



DEPARTAMENTO DE CIÊNCIAS DA VIDA

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Spatial and temporal variation of
Corbicula fluminea (Müller, 1774) in the
Mondego Estuary

Ana Vanessa Pinto Modesto

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor João Carlos Marques (Universidade de Coimbra)

Ana Vanessa Pinto Modesto

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Resumo

Os movimentos geográficos intencionais ou acidentais das espécies promoveram o aumento da sua capacidade de dispersão natural da sua área natural para áreas onde não existiam anteriormente. Estas espécies causam impactos ecológicos e econômicos e são classificadas como espécies invasoras não indígenas (NIS). A *Corbicula fluminea* é conhecida como uma das espécies invasoras mais bem-sucedidas em ecossistemas aquáticos. Nas áreas a montante do estuário do Mondego (áreas oligohalinas e mesohalinas), a *C. Fluminea* (malha de 1 mm) foi estudada durante seis anos, desde o inverno de 2003 ao inverno de 2008. A densidade e a biomassa sazonais variaram entre 11179 ind m⁻² e 8.547 ind. m⁻², e entre 322.9 g PSLC m⁻² e 0.012 g PSLC m⁻², respectivamente. Portanto, as populações de *C. fluminea* apresentaram altas densidades e baixas biomassas que mostraram variabilidade espacial e temporal relacionadas com mudanças sazonais e inter- anuais. Os padrões de variação espacial e temporal de *C. fluminea* pareceram ser determinados pelas variáveis ambientais naturais. A alta temperatura, areia muito grossa, areia grossa e a baixa salinidade foram as principais variáveis ambientais naturais que afetaram a densidade de *C. fluminea*. Relativamente à biomassa, as variáveis ambientais que influenciaram a *C. fluminea* foram alta temperatura e fósforo. Outras variáveis, como as características morfológicas, disponibilidade de recursos alimentares e predação, que não foram estudadas, também podem ter uma contribuição para a estrutura das populações observadas no estuário do Mondego. Relativamente às malhas, a malha 0.5 mm é normalmente utilizada em estudos de comunidades estuarinas bentónicas. A importância do uso da malha de 0.5 mm (estudo desde o inverno

de 2006 a outono de 2008) no que respeita aos dados de densidade foi evidenciada, tendo sido observadas diferenças significativas em relação à malha de 1 mm. No entanto, o mesmo não aconteceu com a biomassa, uma vez que não houve diferenças significativas entre as malhas.

Abstract

The geographical intentional or accidental movements of species promoted the increase of the natural dispersion capacity from its natural range to areas where not existed previously. These species cause ecological and economic impacts and are classified as non-indigenous invasive species (NIS). *Corbicula fluminea* is known as one of the most successful invasive species in aquatic ecosystems. In the upstream areas of Mondego estuary (oligohaline and mesohaline sectors), *C. fluminea* (1 mm mesh) was studied during six years from winter 2003 to winter 2008. Seasonally density and biomass ranged from was 11179 ind. m⁻² to 8.547 ind. m⁻² and 322.9 g AFDW m⁻² to 0.012 g AFDW m⁻², respectively. Therefore, populations of *C. fluminea* presented high densities and low biomasses that showed spatial and temporal variability related regarding seasonal and interannual changes. The spatial and temporal variation patterns of *C. fluminea* seem to be determined by natural environmental variables. High temperature, very coarse sand, coarse sand and low salinity were the main natural environmental variables that affected the density of *C. fluminea*. Concerning biomass, the environmental variables that influenced *C. fluminea* were high temperature and phosphorus. Other parameters like morphological characteristics, availability of food resources and predation, which were not studied, can also have a contribution to the populations structure observed in Mondego estuary. Concerning meshes, the 0.5 mm mesh sieve is commonly used in studies of estuarine benthic communities. The importance of using the 0.5 mm mesh (study since winter 2006 to autumn 2008) was illustrated with regard to density data, which presented significantly different values as compared to the 1 mm mesh. Nevertheless, the same was not observed

regarding biomass, which did not present significant differences between the two mesh size sieves.

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1. INTRODUCTION

1. Introduction

The geographical movement of species is not static in time, and has flowed in a reasonably predictable manner (Sax *et al.*, 2005). However, in the last decades the human activities caused numerous intentional or accidental movements that promoted the increase of the natural dispersion capacity of species from its natural range to areas where not existed previously (Cox, 2004). These species cause ecological and economic impacts and are classified as non-indigenous invasive species (NIS) (Colautti and MacIsaac, 2004). According to Sakai (2001) and Allendorf and Lundquist (2003) the invasive process contemplates two steps and an intermediate phase. The first step - introduction - is where the invasive species reaches into a new environment. Before entering in an exponential growth, the introduced species remains in low densities for a variable period of time, that can last for months, years or decades, called the lag time phase (Crooks, 2005; Sakai *et al.*, 2001). This period of time can be affected by mechanisms of the invader characteristics and the receiving environment (Crooks, 2005). The second step is the dispersion in the invaded ecosystem and the possible replacement of the native species by the invasive species (Allendorf and Lundquist, 2003). Therefore, alien species are recognized by many authors as one of the major threats for biodiversity (Pranovi *et al.*, 2006) promoting significant changes in native communities (Lodge, 1993; Vitousek *et al.*, 1996; Kolar and Lodge, 2001).

The Asian clam, *Corbicula fluminea*, is a freshwater bivalve native of southeast of Asia (Aldridge and Muller, 2001). This clam, as other freshwater species of this family, are the most numerous macroinvertebrates in many

rivers, lakes and estuaries (Byrne *et al.*, 2000) and is known as one of the most successful invasive species in aquatic ecosystems (Cataldo and Boltovskoy, 1999). Its great capacity to colonize new environments explains its almost worldwide colonization in the last century (Cataldo and Boltovskoy, 1999). The earliest record of inter-continental invasion was registered in British Columbia in North America in the 1920s (Counts, 1981; Mouthon, 1981). It was then recorded in Argentina around 1960-1970 (Cataldo and Boltovskoy, 1999). Nowadays, *C. fluminea* can be found more or less extensively in Asia, Australia, Africa, America and European continents (Mouthon, 2001; McMahon, 2002). In the early 1980s the presence of *C. fluminea* in two estuaries of France and Portugal (Mouthon, 1981) was the first register of its invasion in Western Europe. In Portugal, in 1981 *C. fluminea* was firstly observed in the Tagus river estuary (Mouthon, 1981). Then, in 1989 it was registered in Minho estuary (Araujo *et al.*, 1993), in the Lima estuary in 2002 (Sousa *et al.*, 2006) and in the Mondego estuary in 2003 (Vinagre, 2009).

Such success of *C. fluminea* to colonize new habitats might be related with its r-select characteristics: short life span (2–3 years), rapid growth rates, early sexual maturity, high reproductive capacity, free-living juvenile period and extensive dispersion ability (Morton, 1997; McMahon, 1983). Moreover, the spread success has also involved human vectors: aquaculture, recreational activities and transportation are the main causes of freshwater estuarine species dispersion from their native range (Elton, 1958; Ruiz *et al.*, 2000; Sousa *et al.*, 2008a).

Besides r-characteristics and human vectors, the invasion success of *C. fluminea* can also be related to its capacity to adapt to different abiotic factors

variations including sediment type, salinity, temperature, hypoxia, and pH that may facilitate or prevent the rapid establishment of this species in new ecosystems (Elton, 1958). Regarding sediment type, this species can live in a variety of substrates with predominance in coarse sand, gravel or sand-gravel mixture (Cherry *et al.*, 1980) but it is almost absent in habitats with large amount of silt (Sickel, 1986). Even being a freshwater species, *C. fluminea* can occur in brackish waters with range salinity between 3 and 8, but rarely overcome this range (McMahon, 1983). Nevertheless, Morton (1985) reported that *C. fluminea* can osmoregulate for a period of 7 days with salinities below 13, and with salinities higher than 13 mortality increases progressively with salinity and time. According to McMahon (2002), the temperature tolerance limits varies between 2 and the maximum 36 °C. *C. fluminea* has a weak tolerance to elevated temperatures and to moderate hypoxia (McMahon, 2002). In addition, *C. fluminea* can live in environments with a pH ranging from 5.5 and 8.3 (Araujo *et al.*, 1993). Therefore, environmental variables can play a major role in the population regulation and different behaviours of this species are expectable for different habitats (Sickel, 1986).

Although being more effective in colonizing freshwater habitats than brackish waters (Sousa *et al.*, 2006), *C. fluminea* is being recorded and sampled in the Mondego estuary since 2003 (Vinagre, 2009).

Considering the highly invasive behaviour of *C. fluminea* and the impacts that it can have in the ecosystems, it is important to have an approach that combines the spatial and temporal distribution of this species, in order to increase our knowledge and understanding about its invasive behaviour in the Mondego estuary. The knowledge gained with this kind of approach can help to

implement mitigation measures to reduce the potential impacts of this aquatic invasive species in the ecosystem. Therefore, the aims of this work were 1) to study the biology and ecology of *C. fluminea*, 2) to describe the spatial and temporal variations of *C. fluminea* in 1 mm mesh, 3) to identify the most important natural environmental variables influencing the density and biomass of *C. fluminea* and 4) to analyse sieves meshes size (1 and 1+0.5 mm) influence on the characterization of *C. fluminea* spatial and temporal variations in the mesohaline and oligohaline stretches of the Mondego estuary.

2. MATERIAL AND METHODS

2. Material and Methods

2.1. Study area

The Mondego estuary (40°08'N, 8°50'W) is located in a warm temperate region on the Atlantic coast of Portugal and spreads along 21 km upstream from the mouth (Fig. 1). This estuary has a mean water flow of $79 \text{ m}^3 \text{ s}^{-1}$, reaches more than $140 \text{ m}^3 \text{ s}^{-1}$ in rainy years, and drops to $27 \text{ m}^3 \text{ s}^{-1}$ in dry years. At about 7 km from the sea, the Mondego estuary is divided in two arms, North and South, separated by Murraceira Island, connecting again at 1 km from the mouth. These two arms display different hydro-morphological characteristics. The North arm is deeper (5-10 m during high tide, with a tidal range about 2-3 m), low residence times (1-2 days) and constitutes the main navigation channel that supports the harbour of Figueira da Foz, being subjected to regular dredging activities that cause physical disturbance of the bottoms. The North arm margins are artificially armoured with stones as a result of a re-routing process to facilitate the agriculture system in the valley and to prevent damage caused by flood events. This estuarine subsystem presents larger daily salinity oscillations due to the higher tidal penetration together with the greater of freshwater outflow. In addition, the bottom sediments are mainly composed by medium and coarse sand (Marques *et al.*, 2003; Teixeira *et al.*, 2008).

The downstream section has higher marine water influence and the upstream area more freshwater influence. In addition, the upstream part is mainly composed by subtidal areas. According to Teixeira *et al.* (2008), this estuary was divided in five stretches based on salinity and sediment characteristics. The euhaline estuarine area is located near to the mouth, is influenced by high salinities (30-34) and sediment is mostly composed by fine,

medium and coarse sand particles; the polyhaline muddy stretches are characterized by salinities between 18-30 and sediment composed by fine, medium sand particles along with an increase of silt and clay contribution towards inner stations; the polyhaline sand is influenced by salinities between 18-30 and sediment characterized by fine and medium sand particles but with an increment of the percentage of coarse sand towards inner stations; the mesohaline stretch is affected by salinities between 5-18 and sediment is dominated by coarse sand; the oligohaline area by bottom salinities ranging between 0.5-5 and sediment is mostly composed by coarse sand.

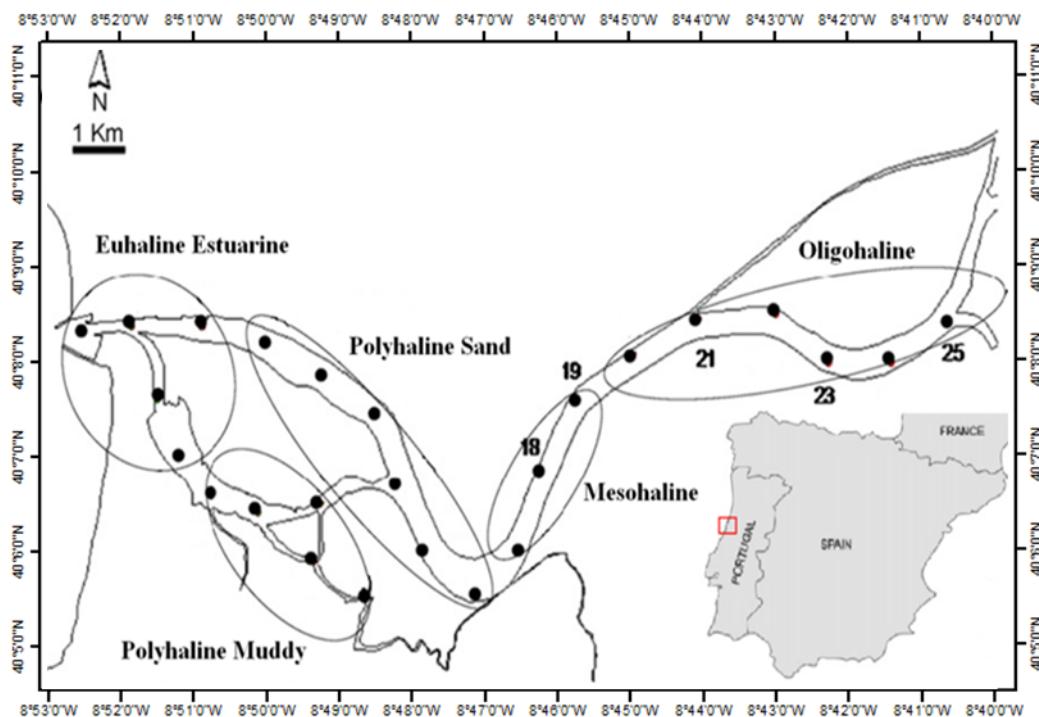


Figure 1. The Mondego estuary and sampling stations localization. Ellipses represent the five stretches based on salinity and sediment type (according to Teixeira *et al.*, 2008).

Since the winter of 2003, the soft-bottom subtidal communities of the Mondego estuary have been regularly monitored, with a network of 25 sampling

stations covering the estuary. A preliminary analysis on *C. fluminea* abundance data was performed in order to determine its presence along the estuary. After this assessment, five sampling stations were selected in the upstream area of North arm in the Mondego estuary: two in the mesohaline area, St 18 and St19, and three in the oligohaline stretch, stations 21, 23, 25 (Fig. 1).

2.2. Sampling and laboratory procedures

2.2.1. *Corbicula fluminea*

Seasonally, from winter 2003 to autumn 2008, specimens of *C. fluminea* were sampled at the five selected stations, during high tide. Three replicates were collected at each station, using a van Veen LGM grab of 0.05 m² (winter 2003 to summer 2003), and 0.08 m² area (autumn 2003 to autumn 2008). The samples were sieved through a 0.5 mm mesh bag and were preserved with a neutralized 4% formalin solution. In the laboratory, samples were sieved (from winter 2003 to autumn of 2005 through a 1 mm mesh sieve; since winter 2006 to autumn 2008 through 1 and 0.5 mm mesh sieve). All the *C. fluminea* specimens were sorted, counted and preserved in ethanol 70%. The biomass of clams was assessed as Ash Free Dry Weight (AFDW). *C. fluminea* specimens were dried at 60 °C until a stable weight was reached (≥ 72 h), weighted (DW, dry weight), combusted at 450 °C for 8h, and reweight (AW, ash weight). The resulting AFDW was determined by difference (AFDW = DW – AW). From autumn 2007 to autumn 2008, AFDW of individuals collected in St 19 and St 21 were calculated based in the allometric equation (Franco *et al.*, 2011):

$$\log(\text{AFDW}) = 3,141 * \log \text{SL (mm)} - 4,906$$

2.2.2. Physicochemical parameters

Simultaneously with *C. fluminea* sampling, one more replicate of sediment was gathered to quantify the sediment organic matter content and to perform the grain size analysis. The sediment organic matter content (%) was quantified by weight difference between sediment's weight after over drying at 60 °C for 72 hours and the sediment weight after combustion at 450 °C for 8 hours, and then expressed as a percentage of total sample weight. Grain size analysis was carried out by mechanical separation through a column of sieves with different mesh sizes, following the Brown and McLachland (1990) system classification (clay: <0.038 mm; silt: >0.038 – <0.125 mm; fine sand: >0.125 – <0.250 mm; medium sand: >0.250 – <0.500 mm; coarse sand: >0.500 mm, very coarse sand: >2.000 mm) and expressed as a percentage of the total sample's weight.

In situ, at each station, during high tide, water temperature (°C), salinity, dissolved oxygen (mg L^{-1}) and pH, were measured close to the bottom, using a WTW 380i sonde. Bottom water samples (1.5 L) were collected at each site for analysis of dissolved inorganic nitrogen (mg L^{-1}), phosphorus (mg L^{-1}), chlorophyll *a* (mg m^{-3}), total suspended solids (g L^{-1}), and particulate organic matter (g L^{-1}). In the laboratory, each water sample was immediately filtered with Whatman GF/C glass fiber filters (one filter each 0.75 L) to obtain two filters for each sample and stored frozen at -18 °C until analyses were done following standard methods described by Limnologisk Metodik (1992) for ammonium and phosphorus, Strickland and Parsons (1972) for nitrate and nitrite. Filter #1 was frozen at -18 °C for Chlorophyll *a* analysis and filter #2 was dried at 60 °C during 48h for total suspended solids and particulate organic

matter analysis. Chlorophyll *a* was extracted from filter #1 according to Parsons *et al.* (1985). Total suspended solids and particulate organic matter were calculated from filter #2. For total suspended solids, the filter #2 (pre-weighted) was dried at 60 °C for 48h, and weighted. After combustion at 450 °C for 8 h, the weight was determined to estimate particulate organic matter suspended in water.

2.3. Data analysis

2.3.1. Spatial and temporal variation of *C. fluminea*

Abundance and biomass data were converted to density (ind. m⁻²) and weight per area unit (g AFDW m⁻²), respectively. The density and biomass data used in the spatial and temporal analysis were obtained by using 1 mm mesh per replica, since winter 2003 to autumn 2008. Data exploratory analysis was performed using the Brodgar 2.6.6 software (www.brodgar.com). Density and biomass were log (x+1) and cubic root transformed, respectively. Data were then imported to PRIMER 6 + PERMANOVA[®] software (software package from Plymouth Marine Laboratory, UK) (Clarke, 2001; Anderson *et al.*, 2008). Each variable was normalized and a permutational PERMANOVA (significance level of $\alpha = 0.05$) was performed to observe if there were statistical significant differences. These statistical significances were analyzed using the Euclidean distance and 9999 permutations. When permutations number was > 100, Permanova-p was applied, if <100, the Monte Carlo-p was used. Pair-wise tests were used when there were statistical significant differences.

In order to test differences in density and biomass between stations we used a one-way PERMANOVA (univariate analysis) - fixed factor "station" (st) with 5 levels: 18, 19, 21, 23 and 25. The null hypothesis was:

$H_{0(1)}$: There are no differences in density and biomass between stations.

Afterward, to analyze differences in density and biomass between seasons we conducted one-way PERMANOVA - fixed factor "season" (se) with 4 levels: winter, spring, summer and autumn. The null hypothesis was:

$H_{0(2)}$: There are no differences in density and biomass between seasons.

Finally, to examine differences in density and biomass between years we performed one-way PERMANOVA - fixed factor "year" (ye) with 6 levels: 2003, 2004, 2005, 2006, 2007 and 2008. The null hypothesis was:

$H_{0(3)}$: There are no differences in density and biomass between years.

2.3.2. Sieves meshes size influence on the characterization of *C. fluminea* spatial and temporal variations

The density and biomass data for meshes analysis were obtained by using 1 and 1+0.5 mm mesh per replica, since winter 2006 to autumn 2008. In these meshes analysis, Brodgar 2.6.6 software (www.brodgar.com) was used to perform exploratory data analysis. Density and biomass were log (x+1) and cubic root transformed, respectively. Then, data were imported to PRIMER 6 + PERMANOVA[®] software (software package from Plymouth Marine Laboratory, UK) (Clarke, 2001; Anderson *et al.*, 2008). Each variable was normalized and a

permutational PERMANOVA (significance level of $\alpha = 0.05$) was performed to observe if there were statistical significant differences. These statistical significances were analyzed using the Euclidean distance and 9999 permutations. When permutations number was > 100 , Permanova-p was applied, if <100 , the Monte Carlo-p was used. Pair-wise tests were used when there were statistical significant differences.

Initially, in order to assessment differences in density and biomass between stations using different meshes, we used two-way PERMANOVA (univariate analysis) - fixed factor "mesh" (me) with 2 levels: 1 and 1+0.5 mm and fixed factor "station" (st) with 5 levels: 18, 19, 21, 23 and 25. The null hypothesis was:

$H_0 (4)$: There are no differences in density and biomass between stations using different meshes.

In order evaluate differences in density and biomass between seasons using different meshes we used two-way PERMANOVA - fixed factor "mesh" (me) with 2 levels: 1 and 1+0.5 mm and fixed factor "season" (se) with 4 levels: winter, spring, summer and autumn. The null hypothesis was:

$H_0 (5)$: There are no differences in density and biomass between seasons using different meshes.

Finally, two-way PERMANOVA was used to test differences in density and biomass between years using different meshes - fixed factor "mesh" (me) with 2 levels: 1 and 1+0.5 mm and fixed factor "year" (ye) with 3 levels: 2006, 2007 and 2008.

H_0 (6): There are no differences in density and biomass between years using different meshes.

2.3.3. Environmental variables

Principal Component Analysis (PCA) was performed in order to analyze environmental variables, which were sampled since winter 2003 to autumn 2008 per station. Data exploratory analysis was performed using the Brodgar 2.6.6 software (www.brodgar.com). Temperature (T), salinity (S), pH, chlorophyll *a* (Chl *a*), dissolved oxygen (DO), dissolved inorganic nitrogen (DIN), phosphorus (P-PO₄), total suspended solids (TSS), particulate organic matter (POM), sediment organic matter (SOM) were not transformed. Very coarse sand and coarse sand (VCS+CS) were submitted to X² transformation and medium sand (MS) were log x transformed. Fine sand (FS), silt and clay (S+C) were log x+1 transformed. Subsequently, data were imported to PRIMER 6 + PERMANOVA© software (software package from Plymouth Marine Laboratory, UK) (Clarke, 2001; Anderson *et al.*, 2008). All environmental variables (except DO, pH, Chl *a*, DIN, TSS, POM, SOM, MS, FS, S+C - correlated) were normalized and used in PCA analysis. In PCA, experimental design was regarded three factors station, season and year.

2.3.4. Influence of environmental variables on *C. fluminea*

Multiple linear regression models were performed in order to evaluate the relationship between physicochemical parameters (explanatory variables) with

density and biomass (1 mm mesh) of *C. fluminea* (response variables) since winter 2003 to autumn 2008 per station. First of all, abiotic and biotic data were transformed. Temperature, salinity, pH, chlorophyll a, dissolved oxygen, dissolved inorganic nitrogen, phosphorus, total suspended solids, particulate organic matter and sediment organic matter were not transformed. Very coarse sand and coarse sand were X^2 transformed and medium sand was log x transformed. Fine sand, silt and clay were log x+1 transformed. Density and biomass were log x and cubic root transformed, respectively. Then, the relationships between density and biomass with environmental variables were tested. The abiotic factors with low relationships (< 20%) to density (pH, DO, Chl a, DIN, P-PO₄, TSS, POM, SOM, MS, FS, S+C) and to biomass (S, pH, DO, Chl a, DIN, TSS, POM, SOM, VCS+CS, MS, FS, S+C) were removed. The correlation and collinearity between biotic and abiotic factors were also tested. Finally, response and explanatory variables were analyzed through multiple linear regression models, using AIC (Akaike Information Criteria) as a selection criterion. In density multiple linear regression model, three points were removed once the standardized residuals with these three points were more than 2.

The Brodgar 2.6.6 software (www.brodgar.com) was used to perform multiple regression model analysis of density and biomass.

3. RESULTS

3. Results

3.1. Spatial and temporal variation of *C. fluminea*

Seasonal average of 1 mm density and biomass of *C. fluminea* at each station, season and year, since winter 2003 to autumn 2008, are represented in figure 2.

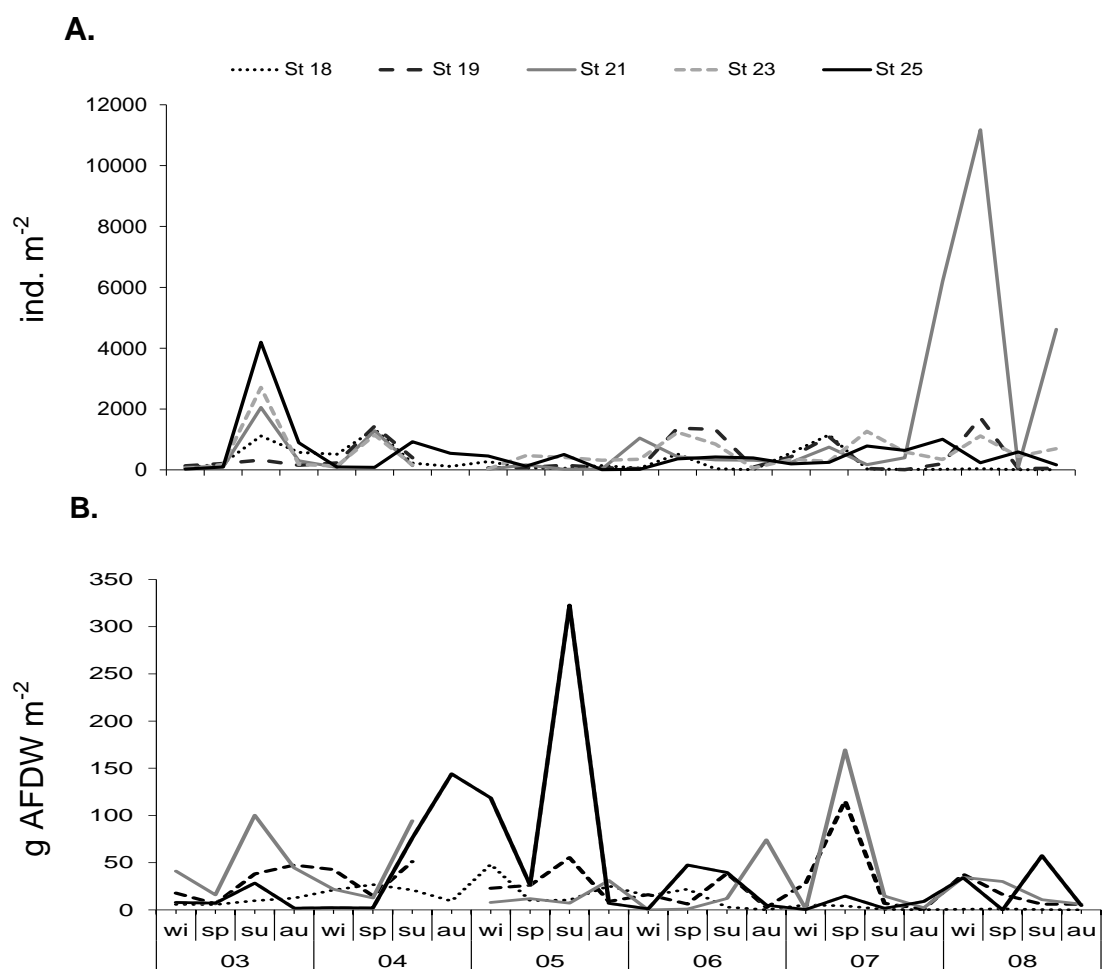


Figure 2. Spatial and temporal variation (average per station) of *C. fluminea* density (A.) and biomass (B.) of 1 mm at each station and seasons of winter (wi), spring (sp), summer (su) and autumn (au) from 2003 to 2008.

Highest density value was 11179 ind. m⁻² (St 21; spring 2008) and the lowest values were 8.547 ind. m⁻² (St 25, autumn 2005; St 18, autumn 2006; St 19, autumn 2007; St 18; summer 2008 and St 18, autumn 2008) (Fig. 2A). Regarding biomass, the highest value was 322.9 g AFDW m⁻² (St 25, summer 2005) and the lowest was 0.012 g AFDW m⁻² (St 23, winter 2007) (Fig. 2B).

PERMANOVA revealed spatial and temporal differences of 1 mm density and biomass since winter 2003 to autumn 2008.

In the first hypothesis ($H_{0(1)}$), density and biomass showed significant differences between stations ($F_{(St)4,346}=5.6326$; $p=0.0002$) and ($F_{(St)4,346}=6.4559$; $p=0.0001$), respectively. Pair-wise tests for density and biomass demonstrated differences between station 18 and the remaining stations. Regarding biomass, there also were significant differences between St 23 and St 25.

Second hypothesis ($H_{0(2)}$), density and biomass showed significant differences between seasons ($F_{(se)3,347}=6.51$; $p=0.0003$) and ($F_{(se)3,347}=4.3152$; $p=0.0052$), respectively. Pair-wise tests for density and biomass demonstrated that significant differences occurred between dry periods (summer and spring) and rainy periods (autumn and winter).

Finally, in the third hypothesis ($H_{0(3)}$), density and biomass presented significant differences between years ($F_{(ye)5,345}= 2.379$; $p=0.0375$) and ($F_{(ye)5,345}=8.3388$ $p=0.0001$), respectively. Density pair-wise tests showed differences between 2005 and the remaining years. Regarding biomass pair-wise tests, all the studied years showed differences, except year 2003 (with 2004 and 2008), 2004 (with 2005 and 2008) and 2006 (with 2007 and 2008).

3.2. Sieves meshes size influence on the characterization of *C. fluminea* spatial and temporal variations

Seasonal average of density and biomass of *C. fluminea* in different meshes: 1 and 1+0.5 mm at each station, season and year, since winter 2006 to autumn 2008, are represented in figures 3 and 4.

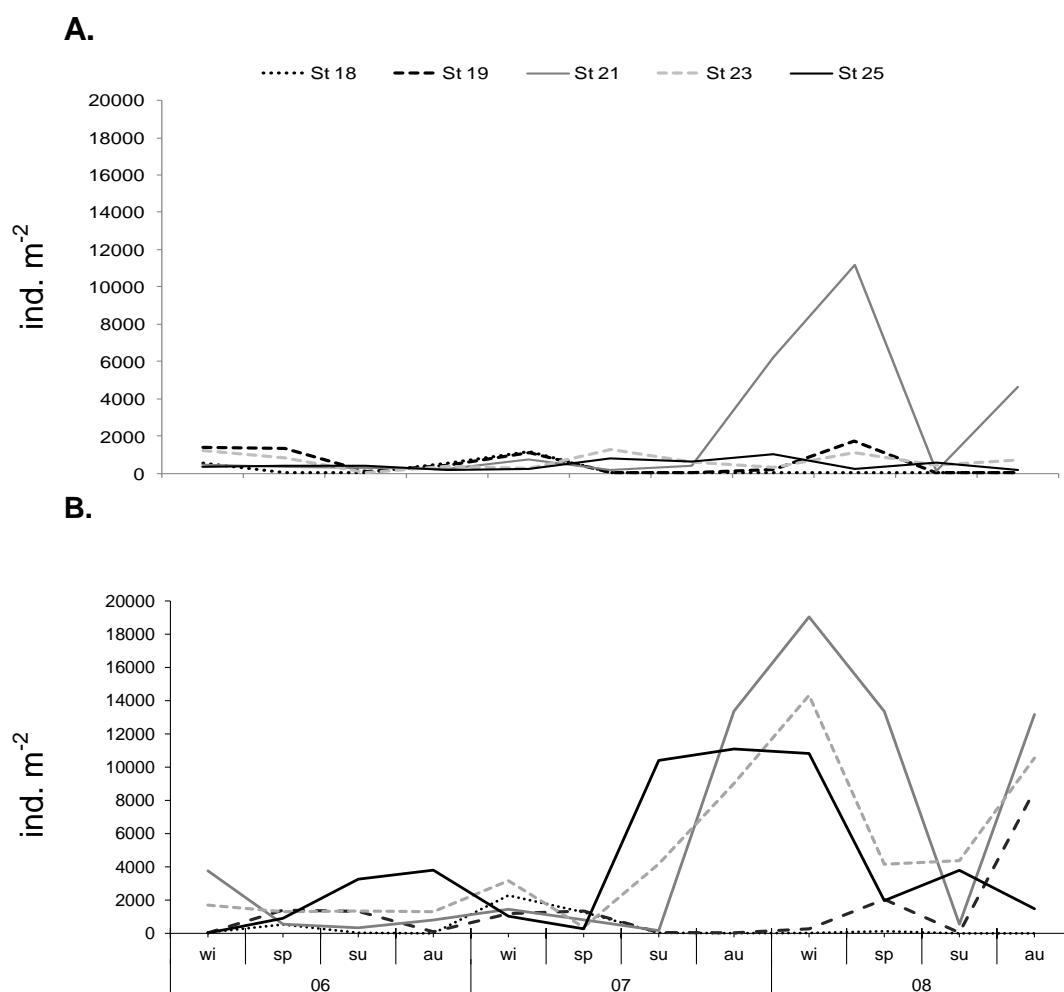


Figure 3. Meshes sieve (A.) 1 mm and (B.) 1+0.5 mm influence in temporal and spatial variation (average per station) of *C. fluminea* density at each station and seasons of winter (wi), spring (sp), summer (su) and autumn (au) from 2006 to 2008.

For density of 1 mm, the highest value was 11179 ind. m⁻² (St 21; spring 2008) and the lowest values were 8.547 ind. m⁻² (St 18, autumn 2006; St 19, autumn 2007; St 18; summer 2008 and St 18, autumn 2008). Concerning 1+0.5 mm density, the highest value was 19038 ind. m⁻² (St 21, winter 2008) and the lowest value was 8.547 ind. m⁻² (St 18, autumn 2006; St 18, autumn 2008).

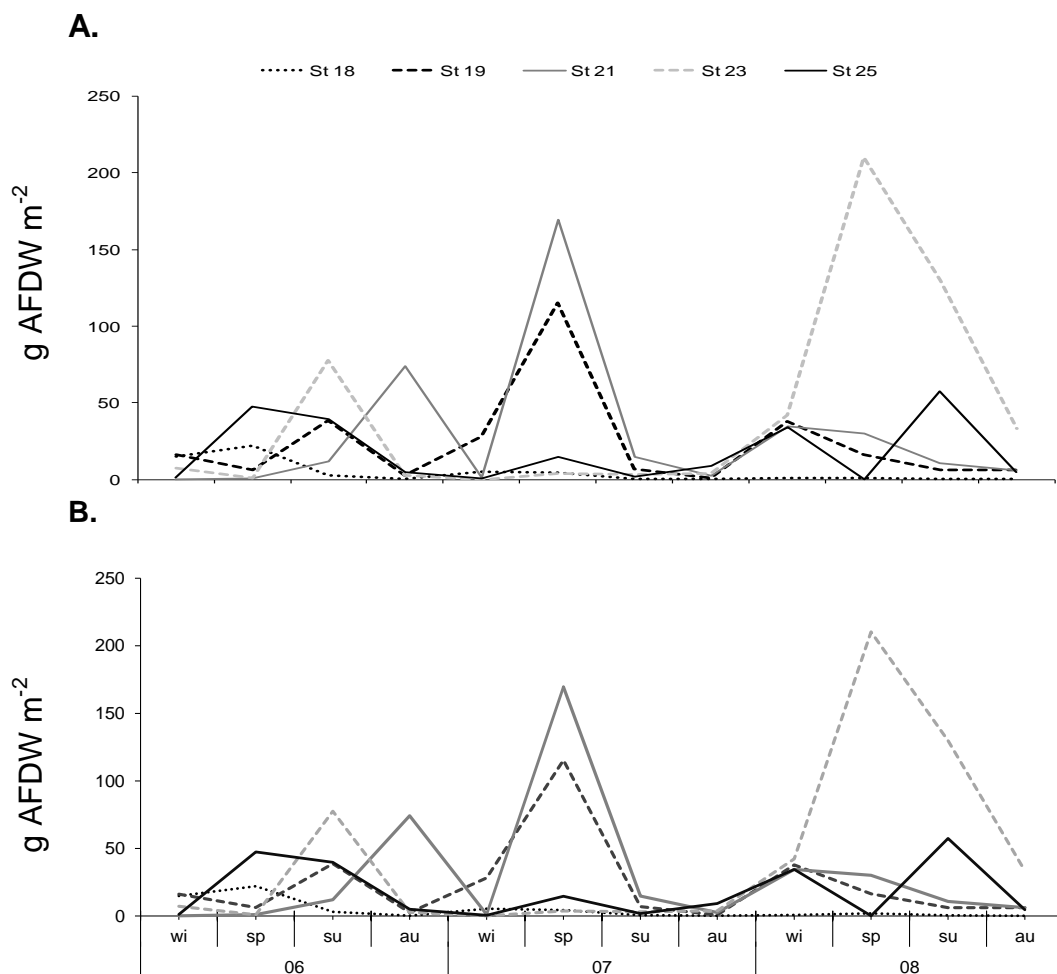


Figure 4. Meshes sieve (A.) 1 mm and (B.) 1+0.5 mm influence in temporal and spatial variation (average per station) of *C. fluminea* biomass at each station and seasons of winter (wi), spring (sp), summer (su) and autumn (au) from 2006 to 2008.

For biomass of 1 mm, the highest value was 210.3 g AFDW m⁻² (St 23, spring 2008) and the lowest value was 0.012 g AFDW m⁻² (St 23, winter 2007).

The highest 1+0.5 mm biomass value was 210.3 g AFDW m⁻² (St 23, spring 2008) and the lowest was 0.077 g AFDW m⁻² (St 18, autumn 2008).

Regarding spatial and temporal variations between meshes (1 and 1+0.5 mm), no significant differences were observed, except for spatial analysis where density showed differences.

In the fourth hypothesis ($H_0 (4)$), density showed significant interaction effect between meshes and stations ($F_{(me*st)4,350}=2.4654$; $p=0.0453$). For the Term “me*st” for pairs of levels of factor “mesh”, stations 21, 23 and 25 showed differences between meshes 1 and 1+0.5 mm. For the Term “me*st” for pairs of levels of factor “station”, 1 mm mesh presented significant differences between: St 18 and all remaining stations; St 19 and stations 18, 21, 23; St 21 and St 25. Concerning 1+0.5 mm mesh, St 18 and St 19 presented significant differences from all remaining stations. Regarding biomass no significant differences between meshes ($F_{(me)1,352}=0.026$; $p=0.869$) were found. In addition, significant differences between stations were obtained ($F_{(st)4,350}=11.042$; $p=0.0001$). Furthermore, no significant interaction effect was found between the factors mesh and station ($F_{(me*st)4,350}=0.004$; $p=1$). For pairs of levels of factor “station”, biomass showed significant differences between St 18 and all other stations; St 25 and stations 19, 23.

Regarding fifth hypothesis ($H_0 (5)$), the density revealed significant differences between meshes ($F_{(me)1,352}=27.224$; $p=0.0001$) and between seasons ($F_{(se)3,352}=4.1837$; $p=0.0061$). No significant interaction effect was found between meshes and seasons ($F_{(me*se)3,352}=1.7367$; $p=0.161$). Pair-wise tests between 1 and 1+0.5 mm meshes indicated significant differences between the density values. Pairs of seasons had significant differences

between spring and summer, spring and autumn. In relation to biomass, there were no significant differences between meshes ($F_{(me)1,352}=0.026$; $p=0.869$). However, seasons showed differences between them ($F_{(se)3,352}=8.570$; $p=0.0002$). Between meshes and seasons were not determined significant interaction effects ($F_{(me*se)3,352}=0.001$; $p=1$). Season pair-wise tests presented differences between dry periods (summer and spring) and rainy periods (autumn and winter).

Finally, the sixth hypothesis ($H_0 (6)$), the density presented significant differences between meshes ($F_{(me)1,354}=27.224$; $p=0.0001$), however, between years did not occur significant differences ($F_{(ye)2,354}=2.479$; $p=0.0824$). There were no interaction effect between meshes and years ($F_{(me*ye)2,354}=0.92437$; $p=0.406$). For pairs of levels of factor mesh, 1 mm showed differences with 1+0.5 mm. Concerning biomass, there were no significant differences between meshes ($F_{(me)1,354}=0.026$; $p=0.869$), but the years presented significant differences ($F_{(ye)2,354}=6.4556$; $p=0.002$). No interaction effect was presented between meshes and years ($F_{(me*ye)2,354}=0.005$; $p=0.9939$). Pair-wise tests between years demonstrated that the differences were significant between 2008 and all the other years.

3.4 Environmental variables

Spatial-temporal analysis of environmental data was performed according to figures 5, 6, 7 and 8.

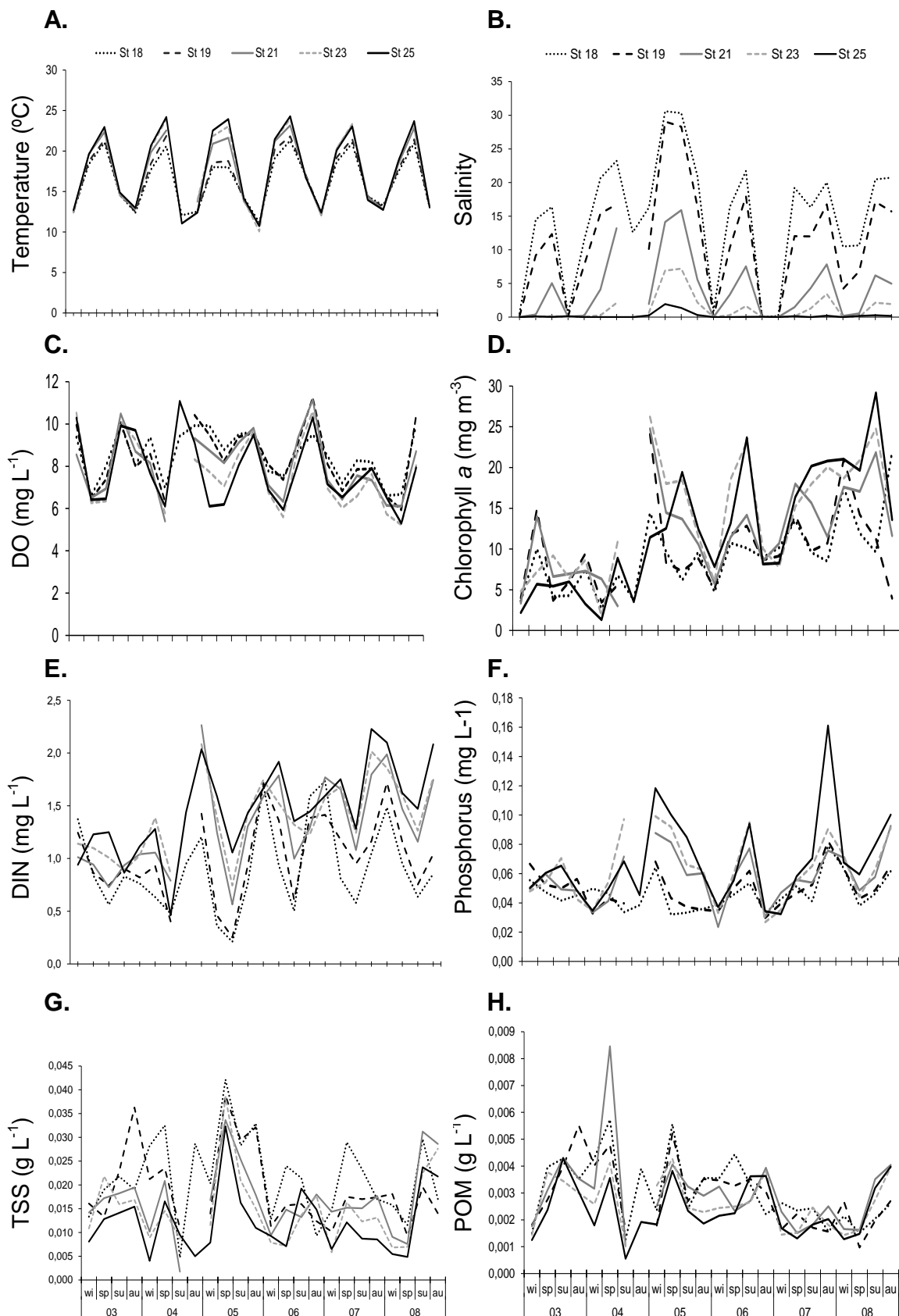


Figure 5. Seasonally variation of temperature (A.), salinity (B.), dissolved oxygen (C.), chlorophyll a (D.), dissolved inorganic nitrogen (E.), phosphorus (F.), total suspended solids (G.) and particulate organic matter (H.) parameters at each station, from winter 2003 to autumn 2008.

In general water temperature changed seasonally being the lowest value recorded of 10.1 °C and the highest value recorded of 24.3 °C. Salinity fluctuated from 0.0 to 30.6, decreasing from station 18 (0.0 - 30.6) to station 25 (0.0 - 1.9). In addition, temperature and salinity variations showed higher values in dry periods (summer and spring) and lower values in rainy periods (autumn and winter). Water dissolved oxygen varied from 5.2 mg L⁻¹ (St 23, summer 2008) and 11.3 mg L⁻¹ (St 19, winter 2007) with the highest values registered in winter. Chlorophyll *a* ranged from 1.3 mg m⁻³ (St 25, spring 2004) and 29.2 mg m⁻³ (St 25, summer 2008). Dissolved inorganic nitrogen varied between 0.210 mg L⁻¹ (St 18, summer 2005) and 2.264 mg L⁻¹ (St 21, winter 2005). The phosphorus ranged from 0.020 mg L⁻¹ (St 23, winter 2009) to 0.161 mg L⁻¹ (St 25, autumn 2007). Total suspended solids ranged between 0.002 and 0.042 g L⁻¹, and particulate organic matter ranged between 0.0006 and 0.0085 g L⁻¹. Total suspended solids and particulate organic matter maximum peaks were observed in St 18 on spring 2005 and in St 21 in spring 2004, respectively.

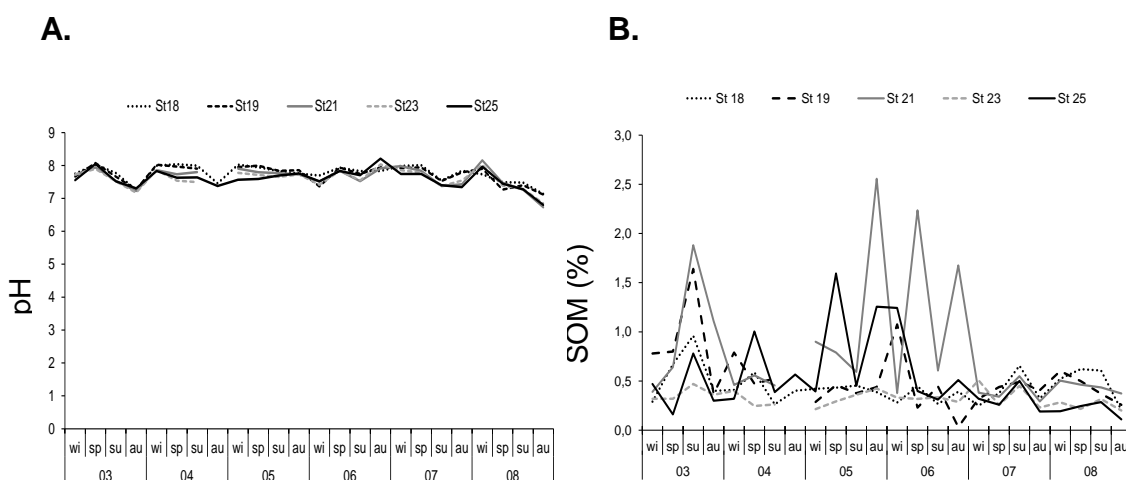


Figure 6. Seasonally variation of pH (A.) and sediment organic matter (B.) parameters at each station, from winter 2003 to autumn 2008.

Variation of pH values ranged between 6.7 (St 21, autumn 2008) and 8.2 (St 25, autumn 2006 and St 21, winter 2008). Sediment organic matter varied between 0.03 and 1.88% with two peaks of 2.24 and 2.55% observed in St 21 on spring 2006 and in St 21 on autumn 2005, respectively.

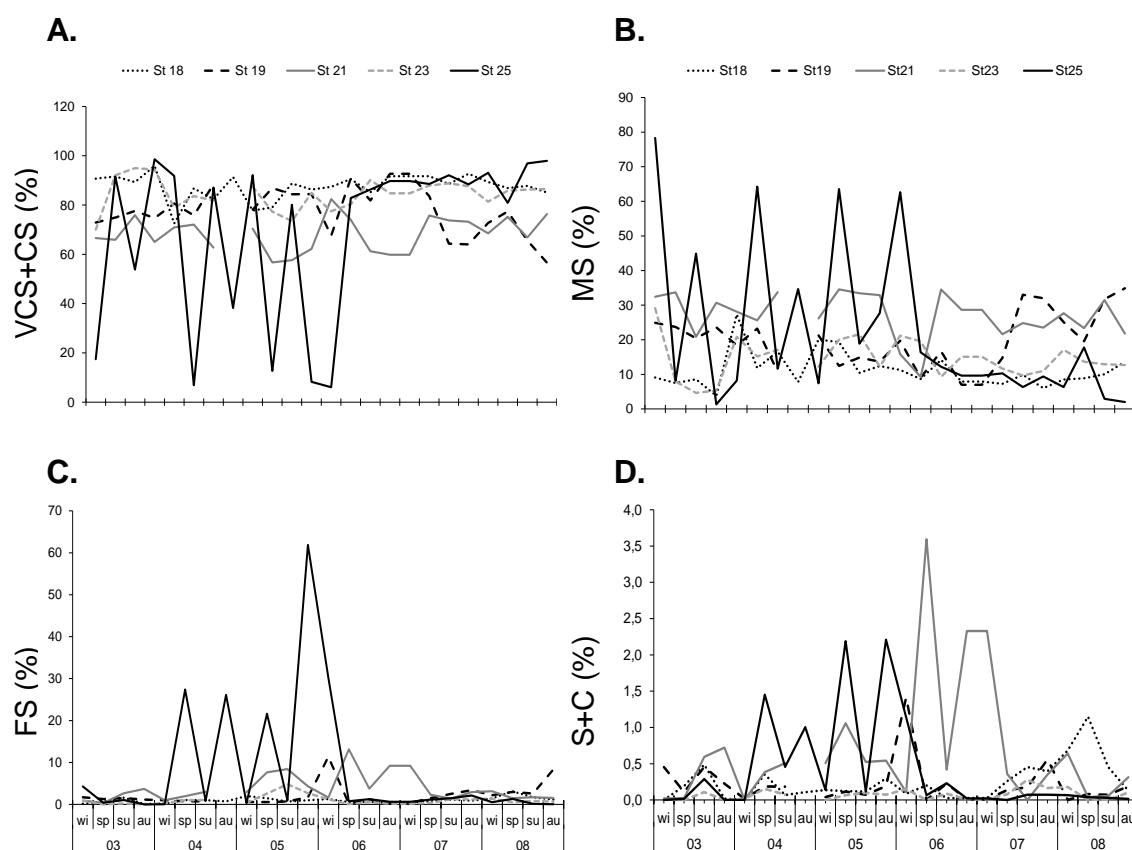


Figure 7. Seasonally variation of very coarse sand + coarse sand (A.), medium sand (B.), fine sand (C.) and silt + clay (D.) parameters at each station, from winter 2003 to autumn 2008.

Very coarse sand, coarse sand and medium sand were the dominant granulometric classes in all stations (ranged between 6.0-98.6%), except in St 25 (spring and autumn 2005 and winter 2006), where fine sand sediments were the predominant class.

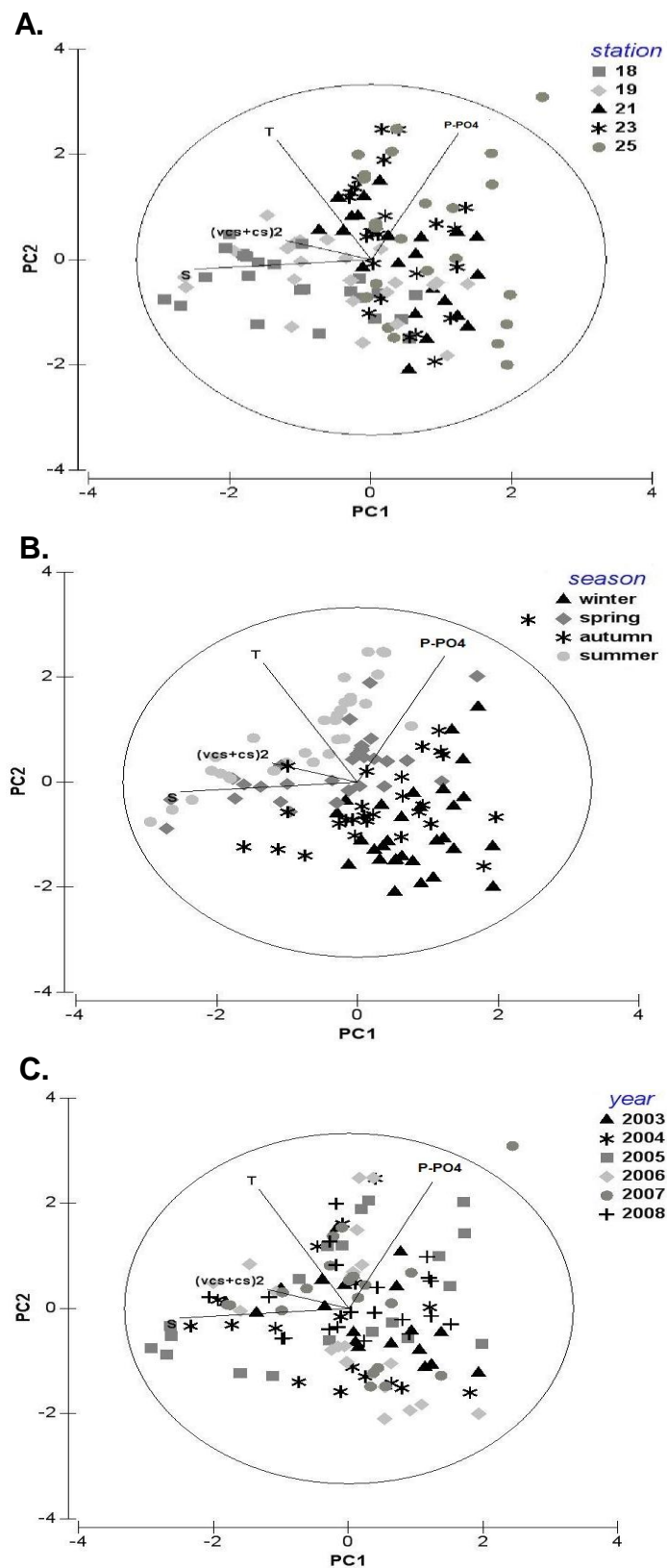


Figure 8. Two-dimensional Principal Component Analysis (PCA) plot of physicochemical parameters for the five stations (A.), in four seasons (B.) since winter 2003 to autumn 2008 (C.) (Axis 1 = 30.5%, Axis 2 = 28.9%). T – temperature, S - salinity, P- PO₄ - phosphorus and VCS+CS - very coarse sand and coarse sand.

The Principal Component Analysis (PCA) of physicochemical parameters showed a pattern in stations and seasons but not in years (Fig. 6). Slight differences between the stations 18 and 19 with 21, 23 and 25 were observed. Regarding seasons, there were differences between dry periods (summer and spring) and rainy periods (autumn and winter). The first two axes, PC1 and PC2, explained 59.4% of the variation. While phosphorus, very coarse sand and coarse sand contributed positively on axis one, temperature and salinity contributed negatively. On axis two, three parameters (T, P-PO₄ and VCS+CS) presented positive contribution. Only salinity contributed negatively to axis two.

3.4 Influence of environmental variables on *C. fluminea*

The multiple linear regression analysis was done to examine how much of the density and biomass variability of the *C. fluminea* data (1 mm) could be explained by the studied environmental variables.

Table I. Multiple linear regression model and respective ANOVA table calculated after the procedure (using AIC as a selection criterion) of natural log density of *C. fluminea* in function of 16 abiotic factors (Adjusted R²= 22%; F= 5.014; p= 0.0009). T – temperature, S – salinity and (VCS+CS)² – very coarse sand and coarse sand.

	Regression				ANOVA			
	Estimate	Std. Error	t value	Pr(> t)	Sum Sq	Mean Sq	F value	Pr(>F)
Intercept	1.077	0.261	4.120	0.00007				
T	0.049	0.012	4.009	0.001	3.856	3.856	12.829	0.0005
S	0.022	0.006	-3.531	0.0006	3.301	3.301	10.983	0.001
(VCS+ CS) ²	0.00008	0.00002	3.338	0.0011	3.348	3.348	11.139	0.001
Residuals					33.06	0.301		

$$\text{Density} = 10^{1.077 (\pm 0.261) + 0.049 (\pm 0.012) T - 0.022 (\pm 0.006) S + 0.00008 (\pm 0.00002) (VCS+CS)^2}$$

The temperature, salinity, very coarse sand and coarse sand explained 22% of the total variation of *C. fluminea* density in the multiple linear regression (Table I). Temperature, very coarse sand and coarse sand presented a positive coefficient, thus *C. fluminea* density was greater for higher values of these two variables, however, the salinity presented a negative coefficient. ANOVA results revealed that temperature influenced more the densities ($p= 0.0005$) than the other variables (salinity (S), $p=0.001$; very coarse sand and coarse sand (VCS+CS), $p= 0.001$). Therefore, temperature explained the majority of the recorded variation in population density.

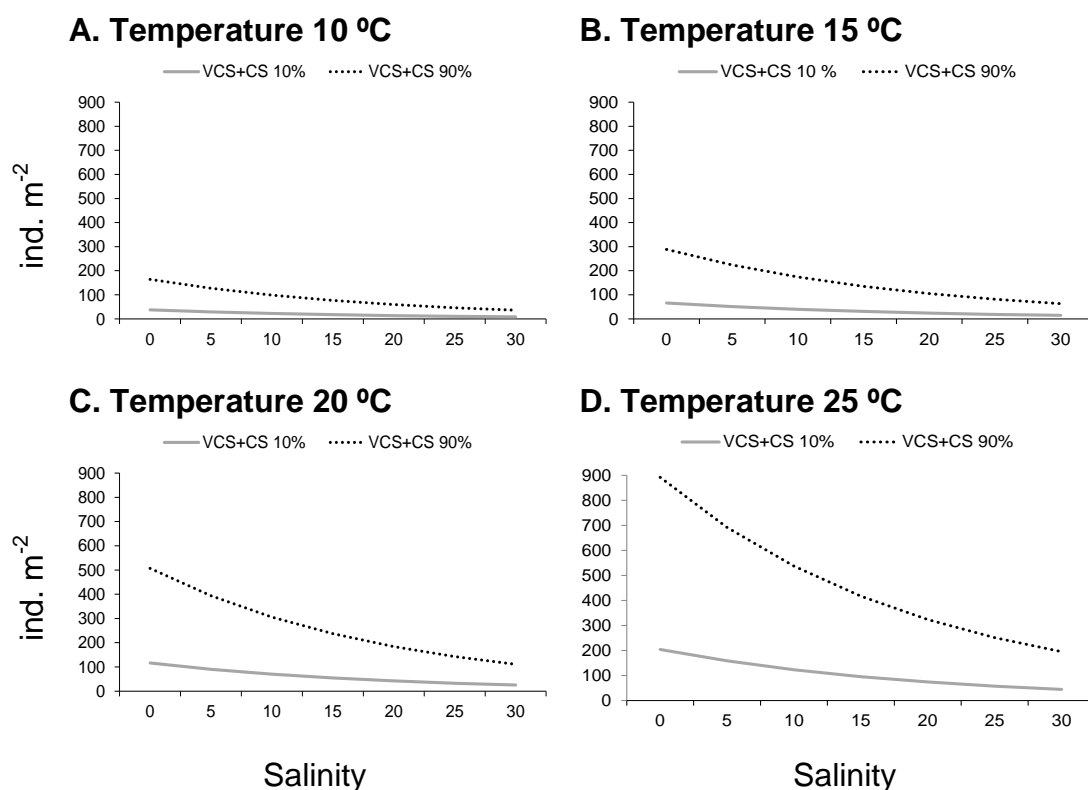


Figure 9. Multiple linear regression of final model between density of *C. fluminea* (ind. m⁻²) and salinity in two different types of very coarse sand and coarse sand (VCS+CS), 10 and 90%, for each temperature, 10, 15, 20 and 25 °C.

The density final model of multiple linear regression (Fig. 9) showed that density increased with the temperature increase. At the lowest temperature (10 °C), the maximum densities were 37.58 ind. m⁻² (VCS+CS 10%) and 164.1 ind. m⁻² (VCS+CS 90%). At the highest temperature (25 °C) the maximum densities were 204.2 ind. m⁻² (VCS+CS 10%) and 891.3 ind. m⁻² (VCS+CS 90%). Regarding salinity, as higher the salinity as lower the density – with high salinity values density tends to 0 ind. m⁻². Moreover, 90% very coarse sand and coarse sand values promote large density changes than 10% of these sediment types.

Table II. Multiple linear regression model and respective ANOVA table calculated after the procedure (using AIC as a selection criterion) of natural cubic root biomass of *C. fluminea* in function of 16 abiotic factors (Adjusted R²= 7.6%; F= 5.79; p= 0.004).
T – temperature and P-PO₄–phosphorus.

	Regression				ANOVA			
	Estimate	Std. Error	t value	Pr(> t)	Sum Sq	Mean Sq	F value	Pr(>F)
Intercept	0.786	0.550	1.427	0.157				
T	0.069	0.028	2.453	0.015	12.381	12.381	7.740	0.006
P-PO ₄	10.84	5.530	1.960	0.053	6.143	2.913	3.840	0.052
Residuals					182.4	1.600		

$$\text{Biomass} = (0.786 (\pm 0.550) + 0.069 (\pm 0.028) T + 10.84 (\pm 5.53) P\text{-PO}_4)^3$$

A multiple linear regression explained 7.6% of the total variation of *C. fluminea* biomass (Table II) through temperature and phosphorus. Temperature and phosphorus presented a positive coefficient where *C. fluminea* biomass was greater for higher values of these two variables. Nevertheless, temperature influenced more the biomass variation (p=0.006) than phosphorus (p=0.052). Therefore, temperature explained the major of the variation in population

biomass and is the most important predictor of abiotic environmental suitability for *C. fluminea*.

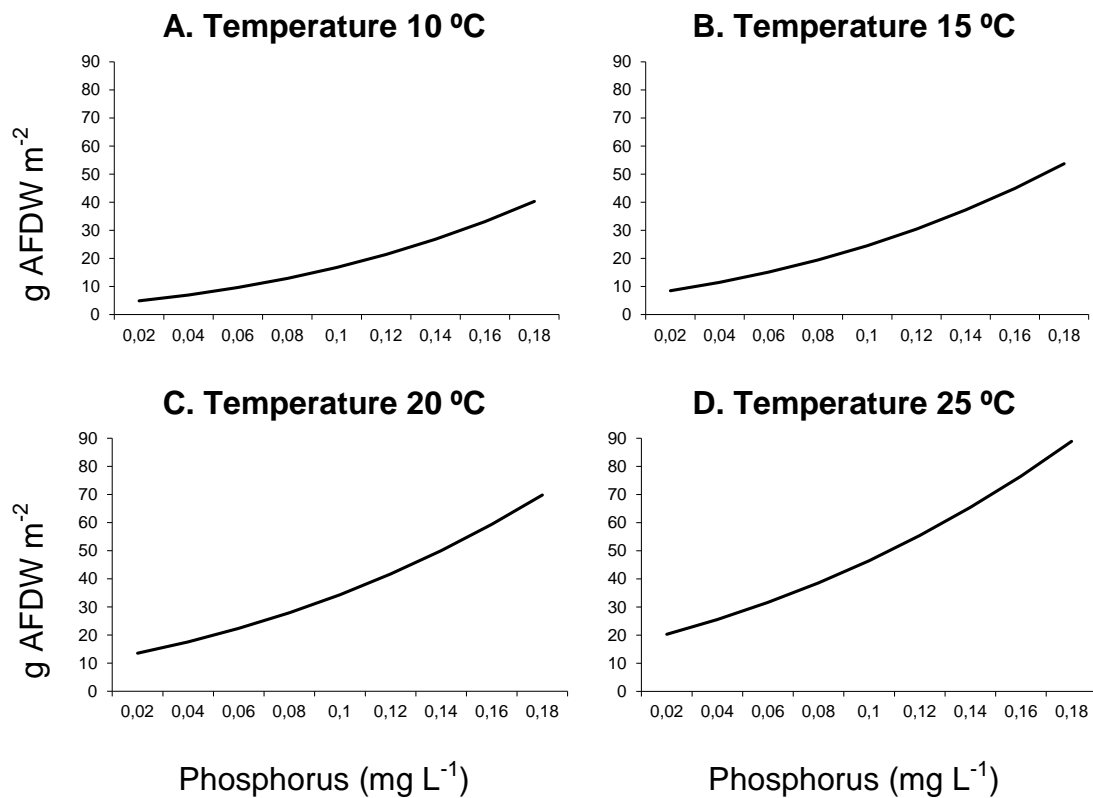


Figure 10. Multiple linear regression of final model between biomass of *C. fluminea* (g AFDW m⁻²) and phosphorus (P-PO₄) for each temperature, 10, 15, 20 and 25 °C.

The biomass final model of multiple linear regression (Fig. 10) showed that biomass had slightly increase with the temperature increase. At 10 °C temperature the maximum biomasses were 40.25 g AFDW m⁻². At 25 °C temperature the maximum biomasses were 88.85 g AFDW m⁻². In addition, the biomass slightly increased when phosphorus values were higher.

4. DISCUSSION

4. Discussion

Since 2003 subtidal soft-bottom macroinvertebrate community in the Mondego estuary has been monitored and for the first time the NIS *Corbicula fluminea* was observed in the mesohaline and oligohaline areas (Vinagre, 2009). However, Ferreira *et al.* (2004) reported its presence in Mondego River tributaries in 2001.

According to Parker *et al.* (1999) and Ricciardi (2003) the impact that a non-indigenous invasive species may have in an ecosystem is significantly associated to its density and/or biomass. Our data (1mm) showed considerable high densities of *C. fluminea* during the six years of survey in Mondego estuary. In fact, these density values are consistent with a previous study performed by Vinagre (2009) that described *C. fluminea* as a dominant species in upstream areas of the estuary. On the other hand, the registered low biomass values compared to the high densities could reflect the continuous dominance of a *C. fluminea* population constituted mainly by small size individuals (Franco *et al.*, 2011). Moreover, Franco *et al.* (2011) suggested that the population pattern observed in the Mondego estuary can be a result of local population dynamics or imported small and large size individuals from upstream areas. Therefore, the high presence of small individuals was probably promoted by the combination of two actions: recruitment in upper part of Mondego River and posterior dispersion of individuals due to the freshwater discharge into estuary and/or from local recruitment of large individuals (higher than 6 mm). Even with a low number of large individuals local recruitment is possible once *C. fluminea* fecundity is extremely high and sufficient to maintain a constant flow of recruits (Aldridge and McMahon, 1978). It should be noted that differences between

large and small size individuals were not measured in this study. However, the presence of individuals in this study with shell length > 6 mm was uncommon (personal communication) and according to McMahon (1983) and Hartog *et al.* (1992), maturity of *C. fluminea* occurs at 3-12 months at a shell length (SL) of 6-10 mm.

In Mondego estuary, *C. fluminea* presented higher densities and lower biomasses when compared to those documented in other European and South American ecosystems (Cataldo and Boltovskoy, 1999; Mouthon, 2001; McMahon, 2002; Sousa, 2008a). According to Franco *et al.* (2011) the observed pattern could be a response of *C. fluminea* to local conditions and invasion of ideal locations in the Mondego estuary. This could corresponded to an early stage of invasion process through the high juvenile productivity, maximum dispersal of juveniles and making use of food when and where it is available seems to making this species on the verge of rapid increases in biomass and distribution.

On the other hand, there are several studies that reported the decadence after the first step of invasion (Crooks, 2005; Sakai *et al.*, 2001). This situation seems have not happened in the Mondego estuary since the population has persisted for at least the last six years. A different pattern, however, has been described on Lima estuary (first record in 2002, similar to Mondego estuary) where the population was composed mainly by large size individuals (likely deficit on recruitment), since that has remained at low densities (Sousa *et al.*, 2006). Therefore, this variance in the bivalve population's structure may express different patterns of invasion and/or different stages of invasion that are

being mediated by environmental and ecological limitations and manifested as invasive species lag times (Crooks 2005).

Naturally estuaries have a highly dynamic environment (due to flotation of the different abiotic factors) that influences spatial and temporal distribution of benthic communities and Mondego estuary is not an exception (Chainho 2006, 2007). Thus, two multiple linear regression models were developed in order to combine the abiotic data with density and biomass of *C. fluminea* in the studied ecosystem. The results of the multiple linear regression models predicted that the habitats of the Mondego estuary with higher values of temperature, very coarse sand and coarse sand together with lower salinity values support higher densities. Moreover, multiple linear regression models also predicted that the habitats with higher temperature and phosphorus support higher biomasses.

According to several studies (Morton 1982, 1985, 1986), salinity is a major environmental factor for the success and velocity of the invasion of new environments by the freshwater bivalve *C. fluminea* in estuarine ecosystems: it is well known that *C. fluminea* is very efficacious in colonizing freshwater ecosystems but that it is considerably less efficient in colonizing brackish water environments. In Mondego estuary, salinity decreased from downstream (St 18) to upstream stations (St 25). This salinity gradient can justify a crescent spatial gradient of density, which was observed from station 18 to 25, except in St 21 which had the highest density values. Therefore, the lower values of density in the downstream stations may be related to salinity influence on *C. fluminea*. Besides salinity, also sediment type is recognized as another factor influencing *C. fluminea* density. According to a previous study (Cherry *et al.*, 1980), the presence of *C. fluminea* individuals was related to the predominance of very

coarse sand and coarse sand which is also the dominant sediment type of the present study. As previously mentioned and opposite to the expected (higher densities on station 25), the high density values were registered on station 21. A possible reason for this exception at station 21 may be due to the presence of sluice near this station, which controls the discharges of water channel with 28 km. According to Franco *et al.* (2011) this sluice can be an important entrance to the estuary of a significant number of individuals coming from a complete freshwater environment (located upstream of St 21), with favourable conditions to *C. fluminea* growth and reproduction. In addition, the majority of the individuals are of small dimensions once biomass did not follow the density increase at station 21. Also biomass values were opposite to expected (higher biomasses on station 25) being the station 23 the site with higher biomass values observed due to the high presence of large size individuals (personal observation). This situation may be related to the transport of *C. fluminea* individuals, especially large sized individuals. Large size individuals can be transported from up to downstream areas aided by the secretion of a long mucus dragline which gives them a lift into the water column (Prezant *et al.* 1984; Boltovskoy *et al.* 1997). This transport might be blocked by local morphological and hydrological characteristics of the St 23. Therefore, these features can provide an ideal place that allows *C. fluminea* growth. However, this theory has not been tested.

Water temperature is also an important factor in the temporal variation and is related with seasonal changes of *C. fluminea* Mondego estuary populations. Maximum density and biomass were observed in spring and/or summer. In fact, the higher temperatures were observed in these two seasons

(range between 17.5 - 23.4 °C). Therefore, the high biomass values in these seasons might be related to higher temperatures and phosphorus availability can promote plankton production leading to higher food sources to *C. fluminea* (Sousa *et al.*, 2008b) and consequent increase of its growth (increase of biomass). In addition, average temperature between 18-22 °C is the ideal temperature for *Corbicula* growth (Cataldo and Boltovskoy, 1999). Other likely consequence of temperature increase and higher food resources availability can be the increase of recruitment activity, thus leading to an increased density (Williams and McMahon, 1986), since these two factors ensure the higher energy required (Doherty *et al.*, 1987) and sufficient feeding conditions for the new-born and for the parents.

Salinity also contributed to interannual changes of the *C. fluminea* density (temporal variation). Regarding all the studied years, the year of 2005 had the lowest density. This situation can be a consequence of severe drought conditions in 2005 that promoted hostile environmental conditions to *C. fluminea* population. The heatwave of 2005 may have promoted the salinity increase and likely contribute to higher mortality of *C. fluminea* individuals, particularly small size individuals which have less salinity tolerance. On the other hand, large size individuals have higher salinity tolerance. Although there is a necessity of a high energetic investment to face the adverse environment conditions (and consequent reduction in reproductive activity), ensuring their survival (Sousa *et al.*, 2006) and thus contribute to higher biomass.

Other variables may also influence the interannual variations of *C. fluminea* biomass. Opposite to density (differences only between 2005 and remaining years), biomass showed significant differences between almost all

the six years. These differences may be explained by limited growth (small/null) or continuous mortality in conjunction with continuous recruitment. Size selective predation can also induce changes in population structure, which was manifested at biomass level (Franco *et al.*, 2011). However, these facts were not quantified in our study, but it may be worth examining.

Concerning meshes, the 0.5 mm mesh sieve is commonly used in studies of estuarine benthic communities (Couto *et al.*, 2010). For that reason, since winter 2006, samples collected on Mondego estuary were separated through 1 and 0.5 mm mesh sieve. The number of *C. fluminea* organisms retained in 1 mm mesh sieve was significantly lower than the retained on 1 and 0.5 mm mesh sieve on the different stations. This density differences observed between meshes can be due to the high number of small size organisms retained on 0.5 mm mesh aperture (Hartley, 1982) and allow a better evaluation of the spatial patterns. For example, station 19 only presented density differences from all other stations when it was added the mesh of 0.5 mm. Thereby, the mesh of 0.5 mm showed that there are differences between the oligohaline and mesohaline zone. Therefore, these results showed the importance of using the 0.5 mm mesh, since it revealed differences in the population's structure at sampling stations level, which 1 mm mesh failed to show. Concerning season patterns, Couto *et al.* (2010) showed that sampling seasons may also explain the density differences, since the density values were significantly higher in the 0.5 mm mesh sieve. However, in our work there were no season's variations neither year's variation. Besides, there were not significant differences between biomass values, and therefore, as a whole, the mesh of 0.5 mm was not changing the information on biomass provided by 1

mm mesh size sieve. This situation is in accordance to other authors which reported that majority of the total biomass was retained by a 1 mm mesh sieve in marine bottom samples (Reish, 1959; Gage *et al.*, 2002). Our particular results are probably related to specific habitat conditions (mesohaline and oligohaline areas mainly composed of very coarse sand and coarse sand sediments), community characteristics (higher number of small size individuals) and short time of sampling (two years). Therefore, it may be worth to study a longer period of 0.5 mm mesh sieve to check whether will occur more differences at spatial and temporal variation of *C. fluminea*.

5. CONCLUSIONS

5. Conclusions

This study showed that *C. fluminea* populations in the Mondego estuary were dominated by small size individuals that seem to be promoted by the combination of two actions: recruitment in upper part of Mondego River and posterior dispersion of individuals due to the freshwater discharge into estuary and/or from local recruitment of large individuals. The spatial and temporal variation patterns of *C. fluminea* seem to be determined by natural environmental variables. Temperature, salinity, very coarse sand, coarse sand and phosphorus gradients appear to be the main environment variables affecting the distribution and populations structure of *C. fluminea*. Other parameters like morphological characteristics, availability of food resources and size selective predation, which were not studied, can also have a contribution. The importance of using the 0.5 mm mesh sieve was illustrated by the analysis of density data, although biomass data did not show significant differences when the 0.5 mm mesh sieve was used. Therefore, in this kind of studies, we consider advisable the utilization of 0.5 mm mesh size sieves.

6. REFERENCES

6. References

- Aldridge D.W., McMahon R.F. (1978). Growth, fecundity, and bioenergetics in a natural population of Asiatic freshwater clam, *Corbicula manilensis* Philippi, from North Central Texas. *Journal of Molluscan Studies* 44: 49–70.
- Aldridge D.C., Muller S.J. (2001). The Asiatic clam, *Corbicula fluminea*, in Britain: current status and potential impacts. *Journal of Chonology* 37 (2): 177.
- Allendorf F.W., Lundquist L.L. (2003). Introduction: population, biology, evolution, and control of invasive species. *Conserv. Biol.* 17: 24-30.
- Anderson M.J. (2008). A new method for non-parametric multivariate analysis of variance. *Aust. Ecol.* 26: 32–46.
- Araujo R., Moreno D., Ramos M.A. (1993). The Asiatic clam *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in Europe. *American Malacological Bulletin* 10: 39–49.
- Boltovskoy D., Correa N., Cataldo D., Stripeikis J., Tudino M. (1997). Environmental stress on *Corbicula fluminea* (Bivalvia) in the Paraná River Delta (Argentina): complex pollution-related disruption of population structures. *Archiv für Hydrobiologie* 138: 483– 507.
- Brown A.C., McLachland A. (1990). *Ecology of Sandy Shores*. Elsevier, Amsterdam, 328p.
- Byrne M., Phelps H., Church T., Adair V., Selvakumaraswamy P., Potts J. (2000). Reproduction and development of the freshwater clam *Corbicula australis* in southeast Australia. *Hydrobiologia* 418: 185–197.
- Cataldo D., Boltovskoy D. (1999). Population dynamics of *Corbicula fluminea* (Bivalvia) in the Paraná River Delta (Argentina). *Hydrobiologia* 380: 153–163.
- Cataldo D., Colombo J.C., Botolvskoy D., Bilos C., Landoni P. (2001). Environmental toxicity assessment in the Parana river delta (Argentina): simultaneous

- evaluation of selected pollutants and mortality rates of *Corbicula fluminea* (Bivalvia) early juveniles. *Environmental Pollution* 112: 379 – 389
- Chainho P., Costa J.L., Chaves M.L., Lane M.F., Dauer D.M., Costa M.J. (2006). Seasonal and spatial patterns of distribution of subtidal benthic invertebrate communities in the Mondego River, Portugal—a poikilohaline estuary. *Hydrobiologia* 555: 59–74.
- Chainho P., Costa J.L., Chaves M.L., Dauer D.M., Costa M.J. (2007). Influence of seasonal variability in benthic invertebrate community structure on the use of biotic indices to assess the ecological status of a Portuguese estuary. *Marine Pollution Bulletin* 54: 1586-1597.
- Cherry D.S., Rodgers J.H. Jr., Granney R.L., Cairns J. Jr. (1980). Dynamics and control of the Asiatic clam in the New River, Virginia. *Bull.-VA. Water Resour. Res. Cent.* 123: 1.72.
- Clarke K.R., Warwick R.M. (2001). *Change in Marine Communities. An Approach to Statistical Analyses and Interpretation*, second ed. Primer-E, Plymouth, UK, 172 pp.
- Colautii R.I., Maclsaac H.J. (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions* 10: 135–141.
- Counts C.L. (1981). *Corbicula fluminea* (Bivalvia: Sphaeriacea) in British Columbia. *The Nautilus* 95: 12–13.
- Cox G.W. (2004). *Alien species and evolution*. Island Press, Washington.
- Couto T., Patrício J., Neto J.M., Ceia F.R., Franco J., Marques J.C. (2010). The influence of mesh size in environmental quality assessment of estuarine macrobenthic communities. *Ecological Indicators* 10: 1162–1173.
- Crooks J.A. (2005). Lag times and exotic species: the ecology and management of biological invasions in slow-motion. *Écoscience* 12: 316-329.
- Doherty F.G., Cherry D.S. & Cairns Jr. J. (1987). Spawning periodicity of the Asiatic clam *Corbicula fluminea* in the New River, Virginia. *Am. Midl. Nat.* 117: 71–82.

- Elton C. (1958). *The Ecology of Invasions by Animals and Plants*. University Chicago Press, 181 pp.
- Ferreira V., Graça M.A.S., Feio M.J., Mieiho C. (2004). Water quality in the Mondego river basin: pollution and habitat heterogeneity. *Limnetica* 23 (3-4): 295-306.
- Franco J.N., Ceia F.R., Patrício J., Modesto V., Thompson J., Marques J.C., Neto J.M. (2011). Population dynamics of *Corbicula fluminea* (Müller, 1774) in mesohaline and oligohaline habitats: Invasion success in a Southern Europe estuary, Estuarine. *Coastal and Shelf Science*, doi:10.1016/j.ecss.2011.07.014
- Fuji A. (1979). Phosphorus Budget in Natural Population of *Corbicula japonica* Prime in Poikilohaline Lagoon, Zyusan-ko. *Bull. Fac. Fish. Hokkaido Univ.* 30(1): 34-49.
- Gage J.D., Hughes D.J., Vecino J.L.G. (2002). Sieve size influence in estimating biomass, abundance and diversity in samples of deep-sea macrobenthos. *Mar.Ecol. Prog. Ser.* 225: 97–107.
- Hakenkamp C.C., Ribblett S.G., Palmer M.A., Swan C.A., Reid J.W., Goodison M.R. (2001). The impact of an introduced bivalve (*Corbicula fuminea*) on the benthos of a sandy stream. *Freshwater Biology* 46: 491-501.
- Hartley J.P. (1982). Methods for monitoring offshore macrobenthos. *Mar. Pollut. Bull.* 13 (5): 150–154.
- Hartog C. Den., F.W.B. Van Den Brink and G. Van Der Velde (1992). Why was the invasion of the river Rhine by *Corophium curvisoinum* and *Corbicula* species so successful? *Journal of Natural History* 26: 1121-1129.
- Kolar CS, Lodge DM. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16: 199–204.
- Lodge D.M. (1993). Biological invasions: lessons for ecology. *Trends in Ecology and Evolution* 8: 133–137.
- Marques J.C., Nielsen N.S., Pardal M.A., Jørgensen S.E. (2003). Impact of eutrophication and river management within a framework of ecosystem theories. *Ecological Modelling* 166: 147–168.

- Marques J.C., Salas F., Patrício J., Teixeira H., Neto J.M. (2009). Ecological Indicators for Coastal and Estuarine Environmental Assessment. A User Guide. WIT Press, Southampton, 183p.
- McMahon R.F. (1983). Ecology of the invasive pest bivalve *Corbicula*. In: Russel-Hunter, WD, The Mollusca, Volume 6, Ecology. Academic Press, Orlando, FL. 505-561.
- McMahon R.F. (1999). Invasive Characteristics of the Freshwater Bivalve *Corbicula fluminea*. In: Claudi R and Leach JH, Nonindigenous freshwater organisms, CRC press LLC, Chapter 22, 315-336.
- McMahon R.F. (2002). Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Canadian Journal of Fisheries and Aquatic Sciences 59: 1235–1244.
- Metodik Limnologisk (1992). Københavns University, Ferksvands biologisk Laboratorium. Akademisk Forlag, Denmark.
- Morton B. (1982). Some aspects of the population structure and sexual strategy of *Corbicula cf. fluminalis* (Bivalvia: Corbiculacea) from the Pearl River, People's Republic of China. Journal of Molluscan Studies 48: 1-23.
- Morton B. (1985). The salinity tolerance of *Corbicula fluminea* (Bivalvia: Corbiculoidea) from Hong Kong. Malacological Review. Vol. 18 (1-2): 91-95.
- Morton B. (1986). *Corbicula* in Asia – an updated synthesis. American Malacological Bulletin Special Edition 2: 113-124.
- Morton B. (1997). The Aquatic Nuisance Species Problem: A Global Perspective and Review. In: D'Itri, F. M. (eds), Zebra Mussel and Aquatic Nuisance Species. Ann Arbor Press, Chapter 1: 1-54.
- Mouthon J. (1981). Sur la présence en France et au Portugal de *Corbicula* (Bivalvia, Corbiculidae) originaire d'Asie. Basteria 45: 109-116.

- Mouthon J. (2001). Life cycle and populations dynamics of the Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) in the Saone River at Lyon (France). *Hydrobiologia* 452: 109–119.
- Parker I.M., Simberloff D., Lonsdale W.M., Goodell K., Wonham M., Kareiva P.M., Williamson M.H., VonHolle B., Moyle P.B., Byers J.E. & Goldwasser L. (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1: 3-19.
- Pranovi F., Franceschini G., Casale M., Zucchetta M., Torricelli P., Giovanardi O. (2006). An ecological imbalance induced by a non-native species: the Manila clam in the Venice Lagoon. *Biological Invasions* 00: 1–15.
- Prezant R.S. & Chalermwat K. (1984). Floating of the Bivalve *Corbicula fluminea* as a Means of Dispersal. *Science* 225: 1491- 1493.
- Sakai A.K., Allendorf F.W., Holt J.S., Lodge D.M., Molofsky J., With K.A., Boughman S., Cabin R.J., Cohen J.E., Ellstrand N.C., McCauley D.E., O'Neil P., Parker I.M., Thompson J.N. and Weller S.G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305-332.
- Sax D.F., Stachowicz J.J., Gaines S.D. (2005). *Species invasions: insights into ecology, evolution and biogeography*. Sinauer, Sunderland.
- Sickel J.B. (1986). *Corbicula* population mortalities: Factors influencing population control. *American Malacological Bulletin, Special Edition* 2: 89-94.
- Sousa R., Antunes C., Guilhermino L. (2006). Factors influencing the occurrence and distribution of *Corbicula fluminea* (Müller, 1774) in the River Lima estuary. *Annales de Limnologie International Journal of Limnology* 42: 165–171.
- Sousa R., Rufino M., Gaspar M., Antunes C., Guilhermino L. (2008a). Abiotic impacts on spatial and temporal distribution of *Corbicula fluminea* (Müller,1774) in the River Minho Estuary, Portugal. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

- Sousa R., Nogueira A.J.A., Antunes C., Guilhermino L., (2008b). Growth and production of *Pisidium amnicum* (Müller) in the freshwater tidal of the River Minho estuary. *Estuarine, Coastal and Shelf Science* 79: 467–474.
- Strickland J.D.H., Parsons T.R. (1972). A practical handbook of seawater analysis, *Bulletin Fisheries Research Board of Canada* (2nd ed) 167: 311.
- Reish D.J. (1959). A discussion of the importance of the screen size in washing quantitative marine bottom samples. *Ecology* 40: 307–309.
- Ricciardi A. (2003). Predicting the impacts of an introduced species from its invasion history: an empirical approach applied to zebra mussel invasions. *Freshwater Biology* 48: 912-981.
- Ruiz G.M., Fofonoff P., Carlton J.T., Wonham M.J. and Hines A.H. (2000). Invasion of coastal marine communities in North America: apparent patterns, processes and biases. *Annual Review of Ecology and Systematics* 31: 481–531.
- Teixeira H., Salas F., Borja Á., Neto J.M., Marques J.C. (2008). A benthic perspective in assessing the ecological status of estuaries: the case of the Mondego estuary (Portugal). *Ecological Indicators* 8: 404-416.
- Vinagre P. (2009). The Mondego estuary (Southwestern Europe) Mesohaline and Oligohaline macrozoobenthic communities. A contribution for their Ecological Quality Status assessment. Master's Thesis, Faculty of Science and Technology, University of Coimbra.
- Vitousek D.M., D'Antonio C.M., Loope L.L., Westbrooks R. (1996). Biological invasions as global environmental change. *American Scientist* 84: 468–478.
- Williams C.J. and McMahon R.F. (1986). Power station entrainment of *Corbicula fluminea* (Müller) in relation to population dynamics, reproductive cycle and biotic and abiotic variables. *Am. Malacol. Bull. Special Edition* 2: 99–111.