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PRIMARY RESEARCH PAPER

Survival of *Corbicula fluminea* (Müller, 1774) in a natural salinity and temperature gradient: a field experiment in a temperate estuary

Daniel Crespo · **Sara Leston** ·
Filipe Martinho · **Miguel A. Pardal** ·
Marina Dolbeth

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Abstract The invasive bivalve *Corbicula fluminea* is described as a freshwater species but can colonize estuarine upper reaches. In a 46-day field experiment, the survival ability of the species along an estuarine gradient was tested. Our goal was to understand its tolerance limits in a transitional area and in which conditions a potential invasion in the estuary could thrive, in order to predict the potential threat of the species distribution and settlement along the freshwater–estuarine continuum. The Lethal Time (LT) method was adapted to this field test with success. The population held an adult stock in the most upstream areas of the estuary (salinity <0.5). Dispersal mechanisms allowed the species to be present in the downstream areas, however, with a different population structure as consequence of the interaction of the diverse physico-chemical gradients of the estuary, in particular different salinity and temperature combinations. During winter, the species was able to survive in all test sites, with practically no mortality, while in summer LT_{50} (median lethal time) changed considerably when comparing the upstream and the most downstream sites [82 days (predicted value) vs. 9 days, respectively]. Still, 100% mortality was never achieved, adding concern to the species invasion potential in this estuary.

Keywords Tolerance limits · Climate change · Invasive species · Dispersal

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D. Crespo (✉) · S. Leston · F. Martinho ·
M. A. Pardal · M. Dolbeth
Department of Life Sciences, Centre for Functional
Ecology – CFE, University of Coimbra, Calçada Martim
de Freitas, 3000-456 Coimbra, Portugal
e-mail: daniel.crespo@uc.pt

S. Leston
Pharmacy Faculty, CNC - Center for Neuroscience and
Cell Biology, University of Coimbra, Azinhaga de Santa
Comba, 3000-548 Coimbra, Portugal

S. Leston
MARE-Marine and Environmental Sciences Centre, IPL,
Escola Superior de Turismo e Tecnologia do Mar,
2520-641 Peniche, Portugal

M. Dolbeth
Biology Department & Centre for Environmental and
Marine Studies (CESAM), University of Aveiro, Campus
Universitário de Santiago, 3810-193 Aveiro, Portugal

Introduction

Despite some unpredictability regarding ecological impacts, human-mediated global changes will impair biodiversity and ecosystems functions, including physiological and metabolic processes (Hicks et al., 2011), species populations and communities (Sorte et al., 2010; Dolbeth et al., 2011; Eggers et al., 2012) and, ultimately, large ecosystems and the biosphere (Schröter et al., 2005; Barnosky et al., 2012).

Biological invasions by non-native species often take place due to human intervention and represent one of the biological/ecological alterations occurring on a worldwide scale as a consequence of globalization (e.g. Crespo et al., 2015).

In the context of current global changes, biological invasions arise as an event of their own (Simberloff et al., 2013). Transitional ecosystems, particularly estuaries, are highly susceptible to events of biological invasion, as several human activities that are major vectors for introductions concentrate in those areas (e.g. fisheries, ballast water, general human occupation, aquaculture) (Cohen & Carlton, 1998; Williams & Grosholz, 2008). Known as fundamental biological areas, estuaries are highly productive habitats (Kennish, 2002; Dolbeth et al., 2011; Hicks et al., 2011) and functionally important (e.g. Sousa et al., 2010, 2012a). Since estuaries are generally characterized by low diversity due to particular physico-chemical conditions (Loo & Rosenberg, 1996; Kennish, 2002; Dolbeth et al., 2011; Hicks et al., 2011), the introduction of novel species with high spreading and outcompeting potential could have a disproportionate effect on ecosystem functioning (Stachowicz & Byrnes, 2006; Sousa et al., 2008b).

The Asian Clam, *Corbicula fluminea* (Müller, 1774), is among the most invasive species of freshwater systems (McMahon, 2002; DAISIE, 2008; Sousa et al., 2008a), presently with a global widespread distribution and high invasive efficiency (Crespo et al., 2015). This species can affect the hydrology, biogeochemical cycling and biotic interactions via two general mechanisms: assimilatory–dissimilatory processes (uptake and release of energy and materials) and physical ecosystem engineering (physical environmental modification by organisms), with impacts ranging from individuals to ecosystems (Hakenkamp et al., 2001; Sousa et al., 2009; Atkinson et al., 2011). This species can create a different habitat matrix for benthic fauna via bioturbation and shell production (Ilarri et al., 2012, 2014) and has high filtration rates, reducing phytoplankton availability for other species (Foe & Knight, 1985; Phelps, 1994; Hakenkamp et al., 2001; Sousa et al., 2008a). It is a highly productive bivalve, which may cause disruption of the food chain, either as a consumer or as food source for higher trophic levels, especially when massive die-offs occur (McMahon, 2002; Sousa et al., 2008b, 2012b). The species is able to dominate the benthic fauna of several lentic and lotic habitats (McMahon, 2002; Crespo et al., 2015). Changes in the biogeochemical cycles have been reported as a consequence of the species pedal feeding, with subsequent alterations in the abiotic characteristics of the top layer of sediments and high rates of nitrogen excretion (Sousa et al., 2008a, and references therein).

Despite being a freshwater species, *Corbicula fluminea* can tolerate salinities up to 10–14 (in PSU, unitless) (McMahon, 1983, 1999), allowing it to colonize the upstream areas of estuaries (Sousa et al., 2008b; Franco et al., 2012; Ilarri et al., 2014). Its life cycle favours the ability to invade other systems, due to fast growth rates, early maturation (McMahon, 1999, 2002; Sousa et al., 2008a) and capability of androgenesis and self-fertilization (Pigneur et al., 2011). Juveniles are released as pediveligers, and their dispersal is aided by a mucilaginous drogue line (Prezant & Chalermwat, 1984; Rosa et al., 2012). Also, the species is known to have a great phenotypical plasticity, indicated by a wide range in shell characteristics and body mass for geographically close systems (Sousa et al., 2007; Vohmann et al., 2010; Rosa et al., 2012), which is another feature contributing to its overall invasiveness potential.

The awareness on the species' impacts has been increasing, since it has led to major economic impacts in North America, due to biofouling (Mattice, 1977; Rosa et al., 2011). The species has been present in North America since the mid-1930s, in South America since the 1960s (Darrigran, 2002) and in Europe since the 1980s (Ilarri & Sousa, 2012; Crespo et al., 2015). Despite the large availability of information on its vital limits, genetics, physiology and metabolism, there is still an information gap on how the species can deal with adverse cyclic conditions such as those found in estuaries. The physico-chemical parameters (e.g. salinity, temperature, water depth, turbidity) change accordingly to cycles of different lengths (tidal, lunar, seasonal, annual and even larger global cycles, McLusky, 1993; Teixeira et al., 2008; Pratt et al., 2014) in estuaries, so it becomes important to evaluate if *C. fluminea* populations are able to develop in such instable environments. Adding to this, as transitional habitats, estuaries show complex gradients (e.g. in a neotropical reservoir, Paschoal et al., 2015), with horizontal, vertical, cross-sectional or other patterns, when referring to the above abiotic factors

(McLusky, 1993). As an invasive species responsible for high secondary production levels (Phelps, 1994; McMahon, 2002; Sousa et al., 2008b), it may capitalize on a large amount of the available energy found in estuaries (Vannote et al., 1980). This could represent an additional threat for native biodiversity, with disruptive effects on functions and processes of native organisms.

This paper reports the results of a field experiment where the invasive species *C. fluminea* was placed along a temperate European estuary following the natural estuarine gradients, in summer and winter. Some of the experimental sites were set in areas where adults of *C. fluminea* were not previously reported (Franco et al., 2012; Modesto et al., 2013). This will allow us to predict if the species represents a threat for the downstream reaches of the estuaries or if the natural gradients and cycles are sufficient to avoid further advance.

Materials and methods

Experimental design

This study took place in the Mondego estuary (Portugal, 40°80′N, 8°50′W), a well-described system with temperate coastal climate of Mediterranean and Atlantic influence. The lower reaches of the estuary comprise two arms, north and south, separated by an alluvium-formed island with a total length of 7 km. This estuary extends for approximately 22 km, and a large portion of its margins are artificially maintained with stones and concrete structures for flood management. The tides are responsible for two large daily salinity oscillations (~3 m tidal range), which in the estuary mouth can reach 30–34 (euhaline) and in the uppermost areas 0.5–5 (oligohaline) (for further information refer to Teixeira et al., 2008; Franco et al., 2012; Modesto et al., 2013).

The north arm of the estuary was surveyed to establish the downstream occurrence limits for *C. fluminea*. Seven sites, designated as survey sites (S1 to S7, Fig. 1), were selected after Franco et al. (2012) and Modesto et al. (2013). At each site, samples were collected with a Van Veen grab, and we measured in situ the following physico-chemical parameters: temperature, salinity, Secchi depth and pH. Sediment samples were also collected for granulometry and organic matter determination. The median grain size was estimated with GRADISTAT 8.0 (Blott & Pye K, 2001).

Sampling sites were selected to account for the downstream distribution limit of *C. fluminea* in this estuary, the population structure and density levels to include in each experimental unit and finally salinity. Three sites were selected (A to C) in summer and four sites in winter (A to D) (Fig. 1) to test the tolerance limits of *C. fluminea*. Site D was added to the experimental set for winter conditions to account for the increase in freshwater discharge that intensifies during this season. *Corbicula fluminea* individuals were handpicked in site A (coincident with S1) in the upstream limit of the estuary where the saline plume can seldom reach, with oligohaline characteristics. This area is still under tidal influence, despite the distance from the estuary mouth (22 km).

At each site, four mesh bags (20 × 30 cm, 1 mm mesh) with individuals were placed in the water and tied to a fixed prop, to guarantee full submersion during low tide. In summer, a total of 13 individuals were placed in each bag, with the following size classes that mimic the population structure found in situ for each season: 3 organisms under 20 mm; 8 organisms between 20 and 25 mm and 2 organisms between 25 and 35 mm. In the winter, a total of 14 individuals were placed in each bag, with the following size classes: 3 organisms under 20 mm; 10 organisms between 20 and 30 mm and 1 organism larger than 30 mm.

The experiment ran for a total of 46 days, in each season. At T0, T1 (1 day), T2 (2 days), T3 (4 days), T4 (6 days), T5 (8 days), T6 (14 days), T7 (22 days), T8 (30 days) and T9 (46 days), mortality levels were verified and temperature, salinity, oxygen (% and mg/l) and pH were measured in low tide conditions.

Statistical analysis

The species density levels per size class of the field survey data were analysed with a 2-way crossed ANOSIM for the ‘sampling stations’ and ‘season’ factors, after square root transformation of the density data and upon a zero-adjusted Bray–Curtis matrix (Clarke & Gorley, 2015). The data were further analysed with distance based linear modelling (DISTLM), where we explored the relationship between species density and physico-chemical

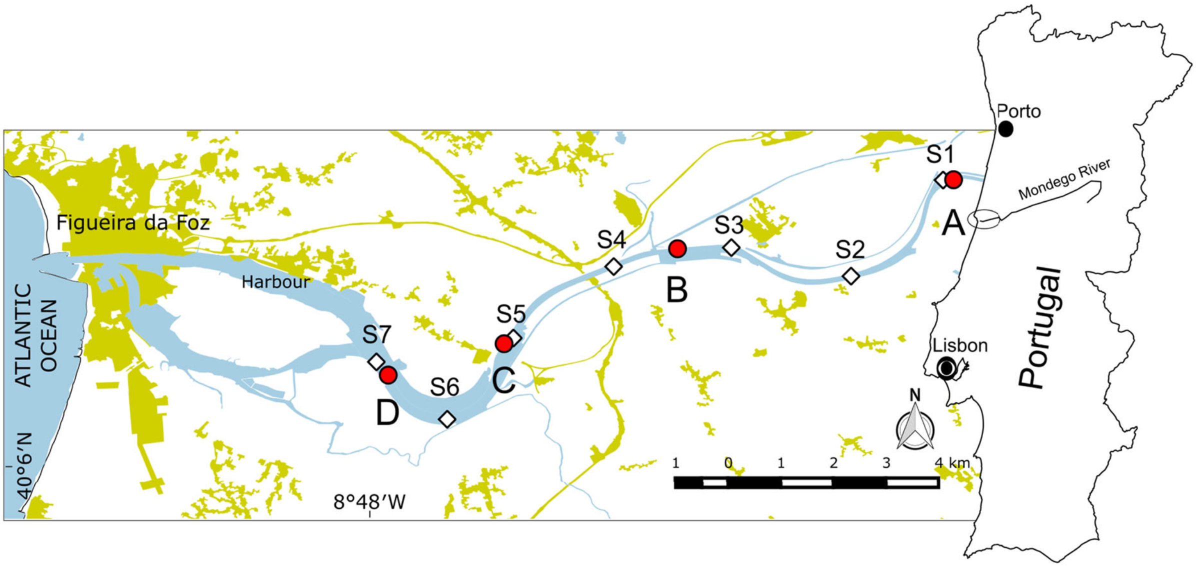

Fig. 1 Location of the field survey [S1 to S7 (*open rhombus*)] and experimental sites [*A*, *B*, *C* and *D* (*filled circle*)] in the Mondego estuary, in the western coast of Portugal

parameters, using AICc as selection criteria for a best model selection procedure (Anderson et al., 2008). These results were also explored with a dissimilarity-based redundancy analyses (dbRDA), to visualize the significant model (Anderson et al., 2008). Prior to the analysis, the physico-chemical variables were checked for collinearity with Draftsman plot, and collinear variables were removed.

A similar procedure was done for the survivorship percentage data from the experimental set (2-way crossed ANOSIM + DISTLM), but upon a Euclidean Distance matrix on non-transformed data.

In addition, with the survival experiment data, samples from each site were characterized with lethal time 25% (LT_{25}) and lethal time 50% (LT_{50}), which represents the moment when mortality reaches 25 and 50%, respectively (Environmental Technology Center Canada, 2007). This method is usually used for ecotoxicological tests, under controlled conditions (Abel & Garner, 1986; Newman & Aplin, 1992; Environmental Technology Center Canada, 2007; Costa et al., 2012), and was adapted for this field experiment. For this study, LT_{25} and LT_{50} provide us with a proxy of the species tolerance to the ecological features of each experimental site, therefore allowing us to compare this tolerance along the estuarine gradient and eventually with other estuarine systems. The LTs were calculated with software package Statistica® 7.0 (http://www.statsoft.com/). Site survival data, in each case, were fitted to the best model among

- logistic model (surviving $= a/(1 + (p/1 - p) * (\text{time}/LT_x)^b)$);
- exponential model (surviving $= a * \exp(\log((a - a * p - b * (1 - p))/a) * (\text{time}/LT_x)) + -b$) and
- Gompertz model (surviving $= a * \exp((\log(1 - p))) * (\text{time}/LT_x)^b$).

These formulas were used to estimate both lethal times (LT_{25} and LT_{50}), where a represents the y-intercept (the control response); LT_p. represents the lethal time for p dead individuals for the data set; p represents proportion of dead individuals (0–1) on the LT of interest; *time* represents exposure time and b represents a scale parameter (estimated between 1 and 4) (Environmental Technology Center Canada, 2007).

Results

Field survey data

According to the field survey data, the downstream limits for *C. fluminea* along the estuarine gradient changed with season, as individuals were found in site S4 during winter and only as far as site S3 (further upstream) for summer conditions (Fig. 2). Different patterns in the density levels and population structure were observed within each site (Fig. 2, ANOSIM for differences between sites across all seasons: global *R*: 0.64, $P = 0.001$). During summer, adults (>20 mm) were found in the three uppermost sites (S1 to S3). Their highest density occurred in S1, yet the largest individuals (>35 mm) were found in S3. Juveniles under 10 mm were dominant at S3, which presented the highest density levels over the whole study period (Fig. 2).

During winter, spatial variation of the population structure was not so clear, as both juveniles and adults

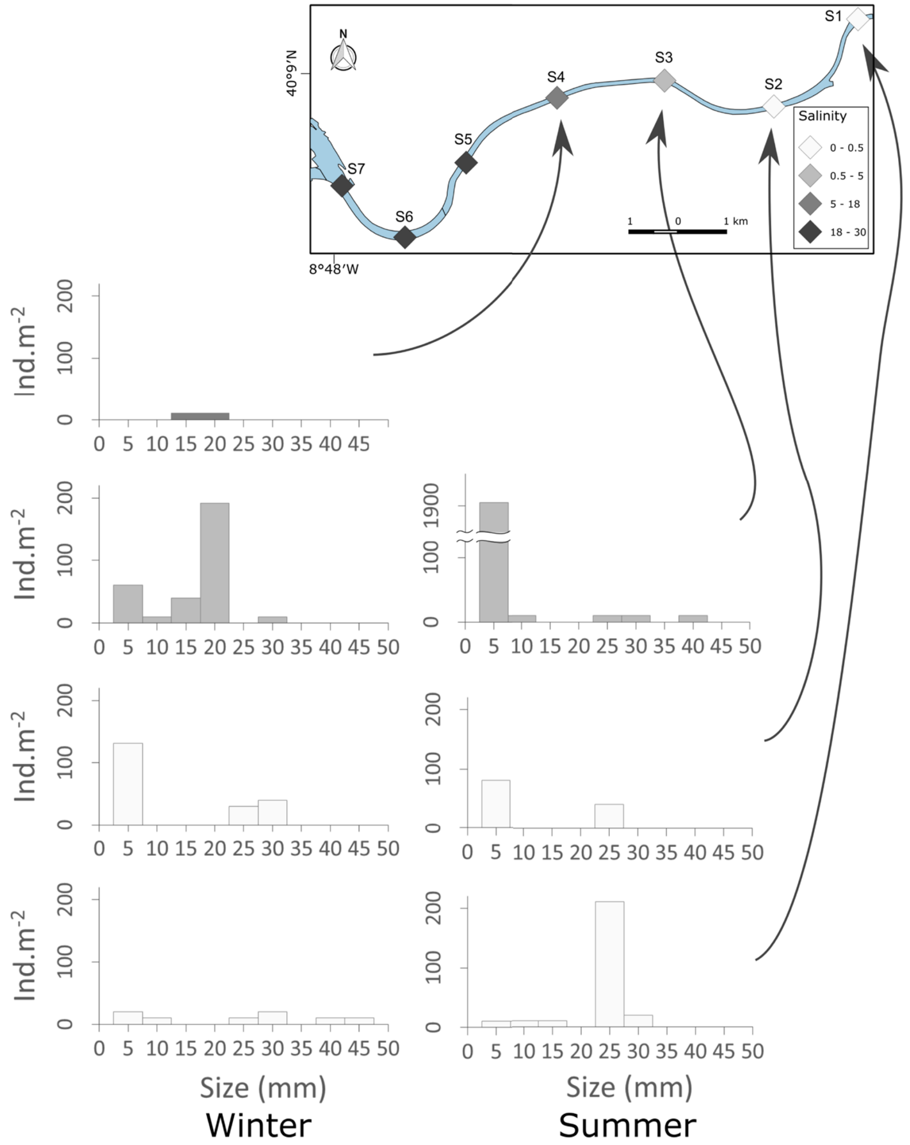

Fig. 2 Field survey results. *Corbicula fluminea* was found in S1, S2, S3 and S4. The population structure in each prospection site in summer and winter is shown in the histograms. The histogram colours refer to the salinity range described for each site. It is clear the downstream shift on the population structure, with adults' strong presence in S1 and a clear dominance of juveniles in S3

(>20 mm) occurred at the three uppermost sites. Juveniles' density was considerably lower when compared to S3 in the summer. In addition, the largest (=oldest) individuals were found in S1.

The best significant model relating population structure density and the environmental data was composed of salinity, temperature and median grain size (MGS), which together accounted for 96% of explained variation. However, only 31% of the total variation was explained by the best fitted model. The downstream site had higher salinity, while higher temperature was associated to the upstream areas, particularly in the summer. MGS was more important in differentiating between the upstream sites during the winter (dbRDA results, plot not presented).

Survival experiment analysis

Practically, all individuals survived in 46 days of the winter experiment, while in summer there were different mortality levels dependent on the site (with different temperature/salinity combinations) (Fig. 3). Nevertheless, 100% mortality was never reached at any site, in the same period length. The survival data and curves are clearly different between winter and summer (Fig. 3, ANOSIM for the difference between seasons, across all sites: global R: 0.972, $P = 0.001$), but not within stations ($P > 0.05$). DistLM showed that the variables most contributing to these differences within season were the maximum water temperature, which explained 91.1% of variation in the survivorship percentage data. High temperature was associated to the summer conditions and to the sites at the uppermost areas (dbRDA results, plot not presented).

Lethal times were only calculated for summer, as mortality in winter was negligible. By the end of the experimental run, more than 30% of the individuals died in the upstream area (site A) and more than 50% in the downstream areas (sites B and C). The lethal times for 50% survivorship followed the natural estuarine gradient, being shorter at site C (9 days) (with higher salinity values) and longer at site A (82 days, predicted value, considering that the test ran for 46 days) (Fig. 4). The confidence intervals decreased as well as the LT_{50} in downstream areas of the estuary. The LT_{25} pattern along the estuary is less clear. These values were very close between 6 and 7 days, but the confidence interval for site C is larger than for LT_{50} (Fig. 4). The average salinity measured in low tide was 0, 0.14 and 2.4 for sites A, B and C, respectively (Fig. 4). The average salinity in high tide was not recorded but previous studies in the same area (Dolbeth et al., 2008) recorded values close to 20, in site C, during the summer.

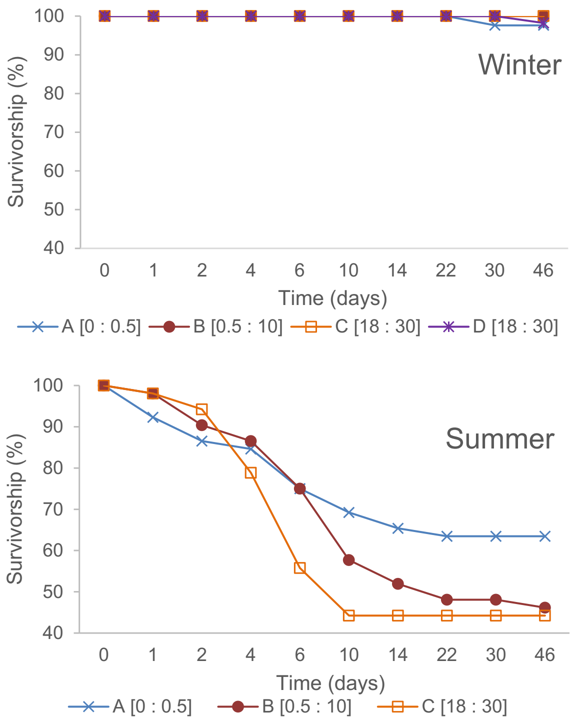

Fig. 3 Average survivorship of *Corbicula fluminea* along the estuarine salinity gradient. Each site shows the values for the Venice classification system of salinity inside the square brackets as an indication. The winter experiment showed very small mortality, while in summer mortality was higher and influenced by salinity. It should be noticed that 100% mortality was never achieved during the 46 days experiments

Discussion

Corbicula fluminea in its natural borders

Corbicula fluminea is a freshwater species that is able to osmoregulate under salinity 13 (Morton & Tong, 1985). As such, it has the potential to spread to middle sections of estuaries, using to its advantage the daily low tides and hence temporary low salinity, when it

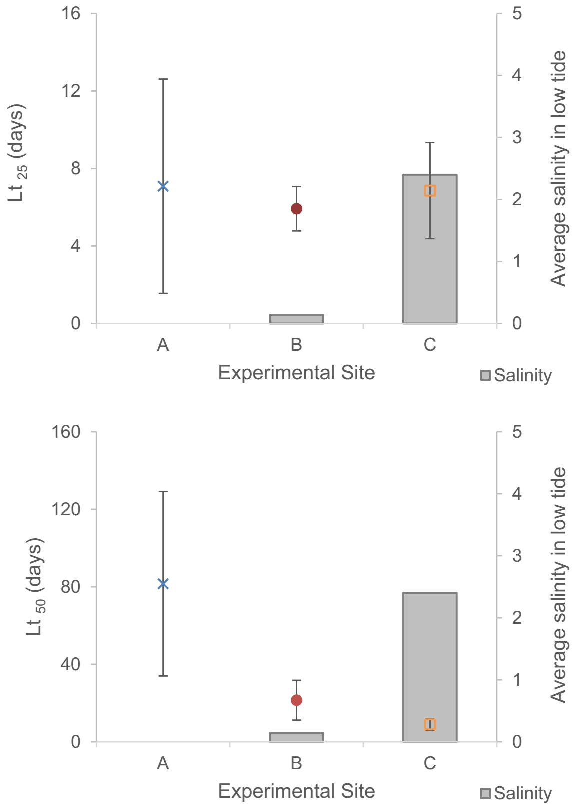

Fig. 4 Lethal time (25 and 50% mortality, and respective confidence intervals) and average salinity recorded during low tide, in summer. LT_{50} showed a larger difference between sites

can perform fundamental tasks for surviving. Therefore, *C. fluminea* can establish in the upstream areas of estuarine systems, as previously recorded in the Mondego estuary. The population distribution and structure along this system respond to the estuarine gradient that includes, among several parameters, salinity as an important driver. The largest densities were found in the oligohaline areas—S2 and S3 (salinity between 0.5 and 5, according to the Venice classification system), with adults between 15 and 20 mm most abundant in S3. Density levels were within the average range reported for other Portuguese estuarine systems (Sousa et al., 2012b), with exception of S3 in summer, which registered higher density (mainly constituted of juveniles, almost 2,000 ind m^{-2}).

The population structure at each surveyed site resulted from the interaction of specific physico-chemical conditions and the strong dispersal abilities of the species. In the summer, when the salinity influence is higher, the adult stock is kept in the uppermost reaches of the estuary (i.e. S1). The population structure shifts when moving downstream, with both young adults and juveniles balanced in the S2, and clearly dominated by juveniles in S3. During winter, *C. fluminea* also occurred in a station further downstream in a mesohaline area—S4, which can be explained by the larger freshwater runoff, able to carry individuals from the upper reaches to downstream areas. McMahon (2002) states that *r*-selected traits such as high reproductive effort and secondary production efficiency could be more important in the invasive potential of a species than the physiological tolerance for a broad environmental range. The field survey data reflect this hypothesis. The shift in the population structure between sites and among seasons may be a consequence of a large investment of *C. fluminea* in the reproduction and dispersal of juveniles, by releasing pediveligers from the stable population at the uppermost area (S1) and drifting along the estuarine gradient. S1 has freshwater characteristics, more suitable for the adult stock population, which is the source of *C. fluminea*'s individuals downstream.

However, high salinity may impair the settlement of the species in the areas closer to the sea, due to physiological constrains, acting as a natural barrier to the invasion. While adults can survive in high salinities during long exposures, juveniles are more sensitive to high salinity and may not have the same energy resources to protect themselves as adults do (McMahon, 1983, 1999; Ilarri & Sousa, 2012). Therefore, even though dispersal mechanisms may be enabling pediveligers of benthic settlement, they cannot handle the estuarine natural change in the physico-chemical conditions and die before the first year. This justifies the absence of large proportions of adults downstream S1 (as shown by Franco et al. (2012) and found in the present field survey campaign), despite the presence of young juveniles in our survey sites 1–4. Juveniles may be able to cope with the higher salinity of S3 and S4 until energetic requirements imply a larger period with the valves open for feeding purposes, therefore becoming more exposed to salinity.

Regarding temporal variability, the shift from winter to summer conditions implies a reduction in freshwater runoff, which might have reduced the survival of the 20 mm class during summer. The reason for the high density of the 5 mm class in the most saline S3 during summer can only be speculated, because no special local characteristics were found in this site. The spring recruitment occurs in the S1 area, where the adult stock is held. The highly effective dispersal mechanism of *C. fluminea*, with the mucilaginous drogue line (Prezant & Chalermwat, 1984), allows the juvenile specimens to drift along the estuary until S3. The water flow in S3 could behave in such a way that these juveniles can no longer drift downstream, accumulating in the site S3. Some of these 5 mm summer individuals can survive until winter, as evidenced by the field experiment (approximately 50% survivorship), growing up to 20 mm. However, to confirm this pattern, the annual cycle of the species in this system should be monitored in more depth, to understand the recurrence of these phenomena.

Surviving beyond the edge

The survival experiment showed that *C. fluminea* adult population was able to survive the estuarine high physico-chemical variability, particularly regarding salinity and temperature conditions. Both the distribution patterns of the species in the estuary (i.e. field survey data) and the survivorship experiment point towards this conclusion. During winter, practically all individuals were able to survive for the whole experimental period in all studied areas, probably due to the decrease in the overall estuarine salinity. Therefore, the Asian clam is potentially able to survive the complete winter season in the middle sections of the Mondego estuary. During summer, mortality occurred and the LT_{50} was shorter, particularly at the downstream and most saline areas. The species faces the most adverse conditions during the summer, due to a combination of both high salinity and temperature. In addition, the species may need to feed for longer periods (i.e. consequence of higher metabolic rates), and therefore can be exposed to salinity during longer time periods than they can handle (McMahon, 1983, 1999; Ilarri et al., 2012).

LT_{25} varied within similar values for all sites in summer. Nevertheless, this sort of mortality did not occur during the winter conditions, suggesting a minimal effect of the experimental manipulation of individuals. At the uppermost site (A), source of all individuals used in the experiment, the confidence intervals both for LT_{25} and LT_{50} were the largest. Potentially, that site has the optimal conditions for the species survival, and thus, mortality might be related to an effect of density/intraspecific competition. LT_{50} values are more relevant to determine whether the species could still represent a threat to estuarine systems, even in the mesohaline areas, as a consequence of its ability to survive in conditions far above its recognized vital limits. It should be noticed that 100% mortality was never found, which adds to the concern about the species invasion potential for this estuary. The salinity barrier seems to be acting on the immature juveniles, unable to survive when submitted to typical estuarine conditions, but the adults could survive and eventually complete reproductive cycles.

With the predicted increase in the number of extreme climatic events such as extreme precipitation (IPCC, 2013), the population of *C. fluminea* in the Mondego estuary may become able to survive during critical life stages and therefore settle in new areas within a frame of full invasion. For example, a summer with abnormal rainfall could allow the species to settle downstream and complete a full life cycle in areas previously inaccessible. Also, small modifications on the physical structure of the estuary can create shelter and context dependent opportunities for the species to thrive in new areas (Früh et al., 2012). The surveyed sites closer to the experimental sites C and D were not invaded yet, but if salinity decreases, for instance due to constant flooding or industrial freshwater runoff, the population may overcome the natural barriers that impair its settlement, and its distribution range may increase downstream. Ultimately, this can drive alterations on the overall biodiversity, by competing with species in close ecological niches and also acting on different trophic levels (Ilarri & Sousa, 2012), with consequences to the ecosystem functioning. Even if the system returns to conditions closer to its previous state and massive summer mortality occurs, the consequences of this invasion cannot be predicted. This potential for invasion could bring several economic nuisances, considering the presence of some industrial facilities along the estuary, as well as drainage systems for agriculture areas, which could be affected by biofouling events, with effective costs for their operation (Nakano & Strayer, 2014).

The use of lethal time to characterize the response of an invasive species in a specific system could be successfully adapted for other estuaries or rivers, thus providing a method for testing and comparing the susceptibility of a water body to invasion. This sort of data could be analysed with focus on species response in different contexts inside a specific water body, or the focus could be directed to the system, with lethal times indicative of ecosystem resistance to invasion. In a framework of management and protection, this method could be used together with recent detection methods for invasive species (Darrigran & Damborenea, 2015), in which molecular techniques allow identify the presence of invasive species before it can be seen, based on plankton samples, thus providing a strong predictive tool of the invasive potential within the binary system/species. The use of in situ survival experiments allows incorporating natural variability to the experimental design regarding habitat and environmental conditions, in turn enabling a more realistic study of the species behaviour towards those changes. This is especially relevant for an estuary, whose environmental conditions change considerably through a daily cycle (McLusky & Elliott, 2004). Nevertheless, in situ experiments may reduce the universality of the results, which may become system specific. This specificity makes sense in the case of this experiment and used methodology, because it was part of the objectives to understand the interaction of the species with this specific system.

There is a fine balance between the ability of species to invade and the susceptibility of the habitat to be invaded. In this case, it seems that despite the species' potential, the habitat is still presenting proper barriers to the progression of the invasion. Salinity, together with the tidal variations in several parameters (e.g. temperature, turbidity, food availability), is likely limiting the settlement of immature adults, and therefore, despite the survival of adults for remarkably long time in these conditions, the invasion has not extended into the downstream reaches of this estuary.

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