that ecosystem engineering activities are one of the most important mechanisms underlying these facilitative interactions (Jones et al. 1994, 1997, Altieri & van de Koppel 2014).

Bivalves are one of the most invasive faunal groups in aquatic ecosystems and can significantly influence biological communities and alter ecosystem structure and functioning through several mechanisms, including ecosystem engineering (Gutiérrez et al. 2003, Sousa et al. 2009, 2014). Several invasive bivalve species in freshwater, estuarine and marine ecosystems have high potential for ecosystem engineering since they possess key attributes such as the production of durable shells, bioturbating and filter-feeding behavior, relatively large size, high densities and widespread distribution (reviewed in Sousa et al. 2009). In fact, the structure provided by their shells might serve as a refuge from biotic and abiotic stress, predation and competition, ameliorate environmental extremes, change abiotic factors and provide a substrate for colonization (Gutiérrez et al. 2003, Sousa et al. 2009).

The Asian clam *Corbicula fluminea* (Müller, 1774) is well recognized for its invasive behavior (e.g. it is listed as one of 100 worst invasive species; DAISIE 2009) and therefore the number of published articles using this species as a model organism has increased in recent years (Sousa et al. 2008a, 2014). In the 20th century, species belonging to the genus *Corbicula* have expanded their distributions to North and South America, Europe and North Africa (reviewed in Ilarri & Sousa 2012). When this IAS is present in high densities, it can cause a wide range of abiotic and biotic impacts, including changes to submerged vegetation, phytoplankton and zooplankton communities and decreases in abundance and diversity of native bivalve species (Vaughn & Hakenkamp 2001, Darrigran 2002, McMahon 2002, Sousa et al. 2008a, Ilarri & Sousa 2012). However, recent studies have shown that the presence of *C. fluminea* could also have a positive influence on the density, biomass and diversity of some faunal groups such as Gastropoda, Crustacea and Insecta in estuarine environments (Ilarri et al. 2012). Yet nothing is known about the mechanisms responsible for the positive effects of *C. fluminea* on estuarine macrozoobenthos. It is expected that the high excretion rates of *C. fluminea*, which result in the release of large amounts of nutrients in the form of feces and pseudofeces, along with the structure provided by the species' shells via ecosystem engineering activities can be relevant to estuar-

ine macrozoobenthos. Building on the results of an earlier study in the Minho estuary (Ilarri et al. 2012), we performed a manipulative experiment to understand and disentangle the main mechanisms explaining changes in the density, biomass and diversity of a macrozoobenthic estuarine assemblage induced by the presence of *C. fluminea* under natural environmental conditions.

MATERIALS AND METHODS

Study area and sampling design

The experiment was carried out in the Minho estuary (NW Iberian Peninsula) (Fig. 1), which has a maximum width of 2 km and is approximately 35 km long. Several studies have been performed in this estuary in the last 2 decades, and a detailed description of the macrozoobenthic and epibenthic communities are available in Sousa et al. (2008c) and Costa-Dias et al. (2010), respectively. Although controversy exists regarding the taxonomic status in the *Corbicula* genus, mainly due to high phenotypic variation in shell shape, an earlier genetic study performed by Sousa et al. (2007a) in 5 different sites along the Minho estuary identified the species found in this estuary as *C. fluminea*. This IAS was first reported in the Minho estuary in 1989, and now represents more than 95% of the total benthic biomass in this estuarine ecosystem and has had several ecological and

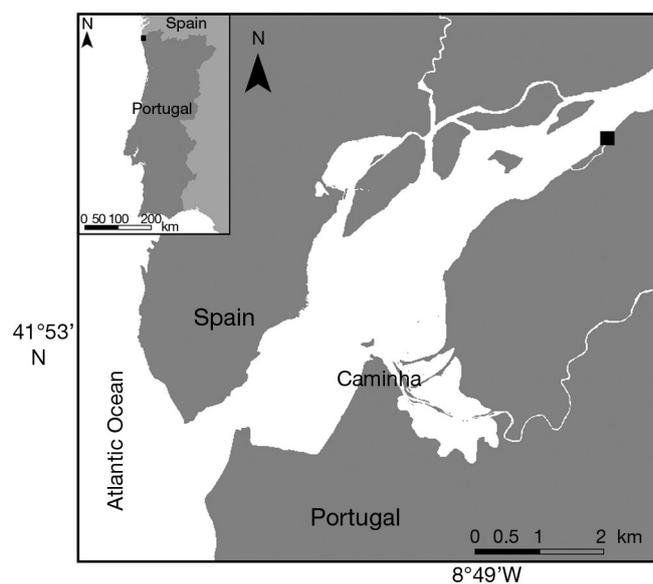


Fig. 1. Study area showing the selected site in the lower Minho estuary, NW Iberian Peninsula

economic impacts (see, for example, Sousa et al. 2008b,c).

The lower intertidal portion of a sandflat in the lower estuary, approximately 8 km upstream of the mouth of the estuary (41° 54' 37" N, 08° 47' 22" W; Fig. 1) was used to determine the main mechanisms responsible for the effects of *C. fluminea* on the estuarine macrozoobenthos (following Ilarri et al. 2012). The studied area was selected due to its low density of *C. fluminea* (<50 ind. m⁻²) when compared to adjacent areas (see Sousa et al. 2008c for comparison) in order to minimize the perturbation caused by the presence of this IAS. Throughout the experiment, abiotic variables were measured at 2 wk intervals during high tide. Temperature, redox potential, salinity, dissolved oxygen and pH were measured 20 cm above the bottom with a multiparameter sea gage (YSI 6820). In addition, 3 random samples of sediment were collected to characterize the granulometry of the sediment in the study area. In the laboratory, sediment samples were oven-dried for 72 h at 60°C, then sieved with Ro-Tap agitation using columns of sieves of different mesh sizes (>2 mm: gravel; 1–2 mm: very coarse sand; 0.5–1 mm: coarse sand; 0.25–0.5 mm: medium sand; 0.125–0.25 mm: fine sand; 0.063–0.125 mm: very fine sand; <0.063 mm: silt + clay). The frequency of each size class was expressed as a percentage of total weight following Sousa et al. (2006).

All adult *C. fluminea* individuals (i.e. clams with shell length >10 mm) were removed from the sandflat 1 wk before the start of the experiment. The experiment was conducted in a complete randomized block design with 6 blocks. Each block contained five 400 cm² boxes (with open tops and laterally lined with a net with a mesh size of 10 mm) corresponding to 5 different treatments: (1) bare sediment (hereafter, control treatment); (2) inanimate substrate consisting of small rocks with a similar oval shape as *C. fluminea* (hereafter, rock treatment); (3) dead *C. fluminea* shells filled with sand and glued together (hereafter, closed treatment); (4) live *C. fluminea* individuals (hereafter, live treatment); and (5) open empty *C. fluminea* shells (hereafter, open treatment). The control treatment was used to recreate a site without *C. fluminea* influence, while the rock treatment functioned as a control for the effect of a physically inert substrate. The closed treatment was used to detect only the effect of colonization on the outside of the shells while clams were alive, and the live treatment was used to detect the total effect of the presence of living *C. fluminea* (shell as a sub-

strate, production of feces and pseudofeces and bioturbation activities). Finally, the open treatment was used to detect the effect of open empty shells after the death of individual clams. All treatments, except the control (no clams), had a density of 1200 ind. m⁻², which reflects mean values in the Minho estuary (Sousa et al. 2008b). All *C. fluminea* individuals and rocks used were measured to minimize possible differences in size and surface area available for colonization between treatments. Boxes were distributed within a grid of ca. 1 m intervals, chosen to minimize habitat variability and inter-plot interactions. The experiment lasted 2 mo (July and August). After that, sediment samples were collected for organic matter determination and macrozoobenthos characterization using cores with an area of 10 and 45 cm², respectively. The organic matter in the sediment was determined by combustion for 24 h at 550°C in a muffle furnace, and was estimated as the weight loss on ignition, expressed as a percentage of the dry weight (DW) of the whole sample, following Sousa et al. (2006). Samples containing biological material were sieved through a 500 µm mesh, and the macrozoobenthos was preserved in 70% ethanol. Organisms were counted and identified to species level whenever possible. To determine biomass, organisms were oven-dried for 72 h at 60°C.

Data analysis

All statistical tests were conducted using the PRIMER analytical software (v.6.1.6, PRIMER-E) with the permutational multivariate analysis of variance (PERMANOVA) + 1.0.1 add-on (Anderson et al. 2008). PERMANOVA tests the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of any distance measure, using permutation methods (Anderson 2001). Prior to PERMANOVA and non-metric multidimensional scaling (NMDS) ordination analyses (see below), all variables were normalized without data transformation, and resemble matrices based on the Euclidean distances were calculated (Clarke & Warwick 2001).

Differences between treatments in the organic matter content of the sediment were tested using a 1-way PERMANOVA (type-III), with treatment as a fixed factor (5 levels: control, rock, closed, live and open). Comparisons between the *C. fluminea* individuals and rock lengths were tested using a 1-way PERMANOVA (type-III), with treatment as a fixed factor (4 levels: rock, closed, live and open).

The NMDS based on the macrozoobenthic density data followed by the PERMANOVA tests were used to discriminate possible differences between treatments. The ecological indexes of species richness (S) and the Shannon-Wiener index (H') were calculated through the DIVERSE analysis (Clarke & Warwick 2001). Differences in overall macrozoobenthic density, biomass, S and H' were tested using a 1-way PERMANOVA (type-III), with treatment as a fixed factor (5 levels: control, rock, closed, live and open). Comparisons of Annelida, Mollusca and Crustacea density and biomass between treatments were made using a 1-way PERMANOVA (type-III), with the same design as described above.

In all PERMANOVA tests, the statistical significance of variance ($\alpha = 0.05$) was tested using 9999 permutations of residuals within a reduced model. When the number of permutations was lower than 150, the Monte Carlo p-value was considered. One-way PERMANOVA pairwise comparisons were also performed for all PERMANOVA tests.

RESULTS

Abiotic characterization

The mean (\pm SD) values of abiotic factors measured in the water column at high tide during the 2 mo experiment were temperature: $20.56 \pm 1.44^\circ\text{C}$; redox potential: 204.31 ± 20.15 mV; salinity: 12.15 ± 3.31 psu; dissolved oxygen: 8.76 ± 0.43 mg l⁻¹ and pH: 7.89 ± 0.14 . The sediment composition of the study area was very homogeneous, with the mean percentage of each size class frequency as follows: >2 mm, $0.1 \pm 0.01\%$; 1–2 mm, $0.2 \pm 0.01\%$; 0.5–1 mm, $1.0 \pm 0.15\%$; 0.25–0.5 mm, $5.5 \pm 0.65\%$; 0.125–0.25 mm, $46.5 \pm 3.10\%$; 0.063–0.125 mm, $34.3 \pm 2.03\%$; and <0.063 mm, $12.4 \pm 1.08\%$.

Organic matter content was highest in the live treatment ($3.5 \pm 0.39\%$) followed by open ($3.2 \pm 0.31\%$), closed ($3.2 \pm 0.43\%$), control ($3.0 \pm 0.33\%$) and rock ($2.9 \pm 0.32\%$) treatments. Significant differences only occurred between the live and rock treatments ($t = 2.54$, $p = 0.04$).

Biotic characterization

The mean (\pm SD) length of *Corbicula fluminea* individuals was 26.8 ± 4.6 mm in the closed treatment, 26.7 ± 5.8 mm in the live treatment, and 26.8 ± 4.9 mm in the open treatment; the mean length of the

rocks in the rock treatment was 26.7 ± 5.0 mm, with no differences between treatments (Pseudo- $F = 0.02$, $p = 0.99$). There was no *C. fluminea* mortality in the live treatment during the experiment.

A total of 12 macrozoobenthic taxa were recorded in all treatments. The 6 most abundant were *Hediste diversicolor* (36.7%), *Corophium multisetosum* (29.7%), *Cyathura carinata* (13.3%), *Spionidae* and *Gammarus* sp. 2 (3.8%) and *Potamopyrgus antipodarum* (3.2%), while the remaining 6 taxa contributed 9.5%. In terms of biomass, the 6 most abundant taxa were *H. diversicolor* (67.5%), *Petromyzon marinus* (6.8%), *C. multisetosum* and *C. carinata* (6.3%), *Nereis cultifera* (5.4%) and *C. fluminea* (3.8%), while the remaining 6 taxa contributed 3.9%.

The NMDS ordination (stress = 0.15; Fig. 2) did not reveal any difference in the macrozoobenthic assemblage that colonized each treatment (Pseudo- $F = 1.26$, $p = 0.10$).

The mean \pm 95% CI macrozoobenthic density (no. ind. 45 cm⁻²) was higher in the open (9.2 ± 4.6) and live (8.5 ± 4.6) treatments, followed by closed (4.7 ± 1.4), control (4.5 ± 1.7) and rock (3.8 ± 1.4) (Fig. 3A). Significant differences in density between the 5 treatments were found (Pseudo- $F = 6.31$, $p < 0.01$). Pairwise tests indicated that these differences were in the comparisons of open with closed ($t = 2.80$, $p \leq 0.05$), open with control ($t = 2.82$, $p \leq 0.05$), open with rock ($t = 3.33$, $p \leq 0.01$), live with closed ($t = 2.87$, $p \leq 0.05$), live with control ($t = 2.83$, $p \leq 0.05$) and live with rock ($t = 3.52$, $p \leq 0.01$) treatments.

Similarly, results for biomass (g DW 45 cm⁻²) showed higher values for the live (0.05 ± 0.056) and open (0.05 ± 0.040) treatments, followed by closed

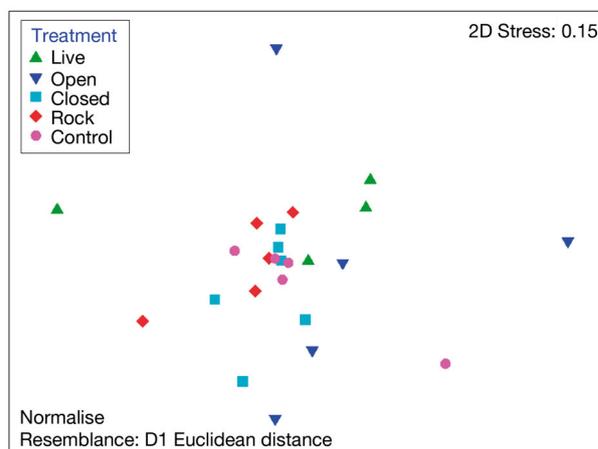


Fig. 2. Non-metric multidimensional scaling (NMDS) plot of the macrozoobenthos associated with the 5 experimental treatments (live, open, closed, rock and control)

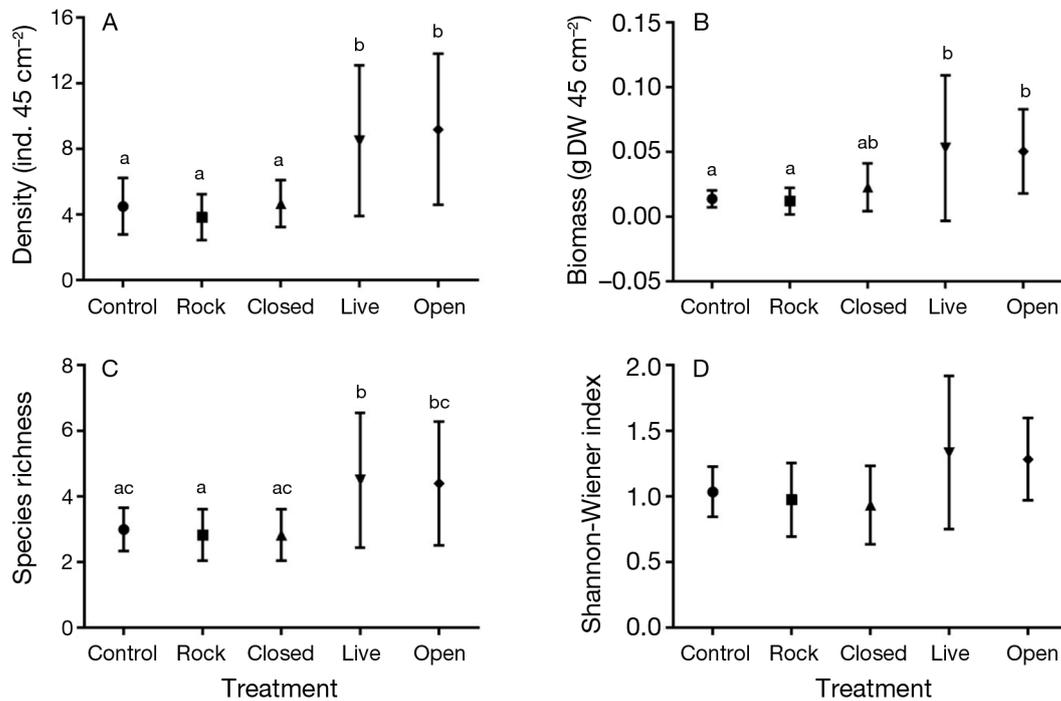


Fig. 3. Macrozoobenthos mean ($\pm 95\%$ CI) (A) density (ind. 45 cm⁻²), (B) biomass (g DW 45 cm⁻²), (C) species richness and (D) Shannon-Wiener index for each treatment (control, rock, closed, live and open). Different lowercase letters indicate significant differences among treatments

(0.02 ± 0.019), control (0.01 ± 0.007) and rock (0.01 ± 0.010) treatments (Fig. 3B). There were significant differences between the 5 treatments (Pseudo- $F = 4.19$, $p \leq 0.05$). Pairwise tests indicated that these differences were in the comparisons of live with control ($t = 2.74$, $p \leq 0.01$), live with rock ($t = 2.76$, $p \leq 0.05$), open with control ($t = 2.72$, $p \leq 0.01$) and open with rock ($t = 2.76$, $p \leq 0.01$) treatments.

The results for *S* showed higher values for the live (4.5 ± 2.1) and open (4.4 ± 1.9) treatments, followed by control (3.0 ± 0.7) and closed and rock (2.8 ± 0.8) treatments (Fig. 3C). There were significant differences between the 5 treatments (Pseudo- $F = 3.69$, $p \leq 0.05$). The pairwise tests indicated that significant differences existed between live and closed ($t = 2.61$, $p \leq 0.05$), live and control ($t = 2.48$, $p \leq 0.05$), live and rock ($t = 2.61$, $p \leq 0.05$) and open and rock ($t = 2.24$, $p \leq 0.05$) treatments.

No significant differences in H' were detected among the 5 treatments (Pseudo- $F = 2.02$, $p = 0.13$) (Fig. 3D).

The results for Annelida density (Fig. 4A) and biomass (Fig. 4B) showed higher values for the live and open treatments. There were significant differences in the density (Pseudo- $F = 2.39$, $p \leq 0.01$), but not in the biomass of Annelida among the 5 treatments

(Pseudo- $F = 2.54$, $p = 0.06$). Within the Annelida taxa, the density and biomass of *H. diversicolor* was highest in the live treatment (Table 1).

Mollusca density (Fig. 4C) and biomass (Fig. 4D) were also highest in the live treatment. There were significant differences in the biomass (Pseudo- $F = 2.09$, $p \leq 0.05$), but not in the density of Mollusca among the 5 treatments (Pseudo- $F = 2.17$, $p = 0.07$).

Crustacea density (Fig. 4E) and biomass (Fig. 4F) were highest in the open treatment. There were significant differences in the density (Pseudo- $F = 3.88$, $p \leq 0.05$), but not in the biomass of Crustacea among the 5 treatments (Pseudo- $F = 1.73$, $p = 0.18$). The density of *C. multisetosum* and *Gammarus* sp. 1 were significantly higher in the open treatment (Table 1).

DISCUSSION

The studied area is subjected to harsh abiotic conditions during the summer, mainly because salinity can oscillate between 0.05 and 15 psu during low and high tide, respectively. These harsh abiotic conditions do not allow for the establishment of a diverse macrozoobenthic assemblage; only true estuarine organisms with the physiological capacity to tolerate

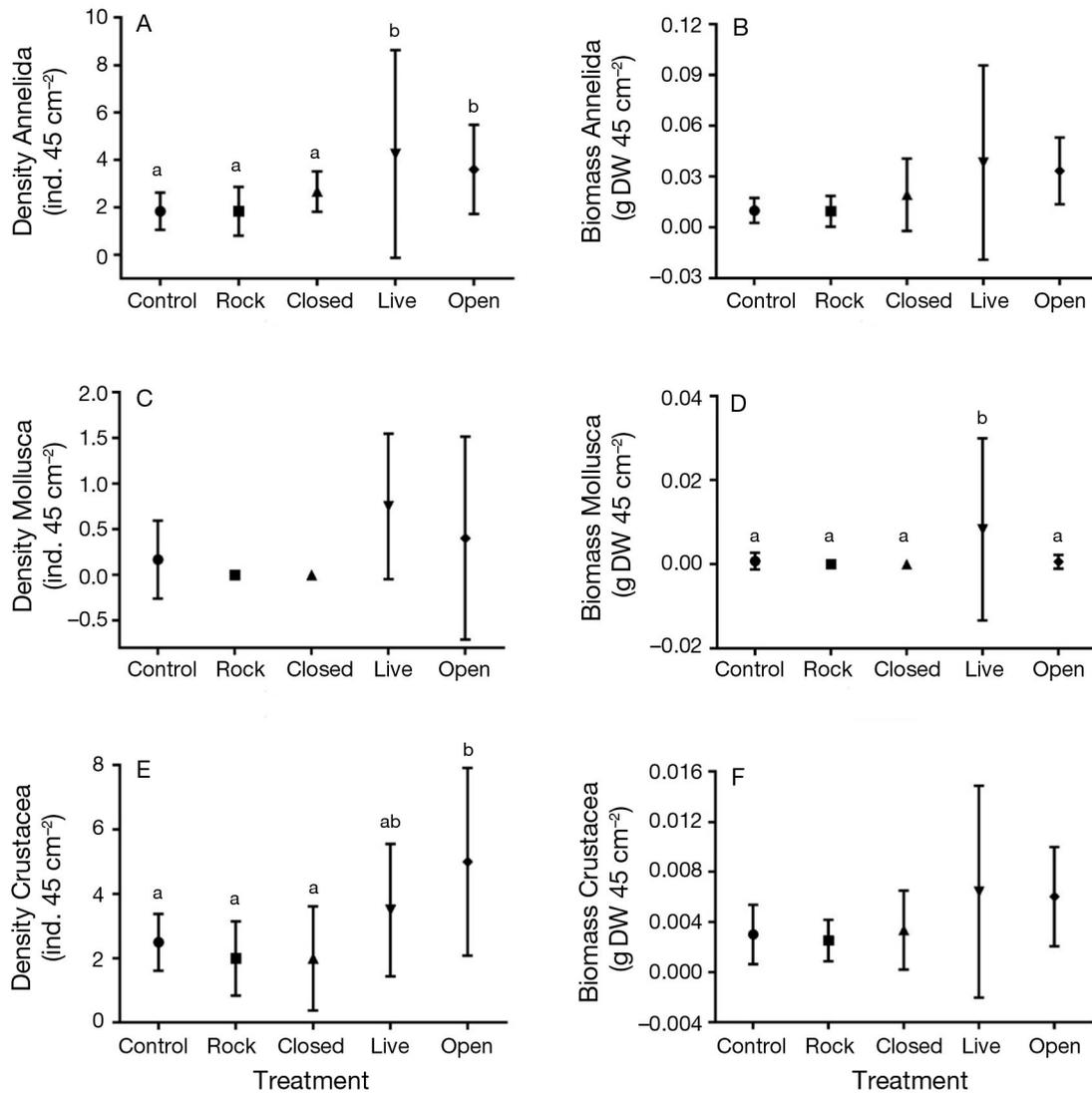


Fig. 4. Mean ($\pm 95\%$ CI) density (ind. 45 cm⁻²) and biomass (g DW 45 cm⁻²) per treatment (control, rock, closed, live and open) for (A,B) Annelida, (C,D) Mollusca and (E,F) Crustacea. Different lowercase letters indicate significant differences among treatments

these changing salinity conditions can thrive in this area. However, the number and composition of species recorded in our experiment were similar to that reported in other studies performed in the same area (see Sousa et al. 2008c, Ilarri et al. 2012, 2014). In addition, the NMDS analysis was not able to distinguish any group in the macrozoobenthic assemblage, which was expected, given that it would be unlikely that the macrozoobenthic composition would differ between the 5 treatments during the 2 mo study period.

Despite the similarity in faunal composition among treatments, our experiment clearly showed that treatments with live and open empty shells of *Corbicula*

fluminea supported higher macrozoobenthos density, biomass and species richness in comparison to the control, closed and rock treatments. Thus, our results suggest that the presence of *C. fluminea* may actually have positive effects on some macrozoobenthic and epibenthic species in invaded estuarine areas, as recently proposed by Ilarri et al. (2012, 2014). In addition, our experimental approach gave further insights into the identification of possible mechanisms underlying the observed changes in macrozoobenthic colonization in areas with and without *C. fluminea*. Indeed, 2 main mechanisms seemed to have influenced the results: (1) the production of feces and pseudofeces by *C. fluminea*,

Table 1. Mean (\pm SD) values of density (ind. 45 cm⁻²) and biomass (g DW 45 cm⁻²) and 1-way PERMANOVA results for the effects of 5 treatments (control, rock, closed, live and open) on the species collected in the lower Minho estuary, NW Iberian Peninsula. * p < 0.05

Species	Density					Biomass						
	Control	Rock	Closed	Live	Open	Control	Rock	Closed	Live	Open	Pseudo-F	p
<i>Hediste diversicolor</i>	1.50 \pm 0.55	1.33 \pm 0.52	2.17 \pm 0.75	3.50 \pm 2.38	2.80 \pm 0.84	0.0099 \pm 0.0070	0.0093 \pm 0.0087	0.0136 \pm 0.0086	0.0382 \pm 0.0360	0.0320 \pm 0.0180	3.17	0.023*
<i>Heteromastus filiformis</i>	0.00 \pm 0.00	0.33 \pm 0.82	0.17 \pm 0.41	0.25 \pm 0.50	0.00 \pm 0.00	0.0000 \pm 0.0000	0.0001 \pm 0.0003	0.0000 \pm 0.0000	0.0000 \pm 0.0001	0.0000 \pm 0.0000	0.74	0.568
<i>Nereis culifera</i>	0.00 \pm 0.00	0.00 \pm 0.00	0.17 \pm 0.41	0.00 \pm 0.00	0.20 \pm 0.45	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0057 \pm 0.0139	0.0000 \pm 0.0000	0.0014 \pm 0.0031	0.77	0.551
<i>Streblospio benedicti</i>	0.33 \pm 0.82	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.20 \pm 0.45	0.0001 \pm 0.0002	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.77	0.548
Spionidae	0.00 \pm 0.00	0.17 \pm 0.41	0.17 \pm 0.41	0.50 \pm 0.58	0.40 \pm 0.89	0.0000 \pm 0.0000	0.0001 \pm 0.0002	0.0000 \pm 0.0000	0.0001 \pm 0.0001	0.0001 \pm 0.0001	0.53	0.833
<i>Corbicula fluminea</i>	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.25 \pm 0.50	0.00 \pm 0.00	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0072 \pm 0.0143	0.0000 \pm 0.0000	1.56	0.220
<i>Potamopyrgus antipodarum</i>	0.17 \pm 0.41	0.00 \pm 0.00	0.00 \pm 0.00	0.50 \pm 0.58	0.40 \pm 0.89	0.0008 \pm 0.0019	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0012 \pm 0.0013	0.0006 \pm 0.0013	0.93	0.468
<i>Corophium multisetosum</i>	1.50 \pm 1.38	0.83 \pm 0.75	1.17 \pm 1.33	2.25 \pm 0.50	3.40 \pm 2.30	0.0012 \pm 0.0014	0.0006 \pm 0.0006	0.0011 \pm 0.0017	0.0031 \pm 0.0031	0.0037 \pm 0.0033	2.13	0.094
<i>Cyathura carinata</i>	0.67 \pm 0.52	1.00 \pm 0.89	0.50 \pm 0.55	1.00 \pm 0.87	0.80 \pm 0.84	0.0017 \pm 0.0019	0.0018 \pm 0.0017	0.0013 \pm 0.0017	0.0026 \pm 0.0022	0.0017 \pm 0.0022	0.27	0.892
<i>Gammarus</i> sp. 1	0.00 \pm 0.00	0.00 \pm 0.00	0.17 \pm 0.41	0.00 \pm 0.00	0.60 \pm 0.55	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0003 \pm 0.0008	0.0000 \pm 0.0000	0.0006 \pm 0.0006	2.03	0.125
<i>Gammarus</i> sp. 2	0.33 \pm 0.52	0.17 \pm 0.41	0.17 \pm 0.41	0.25 \pm 0.50	0.20 \pm 0.45	0.0002 \pm 0.0003	0.0001 \pm 0.0003	0.0007 \pm 0.0017	0.0008 \pm 0.0015	0.0000 \pm 0.0001	0.58	0.825
<i>Petromizon marinus</i>	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.20 \pm 0.45	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0103 \pm 0.0230	1.12	0.366

which increased organic matter content and food resources for some macrozoobenthic species; and (2) ecosystem engineering activities by *C. fluminea*, which created appropriate conditions for the establishment of other species via shell production and bioturbation of sediments.

In the case of Annelida, the density and biomass of *Hediste diversicolor* was higher in the presence of live *C. fluminea*. *H. diversicolor* is usually described as a generalist species that consumes the most abundant food resources available at the local scale (Fidalgo e Costa et al. 2006). In fact, this species has different strategies with which to capture its food, but usually behaves as a filter feeder and a deposit feeder, scavenging organic material and detritus on the sediment surface (Fidalgo e Costa et al. 2006, Olivier et al. 1997). According to Batista et al. (2003), *H. diversicolor* is able to increase its growth and survival rates and to attain higher biomass production in the presence of the clam *Ruditapes decussatus* feces. Therefore, our results suggest that the positive response of *H. diversicolor* to the live treatment may be related to the large amounts of nutrients (in the form of feces and pseudofeces) produced by *C. fluminea* (Vaughn & Hakenkamp 2001). This hypothesis is also supported by the value of organic matter obtained in the live treatment. Although there were no significant differences between treatments (with the exception of live and rocks), the live treatment contained the highest content of organic matter. However, even if changes in organic matter availability serve to increase food resources (which could benefit deposit feeders; Crooks 1998), we cannot discard other explanations for the density increase of *H. diversicolor* in the live treatment. Indeed, bioturbation by *C. fluminea* could also be an important mechanism (Majdi et al. 2014) and it is possible that *H. diversicolor* (being an infaunal organism) responded to the abiotic

changes mediated by the sediment reworking of the live clams.

Similar arguments may also apply to organisms belonging to the phylum Mollusca, since the live treatment had the highest values of density and biomass. It is also possible that Molluscan species (of which only 2 species, *Potamopyrgus antipodarum* and *C. fluminea*, were present) responded to increases in organic matter available in the sediments due to the production of feces and pseudofeces by *C. fluminea*. The density of certain gastropods can increase in response to increases in organic matter content (Osenberg 1989, Brown 1991) and *P. antipodarum* has been described as a consumer of periphyton and fine organic matter (Haynes & Taylor 1984, Broekhuizen et al. 2001, Alonso & Castro-Díez 2012, Krist & Charles 2012). Interestingly, both *C. fluminea* and *P. antipodarum* are non-native in the studied area, and this positive interaction between the 2 species may be seen as an example of the meltdown hypothesis, in which one introduced species facilitates the presence of another (Simberloff 2006). In the same vein, increases in the organic matter available in the sediments may exert a positive feedback to *C. fluminea* because this species, in addition to being a filter feeder, may also behave as a deposit feeder (see Hakenkamp & Palmer 1999, Vaughn & Hakenkamp 2001). Other mechanisms besides the possible use of organic matter may have influenced our results, namely bioturbation activities and the ability of *C. fluminea* adults to release chemical cues that could influence juvenile settlement in areas where adult clams are present (Sardiña et al. 2009). However, future detailed studies are necessary in order to test these hypotheses.

In the case of Crustacea, particularly *Corophium multisetosum* and *Gammarus* sp. 1, higher density was detected in the open empty shells treatment. These results indicate that accumulations of empty *C. fluminea* shells may increase structural complexity, with positive consequences for associated amphipods. In fact, the 3-dimensional structure provided by open empty shells created new microhabitats which provided substrata for attachment, along with a refuge from water flow, predators, competitors, and physical and/or physiological stress (Gutiérrez et al. 2003, Sousa et al. 2009). Interestingly, higher densities were obtained in open empty shells than closed shells, which may be related to the much higher surface area and habitat heterogeneity provided by open empty shells. Based on these results, it is likely that, even after their death, *C. fluminea* can still affect macrozoobenthic assemblages and it will be

important to quantify the decay rates of shells in future studies.

Progress in the study of the impacts of IAS on biodiversity can be facilitated by the implementation of manipulative experiments such as ours, in which an increase in infaunal density, biomass and species richness was attributed to the presence of *C. fluminea*. Treatments with live clams and open empty shells led to similar increases in density, biomass and species richness; however, the species associated with each treatment differed: polychaetes and molluscs were more important in the live treatment, while crustaceans were more relevant in the open treatment. We are aware that in some faunal groups, this positive effect may be modified in the future if biological and environmental conditions change, and that the current positive effect is not generalized to all species present in the River Minho estuary. Indeed, native bivalves have undergone significant declines in density, biomass and spatial distribution following *C. fluminea* introduction (Sousa et al. 2005, 2007b, 2008b,c,d). Interestingly, a recent meta-analysis assessing the impacts of marine invaders on local biodiversity showed that these species typically have negative effects on biodiversity within a trophic level, but positive effects on biodiversity of higher trophic levels (Thomsen et al. 2014). The results of this and earlier studies conducted in the River Minho concerning the impacts of *C. fluminea* on biodiversity seem to follow similar trends to those described by Thomsen et al. (2014).

According to Gutiérrez et al. (2014), assimilation–dissimilation (uptake and release of energy and materials) and ecosystem engineering (physical environmental modifications by organisms) are the 2 main mechanisms explaining the direct and indirect effects of IAS on ecosystem structure and function. Indeed, assimilation–dissimilation involves the uptake (assimilation) of energy and materials (light, water, nutrients etc.) and their release (dissimilation) in the form of dead tissues and waste products (carbon and nutrients in litter, woody debris, feces, urine, carcasses etc.). In the case of bivalves, given their high filtration rates, these organisms are capable of capturing energy from the water column and releasing feces and pseudofeces rich in organic matter into the sediments. This energy may be consumed by benthic organisms, thereby increasing their density, biomass and species richness, at least for deposit-feeding species (Karatayev et al. 1997, Ward & Ricciardi 2007). On the other hand, ecosystem engineering activities can also promote significant changes in biodiversity (Jones et al. 1994, 1997, Anderson &

Rosemond 2007, Altieri & van de Koppel 2014). Concerning IAS, Castilla et al. (2004) found that the introduced ascidian *Pyura praeputialis* (an engineer species) modified the intertidal habitat structure in Antofagasta Bay (northern Chile), by creating broad belts and 3-dimensional matrices, resulting in increased species richness at local and seascape scales. This type of facilitation has also been demonstrated with invasive bivalves (Ruesink et al. 2005, Sousa et al. 2009, 2014). For example, non-indigenous zebra *Dreissena polymorpha* and golden mussels *Limnoperna fortunei* were associated with increased benthic macroinvertebrate density, biomass and taxonomic richness due to increases in the provision of refuges and bed substrate complexity (Beekey et al. 2004, Sylvester et al. 2007). However, in contrast to our manipulative experiment, these studies were not able to disentangle the importance of biodeposition of organic matter on sediments or the importance of the ecosystem engineering activities promoted by bivalves.

CONCLUSIONS

Our results clearly indicate that *Corbicula fluminea* can have positive effects on estuarine macrozoobenthos density, biomass and species richness. These effects are most likely due to an enrichment in organic matter via the production of feces and pseudofeces, along with physical ecosystem engineering (e.g. shell production and bioturbation). However, estuaries are highly dynamic systems, often characterized by high environmental disturbance due to natural or human activities (Day et al. 1989, Little 2000); therefore, the results reported in this study may differ from other aquatic ecosystems (including rivers and lakes) that are less disturbed and that support much higher species richness.

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