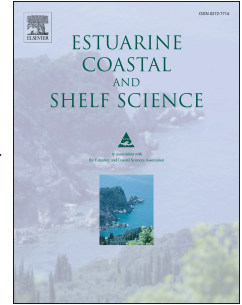


Accepted Manuscript

Effect of the alien invasive bivalve *Corbicula fluminea* on the nutrient dynamics under climate change scenarios

J.P. Coelho, A.I. Lillebø, D. Crespo, S. Leston, M. Dolbeth



PII: S0272-7714(17)30402-X

DOI: [10.1016/j.ecss.2018.03.001](https://doi.org/10.1016/j.ecss.2018.03.001)

Reference: YECSS 5778

To appear in: *Estuarine, Coastal and Shelf Science*

Received Date: 12 April 2017

Revised Date: 20 February 2018

Accepted Date: 4 March 2018

Please cite this article as: Coelho, J.P., Lillebø, A.I., Crespo, D., Leston, S., Dolbeth, M., Effect of the alien invasive bivalve *Corbicula fluminea* on the nutrient dynamics under climate change scenarios, *Estuarine, Coastal and Shelf Science* (2018), doi: 10.1016/j.ecss.2018.03.001.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Effect of the alien invasive bivalve *Corbicula fluminea* on the nutrient dynamics under**
2 **climate change scenarios.**

3
4 J.P. Coelho*^{1,2}; A.I. Lillebø²; D. Crespo³; S. Leston^{3,4,5}; M. Dolbeth^{2,6}

5
6 ¹ Department of Chemistry & CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193
7 Aveiro, Portugal

8
9 ² Department of Biology & CESAM & ECOMARE, University of Aveiro, Campus Universitário de Santiago,
10 3810-193 Aveiro, Portugal

11
12 ³ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de
13 Freitas, 3000-456 • Coimbra, Portugal

14
15 ⁴ CNC-Centre for Neuroscience and Cell Biology, Health Sciences Campus, Pharmacy Faculty, University of
16 Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra – Portugal

17
18 ⁵ MARE-Marine and Environmental Sciences Centre, IPL, Escola Superior de Turismo e Tecnologia do Mar,
19 2520-641 Peniche – Portugal

20
21 ⁶ CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Novo Edifício do Terminal de
22 Cruzeiros do Porto de Leixões, Avenida General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

23 **ABSTRACT**

24 The main aim of this study was to evaluate the impact of the alien invasive bivalve *Corbicula*
25 *fluminea* (Müller, 1774) in the nutrient dynamics of temperate estuarine systems (oligohaline
26 areas) under climate change scenarios.

27 The scenarios simulated shifts in climatic conditions, following salinity (0 or 5) and
28 temperature (24 or 30 °C) changes, usual during drought and heat wave events. The effect of
29 the individual size/age (different size classes with fixed biomass) and density (various
30 densities of < 1 cm clams) on the bioturbation-associated nutrient dynamics were also
31 evaluated under an 18-day laboratory experimental setup.

32 Results highlight the significant effect of *C. fluminea* on the ecosystem nutrient dynamics,
33 enhancing the efflux of both phosphate and dissolved inorganic nitrogen (DIN) from the
34 sediments to the water column. Both drought and heat wave events will have an impact on
35 the DIN dynamics within *C. fluminea* colonized systems, favouring a higher NH₄-N efflux.
36 The population structure of *C. fluminea* will have a decisive role on the impact of the species,
37 with stronger nutrient effluxes associated with a predominantly juvenile population structure.

38

39 *Keywords:* Ecosystem functioning; Invasive alien species; heat waves; droughts; nutrient
40 dynamics

41

42 **Introduction**

43 Invasive alien species (IAS) introduction has increased considerably in aquatic ecosystems in
44 recent decades, as a result of expanding commerce, aquaculture and shipping activities (Sousa
45 *et al.*, 2008b; Chan & Bendell, 2013; Simberloff *et al.*, 2013). Traits such as short life span,
46 early maturity, rapid growth and high fecundity contribute to the success of invasive species,
47 by promoting the development of dense invader populations (McMahon, 2002; Crespo *et al.*,
48 2015). These, in turn, outperform native populations in rapidly repopulating colonized
49 systems, after mortality events in unstable ecosystems. In particular, ecosystem engineers and
50 filter-feeding IAS have been reported to strongly impact biodiversity and ecosystem
51 functioning (Chan & Bendell, 2013).

52 *Corbicula fluminea* O.F. Müller [1774] is one of the most successful invasive species
53 worldwide, and is present in several European estuarine and freshwater areas (Franco *et al.*,
54 2012; McMahon, 2002; Sousa *et al.*, 2008b; Crespo *et al.*, 2015). It can represent as much
55 as 90% of the macrobenthic community production (Sousa *et al.*, 2008a) with abundance and
56 biomass of more than 4000 ind. m⁻² and 550 g AFDW m⁻², respectively (Sousa *et al.*, 2008b).
57 Reported *C. fluminea* ecosystem impacts span from changes in sediment characteristics, via
58 bioturbation and shell production (Sousa, Gutiérrez & Aldridge, 2009), to phytoplankton
59 population control and turbidity decreases due to high filtration rates (Phelps, 1994; Sousa,
60 Antunes & Guilhermino, 2008; Majdi, Bardon & Gilbert, 2014), or the disruption of food
61 chains as a result of its high productivity and massive die-offs (Sousa *et al.*, 2008b; Sousa *et*
62 *al.*, 2012). Additional impacts of *C. fluminea* encompass alterations in the biogeochemical
63 cycles, through a myriad of mechanisms. For instance, pedal feeding promotes alterations in

64 the abiotic characteristics of the top layer of the sediments, resulting in high rates of nitrogen
65 excretion (Vaughn & Hakenkamp, 2001). In a different study, the presence of bivalves
66 (including *C. fluminea*) in the Chesapeake Bay system was modelled to remove 14% to 40%
67 of the carbon load, 11% to 23% of the nitrogen load, and 37% to 84% of the phosphorus load,
68 by reducing net phytoplankton primary production by 31% to 44% (Cerco & Noel, 2010).
69 A distinct mechanism which may also affect biogeochemical dynamics is bioturbation and
70 bioirrigation; the first influences the distribution of sediment particles (by sediment
71 reworking), while the latter will have an impact on solutes (by burrow ventilation) within the
72 sediment and at the sediment–water interface (Majdi, Bardon & Gilbert, 2014). These
73 interactions result in changes in sediment characteristics, such as grain size, organic matter
74 content, sediment stability and aggregation, porosity and water retention capacity, which will
75 affect interstitial water characteristics, oxygen and nutrient concentrations and release to the
76 water column and redox potential, among other abiotic factors that control biogeochemical
77 processes (Sousa, Gutiérrez & Aldridge, 2009).

78 Global climate change, with altered frequencies and intensities of extreme weather events
79 (floods, droughts and heat waves), together with mean sea level rise, are expected to have
80 adverse effects on natural and human systems (IPCC, 2014). The effects of this climatic and
81 environmental instability on biological communities are unpredictable, as are subsequent
82 outcomes to the ecosystem functioning (Wrona *et al.*, 2006). Such effects are especially
83 important for invasive species, because they are often not yet at equilibrium, represent a
84 significant component of aquatic communities and can interact strongly with native species
85 (McDowell, Benson & Byers, 2014). Alterations of environmental conditions due to sudden,
86 dramatic events associated with climate change may also open opportunities for the
87 proliferation of invasive species, previously unable to sustain viable populations due to

88 physical environmental constraints (Montoya & Raffaelli, 2010; Crespo *et al.*, 2015; Crespo
89 *et al.*, 2017).
90 Massive mortality events have been reported for *C. fluminea* (Oliveira, Vilares &
91 Guilhermino, 2015; Sousa *et al.*, 2012) associated with extreme events such as droughts, heat
92 waves, decrease of oxygen concentration in the water, increase of pollution and unmet
93 metabolic demand (Petter *et al.*, 2014; Oliveira, Vilares & Guilhermino, 2015), particularly
94 due to their combined impacts (e.g. Crespo *et al.*, 2017). However, the species may also thrive
95 under some temperature increase scenarios (Rosa *et al.*, 2012) and is known to tolerate some
96 salinity (Crespo *et al.*, 2017). In fact, temperature and salinity have been found to affect rates
97 of oxygen consumption, ammonium excretion, CO₂ emission and mortality (Xiao *et al.*, 2014;
98 Crespo *et al.*, 2017). Yet, little information is available on the possible consequences of
99 climate change on the effect of this IAS for ecosystems functions, such as nutrient release
100 from sediment, its dynamics and changes in the sediment biogeochemistry. Therefore, the
101 main objective of this research was to evaluate the effect of *Corbicula fluminea* in the nutrient
102 fluxes to the water column under extreme climatic events (rise in temperature and rise in
103 salinity following a drought event), and considering distinct population structure (size) and
104 density. Overall, it was expected to gain information on the impact of this species under such
105 climate scenarios, which will predictably increase, to aid for future ecosystem conservation
106 and management purposes.

107

108 **Methods**

109 *Faunal and sediment collection*

110 *Corbicula fluminea* individuals were collected in the well-described Mondego Estuary (Grilo
111 *et al.*, 2011; Dolbeth *et al.*, 2011; Franco *et al.*, 2012). Samples were collected in the
112 oligohaline upper reaches (40° 9'47.91"N, 8°40'12.42"W), still under tidal influence despite

113 the considerable distance to the river mouth. Conditions in the collection site included sandy
114 sediments and low macrofauna diversity which was dominated by *C. fluminea* (Crespo *et al.*,
115 2017). Specimens were collected by hand and transported refrigerated to the laboratory, in
116 water collected *in situ*. Sediment for the experimental tanks was collected in the same site.
117 Fauna were acclimated to laboratory conditions for at least 48h prior to the start of
118 experiments (Magni *et al.*, 2000; Hakenkamp & Palmer, 1999; Atkinson *et al.*, 2011).

119

120 *Experimental tank' assemblage and experimental design*

121 The experimental apparatus (Fig. 1) consisted of glass tanks with 12x12x35 cm (internal
122 dimensions). The tanks were assembled with 10 cm of the collected sediment and filled with
123 20 cm of water (≈ 3 L). Sediment was thoroughly homogenized to assure comparable
124 conditions across tanks, and macrofauna was previously removed by CO₂ enriched water
125 treatments. Two different salinity treatments were selected, in accordance with the usual
126 salinity range measured in the oligohaline estuarine areas (freshwater 0, and 5, synthetic salt
127 water prepared with ultrapure water and Tropic Marin® SEA SALT synthetic sea salt mix),
128 and two water temperature treatments (≈ 24 °C, the reported summer water temperature at the
129 collection site (Modesto *et al.*, 2013), and 30 °C, representing field water temperature in
130 extreme conditions, such as during heat waves occurrence (Mouthon & Daufresne, 2006;
131 Grilo *et al.*, 2011)).

132 Water in each tank was replaced after 24 h to remove excess nutrients associated with
133 sediment manipulation during assembly. Different *Corbicula fluminea* size classes
134 (representing different cohorts) were tested, according to its population structure: small size
135 individuals (<1 cm, ~1 year old), medium size (2-2.5 cm, ~2 years old) and large size (>3.5
136 cm, >3 years). For each treatment, individuals of each size class were stocked at the same
137 total biomass to represent the densities (≈ 18.8 g wet weight per 144 cm⁻² tank area) and the

138 population structure found in the field during summer conditions (Crespo *et al.*, 2017). This
139 corresponded to different density levels: 13 small-sized *C. fluminea* individuals; 2 medium-
140 sized *C. fluminea* individuals; 1 large-sized *C. fluminea* individual (Fig. 1). Each treatment
141 was performed in triplicate. Organisms were not fed during the experiments, but were
142 assumed to feed on the sediment organic matter content (sediment OM= $0.4\pm 0.2\%$).

143 For the small-sized individuals alone, the most abundant size class at the collection site, the
144 density effect was also tested, using a control with no fauna and three density treatments with
145 6, 12 and 24 individuals (mean biomass per tank: 9.0, 18.8 and 35.7 g wet weight per 144 cm²
146 tank area respectively). For this density experiment, tanks were filled with 3 cm of sand and
147 freshwater (8.6 L) at ≈ 24 °C. Each density treatment was done in triplicate.

148 For comparison purposes, in all experiments Control tanks (without *C. fluminea*) were run
149 with the same experimental conditions as those with bivalves (both salinity and temperature
150 treatments). Tanks were permanently aerated and exposed to natural light conditions (≈ 10 h of
151 light with variable intensity, depending on weather conditions). The experiment was held in a
152 constant-temperature room (≈ 24 °C) and the higher tested temperature (≈ 30 °C) was
153 achieved using aquarium heaters (Eheim Jäger 3612 Aquarium Heater). Bivalve mortality was
154 negligible, and was only observed in the first two days of the experiment (assumed to be
155 related with acclimation to the experimental salinity and temperature conditions). When it
156 occurred, the dead bivalve was replaced with a similar sized individual to maintain biota
157 biomass.

158

159 For both experiments, salinity, temperature, pH and dissolved oxygen concentration were
160 monitored in the water column and water samples (10 mL) collected at day 0, day 3, day 6,
161 day 12 and day 18 of incubation from each tank, for nutrient analysis. The concentration of
162 dissolved inorganic nutrients (PO₄-P, NO_x-N and NH₄-N) in the water column was analysed

163 with Continuous Flow Analyzer Skalar Sanplus with Segmented Flow Analysis (SFA), using
164 the Skalar methods: M461-318 (EPA 353.2), M155-008R (EPA 350.1) and M503-555R
165 (Standard Method 450-P I). Changes in the concentrations of dissolved inorganic nutrients
166 were monitored as a proxy for the processes occurring at the sediment water interface due, or
167 not (control), to the presence of the tested population structure of bivalves. In the case of
168 phosphorus, any changes in concentration of the inorganic dissolved form will result from
169 mineralization processes (Zhang *et al.*, 2011) or bivalve metabolism (Vaughn & Hakenkamp,
170 2001), whilst for nitrogen changes in concentration of the inorganic dissolved forms may
171 involve biogeochemical processes taking place at the sediment water interface (e.g. coupled
172 nitrification and denitrification) (Turek & Hoellein, 2015) or excretion of ammonium by
173 living organisms (Liu *et al.*, 2007; Zhang *et al.*, 2011). Nevertheless, the control conditions
174 will account for all these processes taking place at the sediment compartment without the
175 influence of bioturbation and bioirrigation by macrofauna (Turek & Hoellein, 2015; Zhang *et*
176 *al.*, 2011).

177

178 *Data analyses*

179 The data were statistically tested with a Permutational Multivariate Analyses of Variance –
180 PERMANOVA for a three-factor, crossed repeated measures design, to test the response of
181 the concentration of nutrients to the fixed factors size (four levels: control, small, medium and
182 large), salinity (two levels: 0 and 5) and temperature (two levels: 24 and 30), and with time as
183 a repeated measure (3 replicates per time). PERMANOVA is an analysis of variance to test
184 one or more factors, using permutation methods and on the basis of a resemblance matrix
185 (Anderson, Gorley & Clarke, 2008). The only assumption is the exchangeability of samples
186 and therefore it can be used for a repeated measures design (Anderson, Gorley & Clarke,
187 2008). Still, as the analysis was done for each nutrient alone, we treated each time point as

188 separate variable and then performed a multivariate analysis among treatments, using the
189 Euclidean distance as resemblance matrix (Anderson et al. 2008).
190 Prior to the PERMANOVA, the homogeneity of within-group dispersion among tested factors
191 (size, salinity and temperature) was tested with PERMDISP analyses, also with the Euclidean
192 distance as resemblance matrix, as PERMANOVA is sensitive to differences in the
193 multivariate dispersion among groups. For the density experiment, both PERMDISP and
194 PERMANOVA were also applied using the same procedure as with the size experiment (each
195 data point as variable) on a Euclidean Distance basis to test the response of the nutrient
196 content to the fixed factor density, with four levels (control, 6 inds, 12 inds and 24 inds), with
197 replication (3 replicates). All the analyses were performed using PRIMER v6 software with
198 the PERMANOVA add-on package (Anderson, Gorley & Clarke, 2008).

199 Nutrient fluxes to the water column were expressed in terms of concentration change over
200 time. The data obtained per unit of time was modelled by nonlinear regression analysis, using
201 GraphPad Prism 6 (trial version) that uses the least-squares fitting method and the method of
202 Marquardt and Levenberg for adjusting the variables; this method blends the method of linear
203 descent and the method of Gauss-Newton. The one-phase exponential association model (eq.
204 1) was used to fit data from water column nutrient concentrations:

$$205 \quad C_t = C_0 + (C_{ss} - C_0) * (1 - \exp(-k_m t)) \quad (1)$$

206 where C_t and C_{ss} are the concentrations at time t (d) and at steady-state, respectively; k_m is the
207 mineralization rate constant (d^{-1}); C_0 is the concentration at time 0. To assess the goodness of
208 the fit of the experimental data, the coefficient of determination (R^2) and the standard
209 deviation of residues ($S_{x/y}$) were determined. A relatively high R^2 and low value of $S_{x/y}$ were
210 used as criteria for good fit. For each case, the fitting was tested using three replicate
211 concentrations at each studied time.

212 For each nutrient, a half-life (the time it takes to reach half of the equilibrium value) was

213 calculated ($T_{b1/2}$) from the corresponding mineralization (k_m) rate constant, according to the
214 relation $T_{b1/2} = \ln 2/k_m$.

215

216 **Results**

217 *Experimental conditions*

218 Measured salinity for the category “salinity 5” was 4.5 ± 0.3 (mean \pm standard deviation).

219 Regarding temperature, measured values for treatment 24°C were 23.6 ± 1.0 °C, and for the
220 30 °C one 29.8 ± 1.2 °C. Dissolved oxygen concentrations were generally higher for the 24 °C
221 treatment (8.0 ± 0.4 mg L⁻¹; 95%) than for the 30 °C (6.4 ± 0.6 mg L⁻¹; 85% \pm 5), while pH
222 varied within similar values for all treatments (7.1 ± 0.3).

223 Nutrient concentrations at time 0 (C0) were respectively 0.08 mg L⁻¹ PO₄-P, 0.06 mg L⁻¹ NO₃-
224 N, 0.04 mg L⁻¹ NH₄-N and 0.004 mg L⁻¹ NO₂-N. At the last day of the experiment, PO₄-P
225 concentrations ranged between 0.06 and 0.89 mg L⁻¹; NO₃-N concentrations ranged between
226 0.08 and 0.76 mg L⁻¹; NH₄-N concentrations ranged between 0.01 and 0.12 mg L⁻¹; the
227 average NO₂-N concentration was 0.04 mg L⁻¹. However, nutrients showed distinct dynamics
228 and patterns that will be discussed in the following section.

229

230 *Population structure/environmental data experiment*

231 Within-group dispersion was significant for the factor size for both PO₄-P (F=8.59, p-
232 perm=0.001) and NH₄-N (F=24.78, p-perm=0.001). For NH₄-N alone, within group
233 dispersion was also observed for salinity (F=11.06, p-perm=0.03).

234 The 2-way interaction size x temperature was significant for all nutrients, which means that
235 size may have constrained the response to temperature (Table 1). In addition, for PO₄-P and
236 NH₄-N, size x salinity was also significant (Table 1). Time was not included as an
237 independent factor, however, differences with time were visible, with an asymptotic variation

238 pattern for PO₄-P (Fig. 2A) and increasing pattern for NO₃-N through the study period (Fig.
239 2B), and also an increasing pattern for NH₄-N up to day 6 (Fig. 2C). Regarding the other
240 factors, size was the variable with the highest variation component for all nutrients (not
241 shown here), with highest nutrient release generally observed for the small and medium size
242 *Corbicula* treatments.

243 For PO₄-P, significant differences were observed between all size classes' combinations for
244 both salinities, with some exceptions for temperature (Table 1). Generally, PO₄-P release was
245 highest for the small size classes at salinity 0, and for the medium class at salinity 5 and 30 °C
246 (Fig. 2A). As mentioned, PO₄-P release rates were relatively rapid in the first days of the
247 experiment (up to day 6) and progressively diminished until approaching an apparent
248 equilibrium. Dissolved NO₃-N concentrations, in turn, continuously increased until day 12
249 and only started levelling off between days 12 and 18, with less pronounced differences
250 between treatments (Fig. 2B). There were differences between the control and the other size
251 classes, except for the large size individuals at 24 °C (Fig. 2B, Table 1).

252 While salinity was not significant, NO₃-N release was slightly lower for the control at 30 °C
253 and slightly higher for the medium size classes (Table 1, Fig. 2B). Regarding NH₄-N,
254 differences were observed between treatments until day 6 (highest for the medium sized
255 *Corbicula*, with a maximum concentration of 2.0 mg L⁻¹), followed by a sharp decrease
256 towards day 12 (Figure 2C). Consequently, at the end of the experiment no differences were
257 observed between treatments. Nitrate accounted for most dissolved inorganic nitrogen (DIN)
258 in all treatments (89% median value). Considering that time was not accounted as an
259 independent factor, significant differences were detected between control and all size
260 combinations for both salinity and temperatures tested, except for the larger individuals at
261 salinity 0 (Table 1, Fig. 2C). Despite the dispersion in size and salinity, ammonium release
262 was, again, generally highest for the medium and small size class treatments, particularly at

263 the higher salinity and higher temperature (Fig. 2C). Nitrite concentrations did not show a
264 specific pattern, and overall remained low throughout the experiment.

265 A more in-depth study on the kinetics of the nutrient release was performed for phosphorus
266 and nitrate, the prevalent form of nitrogen (Figure 2A, B, Table 2). Generally, a pseudo-first
267 order kinetics model explained nutrient release efficiently, with some exceptions due to the
268 dispersion between replicates or the absence of nutrient release (mainly in Control Tanks).

269 Results for $\text{PO}_4\text{-P}$ confirm the equilibrium at the end of the experimental period, with
270 predicted C_{ss} similar to measured concentrations, and $T_{\text{b}1/2}$ in the range of 2-9 days (Table 2).
271 For $\text{NO}_3\text{-N}$, however, modelling suggests that equilibrium was not attained at the end the 18-
272 day experimental period, with predicted half-life periods of up to 30 days and steady-state
273 concentrations higher than those observed at day 18 (Table 2). Additionally, while no
274 significant differences were observed between most treatments at day 18, kinetic modelling
275 results suggest that if allowed to reach equilibrium, $\text{NO}_3\text{-N}$ concentrations would differ
276 between treatments.

277

278 *Density experiment*

279 Within-group dispersion was non-significant in the density experiment. Density was
280 significant for all nutrients ($p\text{-perm} < 0.02$). However, the pairwise comparisons were not
281 significant ($p\text{-perm} > 0.08$) due to the low number of possible unique permutations (10). Still,
282 some patterns were apparent, with the concentrations of the control generally lower than the
283 ones with fauna (Fig. 3). Phosphate mineralization was faster in the initial 6 days of the
284 experiment, and tended towards equilibrium at the end of the experimental period (Fig. 3A).

285 Density of individuals influenced phosphate release, with the highest concentrations (up to
286 0.9 mg L^{-1}) recorded in the D24 tanks. Inversely, no clear pattern was observed between
287 density treatments for nitrate (Figure 3B) or ammonium (Figure 3C). Generally, nutrient

288 concentrations were within range of the ones observed in the size experiment at equivalent
289 salinity and temperature conditions (i.e. temperature 24 °C and salinity 0, Fig. 3).
290 Kinetic modelling revealed that, similarly to what was observed for the size experiment,
291 *Corbicula* density had distinctive impacts in the nutrient dynamics (Table 3). While the
292 phosphate concentration was positively correlated with bivalve density, with increasing C_{ss}
293 concentrations and half-life times from control to D24 tanks, nitrate concentrations at the end
294 of the experiment were comparable in all treatments. However, the mineralization rates of
295 D12 and D24 treatments were considerably faster, reaching equilibrium before day 3, while
296 D6 and control tanks $T_{b1/2}$ were, respectively, 5 and 9 days (Table 3).

297

298 **Discussion**

299 *The effect of C. fluminea on nutrient dynamics*

300 Considerable scientific attention has been devoted to the impact of *Corbicula fluminea*
301 colonization on invaded ecosystems, in light of its successful geographical expansion and
302 ecological effects (e.g. Crespo *et al.*, 2015). Considering the high population density and
303 productivity of the species, significant biogeochemical effects are expected in the
304 sediment/water column interface, and have been reported by other authors (Majdi, Bardon &
305 Gilbert, 2014; Chen *et al.*, 2016; Zhang *et al.*, 2013; Zhang *et al.*, 2011).

306 Overall, our study reinforces the significant effect of *Corbicula fluminea* on the nutrient
307 dynamics and release into the water column. Despite some within-treatment dispersion, which
308 can be associated with the low number of replicates per treatment, at an ecologically relevant
309 biomass per m^2 , all size classes, salinity and temperature treatments differed, to some extent,
310 from the Control treatment with no fauna. While no sediment or pore-water nutrient
311 concentrations were quantified, differences from control treatments derive from bivalve
312 impact on sediment geochemistry and/or metabolism, in a mass-balance effect between

313 sediment and dissolved nutrient pools, which tends to reach equilibrium.

314 The influence exerted by *C. fluminea* on the nutrient release and dynamics probably results
315 from a combination of mechanisms, comprising both metabolic and physical effects.

316 Still, for the same biomass, the population structure emerges as the main factor governing
317 nutrient release, with enhanced dissolved concentrations in the small and medium sized
318 *Corbicula*' tanks. These enhanced nutrient fluxes associated with smaller-sized clams may
319 result either from their increased sediment reworking activities, when compared with larger
320 individuals, as reported by Majdi *et al.* (2014) and Crespo *et al.* (2018), or by the increased
321 density of individuals in the small size treatments, which occupy a larger surface area. While
322 large clams may induce displacement of particles deeper into the sediment, small clams
323 showed the highest net sediment reworking activity, which should increase the oxic–anoxic
324 sediment interface, and therefore promote nutrient release (e.g. Mortimer *et al.*, 1999;
325 Gilbert, Stora & Bonin, 1998). Bioturbation quantification was performed in this experiment
326 and results seem to support this hypothesis, with much higher sediment reworking rates in
327 medium and small sized *C. fluminea*' tanks, when compared with large sized *C. fluminea* ones
328 (Fig A.1, Crespo *et al.*, 2018). Additionally, smaller clams have been reported to be
329 metabolically more active, exhibiting higher oxygen consumption rates, ammonia excretion
330 rates and CO₂ emission rates than large *C. fluminea* individuals (Xiao *et al.*, 2014; Mortimer
331 *et al.*, 1999). This higher metabolic rate (and higher number of individuals) would favour an
332 increase in the net nutrient release through excretion, which might have substantial effect on
333 river nutrient cycling, especially in summer (Lauritsen & Mozley, 1989).

334 The observed enhancement of phosphorus release by the presence of *C. fluminea* has
335 previously been reported, and attributed to the increased diffusion, enhanced advection
336 between the pore water and overlying water and bivalve metabolism (Zhang *et al.*, 2011). For
337 transitional waters, the increase in the oxic–anoxic sediment interface and the enhancement of

338 sediment total microbial activity may accelerate organic matter degradation, and thus release
339 additional phosphorus to the water column (Lillebø *et al.*, 1999). In a different study, Chen *et*
340 *al.* (2016) observed up to 157% increase in P fluxes from *C. fluminea* bioturbated sediments,
341 associated to the effects of bivalve respiration in the sediment. In addition, an inverse
342 relationship between unionid bivalve size and phosphorus excretion rates has been reported
343 (Vaughn & Hakenkamp, 2001), which may partially explain the higher phosphorus release in
344 medium and small sized *C. fluminea* treatments. Regarding density, phosphate release was
345 favoured by increasing population density, suggesting that metabolic P release may also play
346 a significant role in the phosphorus flux into the water column, as observed by Lauritsen and
347 Mozley (1989).

348 Regarding the dynamics of DIN ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N} + \text{NO}_2\text{-N}$), the presence of *C. fluminea*
349 originated a net increase of DIN release into the water column, especially for medium and
350 small-size treatments. Overall, while at the end of the experiment nitrate was the dominant
351 form, accounting for circa 90% of DIN, in the first days of the experiment ammonium played
352 an important role in the nitrogen cycle. The effect of bivalve presence in the nitrogen release
353 probably resulted from a combination of mechanisms, similarly to what was observed for
354 $\text{PO}_4\text{-P}$. Several reports accredited the increase of dissolved ammonium concentrations to both
355 bivalve metabolism/excretion and bioturbation-mediated release from the sediment (Liu *et al.*,
356 2007; Zhang *et al.*, 2011). Bivalves excrete hypo-osmotic urine, consisting primarily of
357 ammonia, with a variable rate depending on the species, individual size, temperature, stage in
358 reproductive cycle and food availability (Vaughn & Hakenkamp, 2001 and references
359 therein), with consequent increases in the water column ammonium concentration (Mortimer
360 *et al.*, 1999). In parallel, the syphoning activity of *C. fluminea* enhances solute exchange
361 between overlying water and pore water, and thus helps ammonium diffusion from the
362 sediment, while burrowing activities increased mineralization and decomposition rates of

363 organic matter in sediments and ultimately enhanced ammonium efflux (Liu *et al.*, 2007).
364 Ammonium release was favoured in medium and small sized *C. fluminea*' tanks, highlighting
365 the role of metabolic excretion in its dynamics, considering that the mass-specific nutrient
366 excretion rates of animals (i.e. nutrients excreted per unit body mass per unit time) usually
367 declines with increasing body mass (Vanni, 2002). The nitrate dynamics, on the other hand,
368 may result from bioturbation-mediated sediment efflux, and also from the onset of bacterial
369 nitrification (Liu *et al.*, 2007), following the increase of dissolved ammonium in the first days
370 of the experiment. While the presence of *C. fluminea* influenced nitrate release, the effect of
371 bivalve size and density was less pronounced, possibly due to the minor dependence on
372 bivalve metabolism. In fact, bivalve density did not affect the final dissolved NO₃-N
373 concentrations, but rather the kinetics of its release from the sediments, favoured by the more
374 intense bioturbation in higher density treatments.

375 These results highlight the importance of population structure on the dissolved nutrient
376 dynamics, especially considering the die-off events reported for this species (Sousa *et al.*,
377 2012; Oliveira, Vilares & Guilhermino, 2015). Its ability to rapidly repopulate previously
378 colonized habitats following massive population crashes caused by extreme physical
379 conditions will result in dense populations of *C. fluminea* composed mostly of juveniles
380 (Franco *et al.*, 2012; Sousa *et al.*, 2008b), which will result in enhanced nutrient release into
381 the water column when compared with more mature, structured populations.

382

383 *Climate change scenarios*

384 One of the main objectives of the experiment was to evaluate the effect of hypothetical
385 climate change scenarios on the dissolved inorganic nutrient dynamics of *C. fluminea*
386 colonized ecosystems, considering the predictions of the IPCC (2014). Overall, our results
387 suggest that both drought (salinity increase) and heat wave (temperature increase) phenomena

388 may have a significant impact on the nutrient dynamics in aquatic systems colonized by this
389 alien invasive species. Specifically, the drought simulation (salinity increase) revealed an
390 increase of $\text{NH}_4\text{-N}$ release and reduced phosphate release. Ammonium efflux has been found
391 to be salinity dependent, and to increase parallel to overlying water salinity (Giblin *et al.*,
392 2010), through the diffusion of salts into and ammonium out of porewater. Weston *et al.*
393 (2010) found that small increases in salinity in upper estuary sediments (from 0 to 8) may
394 reduce the exchangeable pool of the total $\text{NH}_4\text{-N}$ and increase the fraction of dissolved $\text{NH}_4\text{-N}$
395 relative to adsorbed $\text{NH}_4\text{-N}$. The presence of *C. fluminea* seems to have amplified this
396 diffusion process, probably as a result of bioturbation, and can therefore have a significant
397 impact in DIN release from the sediment to the water column. This increase of ammonium
398 efflux was not reflected on dissolved nitrate concentrations, even after the drop in $\text{NH}_4\text{-N}$
399 concentrations (between day 6 and 12 of the experiment), which is in agreement with a
400 previous study (Turek & Hoellein, 2015). The authors observed that *C. fluminea* significantly
401 increased $\text{NH}_4\text{-N}$ and N_2 flux out of sediments but did not affect $\text{NO}_3\text{-N}$ flux, suggesting the
402 presence of coupled nitrification–denitrification processes (Turek & Hoellein, 2015).
403 Contrary to the most common behaviour in transitional ecosystems with salinity gradients, the
404 increase in salinity did not favor phosphate release into the water column; instead, dissolved
405 $\text{PO}_4\text{-P}$ concentrations decreased in all treatments except in the medium sized *C. fluminea*. P–
406 Fe–S interactions may promote the release of $\text{PO}_4\text{-P}$ from the sediments with increasing
407 salinity, probably due to the dissolution of iron (hydr)oxides, the formation of iron (II)
408 sulfides and the release of iron-bound P (Coelho *et al.*, 2004; Jensen *et al.*, 1995). Given that
409 phosphate desorption from ferric iron (hydr)oxides is enhanced with increasing pH (Spiteri,
410 Cappellen & Regnier, 2008), the stability observed in pH values throughout the different
411 treatments and over time in the present experiment may partially explain the phosphate
412 behaviour pattern. Additionally, the possible formation of ternary Mg-phosphate surface

413 complexes may have prevented, or at least limited the extent of phosphate desorption with
414 increasing salinity (Spiteri, Cappellen & Regnier, 2008).

415 The heat wave scenario (temperature increase), in turn, only enhanced $\text{NH}_4\text{-N}$ release to the
416 water column, in synergy with the salinity effect, while coupled to the depletion of dissolved
417 oxygen concentrations. A similar pattern of increased $\text{NH}_4\text{-N}$ effluxes with temperature in the
418 presence of *C. fluminea* were reported previously (Zhang *et al.*, 2013), coupled with higher
419 sediment oxygen uptake and $\text{PO}_4\text{-P}$ release. This behavior may result from the reductive
420 dissolution of easily reducible Fe oxides due to the depletion of oxygen in the top sediment
421 layers from bivalve respiration (Chen *et al.*, 2016). However, those results reflect a 10°C
422 change in temperature, from 15 to 25°C , while the measured temperature differential in the
423 present study was lower (6°C) and in the upper physiological range of the species (from 24 to
424 30°C). In a different study, Xiao *et al.* (2014) found similar oxygen consumption rates and
425 $\text{NH}_4\text{-N}$ excretion rates for *C. fluminea* at 25°C and 32°C , which may suggest that changes
426 within this temperature range have little effect on the metabolism of this bivalve, and that the
427 increase of $\text{NH}_4\text{-N}$ efflux may have a source other than metabolic, such as bioturbation and
428 bioirrigation. Bivalve activity will enhance nutrient regeneration and enhanced diffusion
429 through the burrow walls, especially with higher water temperatures, which will promote
430 higher $\text{NH}_4\text{-N}$ efflux from the sediments (Kemp *et al.*, 1990). If this is the case, the oxygen
431 depletion in 30°C tanks will not derive from bivalve metabolism, and may have, in turn,
432 constrained the species response during the experiment, due to its sensitivity to low oxygen
433 concentrations.

434 Overall, the present research highlights the significant effect of *C. fluminea* in ecosystem
435 nutrient release and dynamics, enhancing the efflux of both phosphate and DIN into the water
436 column. Concerning the climate change scenarios tested, both drought (salinity increase) and
437 heat wave (temperature increase) events will have an impact on the DIN dynamics within *C.*

438 *fluminea* colonized systems, favouring a higher NH₄-N efflux, especially in the first days after
439 a shift in environmental conditions. The population structure of this IAS on any given system
440 will have a decisive role on the impact of the species, as a predominantly juvenile population
441 was found to promote stronger nutrient fluxes into the water column.

442

443 **Acknowledgements**

444 The authors would like to thank all colleagues who helped in field and laboratory work. This
445 research was supported by FCT (Portuguese Foundation for Science and Technology),
446 through a PhD grant attributed to D. Crespo (SFRH/BD/80252/2011), post-doc grant
447 attributed to S. Leston (SFRH/BPD/91828/2012), the Investigador FCT programme attributed
448 to M. Dolbeth (IF/00919/2015) and BIOCHANGED project (PTDC/MAR/111901/2009),
449 subsidized by the European Social Fund and MCTES (Portuguese Ministry of Science,
450 Technology and Higher Education), through the POPH (Human Potential Operational
451 Programme), QREN (National Strategic Reference Framework) and COMPETE (Programa
452 Operacional Factores de Competitividade). Thanks are also due, for the financial support to
453 CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638) and CIIMAR
454 (UID/Multi/04423/2013), to FCT/MEC through national funds (PIDDAC), and the co-
455 funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. This
456 work was also supported by the Integrated Program of SR&TD ‘Smart Valorization of
457 Endogenous Marine Biological Resources Under a Changing Climate’ (reference Centro-01-
458 0145-FEDER-000018), co-funded by Centro 2020 program, Portugal 2020, European Union,
459 through the European Regional Development Fund.

460

461

462 **References**

- 463 Anderson M., Gorley R.N. & Clarke K.R. (2008) *PERMANOVA+ for PRIMER: Guide to*
464 *Software and Statistical Methods*, PRIMER-E.
- 465 Atkinson C.L., First M.R., Covich A.P., Opsahl S.P. & Golladay S.W. (2011) Suspended
466 material availability and filtration–biodeposition processes performed by a
467 native and invasive bivalve species in streams. *Hydrobiologia*, **667**, 191-204.
- 468 Cerco C.F. & Noel M.R. (2010) Monitoring, modeling, and management impacts of bivalve
469 filter feeders in the oligohaline and tidal fresh regions of the Chesapeake Bay
470 system. *Ecological Modelling*, **221**, 1054-1064.
- 471 Chan K.Y. & Bendell L.I. (2013) Potential effects of an invasive bivalve, *Nuttallia*
472 *obscurata*: on select sediment attributes within the intertidal region of coastal
473 British Columbia. *Journal of Experimental Marine Biology and Ecology*, **444**, 66-
474 72.
- 475 Chen M., Ding S., Liu L., Xu D., Gong M., Tang H. & Zhang C. (2016) Kinetics of phosphorus
476 release from sediments and its relationship with iron speciation influenced by
477 the mussel (*Corbicula fluminea*) bioturbation. *Science of The Total Environment*,
478 **542, Part A**, 833-840.
- 479 Coelho J.P., Flindt M.R., Jensen H.S., Lillebø A.I. & Pardal M.A. (2004) Phosphorus
480 speciation and availability in intertidal sediments of a temperate estuary: relation
481 to eutrophication and annual P-fluxes. *Estuarine Coastal and Shelf Science*, **61**,
482 583-590.
- 483 Crespo D., Dolbeth M., Leston S., Sousa R. & Pardal M. (2015) Distribution of *Corbicula*
484 *fluminea* (Müller, 1774) in the invaded range: a geographic approach with notes
485 on species traits variability. *Biological Invasions*, **17**, 2087-2101.
- 486 Crespo D., Leston S., Martinho F., Pardal M.A. & Dolbeth M. (2017) Survival of *Corbicula*
487 *fluminea* (Müller, 1774) in a natural salinity and temperature gradient: a field
488 experiment in a temperate estuary. *Hydrobiologia*, **784**, 337-347.
- 489 Crespo D., Solan M., Leston S., Pardal M.A. & Dolbeth M. (2018) Ecological consequences
490 of invasion across the freshwater–marine transition in a warming world. *Ecology*
491 *and Evolution*, **8**, 1807-1817.
- 492 Dolbeth M., Cardoso P.G., Grilo T.F., Bordalo M.D., Raffaelli D. & Pardal M.A. (2011) Long-
493 term changes in the production by estuarine macrobenthos affected by multiple
494 stressors. *Estuarine Coastal and Shelf Science*, **92**, 10-18.
- 495 Franco J.N., Ceia F.R., Patricio J., Modesto V., Thompson J., Marques J.C. & Neto J.M. (2012)
496 Population dynamics of *Corbicula fluminea* (Müller, 1774) in mesohaline and
497 oligohaline habitats: Invasion success in a Southern Europe estuary. *Estuarine*
498 *Coastal and Shelf Science*, **112**, 31-39.
- 499 Giblin A.E., Weston N.B., Banta G.T., Tucker J. & Hopkinson C.S. (2010) The Effects of
500 Salinity on Nitrogen Losses from an Oligohaline Estuarine Sediment. *Estuaries*
501 *and Coasts*, **33**, 1054-1068.
- 502 Gilbert F., Stora G. & Bonin P. (1998) Influence of bioturbation on denitrification activity
503 in Mediterranean coastal sediments: an in situ experimental approach. *Marine*
504 *Ecology Progress Series*, **163**, 99-107.
- 505 Grilo T.F., Cardoso P.G., Dolbeth M., Bordalo M.D. & Pardal M.A. (2011) Effects of extreme
506 climate events on the macrobenthic communities' structure and functioning of a
507 temperate estuary. *Marine Pollution Bulletin*, **62**, 303-311.
- 508 Hakenkamp C.C. & Palmer A.M. (1999) Introduced bivalves in freshwater ecosystems:
509 the impact of *Corbicula* on organic matter dynamics in a sandy stream. *Oecologia*,
510 **119**, 445-451.

- 511 Ippc. (2014) Climate Change 2014: Synthesis Report - Contribution of Working Groups I,
512 II and III to the Fifth Assessment Report of the Intergovernmental Panel on
513 Climate Change. (Eds C.W. Team & R.K. Pachauri & L.A. Meyer), p. 151. IPCC,
514 Geneva.
- 515 Jensen H.S., Mortensen P.B., Andersen F., Rasmussen E. & Jensen A. (1995) Phosphorus
516 cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Limnology and*
517 *Oceanography*, **40**, 908-917.
- 518 Kemp W.M., Sampou P., Caffrey J., Mayer M., Henriksen K. & Boynton W.R. (1990)
519 Ammonium recycling versus denitrification in Chesapeake Bay sediments.
520 *Limnology and Oceanography*, **35**, 1545-1563.
- 521 Lauritsen D.D. & Mozley S.C. (1989) Nutrient Excretion by the Asiatic Clam *Corbicula*
522 *fluminea*. *Journal of the North American Benthological Society*, **8**, 134-139.
- 523 Lillebø A.I., Flindt M.R., Pardal M.A. & Marques J.C. (1999) The effect of macrofauna,
524 meiofauna add microfauna on the degradation of *Spartina maritima* detritus from
525 a salt marsh area. *Acta Oecologica-International Journal Of Ecology*, **20**, 249-258.
- 526 Liu J., Chen Z., Xu S. & Zheng X. (2007) Experimental research on the impact of *Corbicula*
527 *fluminea* on DIN exchange at a tidal flat sediment-water interface. *Chinese Journal*
528 *of Oceanology and Limnology*, **25**, 434-443.
- 529 Magni P., Montani S., Takada C. & Tsutsumi H. (2000) Temporal scaling and relevance of
530 bivalve nutrient excretion on a tidal flat of the Seto Inland Sea, Japan. *Marine*
531 *Ecology Progress Series*, **198**, 139-155.
- 532 Majdi N., Bardon L. & Gilbert F. (2014) Quantification of sediment reworking by the
533 Asiatic clam *Corbicula fluminea* Müller, 1774. *Hydrobiologia*, **732**, 85-92.
- 534 Mcdowell W.G., Benson A.J. & Byers J.E. (2014) Climate controls the distribution of a
535 widespread invasive species: implications for future range expansion. *Freshwater*
536 *Biology*, **59**, 847-857.
- 537 McMahan R.F. (2002) Evolutionary and physiological adaptations of aquatic invasive
538 animals: r selection versus resistance. *Canadian Journal of Fisheries and Aquatic*
539 *Sciences*, **59**, 1235-1244.
- 540 Modesto V., Franco J.N., Sousa R., Patrício J., Marques J.C. & Neto J.M. (2013) Spatial and
541 temporal dynamics of *Corbicula fluminea* (Müller, 1774) in relation to
542 environmental variables in the Mondego Estuary (Portugal). *Journal of Molluscan*
543 *Studies*, **79**, 302-309.
- 544 Montoya J.M. & Raffaelli D. (2010) Climate change, biotic interactions and ecosystem
545 services. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
546 **365**, 2013-2018.
- 547 Mortimer R.J.G., Davey J.T., Krom M.D., Watson P.G., Frickers P.E. & Clifton R.J. (1999)
548 The Effect of Macrofauna on Porewater Profiles and Nutrient Fluxes in the
549 Intertidal Zone of the Humber Estuary. *Estuarine, Coastal and Shelf Science*, **48**,
550 683-699.
- 551 Mouthon J. & Daufresne M. (2006) Effects of the 2003 heatwave and climatic warming
552 on mollusc communities of the Saône: a large lowland river and of its two main
553 tributaries (France). *Global Change Biology*, **12**, 441-449.
- 554 Oliveira C., Vilares P. & Guilhermino L. (2015) Integrated biomarker responses of the
555 invasive species *Corbicula fluminea* in relation to environmental abiotic
556 conditions: A potential indicator of the likelihood of clam's summer mortality
557 syndrome. *Comparative Biochemistry and Physiology Part A: Molecular &*
558 *Integrative Physiology*, **182**, 27-37.

- 559 Petter G., Weitere M., Richter O. & Moenickes S. (2014) Consequences of altered
560 temperature and food conditions for individuals and populations: a Dynamic
561 Energy Budget analysis for *Corbicula fluminea* in the Rhine. *Freshwater Biology*,
562 **59**, 832-846.
- 563 Phelps H.L. (1994) The asiatic clam (*Corbicula fluminea*) invasion and system-level
564 ecological change in the Potomac River Estuary near Washington, D.C. *Estuaries*,
565 **17**, 614-621.
- 566 Rosa I.C., Pereira J.L., Costa R., Gonçalves F. & Prezant R. (2012) Effects of Upper-Limit
567 Water Temperatures on the Dispersal of the Asian Clam *Corbicula fluminea*. *PLoS*
568 *One*, **7**, e46635.
- 569 Simberloff D., Martin J.-L., Genovesi P., Maris V., Wardle D.A., Aronson J., Courchamp F.,
570 Galil B., García-Berthou E., Pascal M., Pyšek P., Sousa R., Tabacchi E. & Vilà M.
571 (2013) Impacts of biological invasions: what's what and the way forward. *Trends*
572 *in Ecology & Evolution*, **28**, 58-66.
- 573 Sousa R., Antunes C. & Guilhermino L. (2008) Ecology of the invasive Asian clam
574 *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. *Annales de*
575 *Limnologie - International Journal of Limnology*, **44**, 85-94.
- 576 Sousa R., Dias S., Freitas V. & Antunes C. (2008a) Subtidal macrozoobenthic assemblages
577 along the River Minho estuarine gradient (north-west Iberian Peninsula). *Aquatic*
578 *Conservation: Marine and Freshwater Ecosystems*, **18**, 1063-1077.
- 579 Sousa R., Gutiérrez J. & Aldridge D. (2009) Non-indigenous invasive bivalves as
580 ecosystem engineers. *Biological Invasions*, **11**, 2367-2385.
- 581 Sousa R., Nogueira A.J.A., Gaspar M.B., Antunes C. & Guilhermino L. (2008b) Growth and
582 extremely high production of the non-indigenous invasive species *Corbicula*
583 *fluminea* (Muller, 1774): Possible implications for ecosystem functioning.
584 *Estuarine Coastal and Shelf Science*, **80**, 289-295.
- 585 Sousa R., Varandas S., Cortes R., Teixeira A., Lopes-Lima M., Machado J. & Guilhermino L.
586 (2012) Massive die-offs of freshwater bivalves as resource pulses. *Annales de*
587 *Limnologie - International Journal of Limnology*, **48**, 105-112.
- 588 Spiteri C., Cappellen P.V. & Regnier P. (2008) Surface complexation effects on phosphate
589 adsorption to ferric iron oxyhydroxides along pH and salinity gradients in
590 estuaries and coastal aquifers. *Geochimica et Cosmochimica Acta*, **72**, 3431-3445.
- 591 Turek K.A. & Hoellein T.J. (2015) The invasive Asian clam (*Corbicula fluminea*) increases
592 sediment denitrification and ammonium flux in 2 streams in the midwestern
593 USA. *Freshwater Science*, **34**, 472-484.
- 594 Vanni M.J. (2002) Nutrient Cycling by Animals in Freshwater Ecosystems. *Annual Review*
595 *of Ecology and Systematics*, **33**, 341-370.
- 596 Vaughn C.C. & Hakenkamp C.C. (2001) The functional role of burrowing bivalves in
597 freshwater ecosystems. *Freshwater Biology*, **46**, 1431-1446.
- 598 Weston N.B., Giblin A.E., Banta G.T., Hopkinson C.S. & Tucker J. (2010) The Effects of
599 Varying Salinity on Ammonium Exchange in Estuarine Sediments of the Parker
600 River, Massachusetts. *Estuaries and Coasts*, **33**, 985-1003.
- 601 Wrona F.J., Prowse T.D., Reist J.D., Hobbie J.E., Lévesque L.M.J. & Vincent W.F. (2006)
602 Climate Change Effects on Aquatic Biota, Ecosystem Structure and Function.
603 *AMBIO: A Journal of the Human Environment*, **35**, 359-369.
- 604 Xiao B.-C., Li E.-C., Du Z.-Y., Jiang R.-L., Chen L.-Q. & Yu N. (2014) Effects of temperature
605 and salinity on metabolic rate of the Asiatic clam *Corbicula fluminea* (Muller,
606 1774). *SpringerPlus*, **3**, 455.

607 Zhang L., Liao Q., He W., Shang J. & Fan C. (2013) The effects of temperature on oxygen
608 uptake and nutrient flux in sediment inhabited by molluscs. *Journal of Limnology*,
609 **72**, 13-20.

610 Zhang L., Shen Q., Hu H., Shao S. & Fan C. (2011) Impacts of *Corbicula fluminea* on
611 Oxygen Uptake and Nutrient Fluxes across the Sediment–Water Interface. *Water*,
612 *Air, & Soil Pollution*, **220**, 399-411.

613

614

ACCEPTED MANUSCRIPT

Table 1. Summary of significant terms from the PERMANOVA analyses, using nutrient concentrations as dependent variables and size class (control, small, medium and large), salinity (0 and 5) and temperature (24°C and 30°C) as explanatory variables, with indication of the significant pairwise comparisons. Tested terms were: single ones - size, salinity, temperature, 2-way interactions - Size x Salinity, Size x Temperature, Salinity x Temperature, and the 3-way interaction Size x Salinity x Temperature

Dependent variable	Significant terms	d.f.	Pseudo-F	p-value	Terms/levels of factor	P (perm)
PO ₄ -P	Size x Salinity	3	6.4878	0.001	- Salinity 0 and 5: all size combinations - Control, Small and Large: Salinity 0 vs. Salinity 5	<0.03 <0.01
	Size x Temperature	3	3.4159	0.015	- 24°C: all size combinations, except Control vs. Medium - 30°C: all size combinations, except Small vs. Medium - Large size: 24°C vs. 30°C	<0.01 <0.005 <0.05
NO ₃ -N	Size x Temperature	3	2.7054	0.039	- 24°C: all size combinations, except Control vs. Large - 30°C: all size combinations, except Small vs. Medium - Control: 24°C vs. 30°C - Small size: 24°C vs. 30°C	<0.02 <0.01 0.001 0.018
NH ₄ -N	Size x Salinity	3	5.1773	0.001	- Salinity 0: all size combinations, except Small vs Medium - Salinity 5: all size combinations - All sizes: Salinity 0 vs. Salinity 5	<0.02 <0.03 <0.03
	Size x Temperature	3	4.4135	0.007	- 24°C: all size combinations, except Control vs. Large and Small vs. Medium - 30°C: all size combinations - Medium size: 24°C vs. 30°C	<0.002 <0.01 0.021

Table 2. Estimated kinetic parameters of the nutrient (PO₄-P and NO₃-N) release from the size-class experiment tanks (n=3 for each time point). C₀, concentration at time 0; C_{ss}, concentration at steady state; k_m: mineralization rate constant (d⁻¹); T_{b1/2}: half-life (d); R₂: determination coefficient; SE: standard error;

			C ₀ (mg L ⁻¹) ± SE	C _{ss} (mg L ⁻¹) ± SE	K _m (d ⁻¹) ± SE	T _{b1/2}	R ²	S _{x/y}	
PO ₄ -P	24°C	Sal 0	Control	0.061 ± 0.022	0.18 ± 0.02	0.35 ± 0.21	1.98	0.60	0.039
			Large	0.061 ± 0.032	0.18 ± 0.03	0.31 ± 0.25	2.25	0.44	0.056
			Medium	0.076 ± 0.022	0.32 ± 0.02	0.21 ± 0.06	3.24	0.87	0.038
			Small	0.096 ± 0.024	0.48 ± 0.02	0.23 ± 0.05	2.98	0.93	0.041
	Sal 5	Control	-	-	-	-	-	-	-
		Large	0.082 ± 0.016	0.18 ± 0.05	0.10 ± 0.12	6.92	0.57	0.028	
		Medium	0.095 ± 0.040	0.37 ± 0.23	0.07 ± 0.11	9.23	0.57	0.074	
		Small	0.091 ± 0.030	0.27 ± 0.02	0.35 ± 0.02	1.96	0.67	0.051	
	30°C	Sal 0	Control	0.093 ± 0.025	0.13 ± 0.02	0.41 ± 1.06	1.69	0.08	0.044
			Large	0.050 ± 0.026	0.28 ± 0.02	0.26 ± 0.09	2.67	0.82	0.045
			Medium	0.061 ± 0.040	0.32 ± 0.04	0.21 ± 0.13	3.38	0.71	0.069
			Small	0.089 ± 0.031	0.48 ± 0.03	0.24 ± 0.06	2.92	0.90	0.054
Sal 5		Control	0.068 ± 0.020	0.099 ± 0.014	0.46 ± 1.02	1.50	0.12	0.035	
		Large	0.090 ± 0.026	0.17 ± 0.03	0.18 ± 0.23	3.79	0.30	0.047	
		Medium	0.059 ± 0.099	0.53 ± 0.07	0.41 ± 0.28	1.69	0.57	0.17	
		Small	0.071 ± 0.033	0.25 ± 0.03	0.31 ± 0.18	2.21	0.62	0.057	
NO ₃ -N	24°C	Sal 0	Control	0.038 ± 0.026	2.0 ± 4.2	0.014 ± 0.033	50.4	0.92	0.050
			Large	0.018 ± 0.041	1.9 ± 2.7	0.022 ± 0.038	30.8	0.91	0.078
			Medium	0.031 ± 0.027	0.91 ± 0.08	0.10 ± 0.02	6.66	0.97	0.050
			Small	0.042 ± 0.035	0.83 ± 0.06	0.15 ± 0.03	4.70	0.96	0.063
	Sal 5	Control	-	-	-	-	-	-	-
		Large	0.030 ± 0.031	1.4 ± 0.5	0.044 ± 0.023	15.7	0.96	0.058	
		Medium	0.026 ± 0.042	1.2 ± 0.4	0.057 ± 0.030	12.2	0.94	0.079	
		Small	0.076 ± 0.027	0.97 ± 0.11	0.089 ± 0.021	7.83	0.97	0.049	
	30°C	Sal 0	Control	0.036 ± 0.053	0.18 ± 0.06	0.21 ± 0.27	3.27	0.28	0.094
			Large	-	-	-	-	-	-
			Medium	0.055 ± 0.060	1.0 ± 0.3	0.078 ± 0.046	8.90	0.87	0.11
			Small	0.033 ± 0.052	1.1 ± 0.4	0.062 ± 0.039	11.1	0.90	0.096
Sal 5		Control	-	-	-	-	-	-	
		Large	0.026 ± 0.047	2.1 ± 3.4	0.021 ± 0.039	33.8	0.89	0.090	
		Medium	0.000 ± 0.057	2.5 ± 3.6	0.023 ± 0.040	30.7	0.91	0.11	
		Small	0.005 ± 0.041	1.4 ± 0.6	0.048 ± 0.028	14.6	0.94	0.077	

Table 3. Estimated kinetic parameters of the nutrient (PO₄-P and NO₃-N) release from the density experiment tanks (n=3 for each time point). C₀, concentration at time 0; C_{ss}, concentration at steady state; k_m: mineralization rate constant (d⁻¹); T_{b1/2}: half-life (d); R₂: determination coefficient; SE: standard error;

		C ₀ (mg L ⁻¹) ± SE	C _{ss} (mg L ⁻¹) ± SE	K _m (d ⁻¹) ± SE	T _{b1/2}	R ²	S _{x/y}
PO ₄ -P	Control	0.24 ± 0.03	0.30 ± 0.02	0.59 ± 0.90	1.18	0.29	0.046
	D6	0.21 ± 0.04	0.48 ± 0.04	0.25 ± 0.04	2.81	0.70	0.071
	D12	0.27 ± 0.03	0.73 ± 0.05	0.16 ± 0.05	4.37	0.90	0.059
	D24	0.29 ± 0.04	0.91 ± 0.07	0.14 ± 0.07	4.90	0.92	0.067
NO ₃ -N	Control	0.16 ± 0.05	0.88 ± 0.26	0.077 ± 0.053	8.97	0.84	0.095
	D6	0.17 ± 0.06	0.79 ± 0.12	0.13 ± 0.07	5.18	0.80	0.11
	D12	0.19 ± 0.06	0.70 ± 0.04	0.64 ± 0.31	1.08	0.83	0.010
	D24	-	-	-	-	-	-

Figure captions

Fig. 1. Experimental design schematics.

Fig. 2. The concentration of dissolved inorganic nutrients in the water column: over time for each size class, per salinity and temperature. A fitted regression line was added to plot to clarify the variation patterns. A) $\text{PO}_4\text{-P}$; B) $\text{NO}_3\text{-N}$; and C) $\text{NH}_4\text{-N}$.

Fig. 3. The concentration of dissolved inorganic nutrients in the water column: over time for each density class. A fitted regression line was added to plot to clarify the variation patterns. A) $\text{PO}_4\text{-P}$; B) $\text{NO}_3\text{-N}$; and C) $\text{NH}_4\text{-N}$.

Figure 1

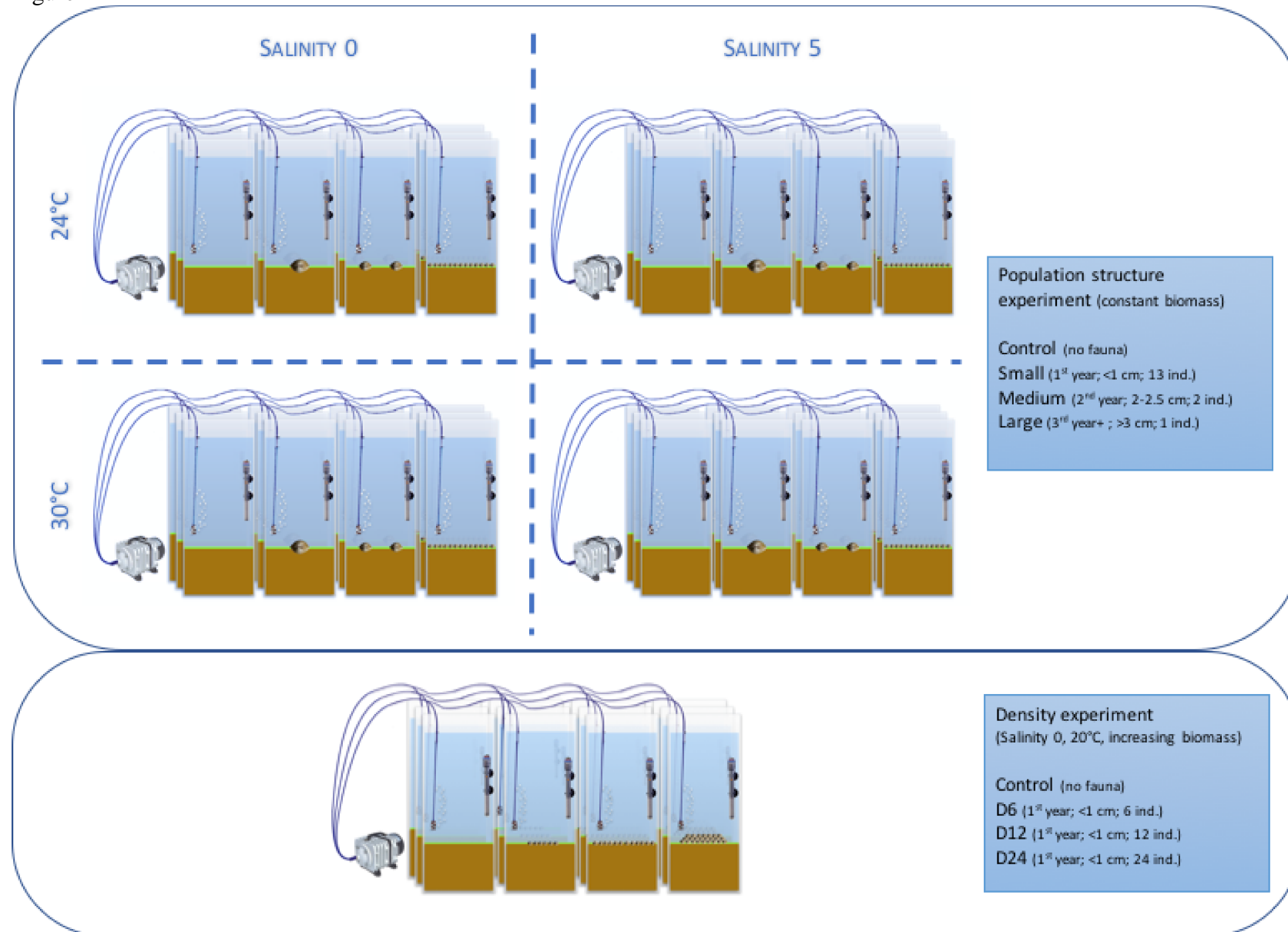


Figure 2A

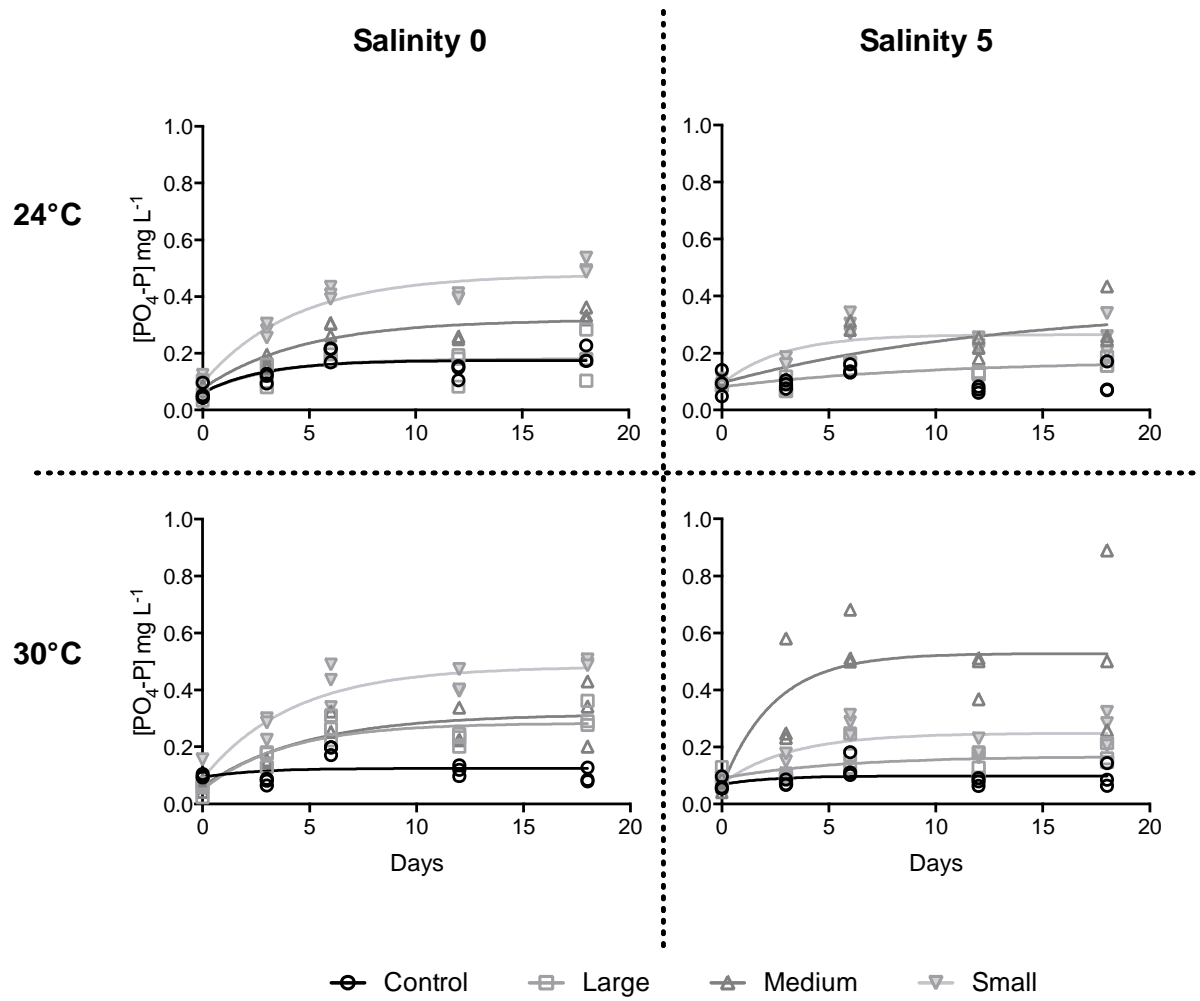


Figure 2B

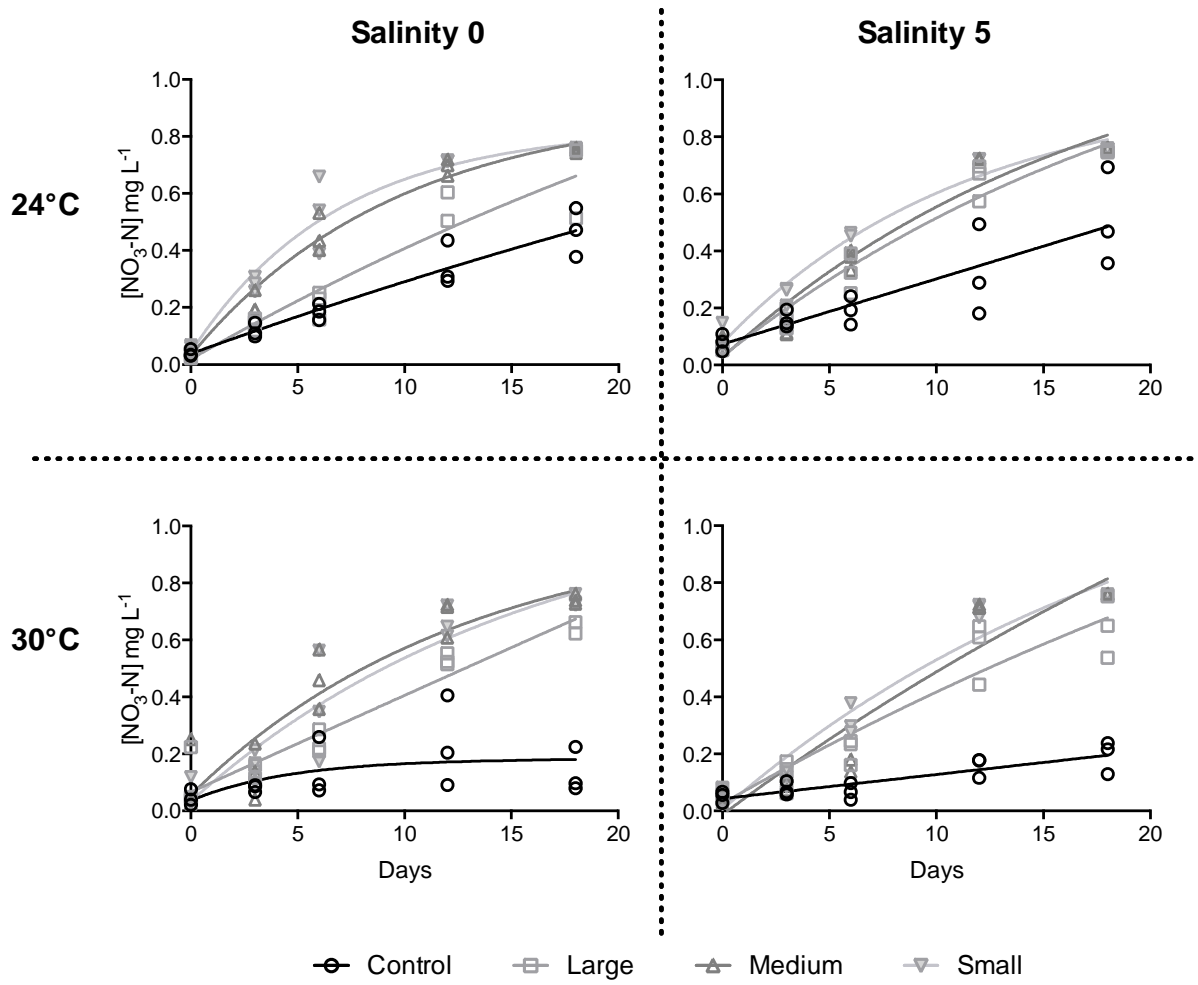


Figure 2C

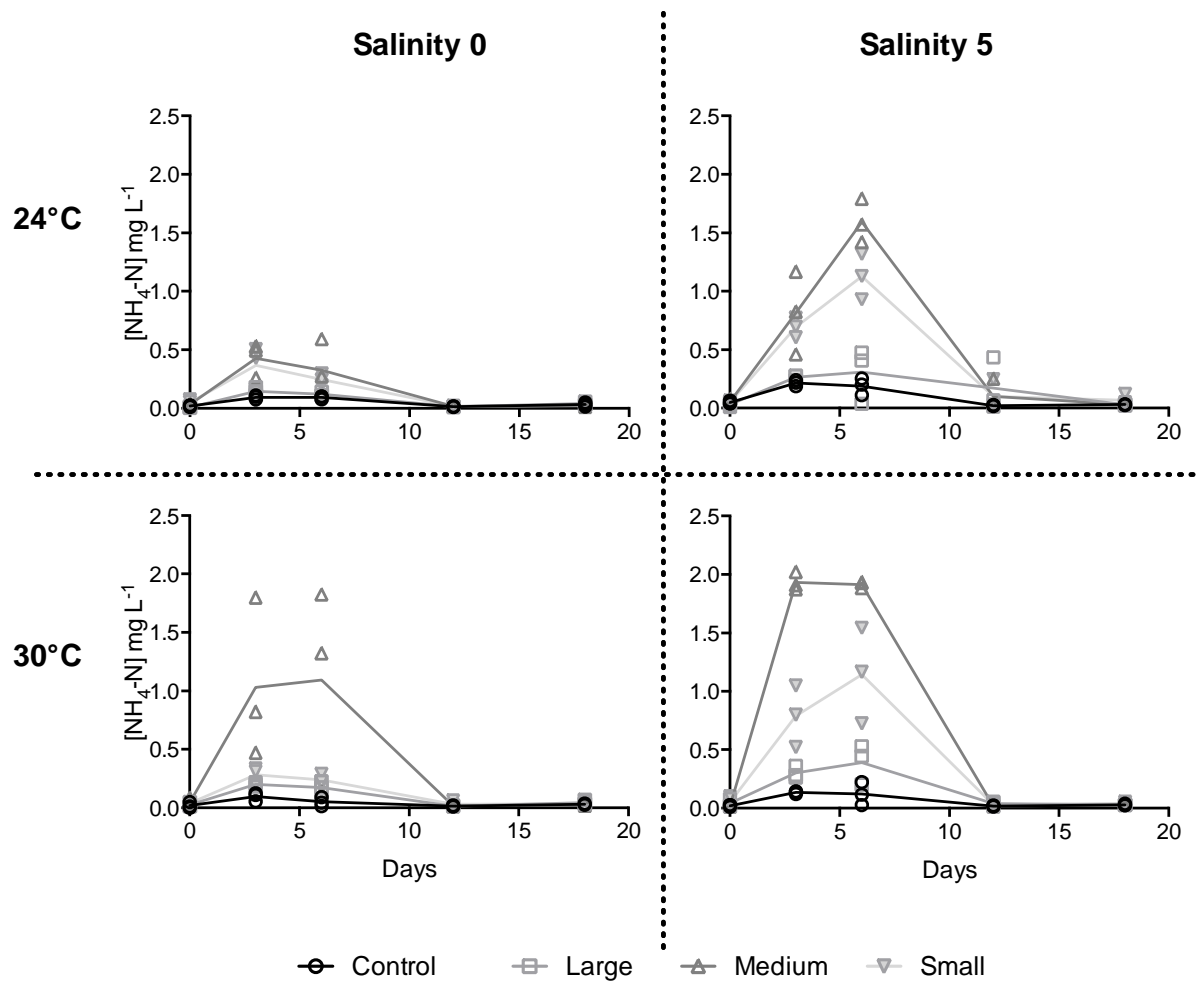
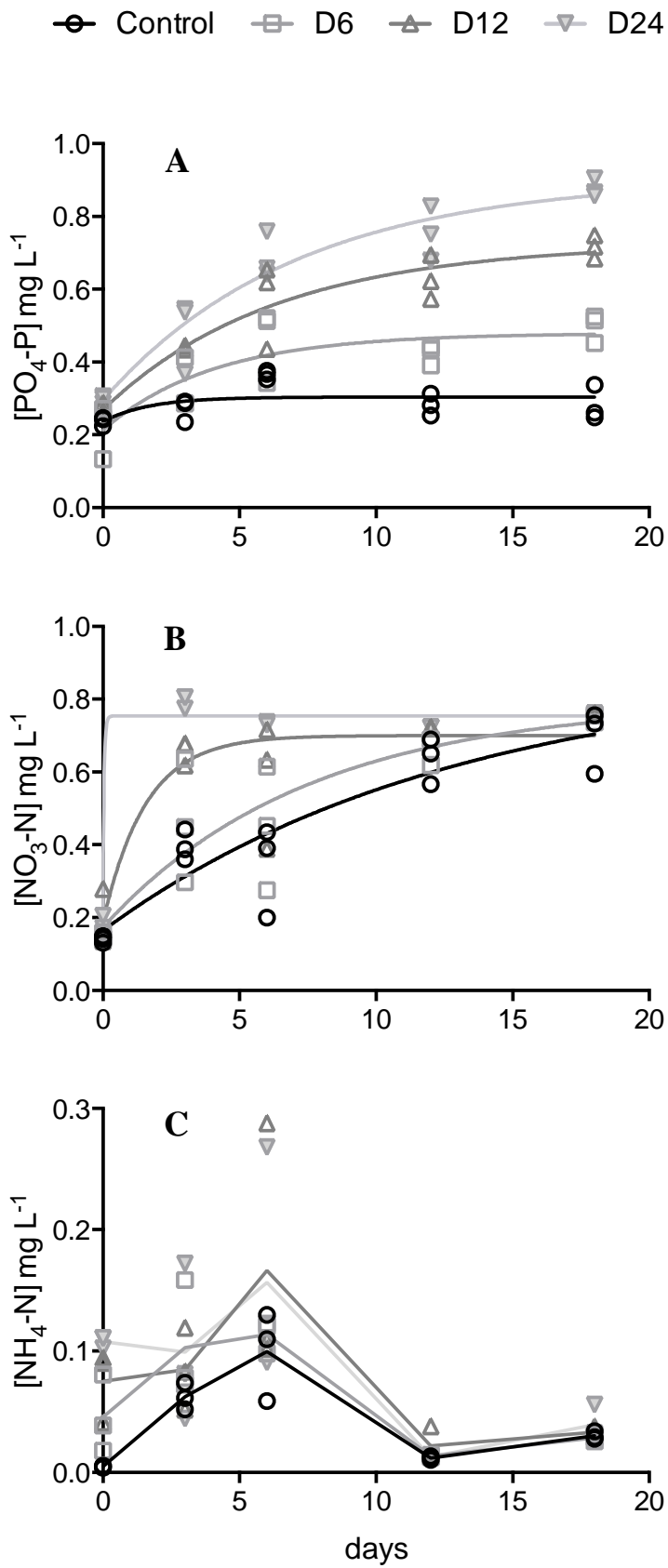


Figure 3



Appendix**Supporting figures**

Fig. A.1 – An example of the sediment remobilization (bioturbation) by *Corbicula fluminea* size treatment tanks. More details on the bioturbation data can be found in Crespo et al., 2018.

