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Factors contributing to the invasive success of *Corbicula fluminea* (Müller, 1774)

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To my family, particularly to the
little princess Maria Inês

Author's declaration

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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.

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Sousa, R., Antunes, C. & Guilhermino, L. 2007. Species composition and monthly variation of the Molluscan fauna in the freshwater subtidal area of the River Minho estuary. Estuarine, Coastal and Shelf Science 75, 90 - 100.

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Sousa, R., Dias, S., Guilhermino, L. & Antunes, C. submitted. River Minho tidal freshwater wetlands: faunal biodiversity at stake? Aquatic Biology.

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Preface

The present work aims to increase the general ecological knowledge about the invasive freshwater bivalve *Corbicula fluminea* (Müller, 1774), with a particular focus on the factors that mainly contribute to the success of its invasive behaviour. An interdisciplinary approach was implemented to investigate the invasions of the Rivers Minho and Lima estuaries by this non-indigenous invasive species (NIS). The data generated from this approach provided important knowledge that can be useful in future sustainable conservation and management strategies taken worldwide to mitigate the impacts of this NIS.

The thesis entitled "Factors contributing to the invasive success of *Corbicula fluminea* (Müller, 1774)" is structured in seven chapters. The first chapter (chapter 1) is a general introduction describing the scope and the main goals of the study, which is followed by a chapter providing essential background information about ecology, distribution, life history and potential impacts of *C. fluminea* in invaded ecosystems (chapter 2). Chapters 3 to 6 address specific research topics in the context of *C. fluminea* ecology, genetics, morphometry and their implication for conservation and management of invaded habitats. In chapter 3 the macrozoobenthic assemblages along the Rivers Minho and Lima estuaries are described. In chapter 4 a genetic and morphometric comparison between the two populations was performed. In chapter 5 the *C. fluminea* populations colonizing the two estuaries are characterised in relation to their abundance, biomass and distribution. Chapter 6 gives special attention to the River Minho estuary, because of the extremely high abundance and biomass of *C. fluminea* in this aquatic ecosystem. Indeed, special emphasis was given to the putative impacts of this NIS on the resident biota, particularly to *Pisidium amnicum* (Mollusca: Bivalvia), and also to conservational and ecological aspects including possible alteration in the ecosystem processes and functions. Sub-chapter 6.4. is a general discussion about the conservational status of the River Minho tidal freshwater wetlands. All these studies merge into a general conclusion, where future areas of research are also proposed (chapter 7).

At the beginning, the PhD project included only a comparison of two *C. fluminea* populations (from the Rivers Minho and Lima estuaries) with the main objective of identifying the factors responsible for the apparently different invasive behaviours adopted by the species in these distinct estuaries. However, during the first phase of the work new ideas emerged and a more holistic approach was considered, emphasising other biotic components, especially other molluscs that directly or indirectly may be affected by *C. fluminea*. This situation was responsible for a huge sampling effort that resulted in a considerable amount of data. Some of these data, already published or accepted for publication (*e.g.* Sousa *et al.* 2005, 2007 d and e), were not included in the presented dissertation since the information provided was not essential for the central question of the thesis. Additionally, all the field work done in the last 4 years resulted in a large abiotic data set that could be used in future research, serving more than just a reference situation of the years 2004 to 2008. Finally, the sampling program increased the profound respect of the author for all the aspects related with ecology and conservation of two of the most important and beautiful Iberian rivers.

During this journey, which includes field expeditions, sampling trips and other research cooperation, many of the working relationships with my colleagues developed into very good friendships. I am particularly grateful to the following institutions, family and friends that contributed decisively for the successes of the present work:

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Abstract

The Asian clam *Corbicula fluminea* is one of the most invasive species in freshwater ecosystems. This species, originally distributed in Asia, is now a common inhabitant of American and European freshwater habitats. This non-indigenous invasive species (NIS) was first reported in the River Minho estuary in 1989 and after a short period it became the major component of the benthic macrofauna. In contrast, in the River Lima estuary its abundance and biomass are considerably lower. The first record of *C. fluminea* in this estuary was in 2002 and until now the species is distributed over a very small area.

Given the completely different invasive behaviours presented by the two populations, the main goal of this study was to identify possible reasons behind the success or failure of this species invasion with potential pay-offs in the prevention of future introductions. To attain this objective, the research started with a main characterization of the macrozoobenthic assemblages colonizing the River Minho estuary (the same information was already available for the River Lima estuary) in order to estimate the dominance of *C. fluminea* in this ecosystem. These studies confirmed a completely different invasive behaviour in the two estuaries. In addition, the two populations showed significant differences in shell shape and colour. However, genetic analysis showed an identical sequence of the 710bp fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) confirming that both populations belong to the species *C. fluminea*. The reasons behind the completely different invasive behaviours presented by *C. fluminea* in the Rivers Minho and Lima estuaries remain unresolved but several hypotheses are discussed.

A special attention was devoted to the River Minho estuary due to the great abundance and biomass of *C. fluminea* in this ecosystem. A model combining several abiotic variables with *C. fluminea* biomass showed that redox potential, nutrient concentrations, hardness, organic matter and sediment characteristics explained almost 60% of the variation in *C. fluminea* biomass in the freshwater subtidal area of the River Minho estuary ($R^2 = 59.3\%$, $F_{[9,\ 86]} = 13.9$, $p < 0.001$). This model improved the understanding of the processes responsible for the distribution and abundance of *C. fluminea* in the River Minho estuary and is essential to inform future management decisions in order to protect local habitats and biodiversity, and to reduce the economic impact of this NIS. In addition, and if used with caution, this model could be a help for ecologists and managers working with this species in other invaded habitats.

In the last part of this dissertation emphasis was placed in addressing the putative impacts of this NIS on the resident biota, particularly to *Pisidium amnicum* (Mollusca: Bivalvia), and also to conservational and ecological aspects including possible alteration in the ecosystem processes and functions. These studies were performed in the River Minho estuary. In this estuary, *C. fluminea* growth was continuous throughout its life span and the annual growth production in 2005 was estimated to be 463.778 g AFDW/m^2/year, and the mean annual biomass was 160.651 g AFDW/m^2, resulting in a $P/\bar{B}$ ratio of 2.89/year and a turnover time of 126.4 days. Comparing the results obtained in this study with values gathered in freshwater ecosystems in which total invertebrate (or high fraction of production) was estimated we can observe that *C. fluminea* production correspond to one of the highest values ever recorded. Therefore, *C. fluminea* is a fundamental element in the River Minho estuary, possibly sequestering a large portion of the available carbon for benthic production. Another aspect studied in the River Minho estuary was the potential impacts of this NIS on the indigenous species. After the introduction of *C. fluminea*, the indigenous bivalve *P. amnicum* population decreased sharply along the River Minho estuary and is now restricted to small patches in the upper limit of the tidal influence. The decrease of *P. amnicum* spatial distribution, abundance and biomass were significant in the last 4 years. A stepwise multiple regression model combining several abiotic variables and *C. fluminea* abundance as independent variables, and *P. amnicum* abundance, as the dependent variable, showed that organic matter and conductivity explained 50.2% of the variation in *P. amnicum* abundance in the River Minho estuary (R^2 = 50.2%, $F_{[2,\ 15]}$ = 7.569, p = 0.005). *P. amnicum* 2005 annual production was estimated to be 2.339 g AFDW/m^2/year, and the mean annual biomass was 1.594 g AFDW/m^2, resulting in a $P/\bar{B}$ ratio of 1.47/year and a turnover time of 248.7 days. These results are of paramount importance in identifying habitats that should be protected in order to preserve this species, and provide a scientific reference that may be useful in the development of management and/or restoration plans.

Finally, an overview of the River Minho estuary diversity and conservation state is provided, including temporal comparisons documenting faunal declines. Probably, other estuarine areas with comparable characteristics are subject to these declines and, therefore, they should also be considered for conservation purposes. The principal threats to this estuarine ecosystem are discussed and some practices that should be implemented to reverse this situation are indicated.

Resumo

A amêijoa Asiática *Corbicula fluminea* é uma das espécies mais invasoras em ecossistemas de água doce. Esta espécie, originalmente distribuída pelo continente Asiático, é hoje em dia um organismo comum nos habitats de água doce Americanos e Europeus. Esta espécie não-indígena invasora foi pela primeira vez descrita no estuário do rio Minho em 1989 e pouco tempo depois tornou-se no maior componente da fauna bentónica. Pelo contrário, no estuário do rio Lima a sua abundância e biomassa é consideravelmente menor. A primeira descrição de *C. fluminea* neste estuário foi em 2002 e a espécie, até ao momento, distribui-se por uma área muito reduzida.

Uma vez que o comportamento invasor apresentado pelas duas populações é distinto o principal objectivo deste estudo foi identificar possíveis razões que expliquem o sucesso ou falhanço da invasão desta espécie com possível recompensa no estudo de futuras introduções. De forma a completar este objectivo, a pesquisa iniciou-se com a caracterização das assembleias macrozoobênticas que colonizam o estuário do rio Minho (o mesmo tipo de informação já existia para o estuário do rio Lima) de forma a estimar a dominância de *C. fluminea* neste ecossistema. Estes estudos confirmaram um comportamento invasor completamente distinto nos dois estuários. Adicionalmente, as duas populações apresentavam diferenças significativas na forma e cor da concha. Contudo, a análise genética mostrou uma sequência idêntica no fragmento 710bp da subunidade I do gene da oxidase do citocromo *c* mitocondrial (mtCOI) confirmando assim que as duas populações pertenciam à espécie *C. fluminea*. As razões por detrás do diferente comportamento invasor apresentado pela *C. fluminea* nos dois estuários permanecem incertas mas várias hipóteses são discutidas.

Uma atenção especial foi dada ao estuário do rio Minho devido à alta abundância e biomassa de *C. fluminea* neste ecossistema. Um modelo combinando variáveis abióticas com a biomassa de *C. fluminea* demonstrou que o potencial redox, concentração de nutrientes, dureza, matéria orgânica e as características do sedimento explicam quase 60% da variância da biomassa de *C. fluminea* no estuário do rio Minho ($R^2 = 59.3\%$, $F_{[9,\ 86]} = 13.9$, $p < 0.001$). Este modelo não só aumentou o conhecimento sobre os processos responsáveis pela distribuição e abundância de *C. fluminea* no estuário do rio Minho bem como será essencial para futuras decisões de gestão que possam ser implementadas de forma a proteger os habitats e biodiversidade local e para reduzir os impactos económicos causados. Adicionalmente, e se utilizado com cautelas, este modelo poderá ser uma ajuda para ecologistas e gestores que trabalham com esta espécie em outros habitats invadidos.

A última parte desta dissertação dá uma especial atenção aos putativos impactos causados por esta espécie no biota residente, particularmente *Pisidium amnicum* (Mollusca: Bivalvia), e também para aspectos ecológicos e de conservação onde se incluem possíveis alterações nos processos e funções do ecossistema. Estes estudos foram realizados no estuário do rio Minho. Neste estuário, o crescimento de *C. fluminea* foi contínuo ao longo do ciclo de vida e a produção anual em 2005 foi estimada em 463.778 g AFDW/m^2/ano, sendo a biomassa anual de 160.651 g AFDW/m^2, o que resulta numa taxa de $P/\bar{B}$ de 2.89/ano e um tempo de *turnover* de 126.4 dias. Comparando estes resultados com valores de estudos realizados em ecossistemas de água doce em que o total de produção de invertebrados (ou uma grande fracção dessa produção) foi estimada verificamos que esta correspondeu a um dos valores mais altos jamais reportados. Consequentemente, *C. fluminea* é um elemento fundamental no estuário do rio Minho, possivelmente sequestrando uma larga porção do carbono disponível para a produção bêntica. Outro aspecto estudado no estuário do rio Minho foi a possível influência desta espécie no biota nativo. Após a introdução de *C. fluminea*, a população do bivalve nativo *P. amnicum* presente no estuário do rio Minho decresceu rapidamente, sendo que hoje em dia a sua distribuição está restrita a pequenas áreas no limite superior da influência tidal. Este decréscimo na distribuição espacial, abundância e biomassa de *P. amnicum* foi especialmente significativo nos últimos 4 anos. Um modelo combinando variáveis abióticas e a biomassa de *C. fluminea* como variáveis independentes, e a abundância de *P. amnicum* como variável dependente mostrou que a matéria orgânica e condutividade explicam 50.2% da variância da abundância de *P. amnicum* no estuário do rio Minho ($R^2 = 50.2\%$, $F_{[2, 15]} = 7.569$, $p = 0.005$). A produção anual para *P. amnicum* no ano de 2005 foi estimada em 2.339 g AFDW/m^2/ano, sendo a biomassa anual de 1.594 g AFDW/m^2, o que resulta numa taxa de $P/\bar{B}$ de 1.47/ano e num tempo de *turnover* de 248.7 dias. Estes resultados são de extrema importância para a identificação de habitats que devem ser protegidos de forma a preservar esta espécie, e providenciam referência científica que pode ser fundamental para o desenvolvimento de planos de gestão e/ou de reabilitação do ecossistema.

Finalmente, um resumo sobre a diversidade e estado de conservação do estuário do rio Minho é fornecido, incluindo comparações documentando declínios faunísticos. Provavelmente, outras áreas estuarinas com características comparáveis estão sujeitas a estes declínios e deverão ser consideradas em futuros trabalhos de conservação. As principais ameaças a este ecossistema estuarino são discutidas e algumas práticas que podem ser implementadas para reverter esta situação são indicadas.

Résumé

Le mollusque Asiatique *Corbicula fluminea* est une des espèces les plus envahissantes dans les écosystèmes d'eau douce. Cette espèce originalement distribuée par le continent Asiatique, est à ce jour un organisme commun dans les habitas d'eau douce Américains e Européens. Cette espèce non indigène envahissante a été pour la première fois décrire dans l'estuaire du fleuve Minho en 1989 e peu de temps après rendre dans le élément dominant de la faune benthique. Par contre dans l'estuaire du fleuve Lima son abondance e sa biomasse est considérablement réduite. La première description de *C. fluminea* dans cet estuaire a été faite en 2002 et l'espèce jusqu'à présent se propage sur une aire très réduite.

Une fois que le comportement envahissant présenté par les deux populations est distinct, le principal objectif de cette étude a été d'identifier les possibles raisons qui expliquent le succès ou l'échec de l'invasion de cette espèce avec le possible récompense dans l'étude de futures introductions. De manière à compléter cet objectif, la recherche a commencé avec la caractérisation des assemblées macrozoobenthique qui colonisent l'estuaire du fleuve Minho (ce même type d'information existait déjà pour l'estuaire du fleuve Lima) de manière à estimer la dominance de *C. fluminea* dans cet écosystème. Ces études confirment un comportement d'invasion complètement distinct dans les deux estuaires. En addition, les deux populations présentaient des différences significatives dans la forme ainsi que sur la couleur du coquillage. Néanmoins, l'analyse génétique montre une séquence identique sur le fragment 710bp dans la subunité I du gène du citocrom c mitochondrial (mtCOI) confirmant ainsi que les deux populations appartenaient à l'espèce *C. fluminea*. Les raisons derrière le différent comportement envahissant présenté par *C. fluminea* dans les deux estuaires restent incertaines, mais plusieurs hypothèses sont discutées.

Une attention spéciale a été donnée à l'estuaire du fleuve Minho dû à la haute abondance et à la biomasse de *C. fluminea* dans cet écosystème. Un modèle combinant variables abiotiques avec la biomasse de *C. fluminea* a démontré que le potentiel redox, concentration des nourrissantes, dureté, matière organique et les caractéristiques du sédiment expliquent presque 60% de la variance de biomasse de *C. fluminea* dans l'estuaire du fleuve Minho (R^2 = 59,3%, $F_{[9,\ 86]} = 13.9$, $p < 0.001$). Ce modèle a non seulement augmenté la connaissance sur les procédures responsables de la distribution et de l'abondance de *C. fluminea* dans l'estuaire du fleuve Minho ainsi comme sera essentiel pour les futures décisions qui puissent être mises en oeuvre de manière à protéger les habitats et biodiversité locale ainsi que pour réduire les impacts économiques causés. En complément et si utilisé avec précautions ce modèle pourra être une aide pour les écologistes et gestionnaires qui travaillent avec cette espèce dans d'autres habitats envahis.

La dernière part de cette dissertation donne une spéciale attention aux supposés impacts causés par cette espèce dans la biote résidante, particulièrement *Pisidium amnicum* (Mollusque: Bivalvia) et aussi pour des aspects écologiques et de la conservation ou s'incluent possibles modifications dans les procédures et fonctions de l'écosystème. Ces études ont étés réalisés dans l'estuaire du fleuve Minho. Dans cet estuaire, la croissance de *C. fluminea* à été continuel tout au long du cycle de vie et la production annuelle en 2005 à été estimée à 463.778 g AFDW/m^2/année étant la biomasse annuelle de 160.651 g AFDW/m^2, ce qui résulte un taux de $P/\bar{B}$ de 2.89/année et un temps de *turnover* de 126.4 jours. En comparant ces résultats avec des valeurs d'études réalisés dans des écosystèmes d'eau douce dans laquelle le total de la production d'invertébrés (ou une grade fraction de cette production) à été estimée nous avons vérifié que cette production à correspondu à une des valeurs les plus hautes jamais reportées. En conséquence *C. fluminea* est un élément fondamental dans l'estuaire du fleuve Minho possiblement séquestrant une large portion du carbone disponible pour la production benthique. Autre aspect étudié dans l'estuaire du fleuve Minho a été la possible influence de cette espèce dans la biote indigène. Après l'introduction de *C. fluminea*, la population du bivalve indigène *P. amnicum* présent dans l'estuaire du fleuve Minho a décru rapidement, étant que de nos jours sa distribution est restreinte à de petits secteurs dans la limite supérieure de l'influence tidal. Cette diminution dans la distribution spatiale, l'abondance et la biomasse de *P. amnicum* a été surtout significative dans les dernières 4 années. Un modèle en combinant variables abiotiques et la biomasse de *C. fluminea* comme variables indépendantes et l'abondance de *P. amnicum* comme variable dépendante a montré que la matière organique et la conductivité expliquent 50,2% de la variance de l'abondance de *P. amnicum* dans l'estuaire du fleuve Minho (R^2 = 50.2%, $F_{[2,\ 15]}$ = 7.569, p = 0.005). La production annuelle de *P. amnicum* pour l'année de 2005 a été estimée à 2.339 g AFDW/m^2/année, soit la biomasse annuelle de 1.594 g AFDW/m^2, ce qui résulte dans un taux de $P/\bar{B}$ de 1.47/année et dans un temps de *turnover* de 248.7 jours. Ces résultats sont d'une extrême importance pour l'identification des habitats qui doivent protégés de manière à préserver cette espèce, et fournissent une référence scientifique qui peut être fondamentale pour le développement de plans de gestion et/ou de réhabilitation de l'écosystème.

Finalement, un résumé sur la diversité et l'état de conservation de l'estuaire du fleuve Minho est fourni, incluant comparaisons documentant déclins faunistiques. Probablement, d'autres systèmes estuariens avec des caractéristiques comparables sont sujets à ces déclins et devraient être considérés dans de futurs travaux de conservation. Les principales menaces à cet écosystème estuarien sont discutées et quelques pratiques qui peuvent être mises en œuvre pour retourner cette situation sont indiquées.

Chapter 1

General introduction and objectives

General introduction and objectives

Non-indigenous invasive species

Few species live only in their region of origin. In fact, the movement of species is one of the most remarkable characteristic of our planet and they are known to expand, contract or change their geographical distribution with time (Sax *et al.*, 2005). However, in the last decades this movement was considerably accelerated due to human activities. These activities were responsible for several intentional or accidental introductions that greatly extended the capacity of many species to disperse to regions outside their natural range (Elton, 1958; Ruiz & Carlton, 2003; Cox *et al.*, 2004). Usually these introduced species in the new range are classified as non-indigenous, non-native, exotic, naturalised or alien species, among other possibilities (Colautti & MacIsaac, 2004).

The seminal book of Elton (1958) brought attention to the impacts caused by these species at the ecological and conservational levels, as well as on human health and economy. In fact, given the magnitude of these impacts, Elton (1958) wrote that "we are living in a world of ecological explosions". Since then, the issue of non-indigenous invasive species (NIS) has been receiving an increasing attention from scientists, policy authorities, environmental managers and the general public. At the present, it is considered one of the main ecological problems at the global level and, therefore, it is one of the most important areas of research in ecology. Given the myriad of detrimental impacts attributed to non-indigenous invasive species (NIS) and limited possibilities for total eradication, predicting NIS establishment and dispersal have fundamental significance (Kolar & Lodge, 2002). In addition, the study of biological invasions can give tremendous insights into ecology, evolution and biogeography since: *i*) invasions allowed the observation of the processes in real time; *ii*) if the exact time, local and characteristics of the introduced species are known, genetic changes and ways of dispersion can be measured directly; *iii*) since several species have been introduced in great numbers and in several different places, the ecological and evolutionary processes at the temporal and spatial scales can be investigated; *iv*) invasions can give information about the importance of dispersal in limiting species distributions and about the role of individual species in structuring ecosystems; and *v*) invasive species can provide information about adaptation, extinction and species saturation (Lee, 2002; Sax *et al.*, 2005; Sax *et al.*, 2007).

Until now, the search for general causes of bioinvasions (*i.e.* the successful establishment and spread of species outside their native range) has been focused mainly on understanding what predisposes a species to become an invader that proliferates in novel habitats and in identifying which are the characteristics of communities favouring the proliferation of NIS (Blackburn & Duncan, 2001). According to Facon *et al.* (2006) several invasion circumstances are possible depending on how migration and other ecological and/or evolutionary forces interact and vary during the invasion process. These authors advance with three theoretical invasion scenarios that represent the extremes of a range of situations. In the first scenario, migration change, a change in the migration processes (the most part of the times related with human activities), is sufficient to trigger such an invasion. The invasive species was previously absent from the novel ecosystem owing to its inability to get there. Such situations, reflecting the so called «empty niche» or «pre-adaptation of the invasive species» are more likely to involve species with low mobility and isolated or unsaturated communities. In this scenario the lack of co-evolutionary history can also be advantageous to the invader. The theory behind this scenario is the core of the enemy release hypothesis, where invaders benefit from the lack of specialized enemies (*e.g.* predators, parasites and competitors) in the recipient community (Keane & Crawley, 2002; Shea & Chesson, 2002; Torchin *et al.*, 2003). In the second scenario, environmental change, if abiotic or biotic conditions of a given area changed (*e.g.* climate change, disturbance) and if these new environmental conditions fit the niche requirement of a non-indigenous species, this species might spread even without acquiring new adaptations. Examples of this scenario are the range expansions of many species after Pleistocene glaciations from southern *refugia* towards the north, and recent poleward and upward movement of species in response to climate change. In addition, human induced disturbance may also facilitate the widespread of NIS (Byers, 2002a). Finally in the third scenario, evolutionary change, invasions start as a result of genetic changes in the invader that are a consequence of a combination of evolutionary forces. The propagule pressure can have a key effect in this scenario (but also in the first scenario). Indeed, the number of individuals introduced and the number of introductions can have a detrimental importance and modern invasion theory is now addressing a special attention to this subject (Lockwood *et al.*, 2005; Colautti *et al.*, 2006).

All ecosystems are susceptible to NIS introductions and possibly no part of the globe is immune to the impacts caused (Mack *et al.*, 2000). Indeed, globalization has facilitated the homogenisation of Earth biota through establishment and spread of NIS (McKinney & Lockwood, 1999). Anyway, aquatic ecosystems are especially vulnerable to these introductions and recently this thematic gained a fundamental importance in aquatic ecology due to unprecedented changes in recipient ecosystems (Grosholz, 2002; Korsu *et al.*, 2007). The transport and subsequent introduction of NIS is a pervasive component of aquatic ecosystems which present a new challenge for the management and conservation of biodiversity of these habitats. This situation is responsible for several environmental, ecological and economic impacts that could not be neglected. Several human activities are clearly responsible for the increased number of introductions in aquatic ecosystems, including: construction of canals connecting different aquatic ecosystems, aquaculture, aquarium releases, recreational activities, fisheries, tourism and transportation by great transatlantic vessels and subsequent release of ballast water (Carlton & Geller, 1993; Cohen & Carlton, 1998). These bioinvasions in aquatic ecosystems are accelerating and can have detrimental consequences, including the erosion of biodiversity and the disruption of ecological processes and functions (Byrnes *et al.*, 2007). This situation can have a disproportional importance in freshwater ecosystems since these areas are perhaps the most impacted habitats in the planet, and their biodiversity is exposed to tremendous risks (Dudgeon *et al.*, 2006).

Species which have great impacts that pose a threat to biodiversity, ecosystem functioning and even human health should be a priority issue in research and environmental management (Mack *et al.*, 2000). The bivalve *Corbicula fluminea* is one of the most invasive species in aquatic ecosystems and is well known by its rapid and extensive spread. This species as its native range in Asia and in the last 80 years invaded several ecosystems worldwide: in 1924, it was introduced in the Pacific coast of the United States of America and in few years dispersed to several states; in the 1970s, it was for the first time recognized in South America and, in 1981, the first paper describing its presence in Europe was published. This NIS can be responsible for significant ecological impacts in the native biota, changes in the environmental characteristics and considerable economic damages due to biofouling. Therefore, it is not surprising that several authors agree that this NIS should be a priority of research and management in aquatic ecosystems (Phelps, 1994, Darrigran, 2002; McMahon, 2002).

Statement of the problem

The bivalve *C. fluminea* is a recent invader in two estuarine ecosystems located in the NW of the Iberian Peninsula: the Rivers Minho and Lima estuaries. According to Araujo *et al.* (1993), the first individuals were collected in the River Minho estuary in 1989. This population suffer a rapid dispersion in the subsequent years and nowadays the River Minho tidal freshwater wetlands (TFWs) have a mean abundance and biomass of more than 1000 ind./m^2 and 100 g AFDW/m^2, respectively. In contrast, the first individuals of *C. fluminea* were collected in the River Lima estuary in 2002 (Sousa *et al.*, 2006a and b). In this ecosystem the population is restricted to a very small area which comprises no more than 2km of river length and the mean abundance and biomass is much lower than in the River Minho estuary. Given the completely different invasive behaviour in the two adjacent estuarine areas the principal aim of this study was to advance with possible explanations for this situation, with potential pay-offs in the prevention of future introductions. Since the River Minho population is widespread over a large area and has great abundance and biomass, special attention was devoted to this ecosystem. Emphasis was given to determining the spatial and temporal distribution of this NIS and trying to find relationships between *C. fluminea* biomass and the principal abiotic factors. In all studies, special attention was given to conservational aspects since freshwater biodiversity is under a severe threat. Indeed, freshwater organisms, including molluscs are suffering distinct but enormous pressures, with the introduction of NIS being one of the factors responsible for their imperilled situation.

Objectives

The overall objective of this study was to identify possible factors responsible for the invasive success (or failure) of the Asian clam *C. fluminea* in estuarine ecosystems. Therefore, an integrative approach combining ecology, genetics and morphometry, and conservation was chosen in order to increase our biological knowledge about the invasive behaviour of this NIS and deduce possible management/mitigation measures easily applied in invaded aquatic ecosystems. As several different deterministic and stochastic factors linked with the species genetics and ecology can all contribute for the invasive dynamics with different intensities, investigations addressing different hypotheses and objectives were carried out. There were several specific objectives, as follows:

- To compare the pattern of the macrozoobenthic composition (with a special emphasis to molluscan fauna) in relation to abiotic conditions in the River Minho estuary. A similar approach was already available for the River Lima estuary (Sousa *et al.*, 2006a; 2007b). This procedure allowed estimations of the abundance, biomass and diversity of subtidal macrozoobenthic assemblages and the dominance of *C. fluminea* in the two estuaries;
- To identify the functionally important species and possible sentinel species that could be used in future ecological and toxicological studies in the River Minho estuary. The same objective was already achieved for the River Lima estuary (Sousa *et al.*, 2006a, 2007b);
- To compare the two populations presented in the Rivers Minho and Lima estuaries, using conventional morphometric measures, geometric morphometric methods and genetic analysis. Additionally, genetic sequences of individuals from both populations were compared with GenBank sequences from other populations in an attempt to identify their origin;
- To investigate the progress of *C. fluminea* abundance, biomass and population structure in the two estuaries;

- To develop a model describing the relationship between abiotic factors and the spatial and temporal distribution of *C. fluminea* in the freshwater tidal area of the River Minho estuary;
- To estimate the abundance, biomass, growth, and growth and elimination production of *C. fluminea* in the River Minho TFWs in order to evaluate their importance for the ecosystem functioning;
- To estimate the abundance, biomass, growth, and growth and elimination production of *P. amnicum* in the River Minho TFWs. According to earlier studies this native species is possibly the most affected after the introduction of *C. fluminea* and therefore a model describing the relationship between abiotic factors and *C. fluminea* abundance and the spatial and temporal distribution of *P. amnicum* abundance in the River Minho TFWs was also developed;
- To discuss possible reasons behind the significant temporal differences observed in the abundance and diversity of faunal species when earlier studies are compared with the current data set.

Chapter 2

Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview

Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview

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Abstract

The Asian clam *Corbicula fluminea* is one of the most invasive species in freshwater aquatic ecosystems. The rapid growth, earlier sexual maturity, short life span, high fecundity and its association with human activities makes *C. fluminea* a non-indigenous invasive species likely to colonize new environments. This species, originally distributed in Asiatic ecosystems, is now a common inhabitant of American and European freshwater habitats. The present paper reviews the information related to the life cycle, ecology and potential ecological and economic impacts caused by *C. fluminea* in the invaded habitats. Furthermore, this paper also proposed future works that may be implemented in order to increase our general knowledge about the ecology of this bivalve.

Introduction

The accidental or deliberate introduction and subsequent spread of non-indigenous invasive species (NIS) has become a serious ecological, conservational and economic problem. These NIS are altering the terrestrial and aquatic ecosystems at unprecedented rates (Carlton & Geller, 1993; Lodge *et al.*, 1998; Cox, 2004) and are now one of the most important environmental problems concerning the scientific community (Sala *et al.*, 2000). In fact, species diversity and distribution were never spatial or temporally stationary. However, in the last years species are being dispersed across their natural geographic barriers through human-mediated activities such as global trade, agriculture, aquaculture, recreational activities and transportation (Cohen & Carlton, 1998; Ricciardi & MacIsaac, 2000; Cox, 2004).

Scientists interested in biological invasions have difficulties describing the fundamental characteristics responsible for the invasive success of some non-indigenous species, and the evolutionary and ecological principles behind the success of invasions in new environments have always been highly debated (Occhipinti-Ambrogi, 2007). Generally, for invasive animal species the most important characteristics to be successful in the new habitat are: great geographical distribution with potential ability to colonize a vast range of habitats; considerable genetic variability and phenotypic plasticity; physiological tolerance to abiotic changes; short generation times, rapid growth, rapid sexual maturity and great fecundity; opportunistic behaviour (r-strategists); fertilized females able to colonize alone; and association with human activities and high dispersal potential (Lodge, 1993; Alcaraz *et al.*, 2005; Céréghino *et al.*, 2005). However, the fundamental role of propagule pressure (*i.e.* introduction effort, which is related to the total number of individuals introduced in conjunction to the number of introductions attempts) is central to the success of NIS establishment and increases the probabilities of dispersion. Despite their significance, this hypothesis only recently gained a determinant importance in the biological invasion theory (Ruiz *et al.*, 2000; Ruesink, 2005; Colautti *et al.*, 2006; Ricciardi, 2007).

The Asian clam *Corbicula fluminea* is considered one of the most important faunal NIS in aquatic ecosystems (McMahon, 2002). In the last few decades, studies of *C. fluminea* have shown both a considerable geographic dispersion and invasive behaviours (Mouthon, 1981; Araujo *et al.*, 1993; McMahon, 1999). The invasive success and subsequent dispersion of *C. fluminea* relies more on their natural characteristics (*e.g.* rapid growth, earlier sexual maturity, short life span, high fecundity, extensive dispersal capacities and its association with human activities) than in its physiological tolerance (McMahon, 2002). In fact, this NIS when compared, for example, with other freshwater bivalve species appears to be less tolerant of environmental fluctuations such as elevated temperatures, hypoxia, emersion, low pH and low calcium concentrations (Byrne & McMahon, 1994; McMahon, 1999; Johnson & McMahon, 1998; McMahon & Bogan, 2001; Sousa *et al.*, 2007c, 2008).

This paper revises the data available for *C. fluminea* discussing the general life cycle characteristics, the potential ecological and economic impacts caused by this NIS in invaded habitats and proposes future works that may be implemented to increase our general knowledge about the ecology of this bivalve.

Invasion history

The original distribution of the *Corbicula* genus was confined, in the beginning of the last century, to Asia, Africa and Australia and since then it has dispersed worldwide (Mouthon, 1981; Counts, 1986; Araujo *et al.*, 1993; Ituarte, 1994; McMahon, 1999). The first documented occurrence of this genus outside its original distribution was in the Pacific coast of United States in the 1920s, possibly being introduced by Chinese immigrants as a food resource (Counts, 1981). Forty years later, its distribution extended to the Atlantic coast of the United States. In South America, this genus was first recognized around the 1970s (Ituarte, 1994) and in Europe its presence was described for the first time by Mouthon (1981). Complicating the picture, species from the *Corbicula* genus were also present in the fossil record of North America and Europe before the last glaciation (Araujo *et al.*, 1993). However, the specific classification of these fossil individuals is very difficult, a fact that may be easily understood considering the taxonomic problems that still exist. Consequently, recent invasions could be seen as a re-colonization process of earlier habitats and not as a true invasion (Pfenninger *et al.*, 2002). If this perspective is correct, it seems that only in the last decades this genus found the necessary conditions to re-colonize the earlier habitats, coupled with increased chances of re-colonization through the vector of human activities. Another alternative hypothesis is the possible retention and a subsequent dispersion of *Corbicula* specimens from *refugia* such as South European ecosystems which were not subjected to glaciations processes. However, given the present rate of dispersion it is difficult to understand the reason why the species did not begin the re-colonization from the *refugia* areas before (but see Karatayev *et al.* (2006) with the suggestion that the spread of freshwater NIS bivalves' species was not a continuous process, but somewhat punctuated by periods of rapid long distance spread).

The introduction and subsequent dispersion of *C. fluminea* in aquatic ecosystems is probably a result of various human activities (*e.g.* ballast water transport, food resource, utilization of specimens as fish bait, aquarium releases, transport of juveniles and/or adults as a tourist curiosity or the juvenile byssal attachment to boat hulls) (McMahon, 1999, 2002; Darrigran, 2002; Lee *et al.*, 2005). Additionally, *C. fluminea* has extensive capacities for natural dispersion since the pediveliger and juveniles are passively transported by fluvial or tidal currents, being also transported on the feet or feathers of aquatic birds (Prezant & Chalermwat, 1984; McMahon, 1999, 2002). This kind of natural transportation may have a fundamental importance in the magnitude of secondary introductions (Figuerola & Green, 2002; Green & Figuerola, 2005).

Morphometry and genetics

Considerable controversy exists about the number of *Corbicula* species present in European and American freshwater ecosystems, to which taxon they belong and where they originated (Pfenninger *et al.*, 2002). This controversy is related to the complicated taxonomical classification in the *Corbicula* genus due to the marked variation in shell morphology, colour and reproductive biology (Komaru & Konishi, 1999; Rajagopal *et al.*, 2000; Renard *et al.*, 2000; Siripattrawan *et al.*, 2000; Qiu *et al.*, 2001; Park *et al.*, 2002; Pfenninger *et al.*, 2002; Park & Kim, 2003; Lee *et al.*, 2005; Sousa *et al.*, 2007a). In French and Dutch rivers, Renard *et al.* (2000) based on morphometric variation and genetic analysis described three morphotypes that were classified as *C. fluminea*, *C. fluminalis* and another species for which a specific name was not assigned (being referred as *Corbicula spec.*). The results of Pfenninger *et al.* (2002), with material collected in the River Rhine recognized the presence of two *Corbicula* lineages: one corresponding to *C. fluminea* and the other to *Corbicula spec.* as defined by Renard *et al.* (2000). Additionally, the results of Sousa *et al.* (2007a) show clear morphometric differences in individuals colonizing two adjacent Portuguese estuarine ecosystems, although the two populations share similar mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) sequences that correspond to *C. fluminea sensu* Renard *et al.* (2000). However, the results obtained by Park & Kim (2003) with specimens from the native distribution range (and comparison with non-native mtCOI sequences) give additional information about the different lineages inside the *Corbicula* genus. According to these authors, *C. fluminalis sensu* Renard *et al.* (2000) belongs also to the freshwater *Corbicula* lineage. These results may introduce several changes in our knowledge about the *Corbicula* distribution in European ecosystems because we may have several lineages belonging to the freshwater clade [e.g. may be the same species: *C. fluminea sensu* Renard *et al.* (2000)] but with several races/morphotypes with origin in Asia and/or North America. In American ecosystems the same controversy still exists and Siripattrawan *et al.* (2000), based in mtCOI gene analysis, established the presence of two species (classified as *C. fluminea* and *C. leana*). However, Lee *et al.* (2005) with a study conducted in 12 sites distributed for North and South American freshwater ecosystems do not attribute a specific name to the different morphotypes analyzed. Given the actual confusion inside this thematic, all these morphometric and genetic complications have to be studied in the future in order to elucidate the number of species inside the *Corbicula* genus. These studies will be also very informative for the clarification of the routes of introduction and for the invasion dynamics management in future invaded ecosystems.

Life cycle

Species from the *Corbicula* genus comprise different reproductive modes which have been related to its large ecological spectrum (Morton, 1986; Rajagopal *et al.*, 2000; Korniushin & Glaubrecht, 2003). Additionally, several unusual features of reproductive biology, such as polyploidy, unreductional biflagellate sperm, androgenesis and clonality were observed in this genus (Komaru & Konishi, 1996, 1999; Komaru *et al.*, 1997; Qiu *et al.* 2001).

C. fluminea (Fig. 2.1. a) is generally described as a hermaphroditic species. The fertilization occurs inside the paleal cavity and larvae are incubated in branchial water tubes (Fig. 2.1. b). However, studies done by Rajagopal *et al.* (2000) in the putative *C. fluminalis* (which is classified by Park & Kim (2003) as another freshwater *Corbicula* morphotype) show that the specimens that colonized the River Rhine are dioecious (with 3% of hermaphrodites). Another interesting characteristic of this species deals with the embryonic nutrition of brooding individuals, which remain uncertain. According to Kraemer

& Galloway (1986) and Byrne *et al.* (2000), eggs of *Corbicula* are rich in nutrients that are essential for the developing embryos. Additionally, the interlamellar junctions of inner demibranchs in *C. fluminea* and *C. australis* were found to be modified, which presumably serve as alternative source of nutrition for embryos (Byrne *et al.*, 2000). After this protective period, larvae are released into the water, settle and bury into the substratum (Cataldo & Boltovskoy, 1999; McMahon, 1999). When *C. fluminea* juveniles are released, they have small dimensions (around 250 µm) but completely formed with a well developed shell, adductor muscles, foot, statocysts, gills and digestive system and have the usual D-shaped configuration (Fig. 2.1. c) (McMahon, 2002). After the water column release, juveniles anchor to sediments, vegetation or hard surfaces due to the presence of a mucilaginous byssal thread. These juveniles can also be re-suspended by turbulent flows and dispersed for long distances, principally in the downstream direction (McMahon, 1999). The maturation period occurs within the first 3 to 6 months when the shell length reaches 6 to10 mm (Fig. 2.1. d).

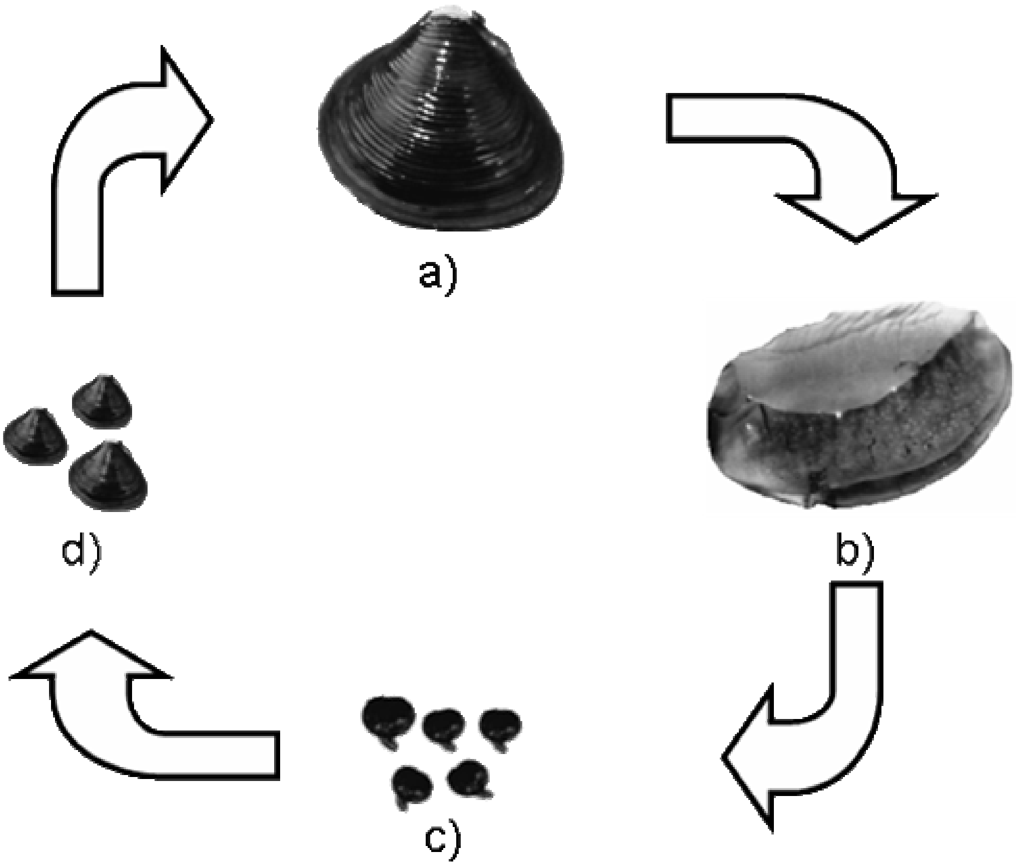

Fig. 2.1. Illustrative representation of the life cycle of *C. fluminea*: a) adult specimen; b) inner demibranch with larvae; c) small juveniles recently released (with a completely developed foot and with the common D-shaped configuration) and d) small adults.

The life span of this species is extremely variable, ranging from 1 to 5 years, with usual bivoltine juvenile release pattern (McMahon, 1999). However, the number of annual reproductive periods changes from ecosystem to ecosystem. The majority of studies concluded that this species reproduces twice a year: one occasion in the spring going through the summer and the other starting in late summer and going through the autumn. In contrast, some studies found only one reproductive period, while in others three were found, with differences among years even in the same site (Doherty *et al.*, 1987; Darrigran, 2002). These fluctuations in the number of reproductive events may be related with water temperature (Hornbach, 1992; Rajagopal *et al.*, 2000; Mouthon, 2001b) and/or with the food resources available in the ecosystem (Cataldo & Boltovskoy, 1999; Mouthon 2001a and b).

C. fluminea grows rapidly, in part due to its high filtration and assimilation rates (McMahon, 2002). The major part of its energy is allocated to growth and reproduction and only a small portion is devoted to respiration (McMahon, 2002). According to this author, this species has the highest net production efficiencies recorded for any freshwater bivalve, reflected by short turnover times of only 73 – 91 days. Like other freshwater bivalve species, *C. fluminea* transferred only a small percentage of assimilated energy to reproduction. Nevertheless, its elevated assimilation rates allow a high absolute energy transfer to reproduction when compared with other freshwater bivalves. *C. fluminea* has a high fecundity but a low juvenile survivorship and a high mortality rate throughout life span. This low adult survivorship leads to populations dominated by high proportions of juveniles (McMahon, 1999, 2002). Anyway, in some ecosystems this population domination by immature juveniles is not so effective and the presence of adults in high abundance and having large sizes has been reported (Boltovskoy *et al.*, 1997; Sousa *et al.*, 2005, 2007c, 2008, in press)

The principal life history characteristics of *C. fluminea* are summarised in Table 2.1.

Table 2.1. Summary of the principal life history characteristics of *C. fluminea* (adapted from McMahon, 2002).

Life history characteristics	***C. fluminea***
Life span	1 to 5 years
Age at maturity	3 to 9 months
Reproductive mode	Hermaphroditic
Growth rate	Rapid
Fecundity	68 678
Juvenile size release	250 µm
Position of broods	Inner demibranchs
Type of released larvae (juveniles)	D-shaped configuration
Type of brooding	Synchronous
Juvenile survivorship	Low
Adult survivorship	Usually low
Number of reproductive events	Usually two but may vary
Assimilated energy respired	11 – 42%
Non-respired energy transferred to growth	58 – 71%
Non-respired energy transferred to reproduction	5 – 15%
Turnover time	73 - 91 days
Habitat requirements	Intolerant to high salinity values and even moderate hypoxia conditions (this species is usually restricted to well-oxygenated areas). Tolerate low water temperatures and prefer sandier sediments mixed with silt and clay (which enhance the organic matter content). However in some ecosystems, this species can be found in all types of sediments (with or without submerged vegetation) (Sousa *et al.,* 2008)

Possible environmental and ecological effects

The introduction of NIS is a serious threat to the native biodiversity and ecosystem functioning with potential repercussions in food webs, biogeochemical cycles and human economy (Kolar & Lodge, 2001; Grosholz, 2002).

The great invasive and reproductive capacity of *C. fluminea* makes this species an important component of aquatic ecosystems. Usually, *C. fluminea* introductions have consequences to other elements of the ecosystem including submerged vegetation, phytoplankton, zooplankton and higher trophic levels (Table 2.2.) (Phelps, 1994; Johnson & McMahon, 1998; Strayer, 1999; Cherry *et al.*, 2005; Cooper *et al.*, 2005; Sousa *et al.*, 2005*,* 2007c, 2008, in press). A revision of several studies shows that the invasion of *C. fluminea* has negatively impacted native bivalve abundance and diversity in North American and European freshwater ecosystems (Araujo *et al.*, 1993; Williams *et al.*, 1993; Strayer, 1999; Aldridge & Muller, 2001; McMahon, 2002; Sousa *et al.*, 2005, 2006a, 2006b, 2007c, 2008, in press). The ancient bivalve fauna of European and North

American rivers was dominated by freshwater mussels from the Margaritiferidae and Unionidae families and small clams from the Sphaeriidae family. For example, the freshwater mussel species were very common in stable substrates but nowadays this ancient bivalve fauna is at risk in the principal European rivers (Reis, 2003; Geist & Kuehn, 2005), being also of conservational concern in North American freshwater habitats (Lydeard et al., 2004; Strayer et al. 2004). In contrast, several worldwide freshwater ecosystems are now colonized by non-indigenous invasive bivalve species (*e.g. C. fluminea*, *Dreissena polymorpha* and *Limnoperna fortunei*) that replaced the native forms very quickly. The principal problem of the recent freshwater bivalve species invasions, such as *C. fluminea*, resides in the potential change in the ecological conditions of the invaded ecosystems. *Corbicula* species potentially affect native bivalve fauna in several ways: burrowing and bioturbation activity, principally at high abundances, may displace and/or reduce available habitats for juvenile unionids and sphaeriids (Vaughn & Hakenkamp, 2001); suspension and deposit feeding by *Corbicula* may negatively impact unionid juvenile recruitment (Yeager, Cherry & Neves, 1994; Hakenkamp & Palmer, 1999); dense populations of *Corbicula* may ingest large numbers of unionids sperm, glochidia and newly metamorphosed juveniles (Strayer, 1999); *Corbicula* may advantageously compete for food resources with sphaeriids and juvenile unionids; *Corbicula* have larger filtration rates, on a per biomass basis, than sphaeriids and unionids and consequently have the potential to limit planktonic food available to native bivalves (McMahon, 1991). However, the reasons behind these negative impacts in the native fauna remain speculative and further manipulative research is needed to clarify these ecological interactions and impacts. Additionally, this invasive species can be a vector of introduction of new parasites and diseases to the biotic components of invaded ecosystems. Other biotic interactions which remain unexplored in *C. fluminea* ecology are the interaction of native parasitic species with this invasive species and the potential for native parasites to control the NIS abundance. Negative interactions with human activities have also been described after the introduction of this species (*e.g.* biofouling of water channels and raw water systems of factories and power stations and problems created for sand companies) (Darrigran, 2002).

Positive effects (Table 2.2.) are also expected to occur in invaded ecosystems since this species can provide habitats to other organisms (*e.g.* empty shells provide substrate or refuge for algae, gastropods, freshwater sponges, or other benthic species) (Crooks, 2002; Gutiérrez *et al.*, 2003) and be a new food resource for several pelagic and benthic species (Cantanhêde *et al.*, 2008). Species from higher trophic levels are expected to consume *C. fluminea* and its high abundance and biomass may be a very important food source in many aquatic ecosystems. Fishes, birds and mammals are potential consumers, although this perspective has not been fully exploited in ecological studies performed with this species in invaded habitats.

Repercussions on biogeochemical cycles are also expected and the classification of these impacts as positive or negative are very difficult and could depend on the specific characteristics of the invaded ecosystem. *C. fluminea* is a very efficient ecosystem engineer, altering the structure and function of invaded ecosystems (Crooks, 2002; Karatayev *et al.*, 2006). When bivalves are the major component of a certain ecosystem they strongly couple the benthic and water column environments, consuming large amounts of primary producers, by filtering water at high rates. Through active feeding on particulate organic matter, filter-feeding bivalves can control phytoplankton standing stocks, primary production, water clarity, nutrient cycling, nature of food webs and sedimentation rates of particulate matter in habitats where they are abundant (Yamamuro & Koike, 1993, 1994; Gerritsen *et al.*, 1994; Phelps, 1994; Dame, 1996; Ricciardi *et al.*, 1997; Strayer *et al.*, 1999; Nakamura & Kerciku, 2000; Gangnery *et al.*, 2001; Kohata *et al.*, 2003; Ruesink *et al.*, 2005). In addition, *C. fluminea* is recognized by their pedal feeding with consequential alterations in the abiotic characteristics of the top layer of the sediments. At the same time, there is growing evidence that bivalves also excrete large amounts of inorganic nutrients, mainly inorganic nitrogen, at the form of faeces and pseudofaeces (Asmus & Asmus, 1991). This release of nutrients can stimulate primary production by submerged vegetation and phytoplankton (Phelps, 1994; Yamamuro & Koike, 1994; Dame 1996). Additionally, in some ecosystems and principally in summer months, the combination of several factors (*e.g.* increasing temperature values, low flow conditions, decrease dissolved oxygen, the presence of great quantities of organic matter, decrease in the redox potential) may cause massive mortalities in benthic species, including *C. fluminea* (Johnson & McMahon, 1998; Strayer, 1999; Cherry *et al.*, 2005; Cooper *et al.*, 2005; Sousa *et al.*, 2007c, 2008). This occurrence can abruptly increase the nutrients concentrations causing massive mortalities in all the benthic fauna, also affecting the water quality. Usually, the *C. fluminea* population rapidly recovers reaching previous abundance and distribution while native species usually take a long time to react (Sousa *et al.*, 2007c, 2008). Therefore, this phenomenon could act in favour of *C. fluminea* and against native species, and may determine and/or accelerate the extirpation of some native species.

Table 2.2. Positive and negative effects that may occur after *C. fluminea* introduction in aquatic ecosystems.

Positive effects	Negative effects
Shelter and substrate for other species (Crooks, 2002; Gutiérrez *et al.*, 2003);	Displace and/or reduce available habitat for other species (Vaughn & Hakenkamp, 2001);
Food resource for pelagic and benthic species (Cantanhêde *et al.*, 2008);	Suspension and deposit feeding by *C. fluminea* may negatively impact the recruitment of other species (*e.g.* juvenile unionids, sphaeriids) (Yeager *et al.*, 1994; Hakenkamp & Palmer, 1999);
Reduce euthrophication processes due to high filtration rates (Phelps, 1994; McMahon, 2002);	Competition for benthic food resources with other species (Sousa *et al.*, 2005);
Increase water clarity due to the high filtration rates which may enhance the submerged vegetation cover (Phelps, 1994);	High filtration rates, which can be responsible to limit planktonic food to other species and may ingest large numbers of unionids sperm, glochidia and newly metamorphosed juveniles (McMahon 1991, Strayer 1999);
Bioindicator species for ecotoxicological studies (Doherty, 1990; Inza *et al.*, 1997; Cataldo *et al.*, 2001b).	Vector of parasites and pathogens introduction;
	Massive mortalities that eventually occurred in specific environmental conditions are catastrophic for other biotic components and water quality (Johnson & McMahon, 1998; Strayer, 1999; Cherry *et al.*, 2005; Cooper *et al.*, 2005; Sousa *et al.*, 2007c, 2008);
	Bioaccumulation and bioamplification of contaminants (Narbonne *et al.*, 1999; Tran *et al.*, 2001; Cataldo *et al.*, 2001a and b; Achard *et al.*, 2004);
	Biofouling (Darrigran, 2002).

Corbicula fluminea as a freshwater bioindicator species

In the last years the utilization of bivalves as bioindicator species became a common tool to assess biological impacts of some pollutants in estuarine and coastal areas. At the same time the use of bivalves in freshwater ecosystems for similar purposes has not been so common. The recent introduction of NIS in some ecosystems makes possible to utilize these species as bioincators because they have great abundance and possess good ecotoxicological characteristics. For example, the zebra mussel *Dreissena polymorpha* has been frequently used to assess potential environmental impacts in freshwater ecosystems. *C. fluminea* seems to be a very interesting species from an ecotoxicological point of view because it has some appealing characteristics that could justified its use in this kind of studies, namely: *i*) this species has become a major component of benthic communities in several lotic and lentic habitats in different regions of the world and, thus, it has a wide spatial distribution; *ii*) it may be found in both pristine and polluted environments *iii*) nowadays presents a very strong invasive dynamics in rivers, channels and lakes where it reaches very high abundance (Phelps, 1994; Sousa *et al.*, 2005, 2007c, 2008, in press); *iv*) this bivalve is easily maintained in the laboratory for several months and may be transplanted into field conditions using caging procedures (Cataldo *et al.*, 2001a); *v*) this species has a great filtration capacity allowing the uptake of large amounts of pollutants, *vi*) several field studies have shown that *C. fluminea* is a good bioindicator of heavy metals or other contaminants (Doherty, 1990; Inza *et al.*, 1997; Cataldo *et al.*, 2001b) and *vii*) the size of adults makes possible the dissection and separation of the main organs allowing specific analysis. The combination of all these traits and its ability to bioaccumulate and bioamplify several contaminants make *C. fluminea* a very convenient model in ecotoxicology (Way *et al.*, 1990; Bassack *et al.*, 1997; Baudrimont *et al.*, 1997a and b, 2003; Inza *et al.*, 1997; Narbonne *et al.*, 1999; Tran *et al.*, 2001; Cataldo *et al.*, 2001a and b; Achard *et al.*, 2004). Additionally, due to their ubiquitous distribution, this species can serve as a basis of worldwide comparisons of environmental monitoring data in freshwater ecosystems as the same manner as *Mytilus spp.* in marine environments.

Conclusion and future studies

C. fluminea is recognized as one of the most important invasive macrozoobenthic species in aquatic ecosystems, colonizing lentic and lotic habitats worldwide. The factors responsible for its great and successful invasive behaviour seem to reside further in their r-strategy and association with human activities than in great physiological capacities.

Given the large invasive potential of this NIS, it is essential to increase the research effort using new methodologies to reduce the negative impact of this NIS in invaded ecosystems, including in biodiversity (particularly in what concerns native bivalves of high conservational importance). General models trying to find patterns of distribution along large scales and establishing relationships between *C. fluminea* abundance and/or biomass and abiotic factors will be very informative for future risk analysis. Manipulative studies are also necessary in order to increase our knowledge about important ecological processes mediated by *C. fluminea* that can be responsible for changes in the ecosystem functioning (*e.g.* ecosystem engineering and facilitation processes, competition, parasitism, predation, filtration rates, secondary production). This information will be vital for the adoption of mitigation measures in early phases of the invasion and to reduce its negative ecological and economic impacts. In habitats where the presence of the species is effective, with great abundance and biomass, works on methods to eradicate or to control this NIS are needed to support management measures. As well, it is essential to minimize any form of transport of this species to other freshwater ecosystems not yet colonized. These situations are almost impossible to resolve, have large economic costs and potential tremendous impacts to the other biota components. However, in the last years some solutions like biological (Zavaleta *et al.*, 2001) or chemical (Aldridge *et al.*, 2006) management have arisen as a possible answer. Future studies have also to resolve some uncertainties in relation to the *Corbicula* genus taxonomy, as well as the origin, sources and pathways of dispersion. An international cooperation is crucial to complement these research efforts. For example, it is fundamental to complete genetic and phylogenetic studies in populations from different ecosystems around the world. Indeed, the systematic of hermaphroditic freshwater *Corbicula* lineages are extraordinarily complex and further research in this topic is necessary. A good cooperation between scientists from the *C. fluminea* native range with scientists from the invaded range will likely yield excellent and unexpected results. In reality, management programs, mitigation measures and eradication efforts on invasive species do only make sense when being undertaken by all affected countries (Gollasch, 2007).

Chapter 3

Characterization of the macrozoobenthic assemblages of the Rivers Minho and Lima estuaries

3.1. Subtidal macrozoobenthic assemblages along the River Minho estuarine gradient (north-west Iberian Peninsula)

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Abstract

The community composition and spatial distribution of the macrobenthic fauna in relation to abiotic factors was investigated at 20 sites along the River Minho estuarine gradient, in the north-west Iberian Peninsula. A total of 68 taxa were identified and the non-indigenous invasive species *Corbicula fluminea* dominated both numerically (43.1%) and by biomass (97.7%). Multivariate analysis identified spatial differences in structure and composition of assemblages and suggests a continuum between five distinct assemblages along the length of the estuary. This situation fits the two-ecocline model in which an ecocline from the sea to mid-estuary overlaps with an ecocline from freshwater to mid-estuary. Each assemblage was found in a particular physico-chemical environment and had a specific composition. The distribution and diversity patterns were linked to salinity. However, inside each assemblage the sediment characteristics (granulometry and organic matter content) played an important role. The highest abundances, biomasses and total species numbers were recorded from upstream freshwater tidal areas, indicating the importance of these habitats within estuarine ecosystems.

Introduction

Estuaries are generally recognized as areas of exceptional biological importance (Maes *et al.*, 1998; Herman *et al.*, 1999; McLusky, 1999; Thiel & Potter, 2001). They are a transition zone between the marine and the freshwater domain, and are characterized by a fauna and flora well adapted to the available habitats. Numerous studies on estuarine ecosystems have noted their high biological productivity and their important ecological and environmental function (*e.g.* biogeochemical cycling and movement of nutrients, mitigation of floods and maintenance of biodiversity) (Meire *et al.*, 2005). However, estuarine ecosystems are demanding habitats because of the unpredictable variation in abiotic conditions, and only some species can exploit their high productivity (Day *et al.*, 1989; McLusky, 1989; Little, 2000). Over the last decades, several studies have highlighted human threats to these ecosystems (*e.g.* heavy metals contamination, increasing eutrophication processes, dredging and harbour activities, land reclamation, hydrological regularizations and over-exploitation of living resources) (Valiela *et al.*, 1997; Raffaelli *et al.*, 1999; Lindegarth & Hoskin, 2001; Alfaro, 2006).

Macrozoobenthic species are the most studied for biological monitoring purposes and these organisms have several characteristics favourable for the study of environmental change including limited dispersal, long life-cycles, ease in identification, abundance, occupation of a great variety of microhabitats, connections with higher trophic levels and economic importance (Pearson & Rosenberg, 1978; Gaston *et al.*, 1998; Constable, 1999; Borja *et al.*, 2000; Warwick *et al.*, 2002). Because of the key ecological roles played by macrozoobenthic communities within estuarine and coastal ecosystems, knowledge on macrozoobenthic diversity patterns is fundamental for the identification of priority areas for conservation (Van Hoey *et al.*, 2004). Benthic community composition studies in European estuarine ecosystems are numerous and have revealed differences in benthic abundance, biomass and diversity along estuarine gradients. These studies also provided initial evidence that different habitats may contribute differentially to biodiversity and food webs (McLusky, 1999).

Recently, the international section of the River Minho (including all the estuarine area) was classified as a Natura 2000 site. The variety of habitats available (*e.g.* saltmarsh, sandflats, mudflats and freshwater tidal habitats) and relatively high species diversity made this estuary an ideal site to investigate faunal assemblage composition along a clear estuarine gradient. Additionally, River Minho estuary is used in ecotoxicological studies as a reference site because of the very low human pressure (Cairrão *et al.*, 2004; Moreira *et al.*, 2006; Quintaneiro *et al.*, 2006; Monteiro *et al.*, 2007). As there was a complete lack of quantitative informative data on the composition and distribution of the subtidal macrozoobenthic assemblages present in the River Minho estuary, reference work was carried out during July of 2006. The major aims of this study were to compare the pattern of the macrozoobenthic composition in relation to abiotic conditions, to estimate the abundance, biomass and diversity of subtidal macrozoobenthic assemblages in order to evaluate the importance of this estuarine area for conservation purposes, and to identify the functionally important species in the River Minho estuary.

Material and methods

Study Area

The River Minho drains a hydrological basin of 17 080 km^2, 95% of which is located in Spain and 5% in Portugal. This river has a length of 300km (the last 70km mark the Portuguese/Spanish border) draining NNE–SSW into the Atlantic Ocean and has a mean annual freshwater discharge of 300 m^3/s. Its estuary is located at the north-west of the Iberian Peninsula and the influence of spring tides extends approximately 40km upstream, creating a total estuarine area of 23km^2. This mesotidal estuary is partially mixed, however; during the period of high floods it tends to evolve towards a salt wedge estuary (Sousa *et al.*, 2005).

Sampling and laboratory analysis

Samples were collected at high tide in early July of 2006 from 20 sites located in the subtidal areas of the River Minho estuary (Fig. 3.1.1.). Five replicates per site (one for sediment analysis and four for biological analysis) were gathered with a Van Veen grab with an area of 500 cm^2 and a maximum capacity of 5000 cm^3.

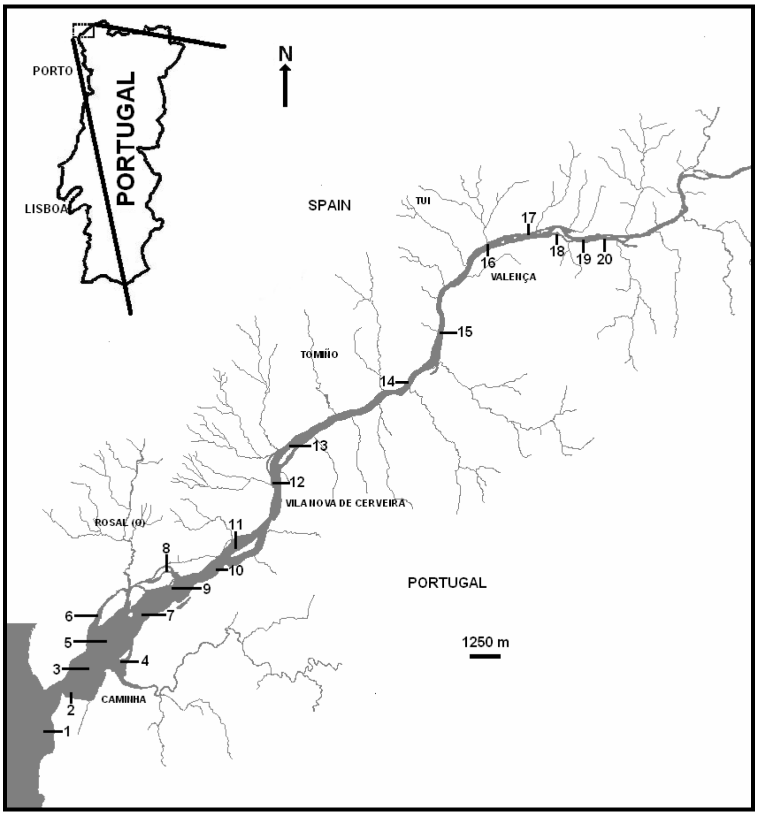


Fig. 3.1.1. Map of the River Minho estuary showing the location of the twenty sampling sites.

During sampling, temperature, total dissolved solids, redox potential, salinity, dissolved oxygen and pH of the water column was recorded close to the bottom, using a multiparameter sea gauge YSI 6820. Samples of the water column were also collected to determine hardness and the concentration of nitrites, nitrates, ammonia and phosphates by colorimetry. Additionally, sediment granulometry and the quantity of organic matter contained in the sediment were assessed using the methodology described in Sousa *et al.* (2006a).

Grab samples were sieved through a 1-mm mesh and fixed with 4% formalin. Macrofauna was sorted and, whenever possible, identified to species. The Ash-Free Dry Weight (AFDW) biomass of the specimens was obtained by drying at 60ºC to constant weight and ashing at 550ºC for 4 h.

Data analysis

Principal components analysis (PCA) in the PRIMER package (Clarke & Warwick, 2001) was used to detect habitat differences based on the abiotic data.

Variation in macrozoobenthic assemblages associated with different sites was assessed using the PRIMER package (Clarke & Warwick, 2001). Individual species abundance and biomass were expressed per square metre. Measures included abundance, biomass, number of species, and Shannon–Wiener diversity (H’) and Pielou’s evenness (J’) indices. Similarity matrices relating pairs of sites were calculated using the Bray–Curtis coefficient and then analysed using multidimensional scaling (MDS) based on the fourth-root-transformed abundance data. Significance tests for differences between macrozoobenthic assemblages defined by MDS analysis were undertaken using ANOSIM. The similarities percentages procedure (SIMPER) was used to assess the species contributing most to similarities within assemblages defined by MDS analysis. In order to establish correlations between biological parameters and abiotic characteristics, indices of abiotic and biotic similarity were compared using BIO-ENV (using the Spearman coefficient) (Clarke & Warwick, 2001).

Finally, the ABC method (abundance/biomass comparisons) was used in order to determine environmental stress (Clarke & Warwick, 2001).

Results

Environmental analysis

The results for the environmental variables are presented in Appendix 3.1.1. Sediment composition comprised sandy deposits with low organic matter content from sites 1 to 14 (with the exception of sites 2 and 10) and also site 20, and fine deposits rich in organic matter content at sites 15 to 19 and also sites 2 and 10. Organic matter content ranged from 0.63% in site 12 to 17.21% in site 17. A significant correlation ($N = 20$; $R = 0.99$; $P < 0.0001$) between the quantity of silt and clay and organic matter was found. The PCA matrices of abiotic factors versus sites (Fig. 3.1.2.) revealed clear spatial patterns. From the projection against the first axis of variability, sites appear distributed along an environmental gradient, with downstream sites along one of the edges (sites 1 to 7) and upstream sites (sites 8 to 20) located in the other edge. The main factors responsible for this separation were temperature, nitrites and nitrates for the negative side and total dissolved solids, salinity and redox potential for the positive side. From the projection against the second axis of variability, sites appear to be distributed along a sedimentological gradient, with the sites with finer sediments and higher organic matter content along one edge and the sites with coarser sediments and lower organic matter content located on the other.

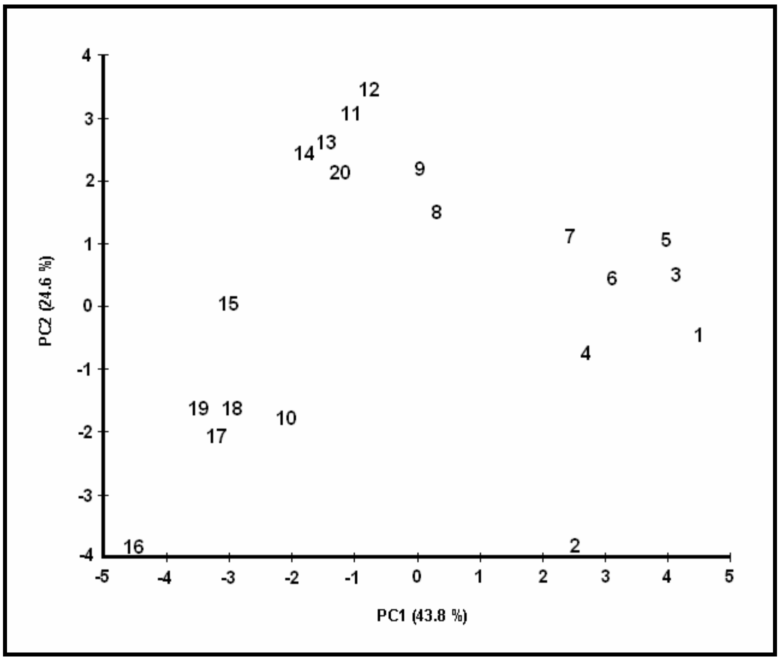


Fig. 3.1.2. Principal Component Analysis (PCA) showing the plotting of the 20 sampling sites. The percentage of variability explained by the principal axes is given.

Biological analysis

Our biological data set consisted of 9310 individuals from 68 taxa belonging to: Annelida, 24; Mollusca, 20; Arthropoda, 20; Vertebrata, 3; Nemertea, 1 (Appendix 3.1.2.). The mean macrozoobenthic abundance was 2327.5 ind./m^2 and was dominated by polychaetes, oligochaetes, bivalves or crustaceans, depending on the assemblage considered (Fig. 3.1.3.). *Corbicula fluminea* was the most abundant species representing 43.1% of the total number of individuals gathered. Lumbriculidae n.i. was the second most abundant taxa with 30.5%. The mean macrozoobenthic biomass was 109.6 g AFDW/m^2 and was dominated by bivalves or crustaceans, depending on the assemblage (Fig. 3.1.4.). *C. fluminea* almost completely dominated the benthic biomass of this estuary (97.7% of the total gathered).

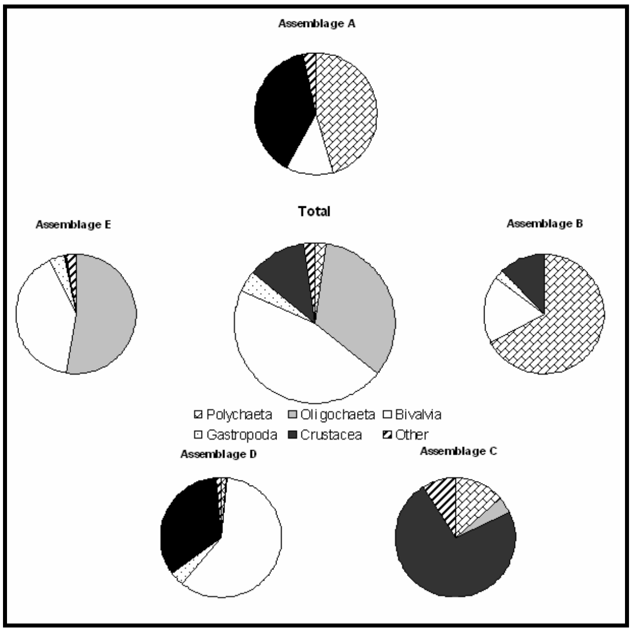


Fig. 3.1.3. Relative abundance at higher taxonomic levels at the total River Minho estuary community and each assemblage defined by MDS analysis.

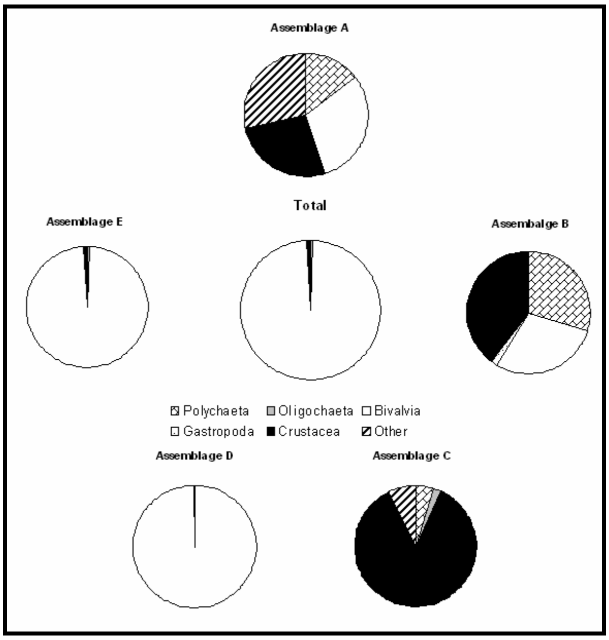


Fig. 3.1.4. Relative biomass at higher taxonomic levels at the total River Minho estuary community and each assemblage defined by MDS analysis.

The MDS analysis (Fig. 3.1.5.) based on the abundance matrix showed a biological community with five distinct assemblages. This procedure applied to the biomass data gave similar results (data not shown). These five faunal assemblages are well separated in the MDS analysis (Fig. 3.1.5.) and the stress value is very low.

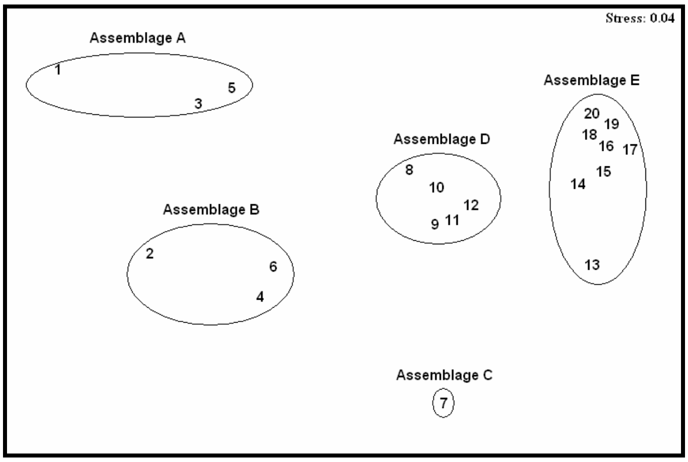


Fig. 3.1.5. MDS plot of faunal similarity among the twenty sampling sites present in the River Minho estuary.

The species responsible for spatial sample grouping (cut-off, 90%) (SIMPER) are given in Table 3.1.1. and show a clear difference along the estuarine gradient. The ANOSIM tests based on abundance and biomass similarities resulted in significant differences between the five faunal assemblages (R = 0.85 and R = 0.65, respectively for abundance and biomass; P < 0.001).

Table 3.1.1. Average similarities for the assemblages defined by MDS analysis. Only species which altogether contribute with more than 90% of total similarity were included.

	Assemblage A	**Assemblage B**	**Assemblage C**	**Assemblage D**	**Assemblage E**
Hediste diversicolor	78.73	35.74	-	-	-
Haustorius arenarius	11.48	-	-	-	-
Cyathura carinata	-	16.86	-	-	-
Scrobicularia plana	-	14.41	-	-	-
Streblospio benedicti	-	8.16	11.61	-	-
Capitella capitata	-	6.03	-	-	-
Spio filicornis	-	5.81	-	-	-
Nephtys hombergi	-	2.13	-	-	-
Hydrobia ulvae	-	1.89	-	-	-
Gammarus chevreuxi	-	-	55.48	4.12	-
Nemertea n.i.	-	-	21.94	-	-
Saduriella losadai	-	-	10.97	-	-
Corbicula fluminea	-	-	-	81.74	46.46
Corophium multisetosum	-	-	-	6.64	-
Lumbriculidae n.i.	-	-	-	-	41.55
Pisidium amnicum	-	-	-	-	3.85

Assemblage A (sites 1, 3, and 5) was located in the navigation channel area, with sandy sediments and low organic matter content and was dominated by the presence of *Hediste diversicolor* plus species originating from the adjacent marine areas (*e.g. Hastorius arenarius*). Abundance and biomass of this assemblage was very low (mean abundance and biomass of 51.7 (±24.8) ind./m^2 and 0.325 (±0.500) g AFDW/m^2, respectively). We found a total of 12 different species and the Shannon–Wiener (H') and the Pielou's evenness (J') indices presented mean values of 2.09 and 0.84, respectively.

Assemblage B (sites 2, 4 and 6) was located in shallow areas with sandy sediments but with lower grain size and higher organic matter content than assemblage A. This was a diverse assemblage (26 species) living near saltmarsh, and was dominated by *H. diversicolor, Cyathura carinata* and *Scrobicularia plana*. Abundance and biomass of this assemblage were low (mean abundance and biomass of 365.0 (±214.4) ind./m^2 and 0.416 (±0.318) g AFDW/m^2, respectively). The Shannon–Wiener (H') and the Pielou's evenness (J') indices presented the mean values of 2.70 and 0.83, respectively.

Assemblage C (site 7) corresponded to sandy habitats with low organic matter content. Abundance and biomass of this assemblage were low (mean abundance and biomass of 110.0 (±108.9) ind./m^2 and 0.151 (±0.141) g AFDW/m^2, respectively). Only 5 species were recorded and the Shannon–Wiener (H') and the Pielou's evenness (J') indices were 1.27 and 0.79, respectively.

Assemblage D (sites 8, 9, 10, 11 and 12) corresponded to sandy habitats with low organic matter content (with the exception of station 10). This assemblage was dominated by the non-indigenous invasive species *C. fluminea*. However, this assemblage was also colonised by macrozoobenthic species well adapted to abiotic oscillations (principally salinity) (*e.g. Gammarus chevreuxi, Corophium multisetosum and H. diversicolor*). Abundance and biomass of this assemblage was high (mean abundance and biomass of 3092.0 (±1816.3) ind./m^2 and 171.262 (±118.140) g AFDW/m^2, respectively) and 17 species were found. The Shannon–Wiener (H') and the Pielou's evenness (J') indices were 1.31 and 0.46, respectively.

Assemblage E (sites 13, 14, 15, 16, 17, 18, 19 and 20) corresponded to upstream areas only exposed to freshwater conditions. These sites had sandy sediments with low organic matter content (sites 13, 14 and 20) and finer deposits rich in organic matter content (sites 15, 16, 17, 18 and 19). In addition, this area experienced the highest nutrient concentrations, principally in the vicinity of the site 16 which was subjected to organic pollution derived from the River Louro tributary. This assemblage was dominated by *C. fluminea* and Lumbriculidae n.i.. Additionally, it was colonised by a diverse macrozoobenthic fauna, well adapted to freshwater conditions (*e.g. Pisidium amnicum, Bithynia tentaculata, Ancylus fluviatilis, Potamopyrgus antipodarum* and *Physella acuta*).

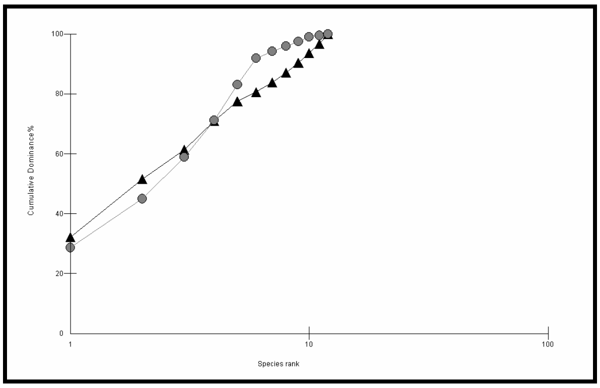
Abundance and biomass were high (mean abundance and biomass of 3716.3 (±2504.1) ind./m^2 and 166.568 (±171.764) g AFDW/m^2, respectively). A total of 28 different species were found and the Shannon–Wiener (H') and the Pielou's evenness (J') indices were 1.36 and 0.41, respectively.

The results of the BIOENV analysis (Table 3.1.2.) indicated that the best correlations occurred with salinity (salinity was also negatively correlated with temperature). However, within each assemblage the sediment characteristics and organic matter content were responsible for the main differences.

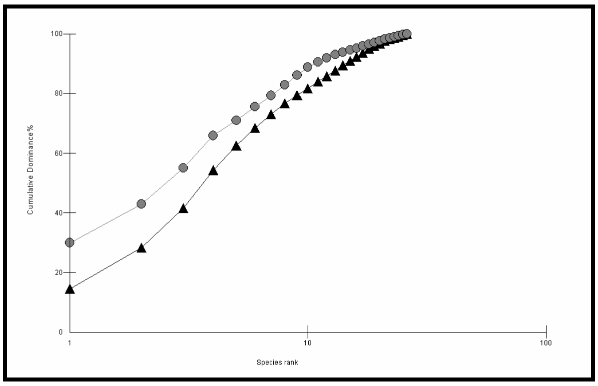
Table 3.1.2. Summary of results from BIOENV analysis – combination of variables (k) giving the highest correlation (using the Spearman rank correlation) between biotic and environmental matrices are shown.

K	Best variables combination			
1	0.851	Salinity	0.847	Total dissolved solids
2	0.854	Salinity Temperature	0.854	Salinity Phosphates
3	0.858	Salinity Temperature Phosphates	0.856	Salinity pH Phosphates

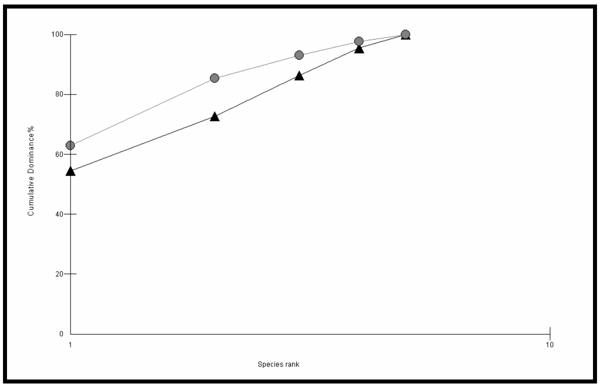
The ABC curves analysis (Fig. 3.1.6.) conducted for the different assemblages identified by MDS analysis, all have a positive W value as the cumulative biomass curves lie above the abundance curve over its entire range (with the exception of assemblage A, where the abundance and biomass cumulative curves cross each other).



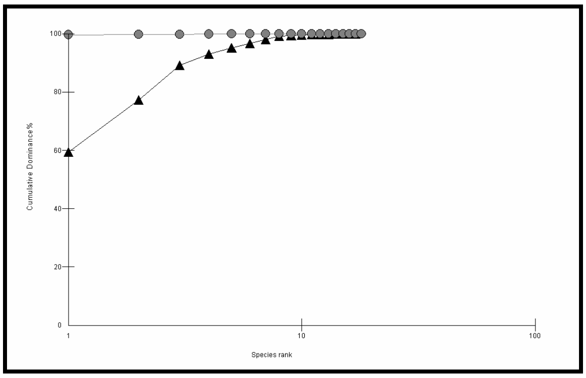
Assemblage A (W = 0.071)



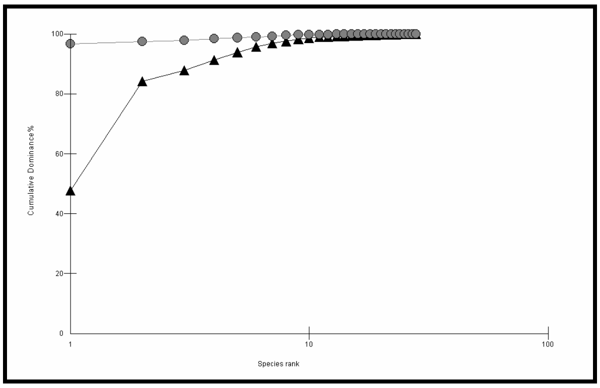
Assemblage B (W = 0.107)



Assemblage C (W = 0.15)



Assemblage D (W = 0.116)



Assemblage E (W = 0.075)

Fig. 3.1.6. ABC curves (triangles represent abundance and circles biomass) for each assemblage identified by MDS analysis (see Fig. 3.1.5.). The W value for each assemblage is given.

Discussion

Environmental characterization

Summer temperature values in the River Minho estuary increased moving upstream from the mouth, as the sea water was cooler than the river water. Salinity also decreased in the inland direction. Spatial fluctuations in dissolved oxygen and pH were never large. The only exception was site 16, which had a low dissolved oxygen concentration owing to the discharge of water from the River Louro (Sousa *et al.*, 2005, 2007c, 2008). When the present data were compared with the results obtained by Sousa *et al.* (2005) no significant temporal changes in sediment characteristics were found.

Spatial biological pattern and similarity with other European estuaries

Five different macrozoobenthic assemblages were distinguished within the River Minho estuarine ecosystem. The abundance, biomass and the specific composition of these macrozoobenthic assemblages were highly correlated with the salinity gradient at the regional estuarine scale and sediment characteristics at the local assemblage scale. Salinity is known to play an important role in estuarine longitudinal distributions (Mannino & Montagna, 1997; Edgar *et al.*, 1999; Josefson & Hansen, 2004; Chainho *et al.*, 2006; Sousa *et al.*, 2006a). However, salinity may be a proxy for other variables that directly affect organisms (*e.g.* substrate type or water column turbidity) (Dethier & Schoch, 2005). In addition, at a smaller scale, the sediment characteristics (granulometry and organic matter content) are key abiotic factors controlling the distribution of organisms and this is not an exception in the River Minho estuary (Sousa *et al.*, 2005, 2007c). Sediment composition within benthic habitats is responsible for the heterogeneity enhancement (Warwick *et al.*, 1991; Hall, 1994; Meire *et al.*, 1994; Ysebaert *et al.*, 2002; Sousa *et al.*, 2006a, 2007b). This situation is responsible, for example, for the difference in abundance, biomass and diversity at different sites holding Assemblage E.

The continuum of assemblages found in this study fits well the two-ecocline model (an ecocline from the sea to mid-estuary overlapping with an ecocline from river to mid-estuary) proposed by Attrill & Rundle (2002). The pattern in the River Minho estuarine community represents a progressive rather than an abrupt change, following the gradual difference in the major environmental variable (salinity). In this estuary the number of marine species decreases in the upstream direction, while the opposite is also true for the freshwater species. According to the van der Maarel's (1990) ecocline definition, it is necessary that a secondary abiotic factor influences the total difference within the gradient, but maintaining all the transitional states. Since only spatial variation was used in this study we were not able to test this secondary environmental influence. However, according to Attrill & Rundle (2002) the freshwater input acts as the secondary factor.

Possibly this is also true for the River Minho estuary since some species (*e.g. G. chevreuxi*, *C. multisetosum* and *H. diversicolor*) migrate seasonally in order to adapt to flow conditions and changes in salinity (Sousa, unpublished).

Biotic factors that were not measured during this study may be of great importance in the moulding of the estuarine macrozoobenthic spatial distribution (Wilson, 1991; Herman *et al.*, 1999). Biotic factors such as predation, intra and inter-competition, adult/larvae interactions, facilitation processes, presence of submerged vegetation, among others may determine macrozoobenthic distributions (Wilson, 1991; Duffy & Harvilicz, 2001). However, the influence of these biotic factors is more pronounced in macrozoobenthic populations with high abundances and/or biomasses (Wilson, 1991). In habitats with low or moderate abundances and/or biomasses, the macrozoobenthos are influenced by recruitment and by the abiotic factors. Therefore, the biotic influence could be an important factor to study, primarily in the two upstream assemblages (Assemblage D completely dominated by the presence of *C. fluminea* and Assemblage E dominated by the presence of *C. fluminea* and Lumbriculidae n.i.). The impact of abundant invasive bivalve species in aquatic ecosystems is well established (*e.g.* capacity to capture substantial amounts of suspended materials by filtering water at high rates, ability to control phytoplankton standing stocks and nutrient cycling, potential to bioaccumulate several contaminants, biotic interactions with native species and alteration in the biodiversity) (Yamamuro & Koike 1993, 1994; Strayer, 1999; Vaughn & Hakenkamp, 2001; Cherry *et al.*, 2005; Cooper *et al.*, 2005; Sousa *et al.*, 2005, 2006b, 2008). Additionally, the presence of great numbers of oligochaetes can also have consequences for the habitat since these species rework and control the geochemical cycles at the sediment surface (Seys *et al.*, 1999).

Comparing our results with other European estuarine ecosystems we conclude that the River Minho estuary is colonized in downstream areas by typical marine species associated with sandy deposits with low organic matter. According to Rundle *et al.* (1998) these marine areas inside estuaries never reach great abundances, biomasses or diversities because sandier habitats with low organic matter content cannot sustain rich assemblages. Assemblages A and B are very common and widely distributed in Portuguese (Marques *et al.*, 1993; Carvalho *et al.*, 2005; Chainho *et al.*, 2006; Sousa *et al.*, 2006a, 2007b) and other European estuarine and coastal areas (Ysebaert *et al.*, 2002, 2003). Assemblage C seems to mark the transition between the marine and the freshwater domain and the two upstream assemblages D and E were dominated by freshwater species and contain several species found in other European estuaries with large freshwater tidal areas (*e.g.* the Thames and Scheldt estuaries) (Attrill *et al.*, 1996; Bruyndoncx *et al.*, 2002). Additionally, the presence of several non-indigenous invasive species (*e.g. C. fluminea*, *Potamopyrgus antipodarum* and *Physella acuta*) also contributes to increasing similarity between different estuaries.

Environmental disturbance and conservation

From ABC curve analyses the macrozoobenthic assemblages in the River Minho estuary had an unpolluted configuration (with the exception of Assemblage A) (Warwick, 1986). Indeed, this estuarine ecosystem has been considered as a low chemical contamination estuary and the actual levels seem not be a serious cause of concern (Cairrão *et al.*, 2004; Moreira *et al.*, 2006; Quintaneiro *et al.*, 2006; Monteiro *et al.*, 2007). However, in recent years it has been exposed to an increasing environmental stress from domestic, industrial and agricultural wastes; recreational and commercial watercraft activities; fishing activities and controls in river flow. Consequently, it is very important to monitor this estuary and take the appropriate preventive and/or mitigation measures.

The present rate of habitat degradation in aquatic ecosystems is alarming (Gray 1997; Lydeard *et al.*, 2004; Strayer *et al.*, 2004), and conservation of biodiversity is of critical importance. The River Minho estuary is within a Natura 2000 site owing to its high ecological and conservational significance and this study reinforces the great importance of this ecosystem. In this estuarine area there are important habitats favourable to the occurrence of economically and/or conservational valuable fish species (*e.g. Platichthys flesus*, *Solea solea*, *Scophthalmus rhombus*, *Dicentrarchus labrax*, *Petromyzon marinus*, *Anguilla anguilla*, *Alosa alosa*, *Alosa fallax* and *Salmo salar*) and several important conservational bird species (*e.g. Ixobrychus minutus* and *Ardea purpurea*) and mammals (*e.g. Lutra lutra*). The macrozoobenthic assemblages of the lower estuarine area (Assemblage A, B and C), although not particularly abundant nor having a high number of species, are essential to support the higher trophic levels. Fish and bird species of conservation importance depend on the favourable condition of the different macrozoobenthic assemblages available in the lower estuarine habitats (*e.g.* saltmarsh, sandflats and mudflats) in order to feed (Little, 2000; Durell *et al.*, 2005). The highest abundances, biomasses and total species numbers were recorded from upstream freshwater tidal areas (Assemblage D and E), indicating the importance of these habitats within estuarine ecosystems. These upstream freshwater tidal estuarine areas have high ecological significance and need enhanced protection. For example, several mollusc species recorded in this study require attention, including the freshwater molluscs *Psilunio littoralis*, *Unio pictorum*, *Anodonta anatina* and *Pisidium amnicum* (Sousa *et al.*, 2007c). Additionally, estuarine freshwater tidal areas are fundamental habitats for several important fish and birds species since they provide nursery areas and corridors for numerous migratory species of commercial and conservational importance and offer refuges from predators and provide essential feeding and drinking grounds (Levin *et al.*, 2001; West *et al.*, 2005).

There is also increasing concern amongst managers and policy-makers about the potential effects of biodiversity loss on the functioning of aquatic ecosystems and the goods and services they provide (Vaughn & Hakenkamp, 2001). Comparing these results with prior studies performed in this estuary, indicate that several species are declining at alarming rates. In the River Minho estuary, species with k-strategies such as freshwater mussels are now restricted to upstream estuarine areas (*e.g. P. littoralis*, *U. pictorum*, *A. anatina* and *Anodonta cygnea* - not found in this study) or have disappeared (*e.g. Margaritifera margaritifera*) and abundances and spatial distributions are now a small fraction of that recorded 20 years ago (Baños, 1978; Araujo *et al.*, 1993, 1999; Maze *et al.*, 1993). These native species are being replaced by non-indigenous invasive species. In recent years, 18 non-indigenous animal species have been recorded, some of them at high abundances and biomasses including *C. fluminea*, *P. antipodarum*, *Procambarus clarkii*, *Cyprinus carpio* and *Micropterus salmoides*. The huge biomass of *C. fluminea* is particularly striking, and is considered a functionally important species in the River Minho estuary. The ecology and possible impact of this species in this and in a neighbouring estuary is discussed by Sousa *et al.* (2005, 2006b, 2007c, 2008).

In conclusion, the present study provides baseline information that can be used in future ecological and conservational studies in this important Iberian estuarine ecosystem. Complementary studies are required to enhance our understanding and it is essential that additional knowledge is acquired of the natural spatial and temporal variability of this macrozoobenthic estuarine community. Additionally, a better understanding of the other trophic components of this estuarine food chain will be essential for the conservation management of this ecosystem. This knowledge will be the most important tool for biodiversity conservation in the River Minho estuary and will help predict and address any future changes that may be caused by man.

3.2. Species composition and monthly variation of the Molluscan fauna in the freshwater subtidal area of the River Minho estuary

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Abstract

Despite their high ecological and environmental importance, little attention has been devoted to the study of freshwater tidal estuarine areas. Information about the biodiversity of these ecosystems remains scarce and very fragmented. In this study, the molluscan fauna of three sites located in the freshwater subtidal area of the River Minho estuary (NW of Portugal) was surveyed monthly between January and December 2005. The molluscan structure showed significant differences between sites and months of the year. A total of 14684 specimens from 15 different species were identified. Abundance ranged from 304 to 3500 ind./m^2, with an annual mean of 1632 ind./m^2. Biomass ranged from 23.4 to 425.4 g AFDW/m^2, with an annual mean of 167.7 g AFDW/m^2. The non-indigenous invasive species *Corbicula fluminea* (Müller, 1774) had a clear predominance in the total abundance and biomass gathered. The multivariate analysis used revealed a community with three distinct groups, principally related to sediment characteristics. Due to great abundance and biomass recorded, *C. fluminea* is a potential key species in this estuarine ecosystem and its possible biological and environmental impacts need urgent investigation.

Introduction

In marine coastal areas, estuarine ecosystems have a high ecological and environmental importance. They offer a considerable variety of habitats, food resources and nursery areas for many species (Day *et al.*, 1989; Herman *et al.*, 1999; McLusky, 1999; Little, 2000; Thiel & Potter, 2001). These ecosystems are frequently subjected to anthropogenic pressures which are reflected in the deterioration of water quality and accumulation of contaminants in the water column, sediments and estuarine food chains (Cave *et al.*, 2005; Chegour *et al.*, 2005; Buggy & Tobin, 2006).

The distribution of macrozoobenthic species in estuarine ecosystems has been intensively studied, at least in the European and North American ecosystems (Gaston & Nasci, 1988; Mannino & Montagna, 1997; Warwick *et al.*, 2002; Ysebaert *et al.* 2002, 2003; Sousa *et al.*, 2006a, 2007b). However, the freshwater estuarine transitional areas in connection with the adjacent fluvial systems are not usually included in these studies

(Attrill *et al.*, 1996; Rundle *et al.*, 1998; Sousa *et al.*, 2005). These limnetic estuarine areas need further investigation to make future predictions concerning potential environmental changes and to increase our knowledge about their biodiversity in relation to environmental factors. Among the benthic species that colonize freshwater tidal estuarine areas, molluscs are one of the most abundant faunal groups frequently being the main food source for higher trophic levels. Additionally, molluscs and bivalves in particular are frequently used in ecological and ecotoxicological studies due to several favourable characteristics, including their capacity to capture substantial amounts of suspended materials by filtering water at high rates, their ability to control phytoplankton standing stocks and nutrient cycling, their potential to accumulate several contaminants and their handling facility (Nakamura & Kerciku, 2000; Gangnery *et al.*, 2001; Kohata *et al.*, 2003; Usero *et al.*, 2005).

The freshwater tidal area of the River Minho estuary is colonized by several species of molluscs. In recent years this diversity has suffered modifications probably related to increased human pressures and the introduction of non-indigenous invasive species (Araujo *et al.*, 1993, 1999; Sousa *et al.*, 2005). Therefore, it is essential to recognise the factors responsible for the spatial and temporal variations in the subtidal molluscan structure and consequently have a better perspective of the biotic and environmental changes that have occurred in the River Minho limnetic estuarine area. In aquatic biomonitoring studies, two main approaches may be used: the conservation and the bioassessment approach. The first is mainly concerned about biodiversity and species conservation, while the second focuses especially on water and sediment quality assessment (Gabriels *et al.*, 2005). Despite some obvious advantages (*e.g.* simplification of the study), such compartmentalization carries a risk of loss of information, especially where integrated interpretation of data from different approaches is concerned and usually reduces the overall comprehension of the system. Consequently, in the present study, these two approaches were integrated in order to study the subtidal molluscan fauna diversity and to perform the environmental characterization of the freshwater tidal area of the River Minho estuary. The aims of this study were to characterise the composition of the molluscan fauna on the subtidal soft bottoms of the River Minho estuary limnetic area, to investigate possible relationships between environmental factors and the spatial and temporal distribution patterns and to identify possible sentinel species that could be used in future ecotoxicological studies.

Material and methods

Study area and sampling analysis

The River Minho estuary extends for about 40km with a tidal freshwater section of near 30 km and covers a total area of 23km^2. A more detailed description of this estuarine area with special emphasis to the limnetic section is in Sousa *et al.* (2005, 2008).

The molluscan fauna present in the subtidal limnetic area of the River Minho estuary was surveyed monthly, from January to December 2005, at three sites during high tide (Fig. 3.2.1.). Six replicates per site (one for sediment analysis and five for biological analysis) were gathered using a Van Veen grab with an area of 500 cm^2 and a maximum capacity of 5000 cm^3. Temperature, conductivity, total dissolved solids, redox potential, salinity, dissolved oxygen and pH were measured simultaneously with molluscan samples collections, using a multi-parametrical probe YSI 6820. Monthly water column samples were also collected to determine hardness and the concentration of nitrites, nitrates, ammonia and phosphates by colorimetry. Additionally, monthly sediment samples were collected for granulometry and organic matter content analysis as described in Sousa *et al.* (2006a).

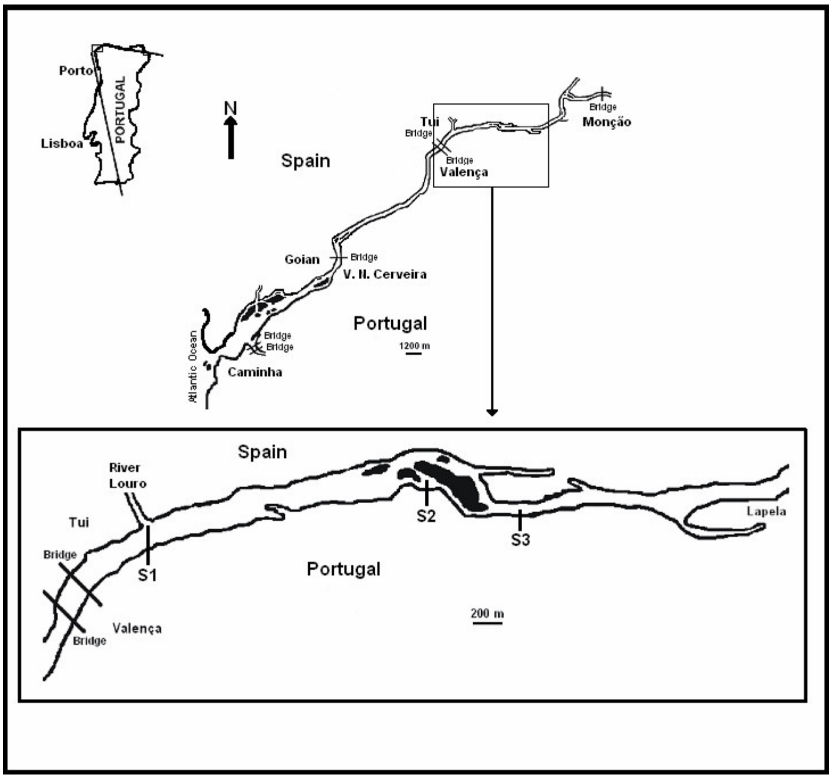

Fig. 3.2.1. Map of the River Minho estuary showing the three sampling stations location.

Biological material was processed through a sieve with a mesh size of 500 µm and animals were separated, sorted, fixed in 70% ethanol and identified to species level. Faunal biomass was calculated using the Ash Free Dry Weight Method - AFDW (Kramer *et al.*, 1994).

Data analysis

Principal Component Analysis (PCA) was carried out for ordination of sites based on the abiotic factors measured. All the abiotic factors were $\log_e$ transformed with the exception of variables in percentage (sediment granulometry and organic matter) which were arcsine transformed.

To compare the similarity among sites (data pooled over five grabs for each site and month) in terms of species composition (abundance and biomass), univariate measures and multivariate analyses were applied using the PRIMER package (Clarke & Warwick, 2001). Individual species abundance and biomass were converted to abundance and biomass *per* m^2. Univariate measures included abundance, biomass, number of species, the Shannon-Wiener diversity index (H') and Pielou's evenness index (J'). Similarity matrices relating pairs of sites were calculated using the Bray-Curtis coefficient and then analysed using multidimensional scaling (MDS) based on the square root transformed abundance data. The BIOENV procedure (using the Spearman coefficient) was employed to investigate possible relationships between biological data and the measured abiotic factors (Clarke & Ainsworth, 1993). Finally, significance tests for differences between sites and months of the year were carried out using a two-way crossed ANOSIM2 (Clarke & Green, 1988). These non-parametric tests compare ranked similarities between and within groups selected *a priori*.

Results

Environmental analysis

The results of the environmental factors are presented in Appendix 3.2.1. Sediment composition of this estuarine area includes fine deposits rich in organic matter. However, the grain size of the sediment decreased from station 1 to the upper stations and no great annual differences in the sediments granulometry were found for each site. Organic matter ranged between 6.0% in station 1 (November) and 19.3% in station 3 (August). A significant correlation ($R = 0.92$; $P < 0.001$) between the quantity of silt and clay and the quantity of organic matter was found. The PCA matrices of abiotic factors versus stations (Fig. 3.2.2.) revealed clear spatial and temporal patterns. From the projection against the first axis of variability, stations appear distributed along an environmental gradient, with the station 1 (with coarser sediments and lower organic matter) along one of the edges and the stations 2 and 3 (with finer sediments and higher organic matter) located in the other edge. In addition to these sediment differences, station 1 also has higher conductivity, salinity and nutrient concentrations, and lower dissolved oxygen values when compared with the others stations; these abiotic factors also contributed to the differences found in the first axis. From the projection against the second axis of variability stations appear distributed along a temporal pattern with the main differences being explained by temperature and redox potential. In this second axis, a clear difference between spring (with the exception of April)/summer months and the rest of the months can be seen.

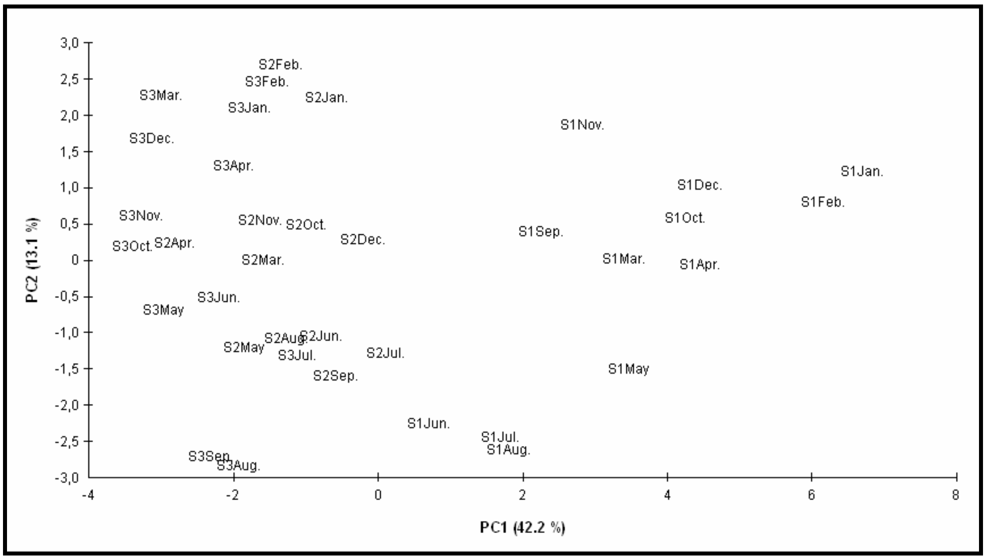

Fig. 3.2.2. Principal Component Analysis (PCA) showing the plotting of the 3 sampling stations from January to December. The percentage of variability explained by the principal axes is given.

Biological analysis

The biological data set consisted of 14684 individuals from 15 molluscan species. From these, eight were bivalves and seven gastropods.

Abundance per site (Table 3.2.1. and Appendix 3.2.2.) ranged from 304 ind./m^2 in station 1 (October) to 3500 ind./m^2 in station 1 (April), with an annual mean of 1632 ind./m^2. *Corbicula fluminea* (Müller, 1774) was the dominant species, accounting to 54.2% of the total specimens gathered, followed by *Pisidium amnicum* (Müller, 1774) corresponding to 20.1%. Biomass per site (Table 3.2.1. and Appendix 3.2.3.) ranged from 23.4 g AFDW/m^2 in station 3 (July) to 425.4 g AFDW/m^2 in station 1 (February), with an annual mean of 167.7 g AFDW/m^2. *C. fluminea* showed a clear predominance in the total biomass, contributing 95.8% to the total gathered, followed by *Bithynia tentaculata* (Linnaeus, 1758) with 1.3%. The species richness also had a time/space variation (Table 3.2.1.). The maximum value of twelve species was registered in station 3 (June) and the minimum of three species was recorded in station 1 (October). Shannon-Wiener index (H') presented low values (Table 3.2.1.). The maximum value was registered in station 3, in May (H'= 1.63), while the minimum was obtained in station 1, in October (H' = 0.47). Also for the Pielou's evenness index (J'), time/space oscillations were found (Table 3.2.1.). The maximum value was registered in station 1, in July, and in station 3 in October (J' = 0.76), while the minimum was recorded in station 1, in February (J' = 0.30).

Table 3.2.1. Monthly total abundance (A-ind./m^2), *C. fluminea* abundance (*C. fluminea* A-ind./m^2), total biomass (B-g AFDW/m^2), *C. fluminea* biomass (*C. fluminea* B-g AFDW/m^2), number of species (S), Shannon-Wiener index (H´) and evenness (J´) in the three sampling stations from January to December of 2005.

Station 1	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
A	1012	1724	1636	3500	1740	2352	1336	1032	628	304	416	612
C. fluminea A	780	1476	792	832	772	744	864	508	440	264	304	372
B	269.71	425.42	249.49	254.13	271.37	238.52	242.69	193.25	179.36	115.19	131.17	161.21
C. fluminea B	268.69	424.86	248.06	249.38	269.33	232.79	241.32	191.88	178.52	115.02	130.32	160.20
S	5	5	4	7	5	8	3	8	6	3	5	8
$H'(\log_e)$	0.73	0.48	0.98	1.09	1.03	1.22	0.83	1.23	0.95	0.47	0.81	1.07
J'	0.45	0.30	0.71	0.56	0.64	0.59	0.76	0.59	0.53	0.43	0.50	0.51

Station 2	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
A	2464	2580	2424	2388	2948	2380	1976	804	1452	512	448	748
C. fluminea A	1712	1708	1536	1820	1864	1972	1500	556	1136	292	292	556
B	149.42	149.01	144.61	155.57	209.21	194.73	158.39	54.88	124.01	27.12	37.40	55.45
C. fluminea B	146.27	145.96	141.28	152.79	204.74	193.19	157.23	53.79	122.78	25.46	35.77	53.69
S	7	7	9	6	8	8	6	6	7	7	5	5
$H'(\log_e)$	0.97	1.02	1.10	0.79	1.12	0.70	0.88	1.04	0.79	1.12	0.87	0.75
J'	0.50	0.52	0.50	0.44	0.54	0.33	0.49	0.58	0.41	0.57	0.54	0.47

Station 3	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
A	2124	2528	1664	2216	2476	2664	560	2480	2016	992	880	720
C. fluminea A	1092	1096	784	744	712	1116	92	940	876	480	524	304
B	188.55	187.96	161.11	140.44	183.01	255.35	23.41	163.86	219.15	115.61	110.15	85.34
C. fluminea B	169.42	165.72	153.97	129.84	162.36	219.18	19.31	152.77	185.57	111.23	106.85	63.91
S	8	8	8	7	11	12	8	8	9	5	6	8
$H'(\log_e)$	1.20	1.18	1.19	1.27	1.63	1.46	1.39	1.20	1.32	1.22	1.13	1.43
J'	0.58	0.57	0.57	0.65	0.68	0.59	0.67	0.58	0.60	0.76	0.63	0.69

The results of the MDS analysis (Fig. 3.2.3.) based on the abundance matrix showed a biological community with three distinct groups: Group A, comprising the stations 1 and 2 from August to December (with the exception of station 2 in September); Group B, comprising the stations 1 and 2, from January until July, and station 2 in September and Group C corresponding to station 3 all year round. ANOSIM2 tests based on abundance similarity revealed significant differences among sites (R = 0.598; P < 0.001) and among months of the year (R = 0.377; P < 0.002).

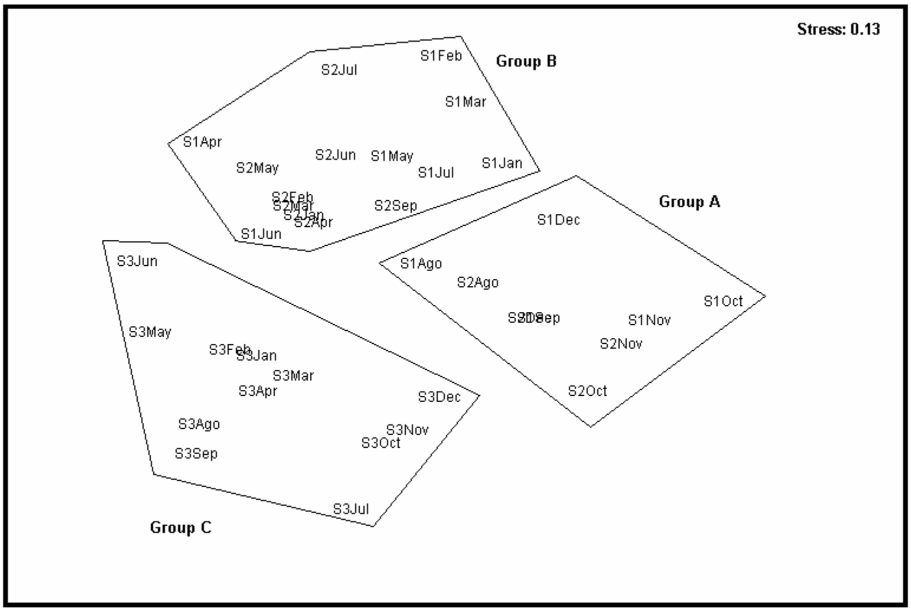

Fig. 3.2.3. MDS plot of the abundance matrix with the three sampling stations from January to December.

BIOENV analysis (Table 3.2.2.) indicated that the best correlations occurred with variables related to the sediment characteristics: granulometry and organic matter content.

Table 3.2.2. Summary of results from BIOENV analysis – combination of variables (k) giving the highest correlation (using the Spearman rank correlation) between biotic and abiotic matrices.

K	Best variable combination	
1	0.499 Organic matter	0.431 Fine sand
2	0.500 TDS Organic matter	0.499 Salinity Organic matter
3	0.501 Salinity TDS Organic matter	0.498 pH TDS Organic matter

Discussion

Environmental characterization

The three sites differed in relation to abiotic conditions. PCA analysis revealed different sediment characteristics and organic matter content among sites. Additionally, the three sites sampled are subjected to different pollution pressures. Station 1 is subjected to high loads of organic contaminants transported from a River Minho affluent (River Louro), which was reflected in the greater nutrient concentrations all year round when compared with the other two sites located upstream. The addition of this water with high concentrations of organic contaminants probably increased the nutrient load leading to high oxygen consumption rates. This hypothesis would explain the low dissolved oxygen concentrations measured in the station 1 all year round. Station 2 is also subjected to organic pollution, since it is located near an agricultural area where fertilisers are used. Additionally, it may also be contaminated with pesticides used in agricultural fields. Station 3 may be considered a clean/pristine site with no visible sources of human impacts. In the vicinity of this site no human activities were found. However, in the summer months, probably due to massive mortalities of *C. fluminea* caused by adverse abiotic conditions (see below), a significant increase of nutrients were found.

Structure of the molluscan community

The structure of the molluscan community present in the River Minho estuary limnetic area showed spatial and temporal oscillations during the study period. Temporal fluctuations may be related in part to recruitment, which in this estuarine area normally happens in spring and summer months. Additionally, in the summer, massive mortalities of *C. fluminea* were registered. These may be due to a combination of factors, including temperature increase, low water flow, presence of high quantities of organic matter and subsequent decrease of redox potential and dissolved oxygen concentration. This situation was particularly pronounced in station 3 and may have been responsible for the abrupt decline of molluscan abundances and biomasses in July. Mortalities of *C. fluminea* subjected to these abiotic conditions have been also described in other studies where they have been considered responsible for the declining of all the other benthic species (Johnson & McMahon, 1998; Strayer, 1999; Cherry *et al.*, 2005; Cooper *et al.*, 2005). Despite the considerable summer decrease, the molluscan assemblage rapidly recovered (Table 3.2.1. and Appendix 3.2.2.), possibly due to rapid migration of organisms from adjacent undisturbed areas but never reached the earlier abundances and biomasses during the studied period. However, the molluscan community maintained some stability with the dominant populations (*C. fluminea* and *P. amnicum*) always present.

Structural differences at the spatial scale were correlated with abiotic factors. Sediment composition and organic matter content appear to be important abiotic factors conditioning the spatial molluscan distribution in the River Minho estuary freshwater tidal area. The relationship between species diversity, abundance and/or biomass and sediment characteristics in estuarine ecosystems is well known. Sediment characteristics, including organic matter content, and salinity are considered the main factors responsible for the distribution of estuarine organisms, including molluscan species (Mannino & Montagna, 1997; Sousa *et al.*, 2005, 2006a, 2007b). In addition to sediment characteristics, other factors (temperature, quantity of nutrients, redox potential, and dissolved oxygen) also influence the molluscan community structure of the River Minho estuary freshwater tidal area. These abiotic factors seem to be responsible for the separation of Groups A and B in the MDS analysis. Additionally, other important factors such as the presence of vegetation, predator-prey interactions, competition, adult-larval interactions and microhabitat characteristics are expected to have an important influence on the molluscan community structure. Unfortunately, our study does not allow analysis of the influence of these factors.

Estuarine macrobenthic species diversity, including molluscs, generally reaches a minimum in the intermediate brackish water, where salinity variation between tides is higher (Attrill, 2002). From this intermediate brackish area, with the lowest diversity, the number of species increases in two opposite directions: (1) towards estuarine saltwater parts and (2) towards freshwater estuarine areas (Attrill, 2002). Comparing our results with other studies conducted in the River Minho estuary (Maze *et al.*, 1993; Sousa *et al.*, in press), the limnetic estuarine area seems to have higher diversity, abundance and biomass of molluscan species than both marine and brackish estuarine areas. In the marine and brackish areas of the River Minho estuary only *Scrobicularia plana* (da Costa, 1778), *Hydrobia ulvae* (Pennant, 1777), *Cerastoderma glaucum* (Poiret, 1789) and sporadic marine species such as *Hinia reticulata* (Linnaeus, 1758) have been found, always occurring in low abundances. In these areas sediments are coarse. Therefore, our findings seem to confirm the suggestions of Rundle *et al.* (1998) that low diversity in marine and brackish estuarine areas may occur in communities associated with coarse sediments. Our results are in clear contradiction with the findings of several studies performed in other estuarine ecosystems where the molluscan abundance, biomass and diversity were higher in estuarine areas with marine influence (Attrill, 2002; Sousa *et al.*, 2006a, 2007b). For example, in the neighbouring River Lima estuary, molluscan species diversity is higher in downstream areas with clear marine influence (Sousa *et al.*, 2006a, 2007b).

Comparison with other limnetic estuarine areas and earlier studies

The studies dealing with the macrozoobenthic species distribution in freshwater tidal estuarine areas are insufficient. The comparison of our results with similar studies conducted in European estuaries shows a similarity in molluscan diversity among them

(Attrill *et al.*, 1996; Bruyndoncx *et al.*, 2002). Bivalves from the Unionidae and Sphaeriidae families are usually present in the European limnetic estuarine areas. The same is true for the gastropods species from the genus *Lymnaea*, *Ancylus* and *Bithynia*. Furthermore, in recent years the presence of several non-indigenous mollusc species [*e.g. C. fluminea*, *Potamopyrgus antipodarum* (Gray, 1843)] have been reported in several European aquatic ecosystems usually with great abundances and biomasses which also contributes to increasing homogenizations and compositional similarity among locations.

Only sites located in the freshwater subtidal area with no submerged vegetation were sampled in the present study. The diversity of the molluscan fauna presented in the River Minho estuary freshwater tidal area would probably have been higher if intertidal areas had also been considered, since the presence of several gastropods is well correlated with the existence of vegetation (Costil & Clement, 1996; Bruyndoncx *et al.*, 2002; Watson & Ormerod, 2004a and b). In addition, the presence of bivalves from the Sphaeriidae family [*e.g. Musculium lacustre* (Müller, 1774), *Pisidium henslowanum* (Sheppard, 1823)] in sheltered habitats of the River Minho estuary were reported in previous studies (Araujo *et al.*, 1993, 1999), but those habitats were not surveyed in this study.

In this study, four different species of the Unionidae family were found. Since the species belonging to this family have been declining in European and North American ecosystems in the recent decades, this finding has ecological and conservational importance. The presence of juveniles was only noticed in the pristine upstream areas such as station 3; therefore, it may be a signal of a recent and/or frequent recruitment. It should be pointed out that the methodology used in this work was not the most suitable to study these freshwater mussels since these species have a very patchy distribution. Thus, it will be important to perform future studies using a more suitable methodology and enlarging the surveyed area to upstream.

Three species belonging to the *Pisidium* genus were also present: *P. casertanum* (Poli, 1791) and *P. subtruncatum* (Malm, 1855), which were rarely found, and *P. amnicum,* which was very abundant. According to Araujo *et al.* (1999) the River Minho is the Southern limit of the European distribution of this species. Therefore, it seems to be a good indicator for studying possible future ecological modifications, such as those related with climatic changes. In addition, the competition with the non-indigenous invasive species *C. fluminea* may affect the population structure and spatial distribution of *P. amnicum*. In a previous study, it was hypothesized that the introduction of *C. fluminea* may be responsible for the almost complete disappearance of *P. amnicum* in lower limnetic estuarine areas (Sousa *et al.*, 2005). In fact, the distribution of *P. amnicum* is restricted to upper estuarine areas having fine sediments and high organic matter content where the probable competition between the two species for food resources is lower.

Conclusion

Due to the great abundances and/or biomasses presented by *B. tentaculata*, *C. fluminea*, *P. antipodarum* and *P. amnicum* these species seem to be suitable for use as sentinel species in future ecological and ecotoxicological studies. *C. fluminea* is clearly the predominant species among the molluscan fauna of the River Minho estuary freshwater subtidal area. This species is recognized as one of the most important non-indigenous invasive species in freshwater ecosystems and their introduction and ecosystem invasion may have considerable ecological impacts on the native species and environmental processes (Phelps, 1994; Cataldo & Boltovskoy, 1999; Darrigran, 2002; McMahon, 2002; Sousa *et al.*, 2005, 2006b, 2008).

In addition to the knowledge that the present study provides, its results may be used as a baseline situation in future studies on the ecology, evolution and conservation of the molluscan community of this important Iberian estuarine ecosystem. Further work is needed to obtain more precise data on species abundance, biomass and distribution principally concerning the endangered freshwater mussels *Psilunio littoralis* (Lamarck, 1801), *Anodonta cygnea* (Linnaeus, 1758) and *Margaritifera margaritifera* (Linnaeus, 1758) (not found in this study but present in upstream areas).

3.3. Subtidal macrozoobenthic assemblages along the River Lima estuarine gradient (north-west Iberian Peninsula)

Published: Sousa R., Dias S. & Antunes C. 2006. Spatial subtidal macrobenthic distribution in relation to abiotic conditions in the Lima estuary, NW of Portugal. Hydrobiologia 559, 135 – 148 and Sousa R., Dias S. & Antunes C. 2007. Subtidal macrobenthic structure in the lower Lima estuary, NW of Iberian Peninsula. Annales Zoologici Fennici 44, 303 – 313.

The characterization of the macrozoobenthic assemblages of the River Lima estuary was already completed when this project starts. In summary these studies were performed in order to compare the pattern of the macrobenthic community's distribution in relation to physical and chemical variables. One of the studies was performed in the summer of 2002 along the total estuarine gradient and identified 54 macrobenthic taxa. Abundance ranged from 212 to 9856 ind./m^2, with an average of 1581 ind./m^2. *Abra alba* presented the highest density corresponding to 39.1% of the total specimens gathered, followed by *Hediste diversicolor* with 31.5%. Biomass ranged from 0.12 to 264.62 g AFDW/m^2, with an average of 17.58 g AFDW/m^2. *Cerastoderma edule* and *A. alba* were the species with a clear predominance in the total biomass, contributing 75.3 and 13.8%, respectively. The multivariate techniques used revealed a macrobenthic community with five distinct groups, particularly related to the sedimentological characteristics and salinity. For the first time the presence of the non-indigenous invasive species *Corbicula fluminea* was described in this estuary.

A second study was also performed in order to investigate possible seasonal variation of the subtidal macrobenthic structure in the lower Lima estuary. Univariate and multivariate analyses were used to establish patterns in species distribution, abundance and biomass and to determine the influence of site and season of the year on the subtidal macrobenthic structure. A total of 101 macrobenthic species were identified and values of diversity indices used were generally low indicating a high degree of dominance of few species. Average abundance and biomass per sampling station ranged from 46.7 to 8 060 ind./m^2 and 0.56 to 28.96 g AFDW/m^2, respectively. *Abra alba* dominated the abundance and biomass gathered. Multivariate ordinations revealed four distinct groups. Abundance/biomass comparison (ABC) curve analyses indicated that the lower part of the estuary is under environmental stress and dominated by opportunistic species. The subtidal macrobenthic structure of the lower Lima estuary showed significant differences between sites but not between seasons of the year.

Chapter 4

Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries

Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries

Published: Sousa R., Freire R., Rufino M., Méndez J., Gaspar M., Antunes C. & Guilhermino L. 2007. Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries. Estuarine, Coastal and Shelf Science 74, 166 – 174.

Abstract

The identification of different species inside the *Corbicula* genus is complicated due to the high variation of shell shape, colour and sculpture of the individuals. The species *Corbicula fluminea* is present in the River Minho estuary (NW Portugal) at least since 1989. More recently, individuals of the same genus colonized an adjacent estuary (River Lima estuary). Although appearing also to be *C. fluminea*, the individuals of the Lima estuary differ from those of Minho estuary in the colour and shape of the shell. Therefore, the two populations were compared by conventional morphometric measures (shell length, width and height), geometric morphometric methods (landmarks analysis using the interior of the shell) and genetic analysis (based on the mitochondrial cytochrome *c* oxidase subunit I gene sequence). Genetic analysis showed an identical mtCOI sequence indicating that both populations belong to the species *C. fluminea*. However, results of conventional and geometric morphometric analysis showed significant differences in shell shape between individuals from the two populations. These differences may be due to *i*) phenotypical plasticity in response to different environmental and/or ecological conditions existing in the two estuaries, *ii*) different origins of the populations and/or distinct routes until reaching the two estuaries and *iii*) inter-population genetic differences caused by processes occurring after the introduction of the species in the two estuaries (*e.g.* differential selection).

Introduction

The original distribution of the *Corbicula* genus in the beginning of the last century was confined to lentic and lotic ecosystems of Asia, Africa and Australia (Pilsbry & Bequaert, 1927 *in* Lee *et al.*, 2005). Since then, some species have spread throughout the world due to a combination of human and natural dispersion mechanisms (Araujo *et al.*, 1993; McMahon, 1999, 2002). The first documented occurrence, outside their native range, was in the United States Pacific coast, in the 1920s. Forty years later, its distribution was extended to the United States Atlantic coast (McMahon, 1999). During the 1970s, specimens belonging to this genus spread into South America (Ituarte, 1994) and, in the 1980s, into Europe (Mouthon, 1981).

Portuguese rivers have been colonized by *Corbicula* at least since the 1980s (see Mouthon (1981) for the first published European description), although local fishermen in the River Tejo recognized the presence of individuals of this genus and used them as bait, in the early 1950s. The principal Portuguese hydrological basins (*e.g.* Minho, Lima, Douro, Vouga, Mondego, Tejo, Sado and Guadiana) have been colonized by this genus. However, the taxonomy of the species present remains uncertain in the most part of the cases. This is not a surprise since the studies that have been performed with European, American and Asiatic populations have been showing that the identification of different species within the *Corbicula* genus is very difficult, due to the marked variation in shell morphology, colour, sculpture and reproductive biology of the individuals (Komaru & Konishi, 1999; Rajagopal *et al.*, 2000; Renard *et al.*, 2000; Siripattrawan *et al.*, 2000; Qiu *et al.*, 2001; Park *et al.*, 2002; Pfenninger *et al.*, 2002; Park & Kim, 2003; Lee *et al.*, 2005). Due to their invasive and dispersal potential, some species of the *Corbicula* genus can cause important ecological and economic impacts, such as changes in food webs, bioaccumulation of environmental contaminants, competition with native bivalves and serious biofouling problems (Phelps, 1994; Darrigran, 2002). Consequently, taxonomical, biological and ecotoxicological studies concerning *Corbicula* species are fundamental to support management actions to overcome problems resulting from their invasion and habitat colonization.

The Minho and Lima estuaries, located in the NW of Portugal, were colonized recently by *Corbicula*: in the Minho estuary, the presence of *Corbicula fluminea* has been recorded since 1989 (Araujo, 1993), while in the Lima estuary, the presence of a bivalve also classified as *C. fluminea* was noticed in 2002 (Sousa *et al.*, 2006a and b). The observation of individuals from both populations suggests that they have morphological differences in the shape and colour of the shells. In addition, they have been showing apparently distinct invasive and dispersal patterns (Sousa *et al.*, 2005, 2006a and b, 2007c, 2008), even considering the earlier introduction of the species in the Minho estuary. Therefore, the main objective of this study was to compare the *Corbicula* populations of Minho and Lima estuaries, using conventional morphometric measures (shell length, width and height), geometric morphometric methods to determine differences in shell shape (based on landmarks analysis using the interior of the shell) and genetic analysis [based on the mitochondrial cytochrome *c* oxidase subunit I (mtCOI) gene sequence]. Additionally, mtCOI sequences of individuals from both populations were compared with pre-existing mtCOI sequences in an attempt to identify the origin of both populations.

Material and methods

Study Area

The Rivers Minho and Lima are located in the NW of the Iberian Peninsula (Fig. 4.1. a and b). These rivers spring in Spain and both hydrological basins have considerable geological and hydrological similarities (Sousa *et al.*, 2005, 2006a, 2007b and c, 2008). The River Minho is about 300km long, with an estuary of about 40km. River Lima is about 108km long and has an estuary of about 20km.

Sampling and laboratory analysis

Individuals were collected using a Van Veen grab in five sites in the Minho estuary (Fig. 4.1. a) and in one site in the Lima estuary (Fig. 4.1. b), since the population of this estuary is restricted to a very small area and so far has not extended its distribution (Sousa *et al.*, 2006a and b). The shells of the collected animals were used for morphometric analysis and their soft parts were carefully removed, immediately preserved in 96% ethanol and stored at a constant temperature until DNA extraction.

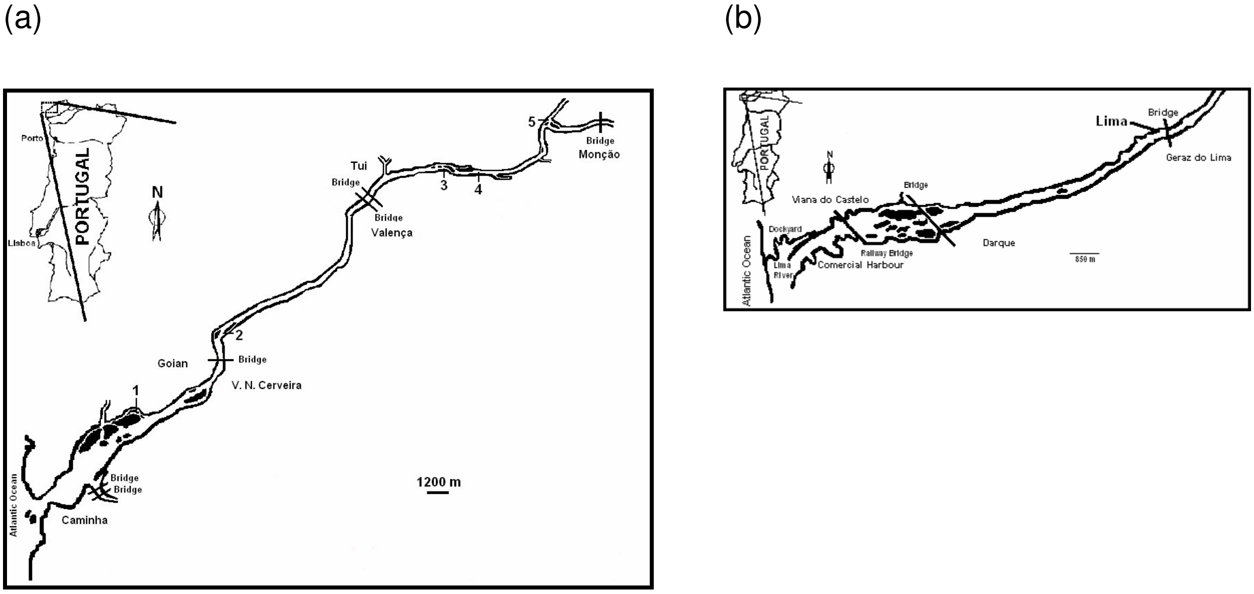

Fig. 4.1. Maps of Minho (a) and Lima (b) estuaries showing the six sites location.

Morphometric analysis

For conventional morphometric analysis, three linear distances in 35 left shells per site were measured with a digital caliper (resolution of 0.01 mm): shell length, shell height and shell width. Additionally, geometric morphological analysis based on landmarks was performed. Each shell was scanned using a previously calibrated HP® Scanjet 5530. For the landmarks analysis, 11 internal homologous points were digitised in each shell using the software tpsDIG (Rohlf, 2003). The first and the last landmarks were placed in the adductor muscles scars and the other landmarks were in the lateral and inner teethes (Fig. 4.2.).

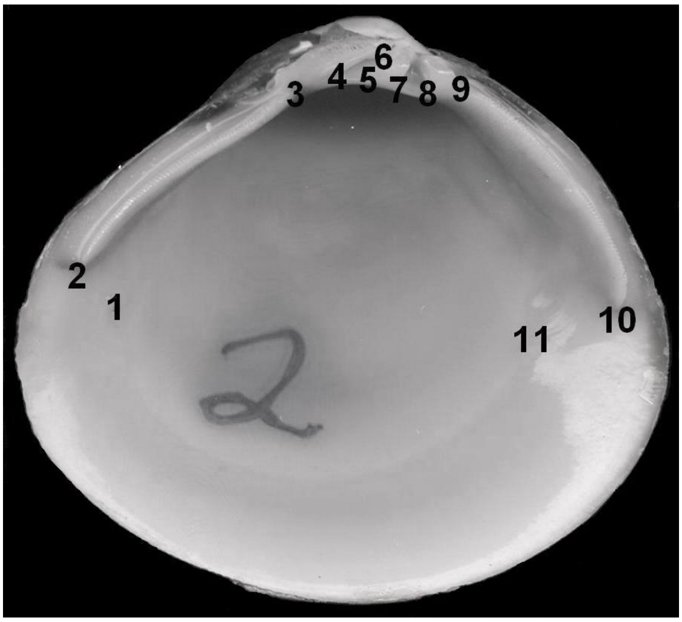

Fig. 4.2. Location of the 11 landmarks selected on the *C. fluminea* shell.

Shape variables generated from the *x, y* coordinates with the effects of any differences in translation, rotation, and scale mathematically held constant were considered. These variables were used to construct a matrix for subsequent statistical analysis and to generate a graphical representation (Adams & Rohlf, 2000). Shape difference was then analysed through a relative warps analysis (similar to Principal Component Analysis) and visualized through Thin Plate Spline. The landmarks showing greatest variation between locations were identified using least squares resistant fit superimposition. Linear discriminant analysis was performed to the partial warps and uniform components, using leave one out procedure to estimate misclassification rates. Significant shell shape differences between sites were tested with a MANOVA on the partial warps plus the uniform components.

Genetic analysis

For the genetic analysis, 30 individuals from the Minho estuary (6 individuals from each site) and 30 individuals from the Lima estuary were used. Total DNA was extracted from each individual according to Winnepenninckx *et al.* (1993) using 20 mg of muscle tissue.

According to Renard *et al.* (2000), a restriction fragment length polymorphism (RFLP) analysis of the mtCOI gene can discriminate different species within the *Corbicula* genus. Therefore, this marker was studied in individuals from both populations in order to confirm the specific filiation. A 710bp fragment of the mtCOI gene was amplified using the primers LCOI490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA) designed by Folmer *et al.* (1994).

Amplification reactions were performed in volumes of 25 μl. The reaction mixture contained 15ng of genomic DNA, 0.2mM of each dNTP, 1μM of each primer, 0.025 U of *Taq* polymerase (Roche Diagnostic)/μl and the buffer recommended by polymerase suppliers. The thermocycler protocol consisted of an initial denaturation of 1 min at 94ºC, 35 cycles of 94ºC for 1 min, 40ºC for 1 min and 72ºC for 1 min and 30 s, and a final extension step of 72ºC for 1 min. PCR products were visualized on an 2% w/v agarose gel. Thirty individuals from each estuary were digested with *Sac*I. Digestions were performed in a 10-20 μl volume, containing 5 μl of PCR product, 5 U of restriction enzyme and the buffer recommended by the restriction enzyme supplier (Roche Diagnostic). After incubation at 37 ºC overnight, another 5 U of restriction enzyme was added, and the digestion was prolonged for another 3 hours. The reaction was stopped by the addition of loading buffer, and restriction fragments were visualized on a 2% w/v agarose gel.

In addition to this analysis, mtCOI from 41 individuals (20 from the 5 different sites of the Minho estuary and 21 from the Lima estuary) were sequenced. The PCR product was purified with ExoSAP-IT® (Amersham Biosciences). Sequencing of the purified PCR product (both strands) was performed employing the CEQ™ DTCS Quick Start Kit and CEQ™ 8000 Genetic Analysis System (Beckman Coulter Inc.). Only a 621bp fragment was selected for further phylogenetic analysis in order to avoid erroneous sequence determination at the 5'- and 3'-end regions. The identity of the sequences obtained was determined by comparison with sequence data from nucleotide databases using the BLAST program (Altschul *et al.*, 1997). Several sequences from nucleotide databases were used in the analyses (Appendix 4.1.). Sequences were aligned using CLUSTALW (Thompson *et al.*, 1994), and haplotypes were determined using the DnaSP program (Rozas *et al.*, 2003). MEGA software version 3 (Kumar *et al.*, 2004) was employed for the construction of the neighbour-joining (NJ) phylogenetic tree, estimating genetic distances according to Tamura's 3`-parameters method (1992). The reliability of nodes on trees was estimated using bootstrap analyses (Felsenstein, 1985) with 1000 replicates.

Results

Colour and Morphometry

The inner shell colour of the two populations is different. Individuals from the Minho estuary have an inner nacre purple surface (primarily in the margin of the shell), whereas the ones from the Lima estuary have a whitish coloration. However, this difference is not as clear as the report example in Park *et al.* (2002).

Shape analysis revealed evident shell differences between the two estuarine populations. The conventional morphometric measures used showed clearly that Lima individuals having more roundness shells and Minho individuals having more oval and elongated shells (Fig. 4.3.).

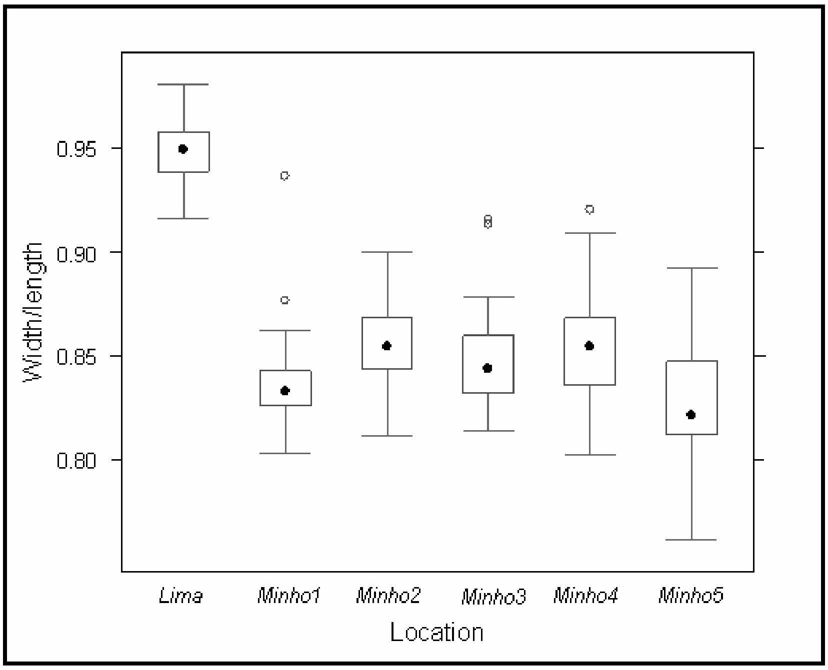

Fig. 4.3. Difference of *C. fluminea* shell roundness (represented as the ratio of shell width/length) across sampled sites.

In relation to the geometric morphometric analysis used we verified that the first relative warp (RW1) explained 42.3%, the second (RW2) 20.8% and the third (RW3) 10.0%, summing 73.14% of variance explained (Fig. 4.4. a and b).

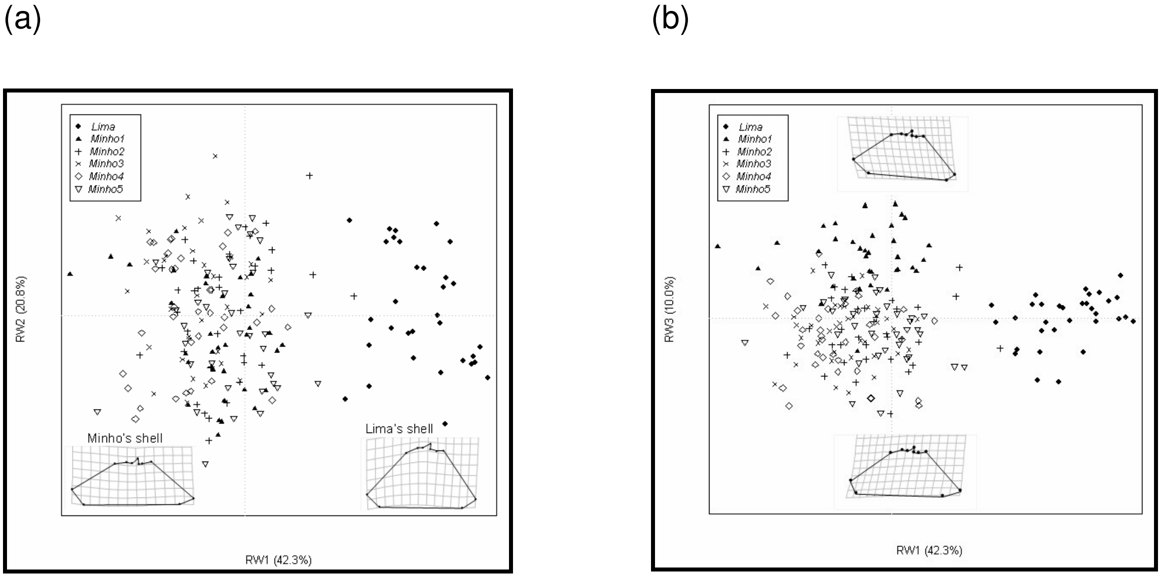

Fig. 4.4. First and second relative warps (a) (RW1 and RW2, and respective percentage of the variance explained) and first and third relative warps (b) (RW1 and RW3, and respective percentage of variance explained) of *C. fluminea* landmarks configuration in different sites. The full circle represents individuals from the Lima estuary and the remaining symbols represent individuals from different sites in the Minho estuary. In the bottom of the figure a Thin Plate Spline representation of each estuary shell shape is shown. See Fig. 4.1. for sites location and Fig. 4.2. for landmarks positions in the inner bivalve shell.

The first relative warp clearly distinguished individuals from the two estuarine ecosystems and these differences are not related with the size of the individuals (Fig. 4.5.a). Resistant fit superimposition in landmarks analysis revealed that this difference is due to landmarks number one, two, ten and eleven that corresponded to areas near the adductor muscles scars and the inferior part of the lateral teethes. Thus, shape differences are probably related to differential outer growth between sites. The third relative warp, revealed a shell shape difference within Minho estuary, where the Minho1 site is clearly apart from the remaining sites (Fig. 4.4.b). This difference is apparent in the positioning and shape of the adductor muscles, and is also not related to shell dimension (Fig. 4.5.b).

Linear discriminant analysis applied to the partial warps plus the uniform components, showed a cross-validation leave-one-out error of 0.314, mainly related to variability across the Minho estuary samples. When considering only two geographical locations (Minho and Lima), the misclassification rate drops down to 0.015. MANOVA results confirmed a highly significant difference between individuals shell shape, across locations (Pillai's lambda = 2.33, $F_{[90, 940]} = 9.09$, $P < 0.001$).

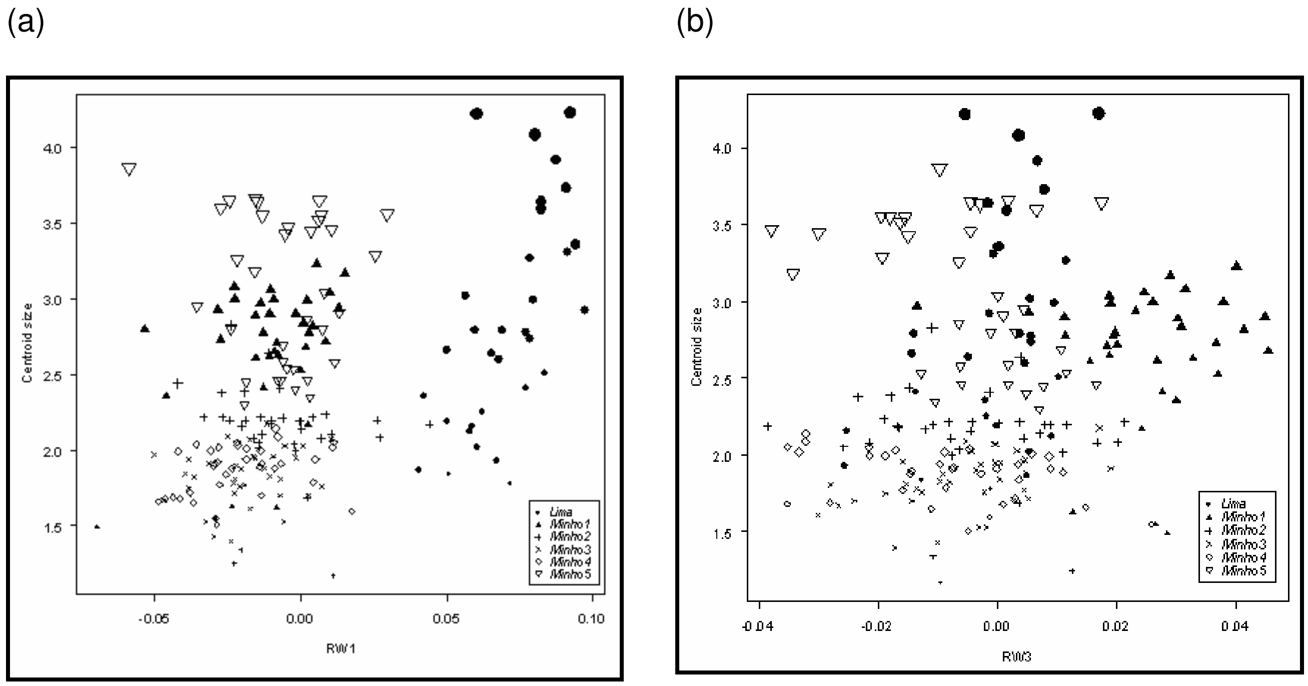

Fig. 4.5. Shape differences (represented by the first (a) and third (b) relative warp) according to *C. fluminea* shell size (centroid size). The size of the symbol is proportional to centroid size.

Genetic analysis

The restriction fragment length polymorphism (RFLP) analysis provided a 710bp fragment for the Minho and Lima individuals. This 710bp fragment was digested by the one-site restriction enzyme *Sac*I and provide two fragments with 200/500bp for all the 60 individuals analysed. According to Renard *et al.* (2000) a clear cut of 200/500bp is specific to *C. fluminea*. Additionally, the sequences analysed were very similar: the alignment revealed five substitutions. Four haplotypes were resolved differing by one substitution (0.16% divergence) or two substitutions (0.32% divergence). Moreover, 38 of the 41 sequences corresponded to one haplotype, and the other three were represented by only one sequence each, all of them from Minho estuary. Comparisons with sequences from databases showed that the main haplotype of the Minho and Lima populations was identical to that described in Europe as haplotype I (Renard *et al.* 2000) and to that described in North America as form A (Siripattrawan *et al.*, 2000; Lee *et al.*, 2005) (Fig. 4.6. and Appendix 4.1.). These two mytotypes appeared grouped with a high bootstrap value into one clade in neighbour-joining tree (Fig. 4.6.) together with Minho and Lima sequences. Additionally, the main haplotype of the Minho and Lima populations is identical to the FW5 (Park & Kim, 2003), one of the most common found across the Asiatic ecosystems.

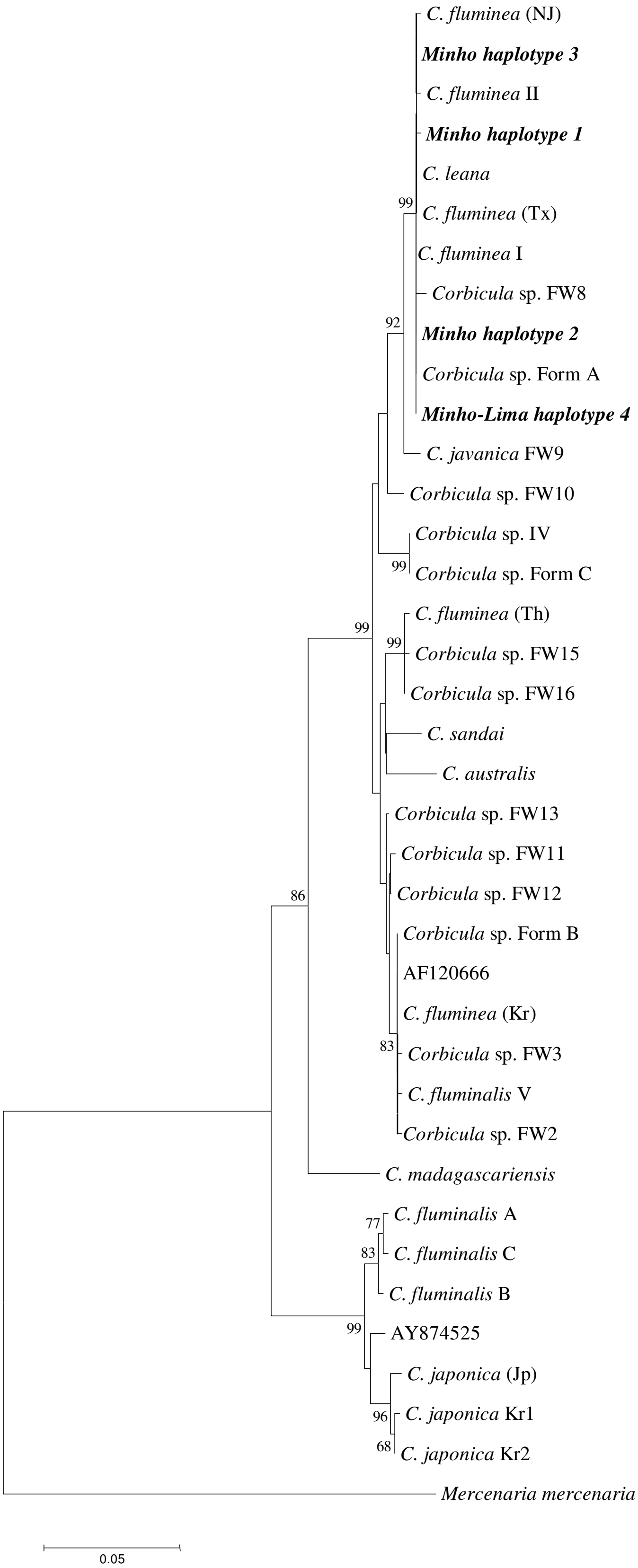

Fig. 4.6. Neighbour-joining tree inferred from mtCOI sequences. Bootstrap values higher than 60 are shown at nodes. Minho haplotype 1: Minho1-1; Minho haplotype 2: Minho2-7; Minho haplotype 3: Minho3-12; Minho-Lima haplotype 4: remaining 38 sequences. See Appendix 4.1. for other abbreviations.

Discussion

In molluscs, shell characteristics have been widely used in species identification. However, their exclusive use for taxonomic and systematic studies is controversial and, at least in some cases, needs to be complemented by genetic analysis (Wilke & Falniowsky, 2001). Consequently, in this study morphometric and genetic analysis was used to compare two *Corbicula* populations colonizing two adjacent Portuguese estuarine ecosystems.

Previous studies performed with *Corbicula* populations showed that this species exhibits considerable shell plasticity (Renard *et al.*, 2000; Pfenninger *et al.*, 2002; Park *et al.*, 2002; Lee *et al.*, 2005). Despite having a similar mtCOI sequence, the populations of Minho and Lima estuaries have clear morphological differences as revealed by conventional and geometric morphometric analysis. Specimens from the Minho estuary are much more oval and elongated, while those from the Lima estuary have a more rounded shape, are taller, have more inflated umbos and coarser shells. According to Monteiro *et al.* (2000) the phenotypes revealed in the final shape of structures, organs and organisms arise from the interfacing and complex combination between morphogenetic rules, ecological conditions and deterministic and stochastic evolutionary forces. It is generally accepted that bivalve shells have great plasticity in order to adapt to the different environmental and ecological conditions and this is very common in freshwater bivalve species (Baker *et al.*, 2003). Comparative studies have shown that bivalve species exhibit several distinct morphological characteristics that allow them to adapt to epifaunal existence, to avoid predation and parasitism, to maintain adequate current flow under crowded conditions and to adapt to wave exposure, type of substratum, salinity and calcium availability (Stanley, 1983; Gardner & Skibinski, 1991; Willis & Skibinski, 1992; Norberg & Tendengren, 1995; Baker *et al.*, 2003). In the particular case of this study, the main abiotic differences between the two estuarine habitats colonized by this NIS are the higher salinity values principally in the summer months in the Lima estuary and possible differences in nutrients and other environmental contaminants (*e.g.* heavy metals) between the two estuaries (Sousa *et al.*, 2006b). Consequently, the different morphological characteristics exhibited by the two studied populations may be related to different abiotic conditions in the two estuaries. In addition, biotic factors may also influence the morphology of the individuals. According to Seed (1968), Gardner *et al.* (1993), Stirling & Okumus (1994), crowding conditions and slower growth rates resulted in more elongated shells. At the present, the Minho estuary population has a great abundance and biomass with some sites having more than 4 000 ind./m^2 and more than 400 g AFDW/m^2, respectively. In 2004 the mean values of abundance and biomass in the total limnetic estuarine area were 1 253 ind./m^2 and

95.2 g AFDW/m^2, respectively (Sousa *et al.*, 2005). So far, in the Lima estuary the abundance and biomass *per* site never exceeded 60 ind./m^2 and 26 g AFDW/m^2, respectively (Sousa *et al.*, 2006a and b). Consequently, the intra-population competition for resources is probably higher in the Minho than in the Lima estuary, and this may result in slower growth rates. Considering that individuals from Minho estuary have more elongated shells, one can hypothesize that this is a result of its high abundance in this estuary. These abundance differences may also explain the morphological distinction registered for the Minho1 individuals, since this site has great abundances when compared with the others 4 sites studied in the Minho estuary (Sousa *et al.*, 2005, 2008). Alternative hypothesis explaining the possible morphometric differences are the different origins of the populations and/or distinct routes until reaching the two estuaries and inter-population genetic differences caused by processes occurring after the introduction of the species in the two estuaries. In the particular case of this study, the hypothesis of ecophenotypic differences between the two populations can be tested by a simple transference of specimens between the two estuaries. However, the ecological risk of this procedure is high since if, by accident, a small number of animals escape in the estuary into which they were transferred, they may spread rapidly and considerably alter the genetic composition of the local population. This also may cause several problems which would be difficult to control, including the anticipation of the exponential growth phase of the Lima estuary population that seems to be in a lag time phase (Sousa *et al.*, 2006b).

The genetic methodology employed in this study has been considered very useful in the distinction of hypothetically different species inside the *Corbicula* genus by simple digestion of PCR products by a one-site restriction enzyme (Renard *et al.*, 2000). According to these authors, the utilization of the enzyme *Sac*I in the restriction fragment length polymorphism (RFLP) analysis of the mtCOI in *Corbicula* specimens is suitable for their rapid specific identification. Since our data revealed a clear cut of 200/500bp in all the 60 individuals analysed we concluded, in agreement with Renard *et al.* (2000), that our specimens, in both estuaries, belong to the species *C. fluminea*. Despite this fact, general uncertainties subsist about the number of species present in several European, American and Asiatic freshwater ecosystems; their taxonomy and their origin(s) (Renard *et al.*, 2000; Siripattrawan *et al.*, 2000; Pfenninger *et al.*, 2002; Park & Kim, 2003; Lee *et al.*, 2005). According to Renard *et al.* (2000), based on conventional morphometric variation and genetic analysis two morphotypes were present in French and Dutch rivers and they belong to the described species *Corbicula fluminea* and *C. fluminalis*. Subsequently, they found another species – *Corbicula spec.* – but they could not assign a specific name to the taxon. The results of Pfenninger *et al.* (2002), with material collected in the River Rhine, confirmed the presence of *C. fluminea* and *Corbicula spec.* as defined by Renard *et al.* (2000). However, posterior results obtained by Park & Kim (2003) provided new insights about the different lineages within the *Corbicula* genus. According to these authors, *C. fluminalis* and *Corbicula spec. sensu* Renard *et al.* (2000) were classified only as freshwater *Corbicula*, without a specific nomenclature

The great difficulties to accede the taxonomic status of *Corbicula* specimens are related to the generally small differences in mtCOI sequences revealed by molecular analyses. This is the case in our study, where the mtCOI sequence performed in 41 individuals of the Minho and Lima estuaries shared an identical sequence with only minor substitutions. In addition, phylogenetic analyses including sequences from databases agree with Lee *et al.* (2005) in the existence of a small number of lineages in *Corbicula* sp. All the sequences analysed in the present study belong to one of the lineages, the morphotype A (Siripattrawan *et al.*, 2000). This clade also includes European sequences of *C. fluminea* haplotype I (Renard *et al.*, 2000; Pfenninger *et al.*, 2002), as well as sequences of the native freshwater *Corbicula* (Park & Kim, 2003). The populations of Minho and Lima estuaries have an mtCOI sequence similar to that of several Asiatic and non native populations distributed worldwide. However, in the present study, the mtCOI was not informative enough to assign the possible introduction source of the *C. fluminea* populations in the Minho and Lima estuaries. Therefore, further research using other methods should be performed to answer this question.

In conclusion, the results of the mtCOI sequence analysis showed that the populations of Minho and Lima estuaries belong to the species *C. fluminea* described by Renard *et al.* (2000). However, significant morphometric differences were found in the two populations. These morphometric differences may be explained at least by three different hypotheses: *i*) adaptations to different environmental and/or ecological conditions during the development in the actual habitats; *ii*) different origins and/or genetic alterations during distinct pathways of migration and *iii*) differential selection processes in the two estuaries. Further genetic studies involving more genes and comparison with other worldwide populations are required to understand the cause of the differences between the populations of Minho and Lima estuaries.

Chapter 5

Distribution of *Corbicula fluminea* (Müller, 1774) in the Rivers Minho and Lima estuaries

5.1. Abiotic impacts on spatial and temporal distribution of *Corbicula fluminea* (Müller, 1774) in the River Minho Estuary, Portugal

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Abstract

The non-indigenous species *Corbicula fluminea* (Müller, 1774) is one of the most invasive bivalves in freshwater ecosystems. This Asian clam was first reported in the River Minho estuary in 1989. After a short period of time, it became the major component of the local benthic fauna in terms of abundance and biomass. In the autumn of 2004 and 2005, spatial and temporal variations in abundance, biomass and population structure of *C. fluminea* were investigated at 16 sites in the freshwater subtidal area of the River Minho estuary. Mean abundance and biomass per site ranged from 80 to 4185 ind./m^2 and 8.5 to 465.9 g AFDW/m^2, respectively. The environmental characterization of the area was performed through multivariate analysis, which revealed three distinct areas subjected to different abiotic conditions. *C. fluminea* population structure is well correlated with these three distinct areas. The combination of several abiotic variables determining *C. fluminea* biomass distribution was evaluated through a stepwise multiple regression. This model showed that redox potential, nutrient concentrations, hardness, organic matter and sediment characteristics explained almost 60% of the variation in *C. fluminea* biomass in the freshwater subtidal area of the River Minho estuary ($R^2 = 59.3\%$, $F_{[9, 86]} = 13.9$, $p < 0.001$). Improved ecological knowledge is essential for future *C. fluminea* management, in order to protect local habitats and biodiversity, and to reduce the economic impact of this non-indigenous invasive species.

Introduction

Since the 1990s, aquatic biological invasions have caught the attention of the scientific community due to their impact on ecosystems and the great economic losses they cause. Sometimes, invasive species induce important alterations on native communities, representing a severe threat to local biodiversity (Lodge, 1993; Vitousek *et al*., 1996; Kolar & Lodge, 2001). Notwithstanding the fact that species distribution is not static in time, human activities have increased the scale of these changes (Ricciardi & MacIsaac, 2000). Many human activities, such as aquaculture, recreational activities and transportation promote the intentional or accidental dispersion of aquatic species across their natural geographical barriers (Carlton & Geller, 1993; Cohen & Carlton, 1998; Grosholz, 2002).

The rapid growth, earlier sexual maturity, short life span, high fecundity and its association with human activities makes *Corbicula fluminea* (Müller, 1774) a non-indigenous invasive species (NIS) able to colonize new environments. These characteristics partially explain its considerable worldwide colonization in the last decades (Araujo *et al.*, 1993; Cataldo & Boltovskoy, 1999; McMahon, 1999, 2002; Darrigran, 2002). The introduction of this NIS is a serious threat to native biodiversity and ecosystem functioning with possible repercussions in food webs and biogeochemical cycles. The invasion of this species has been speculated to have negatively impacted native bivalve abundance and diversity in North American and European freshwater ecosystems (Araujo *et al.*, 1993; Williams *et al.*, 1993; Strayer, 1999). Additionally, repercussions in human economy due to severe biofouling problems may also be expected after *C. fluminea* invasions (Phelps, 1994; Pimentel *et al.*, 2000; Darrigran, 2002).

The River Minho estuary was colonized by this species no later than 1989 (Araujo *et al.*, 1993) and has become the dominant benthic species in terms of abundance and biomass (contributing more than 90% of the macrobenthic biomass) in the limnetic estuarine area (Sousa *et al.*, 2005). Given the high invasive success of *C. fluminea* in American and European ecosystems and the potential damage that can result from its colonization of a new habitat, the prediction of the species distribution in invaded ecosystems is a priority issue in several regions, including in the River Minho estuary. According to Parker *et al.* (1999) and Ricciardi (2003) the impact that a NIS may have in an ecosystem is significantly related to its abundance and/or biomass. Consequently, knowledge about the relationship between NIS abundance and/or biomass and abiotic factors would greatly help ecologists and managers to anticipate which habitats would be most affected by such an invasion (MacIsaac *et al.*, 2000; Palmer & Ricciardi, 2004; Jones & Ricciardi, 2005). Therefore, the principal aims of this study were to investigate the evolution of *C. fluminea* abundance, biomass and population structure between 2004 and 2005, and to develop a model describing the relationship between abiotic factors and the spatial and temporal distribution of *C. fluminea* in the freshwater tidal area of the River Minho estuary.

Material and methods

Study Area

The River Minho originates in Serra da Meira, in the province of Lugo, Spain, and is approximately 300km long. The first 230km are heavily regularized by the presence of numerous impoundments. However, its international section (the last 70km located on the

Portuguese/Spanish border) is not regularized and is in good ecological conditions although some minor foci of organic pollution exist (Sousa *et al.*, 2005).

The River Minho estuary extends for about 40km with a tidal freshwater portion of nearly 30km. This estuary is partially mixed; however, during periods of high floods, it tends to evolve towards a salt wedge estuary (Sousa *et al.*, 2005).

Sampling and laboratory analysis

Samples were collected in the limnetic subtidal area of the River Minho estuary, at high tide in October of 2004 and 2005 (after the *C. fluminea* reproduction season). Samples were gathered by a Van Veen grab with an area of 500 cm^2 and a maximum capacity of 5000 cm^3 in 16 sites (Fig. 5.1.1.).

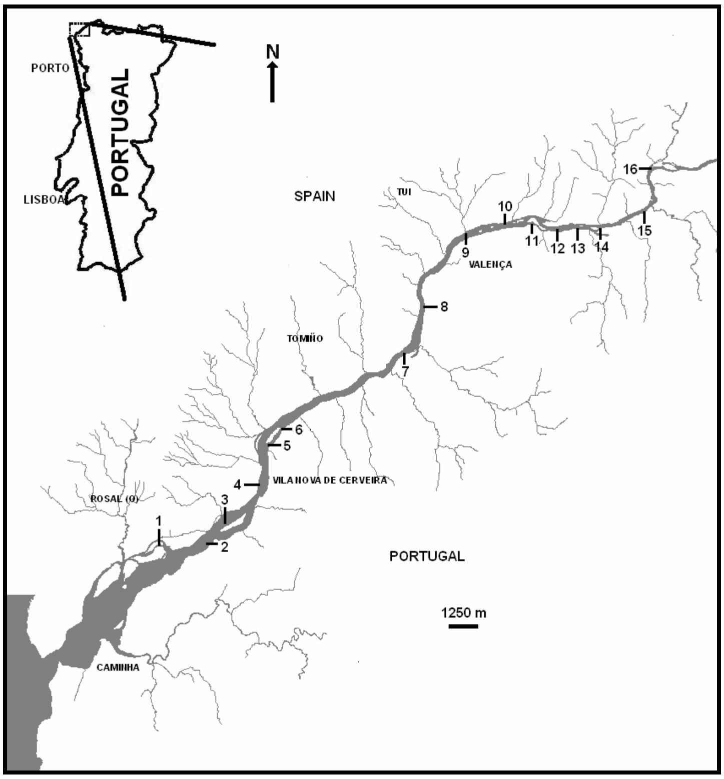

Fig. 5.1.1. Map of Minho estuary showing the sixteen sampling stations location.

At each site, the following water column parameters were measured: temperature (T), conductivity (CND), total dissolved solids (TDS), redox potential (ORP), salinity (S), dissolved oxygen (DO), pH, chlorophyll (Chl), nitrites, nitrates, ammonia, phosphates and hardness. The first eight environmental factors were measured *in situ*, close to the bottom, by the use of a multiparametrical sea gauge YSI 6820. Nitrites, nitrates, ammonia, phosphates and hardness were analyzed in laboratory by colorimetric methods. The granulometry of the sediment (very coarse sand (VCS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS) and silt+clay (SC)) and the organic matter (OM) content of the sediment were also measured. For the granulometry, samples were left in a furnace for 72 hours at a temperature of 60º C. Subsequently a dimensional analysis by sifting with a Ro–Tap agitation, with columns of sieves corresponding to integer values of the Wentworth scale, was performed. The organic matter content was determined by 24h combustion at 550ºC in a muffle furnace. Values were expressed as percentage of each sample weight.

Biological samples from the 16 sites were processed through a sieve with a mesh size of 500µm and *C. fluminea* specimens were separated, sorted and preserved. All organisms were then counted and their shell length measured with a digital dial caliper. *C. fluminea* biomass was calculated using the Ash Free Dry Weight Method – AFDW (Kramer *et al.*, 1994), for each site and year.

Statistical analysis

The relationship between abundance and biomass was estimated through a linear regression. Mean abundance, biomass and shell length and respective standard deviation were also calculated. The environmental characterization of the area was performed through multivariate analysis, using non-metric multidimensional scaling (nMDS), applied to the Euclidean distance matrix of the standardized variables (standardized by the range) (r-project, using package Vegan; Ihaka & Gentleman, 1996).

Differences in bivalve biomass ($\log_e$ transformed) between sample years (2004 and 2005), were tested with a t-test, after verifying homogeneity of variances using the Bartlett's test. For data analysis, bivalve biomasses were $\log_e$ transformed to normalize and stabilize variances. Accordingly, each predictor variable was also observed in detail, to determine if a transformation improved their distribution. Thus, conductivity (log.CND), total dissolved solids (log.TDS), redox potential (log.ORP), salinity (log.S), nitrites (log.nitrites), nitrates (log.nitrates), ammonia (log.ammonia), phosphates (log.phosphates), hardness (log.hardness) and pH (log.pH) were $\log_e$ transformed. Variables in percentage (*i.e.* sediment granulometry: asi.VCS, asi.CS, asi.MS, asi.FS, asi.VFS, asi.SC; and organic matter: asi.OM) were arcsine transformed, as recommended by Zar (1999).

The relationship between bivalve biomass and environmental variables was then analyzed through stepwise multiple regression, using BIC (Schwarz's Bayesian information criterion) as a selection criterion, as recommended by Quinn & Keough (2002). Prior to the multiple regression analysis, Kendall correlation coefficient was calculated for the relationship between all pairs of environmental variables, to determine and avoid collinearity. Total dissolved solids and salinity were correlated with each other, and organic matter was correlated with very fine sand and silt+clay. Thus, total dissolved solids, very fine sand and silt+clay were removed from further analysis. Nitrites (correlated with nitrates) and chlorophyll (only measured in 2004, due to calibration problems in 2005) were also removed from further analysis.

Results

Abiotic characterization

Appendix 5.1.1. shows the physical and chemical parameters measured at each site and year. The nMDS analysis (Fig. 5.1.2.) identified three main areas of distinct environmental characteristics: Group 1 comprising stations 1 to 5; Group 2 comprising stations 6 to 12; and Group 3 comprising stations 13 to 16. These groups appear distributed along a physical and chemical gradient, from the lower to the upper estuarine areas. Group 1 (stations 1 to 5) was characterized by higher values of conductivity, salinity and total dissolved solids compared with the other groups, which is consistent with the more pronounced influence of adjacent marine conditions. In this estuarine area, sandier stations with low organic matter content (with the exception of station 2 with fine sediments and high organic matter) were predominantly found. Group 2 (stations 6 to 12) was characterized by fine deposits with high concentrations of organic matter. In addition, this group showed peak concentrations of nitrates, nitrites, ammonia and phosphates at station 9, in both years. This higher nutrient concentration at station 9 is probably due to the influence of a River Minho tributary (River Louro) with considerable organic pollution. Group 3, including stations 13 to 16, was characterized by sandier deposits with low organic matter, indicating a non-polluted area less impacted by human activities. However, station 16 receives water from a River Minho tributary (River Tea) and in 2004 some organic pollution was detected.

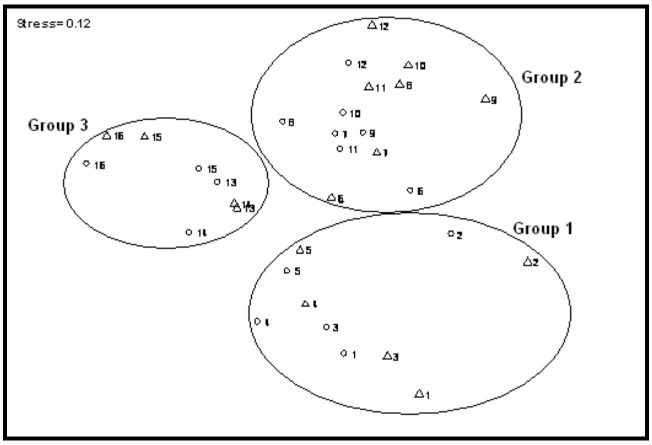

Fig. 5.1.2. nMDS diagram applied to the environmental variables (O: 2004, Δ: 2005).

Abundance, biomass and population structure of C. fluminea

C. fluminea was found in all sampled stations in both years. Mean abundance per site ranged from 80 ind./m^2 in station 10 (2004) to 4185 ind./m^2 in station 13 (2005) (Fig. 5.1.3.).

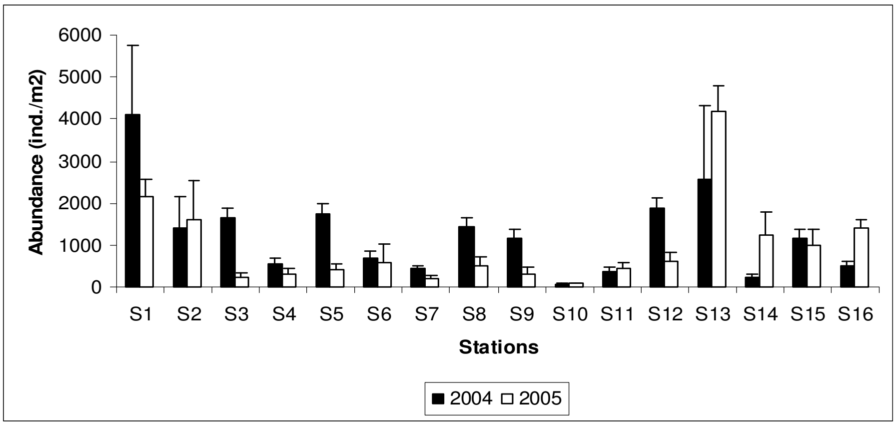

Fig 5.1.3. Annual and spatial variation of *C. fluminea* mean abundance (ind./m^2) (the confidence bands represent the standard deviation).

Mean biomass ranged from 8.5 g AFDW/m^2 in station 10 (2004) to 465.9 g AFDW/m^2 in station 13 (2005) (Fig. 5.1.4.).

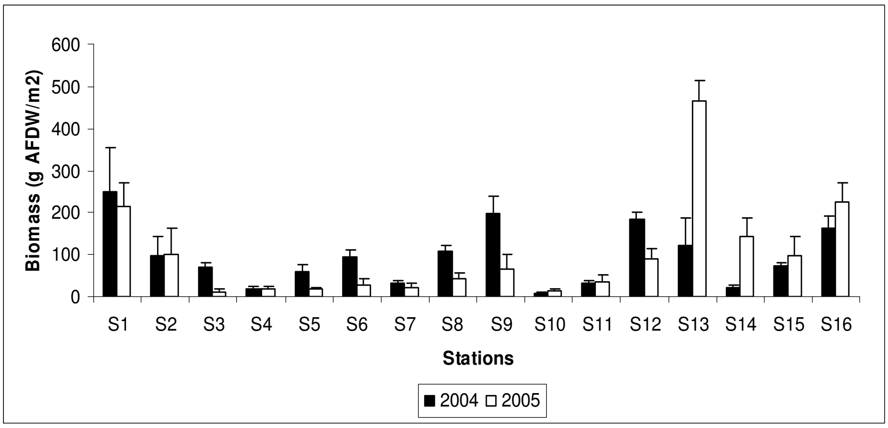

Fig. 5.1.4. Annual and spatial variation of *C. fluminea* mean biomass (g AFDW/m^2) (the confidence bands represent the standard deviation).

There was no heterogeneity of variances between years (Bartlett's test for homogeneity of variances: $K^2 = 1.334$, p-value = 0.248) and *C. fluminea* biomass did not differ significantly between 2004 and 2005 (t-test: t = 0.999, df = 94, p = 0.321). Figure 5.1.5. shows the relationship between abundance and biomass, which was highly significant (ln(biomass) = -2.175±0.358 + 0.955±0.055 × ln(abundance) (coefficient ± SE), $R^2 = 0.77$, $F_{[1, 94]} = 307$, $p < 0.001$). Sampling points below the regression line, represent smaller animals (for a similar abundance, these have smaller biomasses) whether the ones above, the opposite (for a similar abundance, these have larger individuals). Thus, Figure 5.1.5. shows a predominance of smaller individuals in stations closer to the sea (stations 1 to 5).

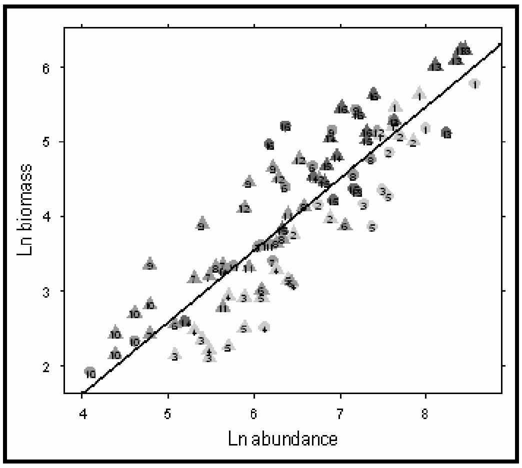

Fig. 5.1.5. Relationship between abundance and biomass (ln(biomass) = -2.175±0.358 + 0.955±0.055 × ln(abundance) (coefficient ± SE), $R^2 = 0.77$, $F_{[1, 94]} = 307$, $p < 0.001$) (the line indicates the model, circles represent samples from 2004 and triangles from 2005; the numbers inside the symbols represent station number; the three grey tones show the station groups evidenced by the multivariate analysis (see Fig. 5.1.2).

These conclusions were confirmed by shell length analysis, shown in Figures 5.1.6 and 5.1.7, where smaller individuals were found in lower stations (from stations 1 to 5) and larger individuals were observed in upper stations (with the exception of station 6 in 2005 and station 13 in 2004, with smaller specimens). Stations in Group 1, where animals with 10-20 mm shell length predominate, corresponds to greater influence of marine waters and sandier deposits with low organic matter, as identified in the multivariate analysis (Fig. 5.1.2.). Group 2 showed a mode at 15-30 mm shell length and Group 3 showed a bimodal distribution, with a peak at 20 mm shell length and a second small peak at 10-12 mm shell length.

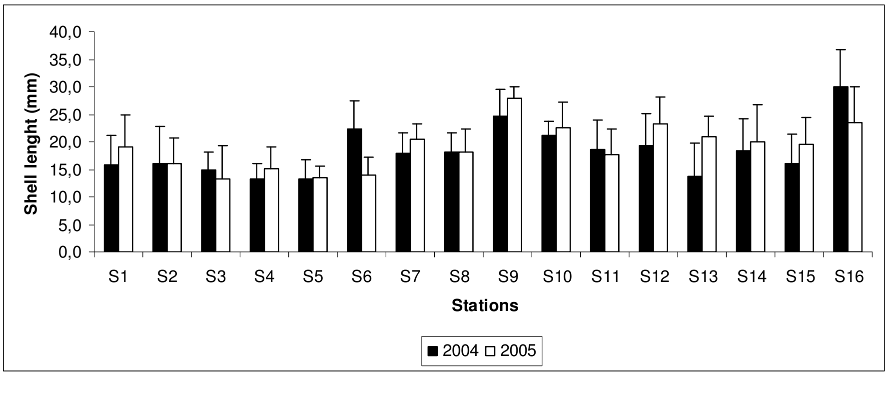

Fig. 5.1.6. Annual and spatial variation of *C. fluminea* shell length mean (mm) (the confidence bands represent the standard deviation).

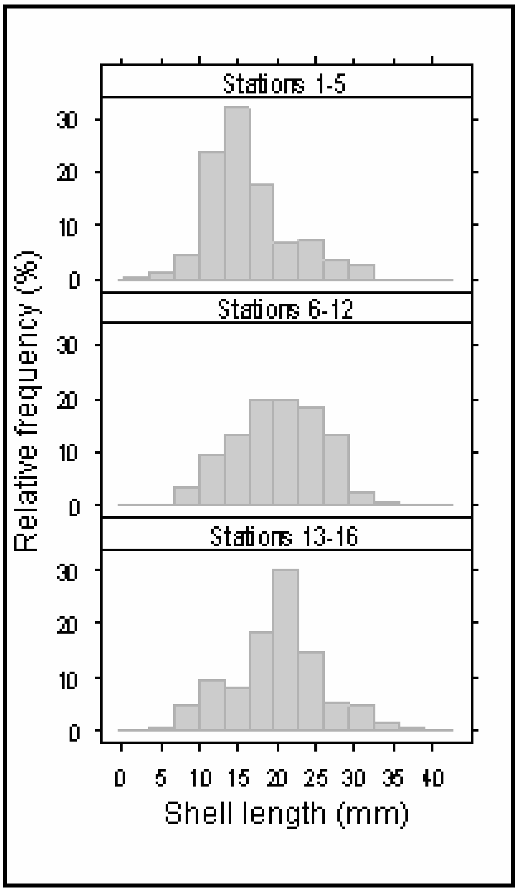

Fig. 5.1.7. Shell length distribution in each area identified by multivariate analysis of environmental data (see Fig. 5.1.2.).

Stepwise multiple regression showed that redox potential, nitrates, ammonia, organic matter, hardness, very coarse sand and fine sand explained 59.3% of the total variation in *C. fluminea* biomass (Table 5.1.1.). Redox potential, organic matter, hardness, very coarse sand and fine sand showed a positive coefficient, thus bivalve biomass was greater for higher values of these variables, whereas with nitrates and ammonia, a negative relationship was observed. However, a more profound analysis of the data presented in Table 5.1.1. indicates that ANOVA results revealed that redox potential, nitrate concentration, very coarse sand and fine sand where much more correlated with population biomass ($p = <0.001-0.009$) than the other variables ($p = 0.01-0.043$). Therefore, these four variables explained the majority of the recorded variation in population biomass and are the most important predictors of abiotic environmental suitability for *C. fluminea*.

Table 5.1.1. Multiple regression model and respective ANOVA table calculated after the stepwise procedure (using BIC as a selection criterion) of natural log *C. fluminea* biomass in function of 17 abiotic factors ($R^2 = 59.3\%$, $F_{[9, 86]} = 13.9$, $p < 0.001$).

	Regression results				**ANOVA results**			
	Coefficient	**Std. Error**	**T value**	**p-value**	**SSq**	**Mean Sq**	**F**	**p-value**
(Intercept)	0.981	0.811	1.21	0.230				
Year	1.315	0.255	5.16	<0.001	1.3	1.3	2.22	0.140
DO	-1.668	0.674	-2.48	0.015	0.6	0.6	1.04	0.311
log.ORP	2.468	0.484	5.10	<0.001	28.3	28.3	50.16	<0.001
log.nitrates	-1.494	0.380	-3.93	<0.001	12.5	12.5	22.17	<0.001
log.ammonia	-1.480	0.701	-2.11	0.038	2.9	2.9	5.14	0.026
asi.OM	0.987	0.287	3.44	0.001	3.9	3.9	6.94	0.010
log.hardness	3.130	0.564	5.55	<0.001	2.4	2.4	4.23	0.043
asi.VCS	3.287	0.580	5.67	<0.001	14.8	14.8	26.25	<0.001
asi.FS	1.532	0.574	2.67	<0.001	4.0	4.0	7.13	0.009
Residuals					48.6	0.6		

Discussion

The nMDS analysis, based on environmental factors, revealed a clear spatial pattern from the lower to the upper estuarine areas, discriminating three main areas. The first area has greater tidal influence and sandier deposits; the second area, probably associated with increased organic pollution and richer in finer sediments and organic matter and the third area, less polluted and with residual tidal influence. This spatial pattern is most likely related with different sediment characteristics and with a clear estuarine gradient, principally in salinity, conductivity and total dissolved solids. Nutrient concentrations seem to have also an important role in the discrimination of the Group 2 (having higher values) from the others.

C. fluminea abundances and biomasses measured in the River Minho estuary were similar to those recorded in other European and American ecosystems (Miller & Payne, 1994; Phelps, 1994; Cataldo & Boltovskoy, 1999; Mouthon, 2001a, b; Mouthon & Parghentanian, 2004). These results were expected since this NIS has been recognized by its great reproductive capacity, elevated population growth, early maturity, short life span and high fecundities (McMahon, 2002). Such characteristics are typically attributed to r-selected species and are, probably, responsible for the great abundances and rapid

C. fluminea worldwide dispersion. However, there are examples in the literature reporting *C. fluminea* population decay after the first phase of invasion where the population rapidly increases (Phelps, 1994). This situation seems not to have occurred yet in the River Minho estuary since the population has persisted at considerable abundances for the last 15 years (Araujo *et al.*, 1993; Sousa & Antunes, personal observation). A different pattern of invasion, however, has been described in a neighbouring estuary (River Lima estuary) with similar hydrological and geological characteristics. Here, *C. fluminea* is a more recent introduction (first record in 2002) and appears to be at the lag time phase (Sousa *et al.*, 2006a and b). Therefore, *C. fluminea* invasive behaviour may be different in distinct ecosystems and abiotic conditions, among other factors, may be determinant for the invasive behaviour of the species. *C. fluminea* preferentially colonizes rivers without considerable seasonal hydrological oscillations, being a common species in the limnetic portions of estuarine ecosystems. In good agreement with this, the results of the present study show considerable values of abundance and biomass in the limnetic area of the River Minho estuary. Thus, freshwater tidal estuarine ecosystems are suitable habitats to the *C. fluminea* introduction and subsequent dispersion and this is confirmed by ours and several other studies (Phelps, 1994; Cataldo & Boltovskoy, 1999; Chainho *et al.*, 2006).

To date no study has attempted to develop stepwise multiple regression models combining abiotic data and *C. fluminea* abundance and/or biomass in freshwater ecosystems. The results of the stepwise multiple regression models predicted that in the limnetic area of the River Minho estuary the habitats with higher values of redox potential, organic matter, water hardness, very coarse sand and fine sand and lower values of nitrates and ammonia support higher biomasses. Redox potential may be an important factor in the distribution of this species, principally in estuarine areas with fine deposits. In summer, due to a combination of factors, mainly related with elevated temperatures, low flow, decrease dissolved oxygen and the presence of large quantities of organic matter, a decrease in the redox potential may occur and in combination these factors can cause massive mortalities in benthic species, including *C. fluminea* (Johnson & McMahon, 1998; Strayer, 1999; Cherry *et al.*, 2005; Cooper *et al.*, 2005). For example at stations 10 and 12 massive mortalities occurred in July and August of 2005, probably due to the higher temperatures and low oxygen concentrations and consequent reduction in the redox potential. Thus, limnetic estuarine areas with higher values of redox potential can support higher biomasses of *C. fluminea*. Organic matter is also likely to be an important factor influencing distribution of *C. fluminea*. Due to its high respiration rates and associated high metabolism this clam can have both filter and pedal feeding (Hakenkamp & Palmer, 1999). Consequently, in addition to the normal filter feeding this species can also have deposit feeding and use the organic matter available in the sediment has a food resource

(Hakenkamp & Palmer, 1999; Hakenkamp *et al.*, 2001; Vaughn & Hakenkamp, 2001). This alternative food source can play an important rule in habitats with low phytoplankton concentrations and the River Minho estuary is a typical oligotrophic estuary. Therefore, this species can have higher biomasses and growth rates in habitats with high organic matter content (Vaughn & Hakenkamp, 2001). Sediment characteristics are an important factor in the distribution of benthic species, including nonindigenous invasive bivalve species (Mellina & Rasmussen, 1994; Byers, 2002b; Jones & Ricciardi, 2005). The model, showed higher biomasses were correlated with higher values of very coarse sand, fine sand and organic matter (with this last abiotic factor correlating with very fine sands and silt and clay). Hardness could have a significant contribution for the shell production and, thus, it should be an important factor for the occurrence and distribution of the species. However, this abiotic factor is a poor predictor of the abundances and/or biomass in other nonindigenous invasive bivalve species (Mellina & Rasmussen, 1994; Jones & Ricciardi, 2005). The concentration in nutrients was negatively correlated with *C. fluminea* biomasses and this species seems to be sensitive to organic pollutants and not well adapted to organic enrichment conditions. This situation seems to be particularly problematic for juveniles' survivorship and/or development since in the areas that surrounded station 9, with high nutrient levels, adults may be found but not juveniles.

There were differences in the *C. fluminea* population structure along the 16 sampling stations in agreement with the distinct abiotic conditions present in the freshwater tidal estuarine area. The lower stations (station 1 to 5) were composed by relatively small individuals when compared with the upper stations and this was, possibly, a consequence of lower organic matter content in stations 1 to 5 (with the exception of station 2) and slower growth rates.

This study was confined to one Portuguese freshwater tidal estuarine area and some results may be restricted to the River Minho estuary *C. fluminea* population. Consequently, caution should be applied in generalizing this model to other invaded areas, since the invasion process may change from ecosystem to ecosystem. Furthermore possible genetic differences at the population level could be important in the invasive behaviour of this species and other important factors such as the propagule pressure, the duration of the lag phase and differences in the community's structure were not taken into account in this model. However, there is no a priori reason for assuming that these results are unique and thus this model should be of great help to ecologists and managers working on invasive species. Although microhabitat variables appear important to the distribution of *C. fluminea* in the River Minho estuary, it could still be influenced by other abiotic factors not measured in this study (*e.g.* current velocity could have an influence on the juveniles' distribution). However, sediment granulometry can give some indication of the prevailing hydrological conditions, including current velocity. In addition, biotic factors such as predation, competition, parasitism and presence of submerged vegetation may also have an effect on the distribution of the species. Future studies should examine these biotic and abiotic factors and possibly be added to the stepwise multiple regression model.

Colonization of *C. fluminea* in the River Minho estuary may be posing a serious threat to native biodiversity and ecosystem functioning with possible environmental and economic repercussions. An adequate understanding of the processes responsible for the distribution and abundance of *C. fluminea* in the River Minho estuary is essential to inform future management decisions. Considering the great abundance and biomass already recorded in the River Minho estuary and the well known reproductive and dispersion capacity of *C. fluminea*, its eradication may be impossible. Biological management is possible and could serve to control population densities (Zavaleta *et al*., 2001) thus reducing both ecological and economic damage resulting from an invasion. It is also important to recognize that the River Minho estuary is a potential source for the introduction of *C. fluminea* to other aquatic ecosystems. Therefore, it is essential to minimize any form of transport of this species to other adjacent aquatic ecosystems.

5.2. Factors influencing the occurrence and distribution of *Corbicula fluminea* (Müller, 1774) in the River Lima estuary

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Abstract

This study evaluated the status of the Asian clam *Corbicula fluminea* (Müller, 1774) in the River Lima estuary (Portugal), which it first invaded in 2002. During the summers of 2004 and 2005, spatial and temporal variations in the abundance, biomass and population structure of *C. fluminea* were investigated in this estuary. This species was present in a limited intertidal area of the estuary and was rarely found in the subtidal zone. Its abundance and biomass were considerably lower than those found in other estuarine systems. Based on these studies it appears that salinity fluctuations and possibly other factors such as nutrients and environmental contaminants are keeping this invasive species from reaching its full potential. Given the lower abundance and biomass recorded and the restrict distribution of the species in this estuary, the eradication of this non-indigenous invasive species is still possible. The findings of this study may be useful for the management of this species not only in the River Lima estuary but also in other locations it may invade.

Introduction

The introduction of non-indigenous invasive species (NIS) has been recognized as a critical environmental problem since the pioneering studies of Elton (1958). The introduction and further dispersion of NIS may produce considerable ecological, genetic, social and economic impacts (Carlton & Geller, 1993; Lodge, 1993; Cohen & Carlton, 1998; Kolar & Lodge, 2001; Grosholz, 2002). These species can function as introductory vectors of diseases, may change the ecosystem, reduce biodiversity and cause great economic damage (Vitousek *et al.*, 1996; Mack *et al.*, 2000; Pimentel *et al.*, 2000). Therefore, the control and management of NIS is a priority issue and will be a great challenge for conservation biology in the next decades.

According to Allendorf & Lundquist (2003), two primary steps in the evolution of invasive populations in new habitats exist. The first one is the introduction, colonisation and establishment of the species in the new habitat. The second step deals with the dispersion and the subsequent replacement of the native species by the introduced species. Between these steps emerge the lag time phase, when several species recently established persist with low or even undetectable densities in a fixed area for a variable period of time, before entering into an exponential growth phase where the population rapidly invades the new environment (Shigesada & Kawasaki, 1997; Crooks & Sole, 1999; Crooks, 2005). This lag time period may have the duration of months, years or even decades (Sakai *et al.*, 2001).

The Asian clam *Corbicula fluminea* (Müller, 1774) is one of the most invasive bivalves in freshwater ecosystems (Phelps, 1994; Cataldo & Boltovskoy, 1999; Hakenkamp & Palmer, 1999; Darrigran, 2002; McMahon, 2002). This species, with origin in Asiatic ecosystems, has been dispersing worldwide over the last 80 years. In the beginning of the last century, *C. fluminea* colonized North America and in the 1980s its presence was reported in Europe for the first time (Mouthon, 1981; Araujo *et al.*, 1993; McMahon, 1999). Given the potential impacts at both ecological and economic levels that the introduction of *C. fluminea* may cause, it is essential to derive demographic models describing its dispersion and population dynamics in new environments. These models may be of considerable help in the adoption of mitigation measures and other management actions that may be required (Phelps, 1994; Darrigran, 2002; McMahon, 2002).

The invasion of a new environment by the NIS, if studied from its beginning, provides a considerable amount of data that may be useful in dealing with future invasions in other ecosystems. The River Lima estuary, in the North of Portugal, was recently invaded by *C. fluminea* (Sousa *et al.*, 2006a). Its presence was noticed for the first time in 2002 and since then, the occurrence of the species has been constant but at low densities. The main objective of this study was to describe *C. fluminea* spatial and temporal distribution in the River Lima estuary in 2004 and 2005 (with a small reference to its first occurrence in 2002). The pattern of distribution and the evolution of the population in this first phase of new habitat invasion may be useful to predict its possible expansion in the ecosystem and to support management measures for the mitigation of the impacts caused by this invasion. In addition, the study of this phase also generates data that may be useful for the management of future invasions in similar environments.

Material and methods

Study Area

The hydrological basin of the River Lima has an area of about 2 480km^2, 1 303km^2 in Spain (53%) and 1 177km^2 in Portugal (47%). The estuary is located in the NW of Portugal (Fig. 5.2.1.) and the influence of spring tides extends approximately 20km upstream. A more detailed description of its physical and chemical characteristics and its benthic organisms is given by Sousa *et al.* (2006a). The studied area was focused in the upper estuarine sector of the River Lima which is known to be colonized by *C. fluminea* at least since 2002 (Sousa *et al.*, 2006a). This area includes subtidal and intertidal areas along no more than 2 km of the river course. In several preliminary studies performed in upstream and downstream areas, *C. fluminea* was never found. Therefore, the study was restricted to the area known as being colonized by this species.

Sampling and Data analysis

Samples were collected in August 2004 and 2005 in both subtidal and intertidal areas of the Lima estuary, at low tide (Fig. 5.2.1.). Ten replicates per sampling station were gathered by a Van Veen grab with an area of 500 cm^2. Stations 1, 2, 3, 4 and 5 were located in intertidal areas and the stations 6, 7, 8 and 9 in subtidal areas.

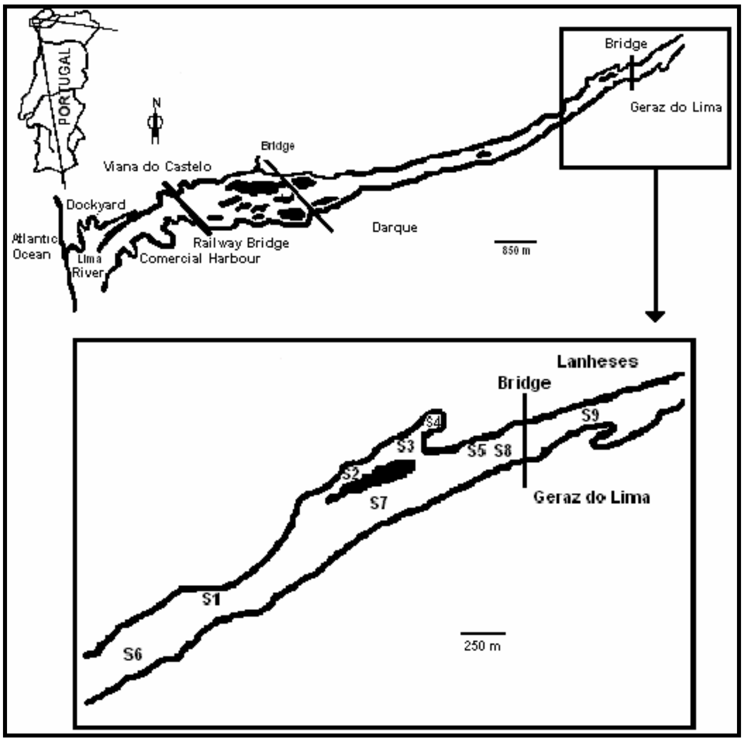

Fig. 5.2.1. Map of the River Lima estuary showing the nine sampling stations.

During fieldwork, the following water column parameters were determined: temperature, salinity, dissolved oxygen and pH. In intertidal areas, they were measured in adjacent low water pools and in the subtidal areas they were determined close to the bottom. This information was gathered using a multiparametrical sea gauge YSI 6820. Particle size and organic matter content of the sediment from each site were also determined. Sediment granulometry was assessed after a drying period of 72 h at 60ºC in an oven.

Dried sediment was sieved through a column of sieves corresponding to integer values of the Wentworth scale and the frequency of each class was expressed as the percentage of total weight. The amount of organic matter in the sediment was determined after a 24h combustion period, at 550ºC, in a muffle furnace.

The *C. fluminea* individuals present in the samples were collected using a sieve with a mesh size of 1 mm. Specimens were immediately separated, sorted and preserved. Subsequently, they were enumerated and their shell length was measured with a digital caliper. Faunal biomass was calculated by the Ash Free Dry Weight (AFDW) method (Kramer *et al.*, 1994) and the relationship between total length and biomass (AFDW) was also estimated for 40 individuals (20 individuals from which year, data pooled). The relationship between total length and biomass AFDW was given by the following equation:

Biomass (g AFDW) = 3E-05*Shell Length (mm)^{2,8353} (N = 40, $R^2 = 0.95$)

For population structure analysis, clams were included in 2 mm class intervals according to their shell length. This approach allows the detection of recent recruitments.
Finally, an environmental variable *versus* sampling station matrix was analysed using Principal Components Analysis (PCA) for grouping sampling stations in relation to environmental factors. This analysis was performed using the PRIMER package (Clarke & Warwick, 2001).

Results

In general, there were no great spatial or temporal variations in the environmental parameters measured in the water column and in the sediment compartment (Appendix 5.2.1.). However, the values of temperature and salinity increased from 2004 to 2005. Water temperature ranged from 20.1ºC (station 2 in 2004) to 22.9ºC (station 3 in 2005), and the salinity from 0.4 psu (station 9 in 2004) to 12.1 psu (station 7 in 2005). Water dissolved oxygen values varied between 7.5 mgl^{-1} (station 3 in 2005) and 8.4 mgl^{-1} (station 5 in 2005), while pH ranged from 7.1 (station 6 in 2005) to 8.8 (station 3 in 2005). Sediment analysis revealed a substratum with coarser sediment with a preponderance of sands. PCA matrices of environmental factors versus sampling stations revealed a clear pattern (Fig. 5.2.2.). From the projection against the first axis of variability, stations appeared distributed along a physical and chemical gradient, with the stations having finer sediments and high organic matter (stations 2, 3 and 4) along one edge and the stations with coarser sediments and low organic matter located on the other. Along the second axis of variability, the separation of the stations was related to the environmental factors measured in the water column principally salinity, pH and dissolved oxygen.

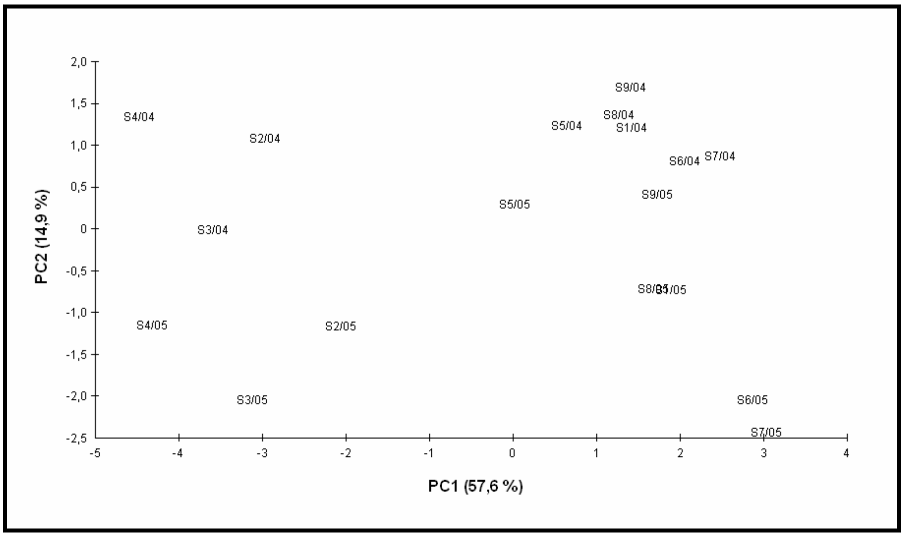

Fig. 5.2.2. Analysis of physical and chemical factors of water column and sediment from PCA of factors x station matrices for the years 2004 and 2005. The percentages of variability explained by the two principal axes are shown.

Abundance and biomass showed variations along the intertidal (with the exception of station 5) and subtidal sampling stations, with higher abundances and biomass values in the intertidal sites. However, these values did not differ greatly between years. Mean abundance per station ranged from 0 ind./m^2 at station 5 in 2005, and stations 6, 8 and 9 in both years to 60 ind./m^2 at station 4 in 2004 (Fig. 5.2.3.). Total mean abundances were 13.3 and 11.6 ind./m^2 in 2004 and 2005, respectively. Mean biomass ranged from 0 g AFDW/m^2 at station 5 in 2005, and stations 6, 8 and 9 in both years to 23.78 g AFDW/m^2 at station 4 in 2005 (Fig. 5.2.4.). Total mean biomass was 6.89 and 6.74 g AFDW/m^2 in 2004 and 2005, respectively.

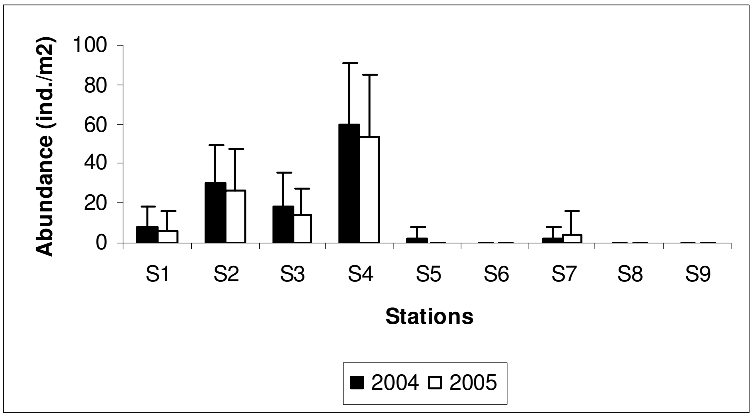

Fig. 5.2.3. Mean abundance of *C. fluminea* (+*SD*) throughout the nine sampling stations in the years 2004 and 2005.

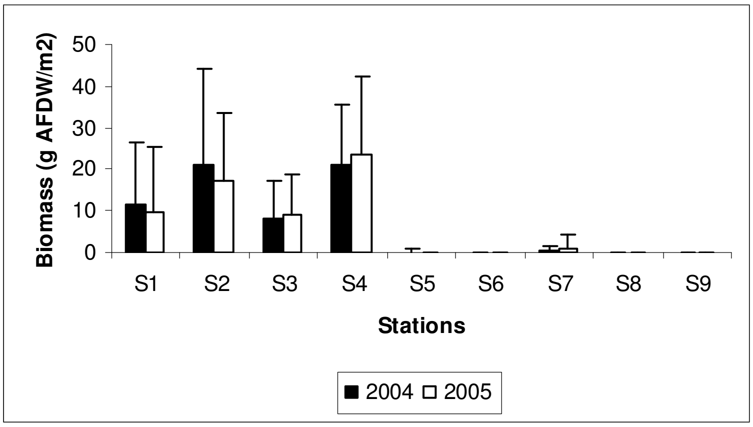

Fig. 5.2.4. Mean biomass of *C. fluminea* (+*SD*) throughout the nine sampling stations in the years 2004 and 2005.

The *C. fluminea* population structure (subtidal and intertidal data pooled) in 2004 is similar to that found in 2005 (Fig. 5.2.5.). However, in 2005 more individuals with higher shell lengths were recorded and only a few small specimens were found. During the study period, the maximum shell length value was found at station 1 (51.6 mm) and the minimum was recorded at station 2 (13.0 mm), both in 2005.

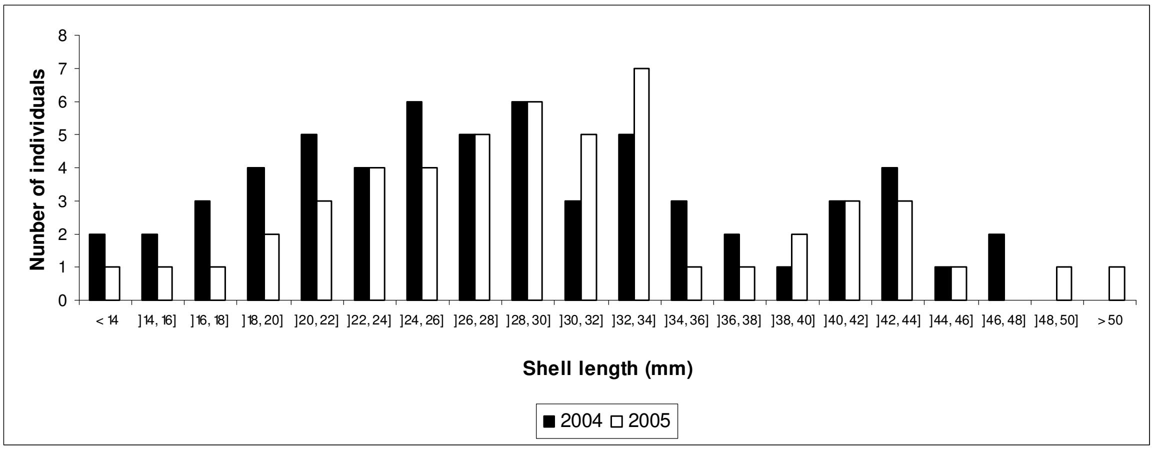

Fig. 5.2.5. Length profiles of *C. fluminea* in the River Lima estuary in the years 2004 and 2005.

Discussion

In the present study, differences in temperature and salinity values were found between 2004 and 2005. The values of these two abiotic factors determined in 2005 were higher than those recorded in 2004. Perhaps, this was a reflection of the severe drought that occurred in 2005, which increased salinity values upstream. The year 2005 was a hot year. Therefore, atmospheric temperatures may have contributed to the higher water temperatures in 2005 compared to 2004. Spatial fluctuations of water dissolved oxygen and pH values were never large which was in good agreement with results gathered in 2002 (Sousa *et al.*, 2006a). The comparison of subtidal sediment characteristics determined in the present study with those reported by Alves (1996) and Sousa (2003) indicates that no significant changes occurred in recent years, and the sediments were predominantly sands. Cumulative curves of the sediment along the nine sites are very similar (data not shown); however, finer sediments were more abundant at stations 2, 3 and 4 than at the others. These stations with finer sediments and high organic matter content correspond to the sites with higher *C. fluminea* abundances and biomasses.

C. fluminea has all the characteristics of an r-selected species, with elevated growth and fecundity and extensive capacity of dispersion that allows rapid population recovery, which is fundamental for invasion success. Once established, this species may have considerable negative ecological and economic impacts (Phelps, 1994; Hakenkamp & Palmer, 1999; Hakenkamp *et al.*, 2001; McMahon, 2002). This NIS was recorded for the first time in the River Lima estuary in 2002 (Sousa *et al.*, 2006a). This study was only performed in the subtidal area, and *C. fluminea* was only found in one sampling station located in the upper estuary (near station 7 of the present study). Since then, this species has been found in the estuary but with low abundances and this study shows that *C. fluminea* abundance and biomass did not have great oscillations during 2004 and 2005. However, *C. fluminea* abundance and biomass in this estuarine ecosystem were very low compared to those found, for example, in the River Minho estuary, an adjacent estuarine area with similar hydrological and geological characteristics and also colonized by this NIS. The presence of *C. fluminea* in the River Minho estuary has been recognized since 1989 (Araujo *et al.*, 1993). The population has been increasing since then, and presently shows a considerable abundance, biomass and distribution (Sousa *et al.*, 2005). In the River Minho estuary, *C. fluminea* is dispersed for more than 30 km, with mean values of abundance and biomass of 1253 ind./m^2 and 95.2 g AFDW/m^2, respectively (Sousa *et al.*, 2005). A model based on a stepwise multiple regression combining several abiotic factors in order to determine *C. fluminea* biomass distribution was applied in the freshwater subtidal area of the River Minho estuary. This model explained almost 60% of the variation in *C. fluminea* biomass ($R^2 = 59.3\%$, $F_{[9, 86]} = 13.9$, $p < 0.001$) and showed that redox potential, organic matter, hardness, very coarse sand and fine sand showed a positive coefficient, thus bivalve biomass was greater for higher values of these variables, whereas with nitrates and ammonia, a negative relationship was observed (Sousa *et al.*, 2008). Given the great environmental similarities between these two adjacent Portuguese estuaries we expected a higher invasive behaviour in the River Lima estuary. Additionally, *C. fluminea* also reached high abundances in other European and North and South American freshwater ecosystems (Phelps, 1994; Cataldo & Boltovskoy, 1999;

Hakenkamp & Palmer, 1999; Hakenkamp *et al.*, 2001; Darrigran, 2002; Mouthon, 2001a, b and 2003; McMahon, 2002; Mouthon & Parghentanian, 2004).

A possible explanation for the low abundances, biomasses and the apparent range expansion stagnation showed by the *C. fluminea* population in the River Lima estuary is the more recent introduction of the species in this ecosystem. In the literature, there are several examples of non-indigenous plants and animals recently established in an ecosystem that persist at low densities for some time (months, years or even decades) before entering an exponential growth phase where they show a high invasive potential (Sakai *et al.*, 2001; Crooks, 2005). This period of apparent stagnation is known as the lag time phase (Shigesada & Kawasaki, 1997; Crooks & Sole, 1999; Crooks, 2005) and is characterized by a very slow or inexistent population growth rate (see Byers *et al.*, 2002). In this period, the number of individuals is reduced and the population is facing the challenge of adapting to the new environment.

When we looked at the River Lima estuary *C. fluminea* population structure we did not find individuals with a shell length less than 13 mm. This suggests a deficient recruitment that may be due, for example, to reduced spawning or to a high mortality rate of larvae and/or juveniles. Reduced spawning may result, for example, from a low investment in reproduction due to the necessity of a high energetic investment to face an adverse environment. High mortality of larvae and/or juveniles may be due to inadequate environmental conditions for their survival and development. Both cases will have a negative impact on population growth and may contribute to a lag time phase if they occur in an initial phase of population development. Additionally, environmental conditions seem to have a considerable influence on lag time phase duration (Crooks & Sole, 1999; Byers *et al.*, 2002 and references therein). *C. fluminea* has a relatively poor physiologic resistance to abiotic factor variations, including temperature, salinity, emersion, pH, calcium concentration and dissolved oxygen changes (McMahon, 2002). Salinity seems to be a major factor influencing the success and velocity of the invasion of new environments by this species 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, even at low salinities values (Morton 1982, 1986). Salinity values in the study area increased in recent years. This factor may be causing difficulties for the adaptation of the species to the new environment and slowing the colonisation process. While the species could be well adapted to the other environmental factors, the overriding influence of salinity could be a key factor in limiting the invasive behaviour of the species. Salinity values increased from 2004 to 2005 together with an apparent decrease of *C. fluminea* abundance and biomass. Abundances recorded in subtidal stations, with more pronounced changes in salinity between low and high tide, were lower than in intertidal sites. As well, 2004 and 2005 subtidal abundances were inferior to those found in 2002 when salinity values were considerably lowers (Sousa *et al.*, 2006a).

Alternative hypotheses for the lower abundances, biomass and failure in dispersal of the River Lima estuary *C. fluminea* population are nutrient enrichment, higher values in heavy metals and other environmental contaminants and possible genetic characteristics. We have no data that support the first two hypotheses in the study area but downstream areas are subject to eutrophication processes, heavy metals contamination and dredging activities (Sousa *et al.*, 2006a and 2007b). In fact, the lower River Lima estuary is considerably more contaminated with chemicals resulting from anthropogenic activities than the River Minho estuary (Antunes *et al.*, unpublished data). Additionally, the *C. fluminea* habitat in the River Lima estuary is near a pulp mill with possible contamination. In relation to genetic characteristics, recent studies done by the authors revealed that the *C. fluminea* populations of Rivers Minho and Lima estuaries have similar mitochondrial cytochrome *c* oxidase subunit I gene sequences (Sousa *et al.*, 2007a). Furthermore, we have no data that supports a different propagule pressure and the existence of potential predators and/or parasites that controlled the River Lima estuary *C. fluminea* population in a different away than for example the River Minho estuary population.

The evidence described above suggests that salinity variation, and probably also other environmental factors (*e.g.* nutrients, environmental contaminants), found in the River Lima estuary are not the most suitable for *C. fluminea* development and may have been hindering population growth and expansion in the ecosystem. However, if the conditions became more favourable and/or the species successfully adapts to the environment, the population may rapidly enter exponential growth and reach the carrying capacity of the ecosystem.

At this moment, the *C. fluminea* population of the River Lima estuary is restricted to a very small area and has a low abundance and biomass. Therefore, this specific phase of population development is the more efficacious period to implement measures to control and reduce the impacts resulting from its introduction since the population is still restricted to a small area and has a low density (Byers *et al.*, 2002). However, one must keep in mind that complete eradication is often difficult and in the case of *C. fluminea*, small juveniles are easily transported by humans and/or aquatic birds which can function as dispersion vectors.

Chapter 6

Colonization of the River Minho estuary by *Corbicula fluminea* (Müller, 1774): implications for ecosystem functioning, impacts on indigenous molluscs and conservation

6.1. Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774)

Submitted: Sousa R., Nogueira A.J.A., Gaspar M.B., Antunes C. & Guilhermino L. submitted. Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774). Biological Invasions.

Abstract

The Asian clam *Corbicula fluminea* (Müller, 1774) is a major component of the River Minho estuary, almost completely dominating the benthic biomass. As part of a major study into the ecology of *C. fluminea*, benthic samples were collected monthly from January 2005 to August 2006. This data was then used to estimate the abundance, biomass, growth, and growth and elimination production of this non-indigenous invasive species. *C. fluminea* growth was continuous throughout its life span. The annual 2005 growth production was estimated to be 463.778 g AFDW/m^2/year, and the mean annual biomass was 160.651 g AFDW/m^2, resulting in a $P/\bar{B}$ ratio of 2.89/year and a turnover time of 126.4 days. The estimated annual elimination production in 2005 was 855.219 g AFDW/m^2/year, resulting in an $E/\bar{B}$ ratio of 5.32/year. In light of these results we conclude that *C. fluminea* is a fundamental element in the River Minho estuary, possibly sequestering a large portion of the carbon available for benthic production and altering the ecosystem functioning. This species should be considered when modelling the nutrient cycles and energy flow in this estuary and in similar aquatic ecosystems where the species can reach high abundance and biomass.

Introduction

The introduction of non-indigenous invasive species (NIS) in freshwater ecosystems has increased dramatically in recent decades (Ricciardi & MacIsaac, 2000; Light & Marchetti, 2007). Freshwater ecosystems are being extremely modified by human activities, resulting in a decline of the native species with a subsequent replacement and spread of NIS (Ricciardi & Rasmussen, 1999; Holeck *et al.*, 2004; Marchetti *et al.*, 2004). In addition, genetic, social and economic impacts provoked by these NIS are usually unpredictable and occasionally result in serious damage (Pimentel *et al.*, 2000; Byers, 2002a). Therefore, measuring and predicting the impact of NIS deserves attention so that managers and stakeholders can prioritize efforts to prevent invasion or control the impacts of the invader (Sakay *et al.*, 2001; Sax *et al.*, 2007). Special consideration should be given to invasive species that carry new attributes to ecosystems and species that dominate communities as virtual monocultures and thereby have the potential to disrupt ecosystem processes (Cox, 2004; Hall *et al.*, 2006).

The Asian clam *Corbicula fluminea* (Müller, 1774) is recognized as one of the most important NIS in aquatic ecosystems (McMahon, 2002). In the last 80 years, species of the *Corbicula* genus extended their habitat from Asia, Oceania and Africa to American and European ecosystems (Araujo *et al.*, 1993; Mouthon, 2001a and b). Due to their invasive behaviour it is essential to study the population structure in invaded ecosystems to assess the potential impacts provoked by this NIS. Indeed, this species may reach considerable abundance and biomass and may play a significant role in nutrient cycling and benthic-pelagic coupling which are potentially responsible for the alteration of ecological structure and functions (McMahon, 2002; Sousa *et al.*, 2008). Consequently, this bivalve is the core of several studies carried out in European and American aquatic ecosystems dealing with biological invasions. However, this kind of information differs from ecosystem to ecosystem and it is essential to develop local studies, principally in areas with great abundance and biomass, such as River Minho estuary. In this estuary, *C. fluminea* was first recorded in October 1989 and in the subsequent years the population underwent a massive expansion with large number of juveniles and adults (Araujo *et al.*, 1993). Nowadays, this species is a persistent component of this ecosystem colonising all the middle and upper part of the estuarine area and reaching considerable abundance and biomass (*e.g.* some sites with an abundance and biomass of more than 4000 ind./m^2 and 550 g AFDW/m^2, respectively) (Sousa *et al.*, 2005, 2008, in press). The introduction of *C. fluminea* to the River Minho estuary was most likely the result of human activities, as has occurred in other American and European ecosystems (McMahon, 1999). A secondary introduction from another habitat previously colonised is the most plausible explanation for the presence of *C. fluminea* in this estuary and the most likely ones are the Rivers Tejo and Douro (Portugal) which were described as primary European habitats invaded by this species (Mouthon, 1981; Araujo *et al.*, 1993). A direct introduction from the native range or even a North or South American source is improbable since the River Minho estuary is not subjected to water ballast discharges or aquaculture activities. Therefore, human activities such as the use of specimens as fish bait, transport of individuals as a curiosity and also the extensive capacities of this species to natural dispersion are probable vectors of introduction (McMahon, 1999).

The aims of this study were to estimate the abundance, biomass, growth and growth and elimination production of *C. fluminea* in the freshwater tidal area of the River Minho estuary.

Material and methods

Study area and sampling strategy

The characterization of the River Minho estuary, with a particular emphasis on the macrobenthic assemblages existent along the estuarine gradient was previously described by Sousa *et al.* (2005, 2007c, in press).

Between January 2005 and August 2006 (always at the end of each month), quantitative monthly samples were carried out in three sites located in the River Minho tidal freshwater wetlands (TFWs), at high tide (Fig. 6.1.1.).

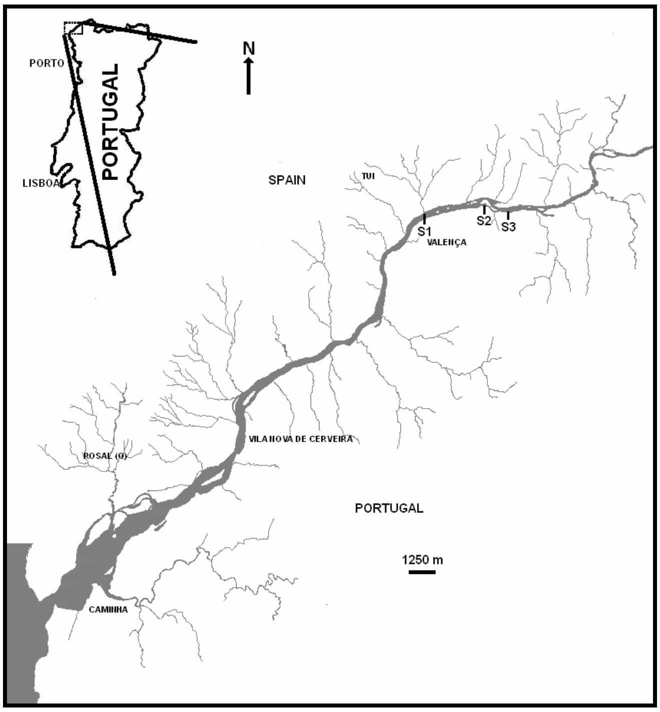

Fig. 6.1.1. Map of the River Minho estuary showing the three sites location.

For each site, six replicates (one for sediment analysis and five for biological analysis) were gathered using a Van Veen grab with an area of 0.05 m^2.

The following abiotic parameters were measured *in situ*, close to the bottom using a multiparametrical sea gauge YSI 6820: temperature, total dissolved solids, redox potential, salinity, dissolved oxygen and pH. In addition, the concentration of nitrites, nitrates, ammonia, phosphates and hardness were measured in the laboratory by colorimetry after collecting water samples in the field, using a Palintest 270 standard photometer 7000. Finally, sediment samples were collected and analysed for granulometry and organic matter content following the procedure described in Sousa *et al.* (2007a).

Biological material was processed through a sieve with a mesh size of 500µm and all individuals were measured with a vernier caliper. Length measurements were converted to estimates of individual biomass expressed as g of ash free dry weight (AFDW). Thus, 24 individuals were dried to 60ºC for dry weight estimations every month during 2005 and AFDW was obtained by ignition at 550ºC for 4 hours following the procedure described in Sousa *et al.* (2006a).

Growth and production estimations

Growth rates were estimated following recognisable cohorts with size frequency distributions from the successive twenty monthly sample dates. Size–frequency analysis was carrying out using the ANAMOD software package (Nogueira, 1992). The analysis reliability was always tested using the χ^2 and the G test ($P = 0.05$). Since growth rates were not constant throughout the year, empirical data was adjusted to a model that takes into account seasonal changes (Gaschütz *et al.*, 1980), expressed as:

$$L_t = L_\infty \left\{ 1 - e^{-\left[kD\left(t - t_0\right) + C\left(\frac{kD}{2\pi}\right) \sin\left[2\pi\left(t - t_s\right)\right]\right]} \right\}^{\frac{1}{D}}$$

L_t: length of the organism at a given moment t;

L_{oo}: maximum possible length of the organism;

t: given instant;

t_0: instant when the organism would have a length equal to 0;

ts: time interval between growth start (when t=0) and the first growth oscillation; growth is expressed by a sine curve which the period is one year;

k: intrinsic growth rate;

C: constant and the values can vary between 0 and 1;

D: parameter that express metabolic deviations from the Von Bertalanffy rule.

Annual production estimates were based on cohort recognition. Growth production (P) and elimination production (E) were calculated with a method derived from Allen (1971) with total values of P and E for the population expressed as:

$$P_t = \left(\frac{N_t + N_{t+1}}{2}\right) \times \left(\bar{W}_{t+1} - \bar{W}_t\right) \text{ for } \bar{W}_{t+1} > \bar{W}_t$$

$$E_t = \left(\frac{\bar{W}_{t+1} - \bar{W}_t}{2}\right) \times \left(N_t + N_{t+1}\right) \text{ for } N_t > N_{t+1}$$

N: density of the cohort in each sample date;

$\bar{W}$: mean individual biomass in each sample date;

t and t+1: consecutive sample dates.

Total values of P and E for each cohort are expressed as:

$$P_i=\sum_{t=0}^{n}P_t \text{ and } E_i=\sum_{t=0}^{n}E_t$$

Total values of P and E for the population are expressed as:

$$P=\sum_{i=1}^{N}P_i \text{ and } E=\sum_{i=1}^{N}P_i$$

P_i and E_i are the growth and elimination production of the cohort i.

$P/\overline{B}$ and $E/\overline{B}$ ratios were determined and $\overline{B}$ (mean population biomass) is expressed as:

$$\overline{B}=\frac{1}{T}\sum_{i=1}^{N}\overline{B}_i \times t_i$$

T: period of study;

N: number of successive cohorts in the period T;

$\overline{B}_i$: mean biomass of the cohort i;

t_i: duration of the cohort i.

Data analysis

Principal Component Analysis (PCA) was carried out for ordination of sampling sites based on the abiotic parameters measured using the CANOCO package (ter Braak & Smilauer, 2002). Previously, all values were transformed using the log(X+1) as part of the analysis.

A length–biomass regression was used to estimate *C. fluminea* AFDW.

Results

Abiotic characterization

The results of the abiotic parameters for each site can be made available on demand to the corresponding author.

The PCA (Fig. 6.1.2.) revealed clear spatial patterns. From the projection against the first axis of variability, sites appear distributed along an environmental gradient: site 1 (with coarser sediments and lower organic matter content) always along one of the edges and the sites 2 and 3 (with finer sediments and higher organic matter content) located in the other edge. The sediment composition of the sampled estuarine area includes fine deposits rich in organic matter. However, site 1 has coarser sediments than the 2 upper sites, being site 3 the local with finer sediments. Organic matter ranged between 5.8% in site 1 (August 2006) and 19.3% in site 3 (August 2005) and a significant correlation ($n = 60$, $R^2 = 0.83$; $P < 0.001$) between the quantity of silt and clay and the quantity of organic matter was established. In addition to these sediment differences, site 1 also has higher salinity and nutrient concentrations, and lower dissolved oxygen and pH values when compared with the others two upstream sites. From the projection against the second axis of variability sites appear distributed along a temporal pattern with the main differences being explained by hardness and temperature from the negative side. This second axis shows differences between spring/summer months and autumn/winter months.

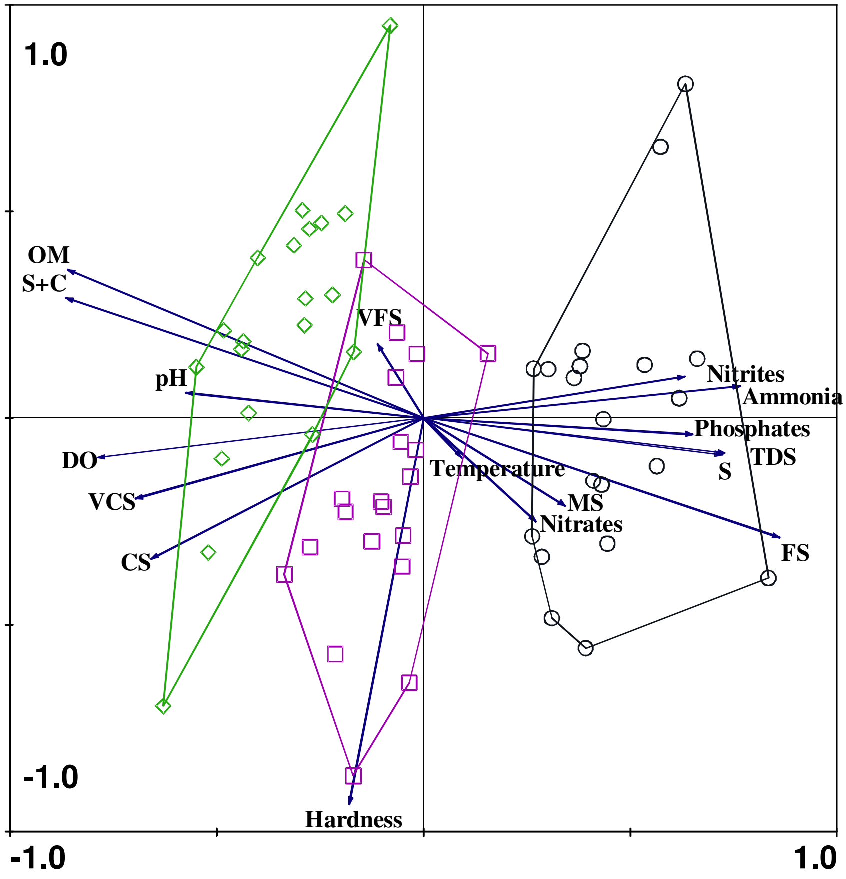

Fig. 6.1.2. Principal Component Analysis (PCA) of the abiotic factors measured monthly in the 3 sites (site 1 - ○; site 2 - □ and site 3 - ◊) from January 2005 to August 2006. The first and second axes explain 40.2% and 20.2% of the total variability, respectively. Temperature (T, ºC), total dissolved solids (TDS, mgl^{-1}), redox potential (ORP, mV), salinity (S, psu), dissolved oxygen (DO, mgl^{-1}) pH, nitrites (mgl^{-1}), nitrates (mgl^{-1}), ammonia (mgl^{-1}), phosphates (mgl^{-1}) and hardness (mgl^{-1}) of water column and organic matter (OM, %), very coarse sand (VCS, %), coarse sand (CS, %), medium sand (MS, %), fine sand (FS, %), very fine sand (VFS, %) and silt+clay (S+C, %) of the sediment.

Abundance and biomass

The total monthly abundance and biomass per site from January 2005 to August 2006 are given in the figures 6.1.3. and 6.1.4., respectively. The mean abundance along the 20 months of sampling was 521, 1320 and 680 ind./m^2 for the sites 1, 2 and 3, respectively. The minimum abundance value was gathered in site 3 in July 2005 with 92 ind./m^2 and a maximum of 2152 ind./m^2 in site 2 in April 2006. The mean biomass was 187.1, 124.3 and 133.9 g AFDW/m^2 for the sites 1, 2 and 3, respectively. The minimum biomass value was gathered in site 3 in July 2005 with 19.3 g AFDW/m^2 and a maximum of 424.9 g AFDW/m^2 in site 1 in February 2005.

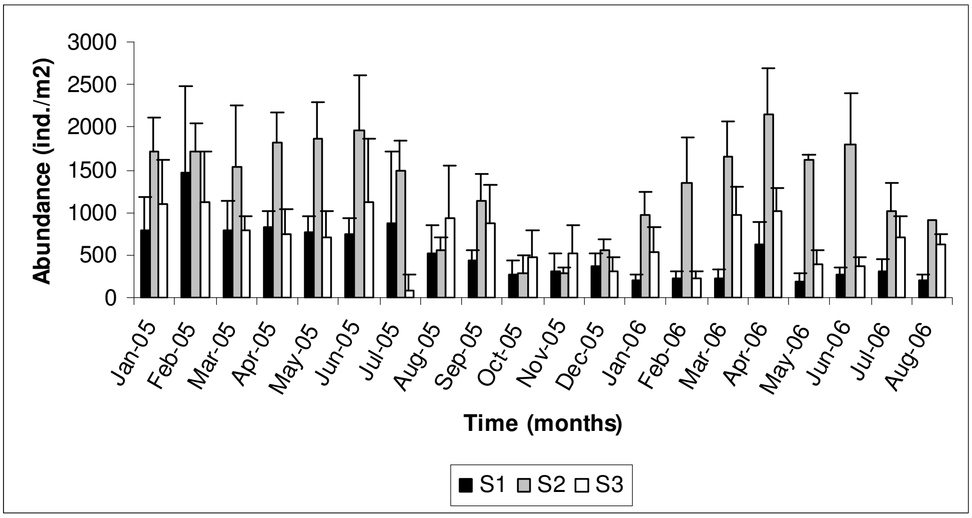

Fig. 6.1.3. Monthly variation of *C. fluminea* mean abundance (ind./m^2) in the 3 sites from January 2005 to August 2006 (the confidence bands represent the standard deviation).

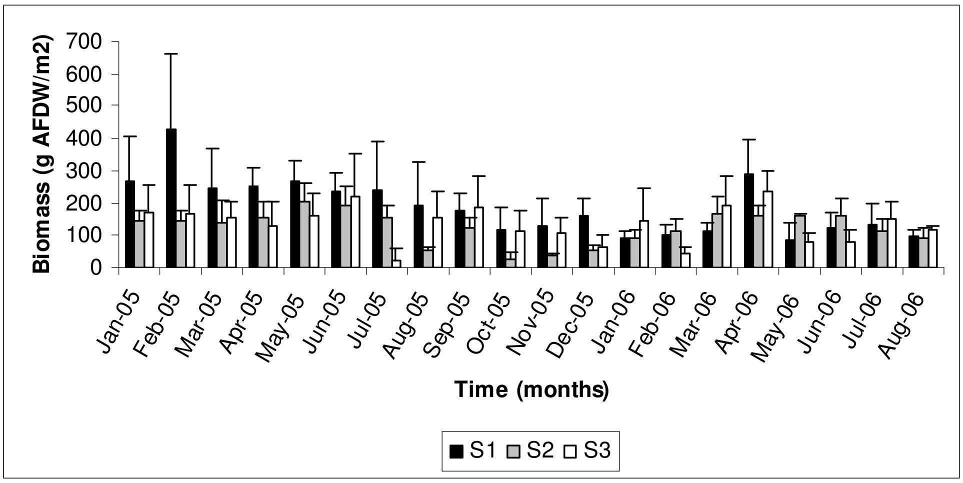

Fig. 6.1.4. Monthly variation of *C. fluminea* mean biomass (g AFDW/m^2) in the 3 sites from January 2005 to August 2006 (the confidence bands represent the standard deviation).

Growth and production

Size-frequency distributions were analysed for recognisable cohorts (Fig. 6.1.5.). In the 20 months of sampling, the presence of 18 different cohorts was observed. The biggest clam gathered in this study had a length of 41.83mm.

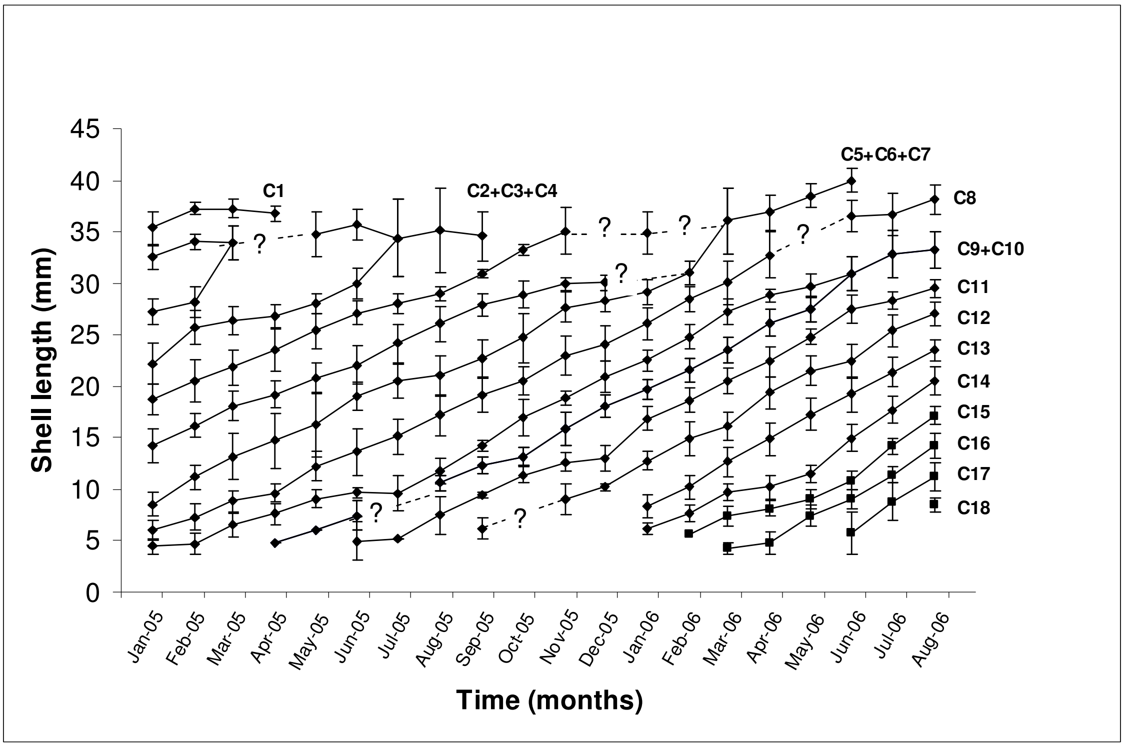

Fig. 6.1.5. Estimated growth of cohorts (mean shell length) from January 2005 to August 2006 (the confidence bands represent the standard deviation). Broken lines indicate probable evolutions.

Growth rates were estimated using the mean size of the recognizable cohorts identified. Growth was continuous throughout the life cycle and life span may vary between 24 and 36 months. A growth mathematical model (estimated with seasonal adjustment) was used on data derived from the recognizable cohort 5 + cohort 11 (Fig. 6.1.6.) and the growth parameters were: L_{∞} = 49.8 mm and K = 0.68.

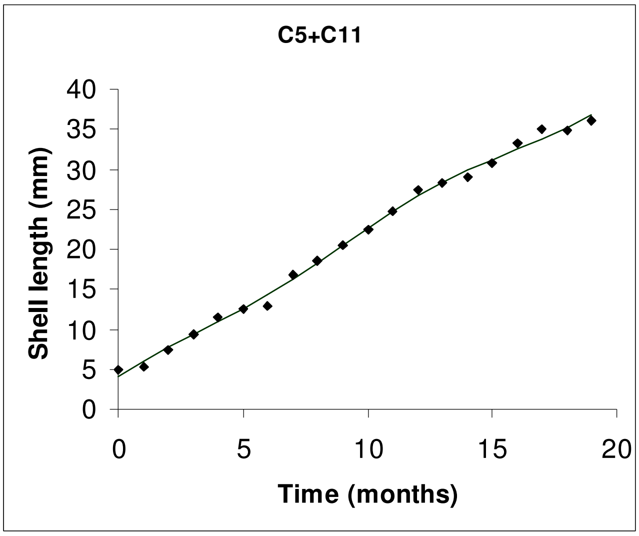

Fig. 6.1.6. Graphic adjustment of the growth curves of cohort 5 + cohort 11.

The relationship between biomass (B) and shell length (SL) was given by the following equation, where B is expressed as g AFDW and SL is expressed in mm:

$$B = 0.00001 \times SL^{3.2} \text{ (n=288; } r^2 = 0.98)$$

Monthly growth production ranged from 12.272 to 77.514 with a mean value of 41.100 g AFDW/m²/month (Fig.6.1.7.). The higher growth production was obtained in spring months in both years, with the exception of January 2005. Monthly elimination production ranged from 20.153 to 187.719 with a mean value of 72.631 g AFDW/m²/month (Fig. 6.1.7.).

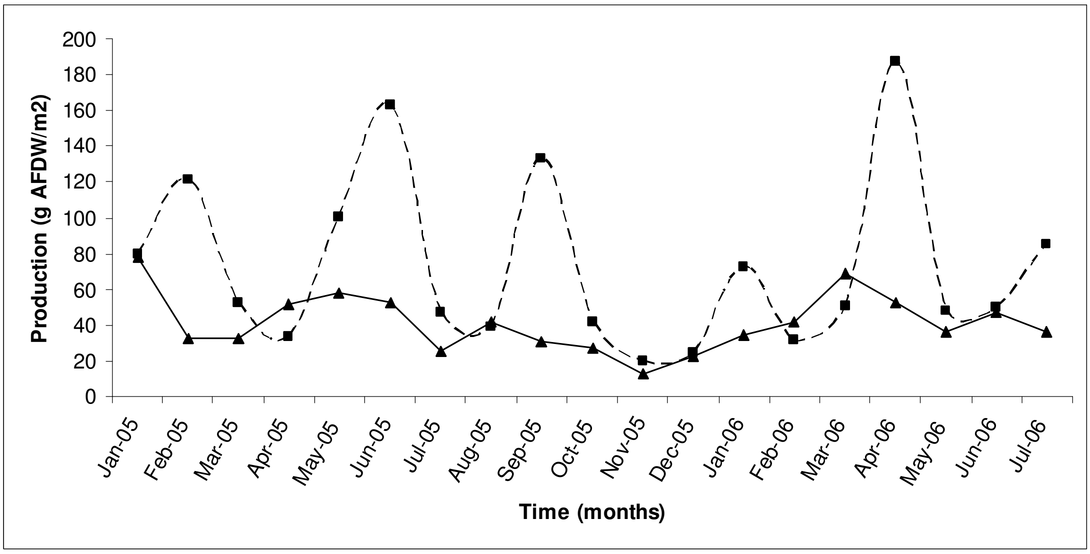

Fig. 6.1.7. Monthly variation of *C. fluminea* growth (▲) and elimination (■) production from January 2005 to August 2006.

For the elimination production, the higher values were also obtained in the spring months. Clear relationships were established between biomass and annual growth and elimination production estimated for each cohort (Fig. 6.1.8.).

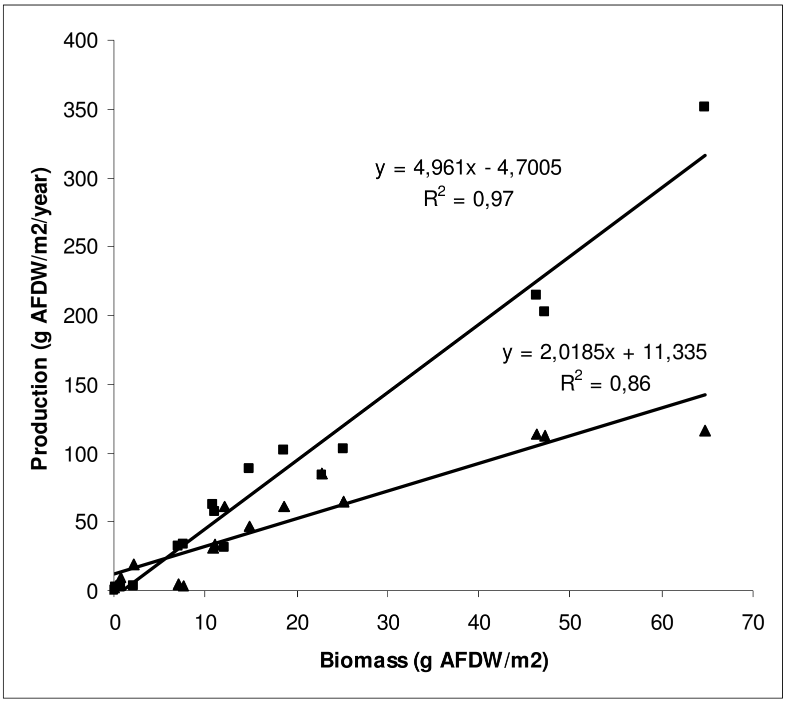

Fig. 6.1.8. Relationships between biomass and annual growth (▲) and elimination (■) production estimated for each *C. fluminea* cohort.

The annual 2005 growth production of *C. fluminea* estimated was 463.778 g AFDW/m^2/year, and the mean 2005 annual biomass was 160.651 g AFDW/m^2, resulting in a P/$\bar{B}$ ratio of 2.89/year and a turnover time of 126.4 days. The annual 2005 elimination production estimated was 855.219 g AFDW/m^2/year, resulting in an E/$\bar{B}$ ratio of 5.32/year. Comparing the secondary production results obtained in this study with values gathered in freshwater ecosystems in which total invertebrate (or high fraction of production) was estimated, we can observe that *C. fluminea* production in the years 2005 and 2006 correspond to the third and second higher values ever recorded, respectively (Fig. 6.1.9.). In addition, the values obtained in this study correspond to single species estimation and not to the total secondary production as the majority of the other studies.

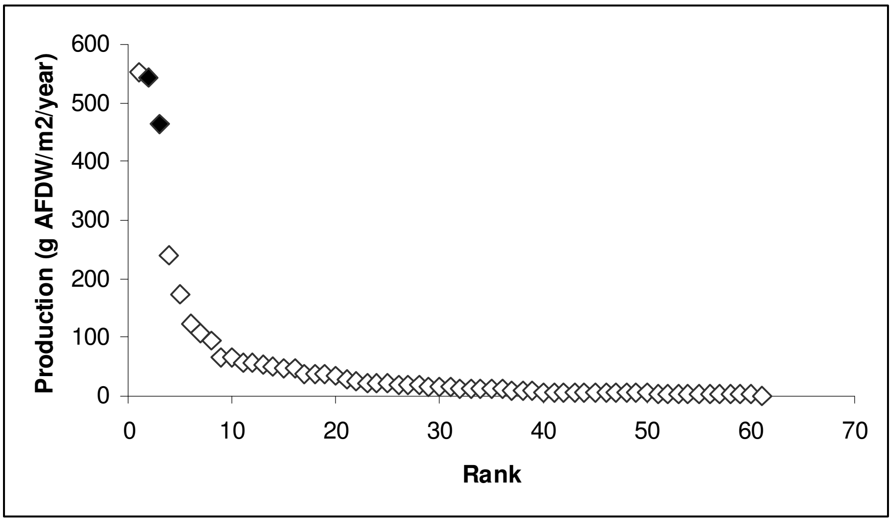

Fig. 6.1.9. Ranking of secondary production values in freshwater ecosystems in which total invertebrate (or high fraction of production) were estimated. Solid quadrates correspond to *C. fluminea* production in the years 2005 (lower value) and 2006 (higher value). Data from Benke (1993) and Hall *et al.* (2006).

Discussion

Abiotic characterization

Spatial differences in the abiotic conditions measured in the 3 sites were established. PCA analysis revealed differences in sediment characteristics and organic matter content among sites. Additionally, the three sites are under different human pressures: site 3 may be considered a clean area with little human impact; site 2 is located near a small agricultural area where fertilisers and pesticides are used and these may reach the river at least in some periods of the year; while site 1 is subjected to higher loads of organic contaminants (and possibly other contaminants such as heavy metals) transported from a River Minho tributary (River Louro). This situation resulted in higher nutrient concentrations measured in site 1 when compared with the other two upstream sites. It is therefore likely that the addition of these organic contaminants increased the nutrient load and reduced the dissolved oxygen concentrations in site 1.

Biotic characterization

The bivalve *C. fluminea* was always present in the 3 sampled sites and the population abundance and biomass showed spatial and temporal variations. In an earlier study, it was found that the biomass and abundance of this NIS in this estuarine ecosystem are well correlated with the abiotic factors, principally redox potential, nutrients, hardness, organic matter and sediment characteristics (Sousa *et al.*, 2008). Therefore, the abundance and biomass differences in the 3 sites may also be an answer to the different abiotic conditions available. The values of abundance and biomass presented by *C. fluminea* in the 3 sites were not significantly different from other sites already surveyed in this estuary (for a comparison see Sousa *et al.*, 2005, in press). However, the persistence of this high abundance (principally of adult specimens) and biomass for several years after the introduction is not common (Phelps, 1994); mean abundance is usually much lower, especially if large scale sampling as opposed to specific patches is carried out (Mouthon, 2001a and b; Mouthon & Parghentanian, 2004; Sousa *et al.*, 2006a and b). Indeed, this species can achieve high abundance and biomass in the first years of invasion but after that generally undergo a decline (Phelps, 1994). In the River Minho, the *C. fluminea* population has persisted in the ecosystem in high abundance and biomass for more than 15 years and there is no sign of recent decline (Sousa *et al.*, 2005, 2007c, 2008, in press, this study).

In the River Minho estuary, *C. fluminea* growth is continuous throughout its life cycle and its life span seems to oscillate between 24 and 36 months, which is similar to correspondent values that have been estimated for several populations worldwide (Britton & Morton, 1986; Mouthon & Parghentanian, 2004). However, there are several exceptions to this with some populations with shorter (Aldridge & McMahon, 1978; Hornbach, 1992) or longer (Cataldo & Boltovskoy, 1999; Mouthon 2001a and b) life spans. These differences are probably related to latitude, temperature, and available food resources. In this study the estimation of the life span was made indirectly since during the 20 months of sampling we were not able to follow a cohort from the beginning until the end. In addition, juveniles recently released from the parents (a true recruitment period) were not found. This situation was not caused by sampling error because in the same samples we found little juveniles from the bivalve *Pisidium amnicum* (chapter 6.3.). A possible explanation is that *C. fluminea* juveniles settle in sites not surveyed in this study, possibly in intertidal or very shallow areas with submerged vegetation, and after that migrate to deeper sites. Alternatively the recruitment sites may be located in upstream areas and the clams migrate downstream after a time. Due to great river discharge the migration of *C. fluminea* inside the estuarine area is possible, even for adults. These uncertainties do not allow us to estimate an accurate life span and also create some problems for the estimation of the growth rates due to the absence of small juveniles.

Growth and elimination production values gathered in this study can be considered to be extremely elevated for freshwater species (for comparisons see Benke (1993) and references therein) (Fig. 6.1.9.). Given these values two fundamental points have to be questioned: *i*) how the ecosystem supports so high *C. fluminea* abundance and biomass? and *ii*) what is the fate of this NIS production? To answer first question it is fundamental to know how large is the primary production based on phytoplankton, the *C. fluminea* consumption rates and whether this abundant population may be able to control primary production as already observed in populations from temperate waters (Phelps, 1994). In relation to primary production this estuary is classified as oligotrophic and has a low residence time (no more than 36 hours). Consequently, the primary production based on phytoplankton is probably far too low to support the *C. fluminea* population. The most plausible explanation is that this NIS explores additional food sources coming from the organic matter contained in the sediments which are rich in macroalgal detritus, submerged vegetation detritus, and bacteria, among others. This detritus may constitute large parts of the food available for *C. fluminea* and this organic matter can be the base of consumption for this NIS (Hakenkamp & Palmer, 1999). In relation to the *C. fluminea* consumption rates, several authors agree that this bivalve has one of the greatest filtration rates worldwide which also results in high assimilation rates (McMahon, 2002 and references therein). Given the abundance and biomass present in the River Minho TFWs it is possible that this NIS control the primary production and have extreme importance in the benthic processes. For the second question, several uncertainties exist about the possible predators that could exploit the high abundance and biomass present in this estuary. According to an earlier study, *C. fluminea* is responsible for almost 98% of the benthic biomass along the total estuarine gradient (Sousa *et al.*, in press). In theory, several species of birds, fishes and mammals could consume these clams. Indeed, stomach content analyses done in some fish species present in this estuarine ecosystem show that *C. fluminea* is consumed by *Platichthys flesus* and *Cobitis paludica* (Dias, unpublished data). Certainly, this large abundance and biomass is not fully exploited by the higher trophic levels and a great part goes directly to the detritus food-web. For example, in the summer of 2005, due to specific abiotic conditions (*e.g.* high temperatures, low dissolved oxygen, low redox potential, low flow conditions), great *C. fluminea* mortalities occurred and a great portion of biomass was not consumed and went directly to the sediments (Sousa *et al.*, 2007c, 2008).

Secondary production estimation is affected by numerous biotic variables and environmental conditions (Benke, 1993; Cusson & Bourget, 2005). According to these authors, benthic production and $P/\bar{B}$ ratios are known to be affected by life history characteristics such as population biomass and density, recruitment, age and life span. In addition, estuarine ecosystems offer a great diversity of substrates: from coarse to fine sediments and areas with or without submerged vegetation. These differences can, for example, influence the quantity and quality of food available for the organisms and be responsible for different production levels even at a small scale. Taking these factors into account, several cautions have to be engaged in order to generalize our production estimation for the entire River Minho TFWs and also take into account that these estimations tend to vary temporally. However, it is our opinion that these values can be considered conservative for several reasons. First, the mean biomass in the 3 sites sampled is comparable with mean values already gathered for all the River Minho TFWs (Sousa *et al.*, 2008, in press). Second, in 2005 a strong heatwave occurred which was responsible for the decrease of *C. fluminea* biomass (at least in sites rich in fine sediments and organic matter content as site 2 and 3 of this study). We hypothesised that this situation was responsible for decreases in the production, mainly in the second half of 2005. Indeed, and if we use data from 2006 (from January to August) to calculate the annual 2006 growth production we will have greater production values and a lower turnover time (P = 543.648 g AFDW/m^2/year; P/$\bar{B}$ = 4.05/year; turnover time of 90.2 days). This 2006 turnover time is in agreement with other studies and confirms that *C. fluminea* has the highest net production efficiencies recorded for any freshwater bivalve (McMahon, 2002). Third, mean 2005 biomass in all the River Minho TFWs [the 16 sites surveyed in Sousa *et al.* (2008)] was 99.1 g AFDW/m^2. However, in 2006 and 2007 this biomass was substantially higher with mean values for all the 16 sites of 121.4 and 161.0 g AFDW/m^2, respectively. Since high *C. fluminea* production was driven by high biomass, certainly the values of 2005 can be considered conservative given the great biomass of 2006 and 2007.

Ecological and conservational significance

The ecological importance of *C. fluminea* in the River Minho estuary is defined by its position in the food web, by the amount of energy flowing through this invasive population and the possible changes in ecosystem functioning which occurred after their introduction. Since *C. fluminea* is a filter and pedal feeder, it represents a direct link between pelagic primary production and higher trophic levels. Therefore, given the abundance, biomass and production of this NIS in the River Minho TFWs, *C. fluminea* can be regarded as a fundamental species in this estuarine ecosystem. A before–after control–impact is impossible to perform in order to quantify the *C. fluminea* invasion effects in the River Minho TFWs due to a complete lack of pre-invasion data and inexistence of suitable reference sites (Hall *et al.*, 2006). Indeed, there were no pre-invasion quantitative (only qualitative) data on invertebrate assemblages or primary production from which to estimate the impact of *C. fluminea*. Additionally, and since places located in the estuary are already colonised by this clam (Sousa *et al.*, 2005, 2007c, 2008, in press) potential control sites are only available in the tributaries which have completely different environmental and ecological conditions. Even considering these limitations, we suggest that impact has been large for several reasons: *i*) the vast majority of benthic invertebrate abundance and biomass was contributed by *C. fluminea* (Sousa *et al.*, 2007c, in press); *ii*) production was extremely high for freshwater ecosystems and can only be comparable to that of dominant taxa in highly eutrophic habitats (Benke, 1993 and references therein); *iii*) given the lower abundance and biomass in this estuary, production of native invertebrates is low [for example *P. amnicum* (chapter 6.3.)] and *iv*) great declines in abundance, biomass and spatial distribution occurred in several native molluscan species after *C. fluminea* invasion (Sousa *et al.*, 2007c, 2008, in press). Invasive animals do not usually form monocultures but their dominance might be an indicator of ecological impact within and between trophic levels (Hall *et al.*, 2006). In the particular case of the *C. fluminea* population present in the River Minho TFWs we have no doubts that the overall benthic production increased after the introduction. This situation may impact ecosystem functioning via the additional secondary production and possibly reduce the native production, at least in other mollusc species. Others non-indigenous bivalves that invaded aquatic ecosystems (*e.g. Dreissena polymorpha*, *Limnoperna fortunei, Corbula amurensis*) have also been described to impact the primary production due to high filter-feeding capacity, the secondary production, and are responsible for changes in the ecosystem processes and community structure (Nichols *et al.*, 1990; Kimmerer *et al.*, 1994; Caraco *et al.*, 1997; Strayer *et al.*, 1999; Strayer & Smith, 2001). Similar impacts are possible for *C. fluminea* and some studies have already shown alterations in the ecosystem processes (*e.g.* changes in the abiotic factors, changes in ecosystem engineering and facilitation processes and changes in biotic processes such as competition, predation and secondary production) (Phelps, 1994; Darrigran, 2002; Cantanhêde *et al.*, 2008; this study).

Conclusion

A proper estimation of impact from invasions is fundamental, as many NIS have negligible consequences while others will require management actions if they strongly affect ecosystem processes or economic interests (Hall *et al.*, 2006). Given the high abundance, biomass and secondary production by *C. fluminea* in the River Minho TFWs, preventing the spread and future introductions into aquatic ecosystems should be a conservational priority. Currently, there is no way to eradicate these clams and so the control is the only management option.

C. fluminea dominated the abundance, biomass and secondary production in River Minho TFWs and the same is potentially true for several worldwide aquatic ecosystems. They had among the highest secondary production values ever measured for an animal species colonising a freshwater ecosystem, and this situation may be responsible for changes in the benthic assemblages and/or in the ecosystem functioning. The mechanism behind their ecological dominance may be the higher competition ability, either because they are better at exploiting resources or because their abundance is so high that they interfere with the capacity of native invertebrates to acquire resources (Byers, 2000; Hall *et al.*, 2006). In addition, this species has an r-strategy life cycle (*e.g.* short life span, early maturity, rapid growth and high fecundity) which can be an advantage in ecosystems subject to periodical disturbances (McMahon, 2002). In fact, in this estuary *C. fluminea* rapidly recovered from the impacts caused by the 2005 heatwave but the native bivalves almost disappeared (Sousa *et al.*, 2007c, 2008).

Given the results of this study we conclude that *C. fluminea* is a fundamental element in the River Minho estuary and this NIS should be considered when modelling the nutrient cycles and energy flow in aquatic ecosystems where the species can reach high abundance and biomass.

6.2. Factors affecting *Pisidium amnicum* (Müller, 1774) (Bivalvia: Sphaeriidae) distribution in the River Minho estuary: consequences for their conservation

Submitted: Sousa R., Morais P., Antunes C. & Guilhermino L. submitted. Factors affecting *Pisidium amnicum* (Müller, 1774) (Bivalvia: Sphaeriidae) distribution in the River Minho estuary: consequences for their conservation. Estuaries and Coasts.

Abstract

The bivalve *Pisidium amnicum* (Müller, 1774) is a common species in several European freshwater ecosystems. However, few Iberian watersheds are colonized by this species and the River Minho estuary is possibly the Iberian aquatic ecosystem with the larger population. In October 2004, 2005, 2006 and 2007, investigations on spatial and temporal variations in *P. amnicum* abundance and biomass were carried out at sixteen sites along the River Minho tidal freshwater wetlands (TFWs). Mean abundance and biomass per site ranged from 0 to 750 ind./m^2 and 0 to 7.42 g AFDW/m^2, respectively. A clear decrease in the spatial distribution, abundance and biomass was observed during the 4 years assessment. Furthermore, a stepwise multiple regression model showed that organic matter and conductivity explained 50.2% of the variation in *P. amnicum* abundance (R^2 = 50.2%, $F_{[2,\ 15]}$ = 7.569, p = 0.005). Ecological knowledge is essential to the implementation of future conservation plans for *P. amnicum* and the results of this study are of paramount importance to identify habitats that should be protected in order to preserve this species and provide scientific reference that may be useful in the development of management and/or restoration plans.

Introduction

Threats to animal freshwater species (*e.g.* habitat loss, climate change, pollution, introduction of non-indigenous invasive species (NIS), presence of impoundments, flow regularisations, fisheries) are accelerating and are important enough to change ecosystems functioning and drive species to extinction (Dudgeon *et al.*, 2006). Indeed, the loss of biodiversity in freshwater ecosystems constitutes a relevant topic in international conservational studies and several works conducted in the last years claimed an urgent increase of interest on this subject (Dudgeon, 2000; Strayer *et al.*, 2004). Although biodiversity seems to be decreasing in freshwater ecosystems, there is still a general unawareness about this issue, which is much more evident when we are dealing with invertebrates that have a disproportional minor attention than vertebrates (Strayer, 2006). This apparent lack of concern might be disastrous because of the essential functions performed by invertebrate species in aquatic ecosystems (*e.g.* food resource to higher trophic levels, involvement in facilitation and ecosystem engineering processes, regulation of primary production, decomposition, water clarity, nutrient cycling).

Within the freshwater benthos, molluscs are fundamental (and threatened) taxa in terms of biodiversity, being also responsible for important ecological functions (Gutiérrez *et al.*, 2003; Spooner & Vaughn, 2006; Vaughn & Spooner, 2006). Despite their importance, the number of studies carried out with these animals continues to be scarce and numerous ecological gaps still exist. Nevertheless, since the 1990's a growing interest on these animals seems to be reappearing, mainly focusing on freshwater mussels. These remarkable organisms are now the core of conservational and ecological studies, particularly in North America (Lydeard *et al.*, 2004; Strayer *et al.*, 2004). Other freshwater bivalves such as the species that belong to the family Sphaeriidae also deserve special attention. The number of studies about these small bivalves is insufficient and as a result, the availability of information is still limited (Watson & Ormerod, 2005).

The Sphaeriidae *Pisidium amnicum* is a common freshwater European species, occurring mainly in the northern countries (Holopainen, 1979). This bivalve is described as having the European southern limit in the Iberian Peninsula, where the River Minho corresponds to its southernmost distribution limit. In fact, the larger Iberian population of this species was found in the River Minho tidal freshwater wetlands (TFWs) (Araujo *et al.*, 1999). *P. amnicum* is hermaphroditic, incubating their eggs in brood sacs inside the inner gills and each bivalve releases several juveniles, with nearly 2 mm, directly to the sediment (Dillon, 2000). The number of incubated larvae has a latitudinal difference. The southern populations (Portugal and Spain, River Minho) have a maximum of 73 (Araujo *et al.*, 1999), and the northern population has a maximum of 12 (Finland, lake Pääjärvi) (Holopainen, 1979). Their size and life span also have latitudinal distinctions (Holopainen, 1979; Araujo *et al.*, 1999).

One of the conservational problems identified by previous studies performed in the River Minho estuary, is the loss of native molluscan diversity, particularly after the introduction of the non-indigenous bivalve *Corbicula fluminea* (Müller, 1774) (Sousa *et al.*, 2005, 2007c, in press). The main goal of this study was to investigate the evolution of the abundance and biomass of *P. amnicum* between 2004 and 2007. This data was used to develop a model describing the relationship between abiotic factors and abundance of *C. fluminea* with abundance of *P. amnicum* in the River Minho TFWs. This information will be valid to identify environmental stressors and favourable abiotic conditions that control the presence of *P. amnicum* and to propose conservational measures for future native species reestablishment.

Material and methods

Study area

The River Minho estuary has a maximal length of 40km with a tidal freshwater portion of nearly 30km. This mesotidal estuary is partially mixed; however, during periods of high floods it tends to advance towards a salt wedge estuary (Sousa *et al.*, 2005). Its main environmental and ecological characteristics were object of several recent studies (Sousa *et al.*, 2005, 2007c, 2008, in press). Special attention has been paid to impacts on this estuarine ecosystem (*e.g.* habitat loss, introduction of NIS, climate change, flow regularization, fisheries, pollution) and on estuarine macrozoobenthic assemblages' distribution and their relation with the abiotic factors.

Sampling and laboratory analysis

Samples were collected at 16 sites along the River Minho TFWs (exactly the same sites of Fig. 5.1.1.) at high tides in October 2004, 2005, 2006 and 2007 (after the *P. amnicum* recruitment season). Five replicates for each site (one for sediment analysis and four for biological analysis) were collected with the help of a 0.05m^2 van Veen benthic grab (maximum volume of 0.005m^3) except for the 2004 samples, when three replicates were collected per site (one for sediment analysis and two for biological analysis).

At each site, water parameters temperature (T), conductivity (CND), total dissolved solids (TDS), redox potential (ORP), salinity (S), dissolved oxygen (DO) and pH were registered close to the bottom with the multiparameter probe YSI 6820.

Water samples were collected at intermediate depths to determine concentration of nitrites, nitrates, ammonia, phosphates and water hardness. They were kept at cool temperatures in the dark until subsequent laboratorial processing. The concentrations relative to each of these parameters were determined by colorimetric methods using the Palintest 270 standard photometer 7000.

Sediment granulometry, which was divided in six size classes [very coarse sand (VCS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS) and silt+clay (SC)] and the organic matter (OM) content of sediment were determined using the methodology described in Sousa *et al.* (2007b). All values are expressed as percentage of each sample weight.

Biological samples obtained from the 16 sites were sieved through a mesh of 500µm and *P. amnicum* specimens were sorted. The abundance was determined and the shell length of each specimen was measured with a digital calliper (± 0.01 mm). *P. amnicum* biomass was calculated using the Ash Free Dry Weight Method (AFDW) as in the methodology described in Sousa *et al.* (2006a). Abundance and biomass (AFDW) of the non-indigenous bivalve *C. fluminea* were also determined.

Statistical analysis

A cluster analysis was performed to the monthly River Minho inflow measured at Foz do Mouro hydrometric station (01G/02H) (Water Institute of Portugal - INAG) to determine the degree of annual river inflow, using the available data from 1990 onwards. The Ward's method was the chosen amalgamation rule and the joining clustering was applied using the dissimilarities or distances between variables to form the clusters. The Euclidean distance was used because the distances between any two objects are not affected by outliers (StatSoft, 2004).

Significance tests for variations in the abiotic factors measured between sites and years were undertaken using a two-way crossed ANOSIM2. Additionally, environmental characterisation of the area was performed using Principal Components Analysis (PCA), applied to the mean values measured during the four years. These analyses were performed with PRIMER 5.0 (Clarke & Warwick, 2001).

A two-way ANOVA followed by *a posteriori* Tukey HSD test was performed to check differences in *P. amnicum* abundance between sites and years, using SigmaStat 2.03. Only the 3 sites where the abundance of this bivalve was higher (sites 11, 12 and 15), were used to assess significant changes in abundance. Raw data was used in the two-way ANOVA analysis. In order to guarantee normal distribution and variance homogeneity data was $\sqrt{abun+1}$ transformed. A confidence interval of 95% was set and the power of the analysis was assessed with $\alpha = 0.05$.

A stepwise multiple regression was computed to estimate the coefficients of linear equation, involving the independent variables that best predict the value of *P. amnicum* abundance, using the software SPSS 15. To avoid violating assumptions underlying regression analysis and to directly test for the effect of environmental variables on *P. amnicum* abundance variation, the sites with zero *P. amnicum* abundance were removed (Jones & Ricciardi, 2005). For data analysis, bivalve abundance was $\log_{10}$ transformed to normalize and stabilize variances. Accordingly, each predictor variable was also observed in detail, to determine if a transformation improved their distribution. Thus, temperature (log.T), conductivity (log.CND), total dissolved solids (log.TDS), redox potential (log.ORP), salinity (log.S), dissolved oxygen (log.DO) pH (log.pH), nitrites (log.nitrites), nitrates (log.nitrates), ammonia (log.ammonia), phosphates (log.phosphates) and hardness (log.hardness) were $\log_{10}$ transformed. Variables in percentage (*i.e.* sediment granulometry: asi.VCS, asi.CS, asi.MS, asi.FS, asi.VFS, asi.SC; and organic matter: asi.OM) were arcsine transformed, as recommended by Zar (1999). In addition to the abiotic factors, abundance of *C. fluminea* was added to the analysis, as well as the type of hydrological year, i.e. very low, low, mean, high and very high river inflow, to which a numerical score was attributed varying between 1 and 5, respectively.

Results

Abiotic characterization

From January 2004 to December 2007, the river inflow varied between 133600 dam^3 (September 2005) and 2918399 dam^3 (December 2006) (Fig. 6.2.1.).

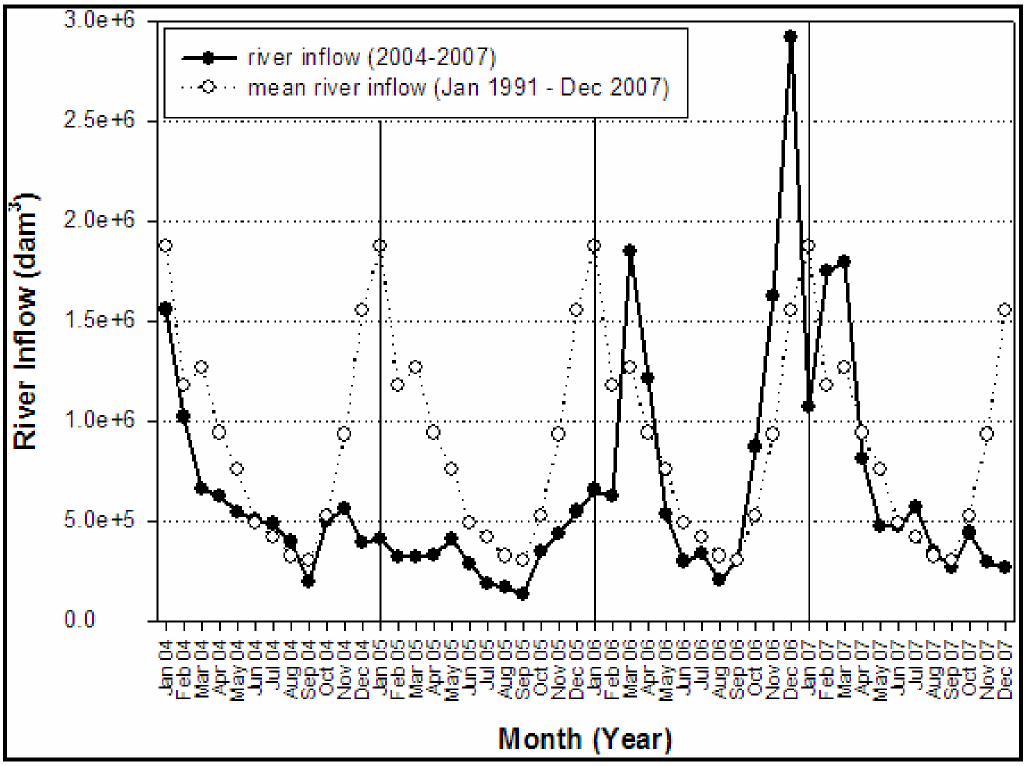

Fig. 6.2.1. Monthly variation of the river inflow measured at Foz do Mouro hydrometric station between January 2004 and December 2007. The annual river inflow based on mean data collected between January 1991 and December 2007 was also given.

The cluster analysis identified 2005 as a year of very low river inflow (score 1), 2004 and 2007 as low inflow years (score 2) and 2006 as a mean river inflow year (score 3) (Fig. 6.2.2.).

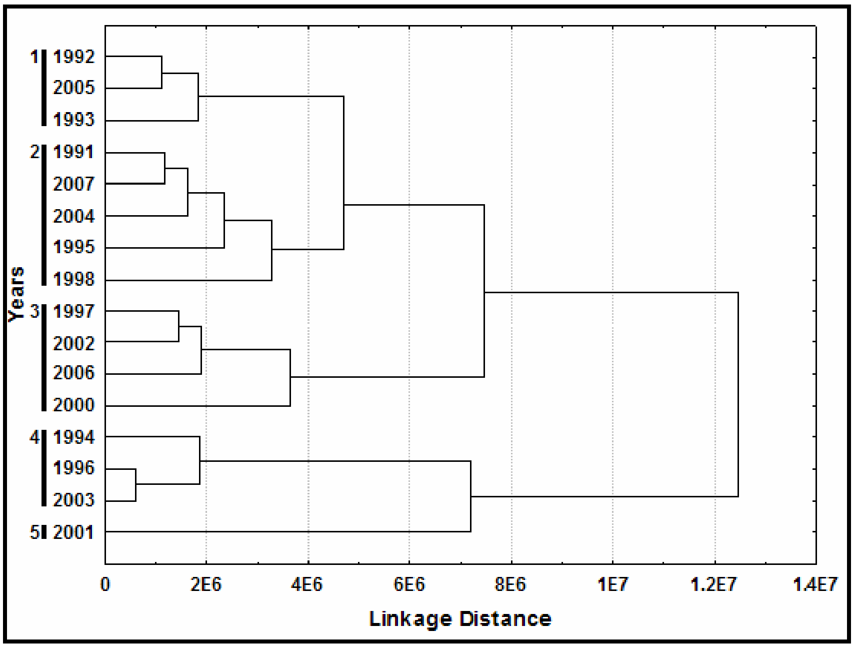

Figure 6.2.2. Cluster diagram applied to the annual river inflow measured at Foz do Mouro hydrometric station.

Appendix 6.2.1. shows the mean (±standard deviation) values for the abiotic factors measured at each site along the four years of sampling. The ANOSIM2 tests based on abiotic factors similarities showed significant differences between sites ($R = 0.582$; $P < 0.001$), but not between years ($R = 0.065$; $P = 0.103$). Thus, since the ANOSIM2 test did not detected differences between years, mean values were used in the PCA analysis (Fig. 6.2.3.). Three main areas were distinguished along the estuarine gradient: Group 1 - comprising sites 1 to 5; Group 2 - comprising sites 6 to 12; and Group 3 - comprising sites 13 to 16. These groups appeared distributed along an abiotic gradient, from the lower to the upper estuarine areas. Along the PC1, the Group 1 could be distinguished (sites 1 to 5) as it was characterised by high values of conductivity, salinity and total dissolved solids. In this estuarine area, sandier sites with low organic matter content were predominant, except for the site 2, which presented fine sediments and high level of organic matter. Along the PC2, the Group 2 could be distinguished (sites 6 to 12) as it was characterised by fine deposits with high organic matter content. In addition to this, the Group 2 showed peak concentrations of nitrates, nitrites, ammonia and phosphates at site 9. Finally, the Group 3 (sites 13 to 16) was characterised by sandier deposits with low organic matter, low nutrient concentrations and low tidal influence.

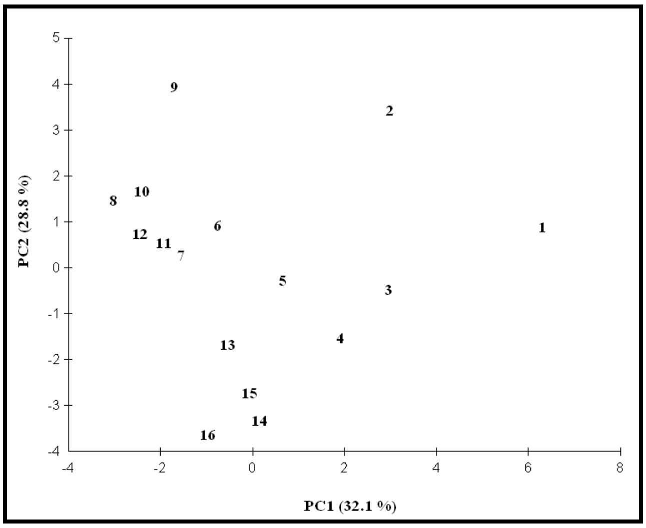

Fig. 6.2.3. Principal Component Analysis (PCA) showing the plotting of the 16 sites. The percentage of variability explained by the principal axes is given.

Biotic characterization

P. amnicum was only collected in areas upstream to site 5. Mean abundance per site ranged between 0 and 750 ind./m^2 (site 12, 2004) (Fig. 6.2.4. A) and mean biomass ranged between 0 and 7.42 g AFDW/m^2 (site 12, 2004) (Fig. 6.2.4. B).

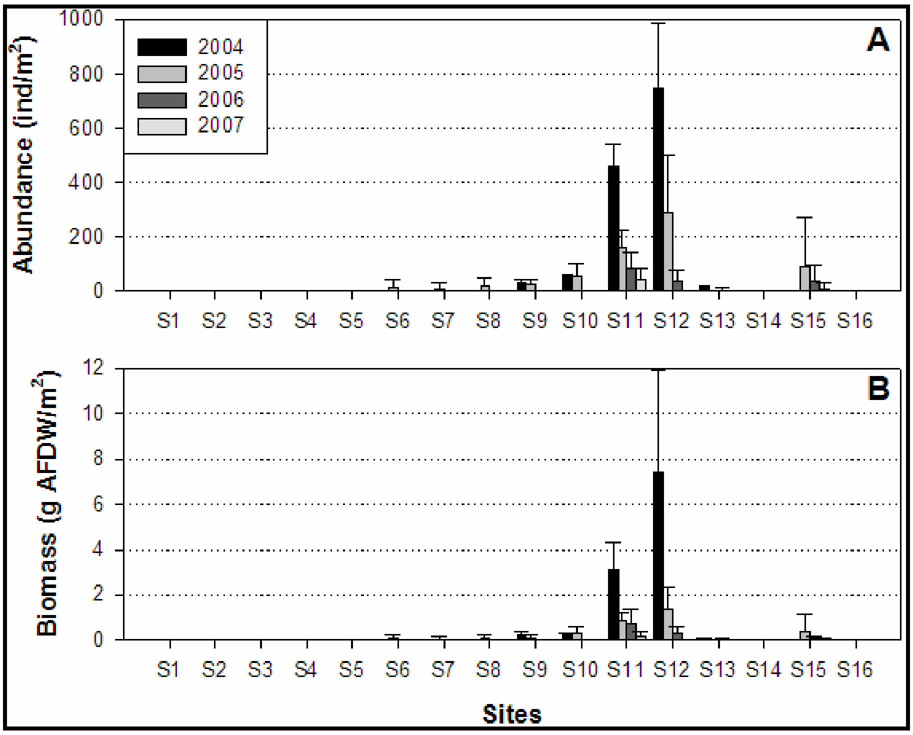

Fig. 6.2.4. Annual and spatial variation of *P. amnicum* mean abundance (ind./m^2) (A) and mean biomass (g AFDW/m^2) (B). The confidence bands represent the standard deviation.

There were significant differences in the mean value of *P. amnicum* abundance between years (P < 0.001), sites (P < 0.001) and “Year *vs.* Site” (P < 0.001) in the 3 sites where the abundance was higher - sites 11, 12 and 15 (Table 6.2.1).

Table 6.2.1. Results of two-way ANOVA tests for differences in *P. amnicum* abundance between sites and years.

Source of variation	df	SS	MS	F	P
Year	3	885.789	295.263	14.827	< 0.001
Site	2	659.853	329.927	16.568	< 0.001
Year x Site	6	653.032	108.839	5.466	< 0.001
Residual	30	597.399	19.913		
Total	41	2.558.008	62.390		

There was a significant difference between 2004 and 2006 (P < 0.001) and 2007 (P < 0.001) and between 2005 and 2006 (P = 0.050) and 2007 (P < 0.001) (Table 6.2.2).

Table 6.2.2. Results of Tukey-tests for differences in *P. amnicum* abundance between years.

Comparison	Diff of means	P	Q	P
2004 *vs.* 2005	5.162	4	3.272	0.118
2004 *vs.* 2006	9.993	4	6.334	< 0.001
2004 *vs.* 2007	13.468	4	8.573	< 0.001
2005 *vs.* 2006	4.831	4	3.750	0.050
2005 *vs.* 2007	8.306	4	6.448	< 0.001
2006 *vs.* 2007	3.475	4	2.698	0.246

There was also a global significant difference between sites 15 and 11 (P < 0.001) and 12 (P < 0.001) (Table 6.2.3.).

Table 6.2.3. Results of Tukey-tests for differences in *P. amnicum* abundance between sites.

Comparision	Diff of means	P	Q	P
Site 11 *vs.* Site 12	0.343	3	0.275	0.979
Site 11 *vs.* Site 15	8.617	3	6.909	< 0.001
Site 12 *vs.* Site 15	8.960	3	7.184	< 0.001

Within each site, it was possible to identify significant differences between 2004 and 2006 ($p = 0.014$) and 2007 ($p = 0.002$) at site 11. At the site 12, significant differences were detected between all years ($p < 0.036$), except between 2006 and 2007 ($p = 0.475$). At the site 15, no significant differences were detected between years ($p > 0.653$) (Table 6.2.4.).

Table 6.2.4. Results of Tukey-tests for differences in *P. amnicum* abundance between years in sites 11, 12 and 15.

Comparision	**Diff of means**	**P**	**Q**	**P**
Site 11				
2004 *vs.* 2005	8.918	4	3.264	0.119
2004 *vs.* 2006	12.642	4	4.626	0.014
2004 *vs.* 2007	15.530	4	5.683	0.002
2005 *vs.* 2006	3.724	4	1.669	0.644
2005 *vs.* 2007	6.611	4	2.963	0.178
2006 *vs.* 2007	2.887	4	1.294	0.797
Site 12				
2004 *vs.* 2005	11.068	4	4.050	0.036
2004 *vs.* 2006	21.628	4	7.915	< 0.001
2004 *vs.* 2007	26.226	4	9.597	< 0.001
2005 *vs.* 2006	10.559	4	4.733	0.011
2005 *vs.* 2007	15.158	4	6.793	< 0.001
2006 *vs.* 2007	4.598	4	2.061	0.475
Site 15				
2004 *vs.* 2005	4.500	4	1.647	0.653
2004 *vs.* 2006	4.291	4	1.570	0.686
2004 *vs.* 2007	1.351	4	0.494	0.985
2005 *vs.* 2006	0.209	4	0.094	1
2005 *vs.* 2007	3.149	4	1.411	0.752
2006 *vs.* 2007	2.941	4	1.318	0.788

The multiple stepwise regression model showed that organic matter (expressed as asiOM) and conductivity (expressed as logCND) were the independent variables that best explained the variation of *P. amnicum* abundance (expressed as logAbundance) ($R^2 = 50.2\%$, $F_{[2, 15]} = 7.569$, $p = 0.005$) (Table 6.2.5.).

Table 6.2.5. Stepwise multiple regression models developed to predict (log) *P. amnicum* abundance across sites from two independent predictor variables, (asi)OM and (log)CND ($R^2 = 0.502$, $F_{[2, 15]} = 7.569$, p = 0.005). The respective ANOVA results are also shown.

Model	B	St. Error	Beta	T	P	r^2
Constant	5.986	2.111		2.836	0.013	0.502
asi. OM	5.987	1.920	0.568	3.117	0.007	
Log CND	-2.422	1.072	-0.412	-2.260	0.039	

Source of variation	SS	Df	MS	F	P
Regression	2.625	2	1.312	7.569	0.005
Residual	2.601	15	0.173		
Total	5.225	17			

The asiOM explained 29.9% of the variation of *P. amnicum* $\log_{10}$ abundance and showed a positive coefficient, thus its abundance was greater for higher values of OM (Fig. 6.2.5. A). The logCND explains 19.1% of *P. amnicum* $\log_{10}$ abundance variation (Fig. 6.2.5. B) and a negative relationship was established.

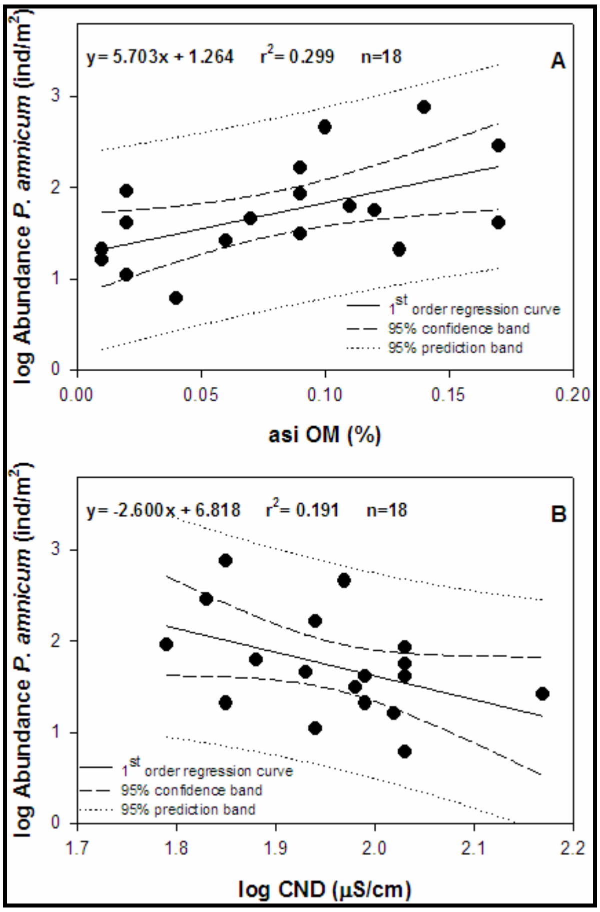

Fig. 6.2.5. Relationship between *P. amnicum* abundance (log transformed) and organic matter content (asi transformed) (A) and conductivity (log transformed) (B).

Discussion

Abiotic characterization

In the last 17 years, three years (1992, 1993 and 2005) recorded very low river inflow. In 2005, a severe drought occurred in Portugal, causing serious deleterious impacts on many estuarine communities including planktonic (Marques *et al.*, 2007), benthic (Cardoso, Raffaelli & Pardal, in press) and ichthyic (Dolbeth *et al.*, 2007, Martinho *et al.* 2007). This drought probably affected the abundance of *P. amnicum* in the River Minho TFWs (see below).

The PCA analysis revealed a clear spatial pattern along the estuarine area, discriminating three main groups, which are in accordance with earlier studies (Sousa *et al.*, 2008, in press). This spatial pattern is related with different sediment characteristics and with a clear estuarine gradient, mainly related with salinity, conductivity and total dissolved solids. Nutrient concentrations also support the discrimination of the Group 2 (presenting higher values) from the others. In detail, Group 1 has greater tidal influence and sandier deposits; Group 2 is probably associated with increased organic pollution due to the influence of a River Minho tributary (River Louro) and is richer in finer sediments and organic matter content; finally, Group 3 is less polluted and have residual tidal influence.

Biotic characterization and ecological and conservational significance

P. amnicum preferentially colonises lakes and rivers that do not present considerable seasonal hydrological oscillations, being a common species in lowland areas and limnetic portions of estuarine ecosystems (Killeen *et al.*, 2004). In accordance with this, the results of the present study showed higher values of abundance and biomass in the upstream areas of the River Minho estuary. The results of the stepwise multiple regression model combining abiotic data, *C. fluminea* abundance and *P. amnicum* abundance indicated that sites with high organic matter and low conductivity supported higher abundances. Organic matter is likely to be an important factor influencing the distribution of *P. amnicum* because this clam utilises pedal feeding to extract organic matter from the interstitial water and sediment, which can be rich in macroalgae detritus, submerged vegetation detritus and bacteria (Holopainen, 1979; Hakenkamp & Palmer, 1999; Hakenkamp *et al.*, 2001; Vaughn & Hakenkamp, 2001). Hence, sites with high organic matter content can sustain high abundance and biomass of *P. amnicum*. Furthermore, one should observe the correlation existing between organic matter and fine sediments (data not shown). So, sediment characteristics could be also important in the distribution of this benthic species, a fact already established for other freshwater bivalves (Mellina & Rasmussen, 1994; Jones & Ricciardi, 2005; Sousa *et al.*, 2008). In this study, conductivity was negatively correlated with *P. amnicum* abundance and this species seems to prefer the upper estuarine areas where conductivity is lower because of a decreased tidal influence. In fact, high conductivity can be responsible for gross osmoregulatory problems and changes in the ionic ratios of freshwater molluscs (Dillon, 2000).

Although organic matter and conductivity appear to be important to the distribution of *P. amnicum* in the River Minho TFWs, it could also be influenced by other abiotic factors not assessed in this study. For instance, current velocity could exert potential influence in juveniles' distribution and migration rates. Nevertheless, sediment granulometry reflect the general hydrological conditions, including current velocity. In addition to this, biotic factors such as predation, competition, parasitism and presence of submerged vegetation may also affect the distribution of *P. amnicum*. Future studies should examine these abiotic and biotic factors and possibly add them to the stepwise multiple regression model. On the other hand, since this study was confined to one Iberian aquatic ecosystem and some results may be restricted solely to the River Minho TFWs, caution should be taken when applying this model to other areas, as this population possibly corresponds to the southernmost distribution limit and these TFWs are almost completely dominated by *C. fluminea*.

During the execution of this study, *P. amnicum* could not be found in the lower areas of the River Minho TFWs (sites 1 to 5). At the sites 6, 7 and 8, its abundance was very low. However, previous studies, report the occurrence of this mollusc in all TFWs until the 1990s (Nobre, 1941; Araujo *et al.*, 1993, 1999). Thus, *P. amnicum* appears to have suffered a dramatic reduction of its spatial distribution since this time. The reasons behind this fact are yet to be investigated. In addition, these earlier studies only registered the presence/absence of *P. amnicum* and the abiotic characterization was very poor, which makes the evaluation of the ecological and environmental changes rather speculative. Pollution and loss of habitat do not seem to represent important pressures in this estuarine area. In contrast, this estuary has been suffering the impacts caused by the introduction of several NIS, particularly *C. fluminea* (Araujo *et al.*, 1993; Sousa *et al.*, 2005, 2007c, 2008, in press). Although this invasive clam could be affecting the abundance, biomass and spatial distribution of *P. amnicum*; up to now, no study investigated the existence of competition between these two bivalves or other kind of interspecific relationship that could result in the decline of the indigenous species. However, a previous study showed a clear decrease in the abundance and distribution of *P. amnicum*, mainly observed in the lower areas of the River Minho TFWs, concomitant with the introduction and rapid expansion of *C. fluminea* in 1989 (Araujo *et al.*, 1993). Another alternative or cumulative hypothesis is a possible increase of conductivity due to decrease in the river discharge. This situation is probably an outcome of dams' construction and climate change and might extend the influence of sea water to upstream areas. Since the occurrence of this clam is negatively correlated with conductivity, the possible increase in the levels of this abiotic factor might have intensified the environmental stress on this population.

A rapid reduction of the *P. amnicum* abundance and biomass was noticed during the last 4 years. The pressures imposed by the strong drought of 2005 probably were responsible for this decline. The alterations in the abiotic features caused by this drought included: low flow, high water temperatures, low level of dissolved oxygen and decrease in the redox potential (Sousa *et al*., 2007c, 2008, in press). These environmental changes were responsible for substantial mortality of benthic species, including *P. amnicum* (Sousa *et al*., 2007c). The synergistic effects of these abiotic alterations together with the fast recover of the NIS *C. fluminea* probably had detrimental effects on the local *P. amnicum* population. These two bivalves have different life cycle strategies and *C. fluminea* could have taken advantage over *P. amnicum* when these changes occurred, as this invasive clam presents high reproductive rates, a typical characteristic of species with r-strategy life cycle (McMahon, 2002). Indeed, a unique Asian clam can release almost 70 000 live offspring/year, while *P. amnicum* releases no more than a few dozens (Araujo *et al*., 1999; Keller *et al*., 2007). Furthermore, after the impact the system would be rapidly occupied by *C. fluminea* possibly interfering with the success of *P. amnicum* recruitment. The current data shows a significant decline of *P. amnicum* abundance during the last 4 years. Since there were no signs of recovery from this dwindling trend, the extirpation of *P. amnicum* from the River Minho basin is a scenario to be taken into consideration.

In the last years, special attention has been paid to the decrease of the biodiversity and the consequences of that on the ecosystem functioning (Daily *et al*., 2000; Loreau *et al*., 2002; Covich *et al*., 2004). The theory states that the ecosystem functioning is less affected by the loss of species in systems with great biodiversity than in systems with low biodiversity (Tilman, 1999; Yachi & Loreau, 1999). Considering the River Minho TFWs does not shelter a great biodiversity, the disappearance of *P. amnicum* from this system could cause the loss of important functions and services provided by this organism (*e.g.* food resource for higher trophic levels, bioturbation of the top layer of the sediments, nutrient cycling through excretion, biodeposition of faeces and pseudofaeces, shells can be important for ecosystem engineering processes) (Holopainen, 1979; Vaughn & Hakenkamp, 2001). The multiple regression model explaining *P. amnicum* abundance identify the upstream areas of River Minho estuary, with high organic matter content and low conductivity, as habitats that should be preserved. These habitats are of vital importance not only for *P. amnicum*, but also for several other species that occur in the same patches, which include freshwater mussels (*Psilunio litorallis*, *Anodonta anatina*, *Unio pictorum*) and gastropods (*Lymnaea peregra*, *Bithynia tentaculata*, *Valvata piscinalis*, *Ancylus fluviatilis*) (Sousa *et al*., 2007c; in press).

Conclusion

Until around twenty years ago, *P. amnicum* colonized all the River Minho TFWs. Nowadays, this species has almost totally disappeared from the lower estuarine areas, subsisting in small patches located mainly in the upper limit of the tidal influence, in very low abundance and biomass. The reasons that may have caused this declining trend are not clear. Nevertheless, the introduction of *C. fluminea* may be posing a serious threat to this indigenous bivalve, probably by direct competition (*i.e. P. amnicum* is restricted to sites having high organic matter content where the probable competition between the two species for food resources is lower) or by altering the ecological characteristics of this ecosystem. In addition, the synergistic effects produced by climate change and decrease in the river discharge may have also contributed to this decline.

The results of this study enhanced the knowledge on *P. amnicum* ecology and provided scientific reference that may be important for future conservation plans and of paramount importance to define habitats that should be protected. This information is essential in the development of management and/or restoration plans to be implemented in the River Minho TFWs.

6.3. Growth and production of *Pisidium amnicum* (Müller, 1774) in the freshwater tidal area of the River Minho estuary

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Abstract

Pisidium amnicum (Müller, 1774) is an important indigenous species in the freshwater tidal portion of the River Minho estuary and until the introduction of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774) was the most abundant bivalve in this ecosystem. Between January 2005 and August 2006 monthly samples were collected in three sites to study the abundance, biomass, growth and production of *P. amnicum*. Results indicate that *P. amnicum* has continuous growth throughout its life span and growth rates were higher during the early phases of development, coincident with the spring and summer months. Life span estimated was 24 months. The annual 2005 growth production of *P. amnicum* estimated was 2.339 g AFDW/m^2/year, and the mean annual biomass was 1.594 g AFDW/m^2, resulting in a $P/\bar{B}$ ratio of 1.47/year and a turnover time of 248.7 days. The annual 2005 elimination production estimated was 7.541 g AFDW/m^2/year, resulting in an $E/\bar{B}$ ratio of 4.73/year. According to these results we conclude that *P. amnicum* continues to play an important role in the food web in areas where it coexists with *C. fluminea*. Previous studies showed declines in the indigenous population after the introduction of *C. fluminea* and currently, areas of high abundance are restricted to small patches in the upper limit of the tidal influence. Consequently, *P. amnicum* is now under considerable pressure and this situation requires ecological and conservational attention.

Introduction

Over the last decades ecological studies done in aquatic ecosystems increased enormously. However, tidal freshwater wetlands (TFWs) seem to be a forgotten area of research where marine and/or freshwater ecologists continue to ignore the extremely important functions completed by these estuarine ecosystems (Strayer *et al.*, 1994; Ewel *et al.*, 2001; Findlay *et al.*, 2006).

Despite the relevance of bivalves in freshwater ecosystems, a lack of knowledge about their ecology still exists, in particular for species belonging to the Sphaeriidae family (Watson & Ormerod, 2005). This results in a significant uncertainty about the ecological role of this family in aquatic ecosystems. Nevertheless, several of these species are ubiquitous and have great ecological importance. For example, they are a food resource for higher trophic levels, contribute to the bioturbation of sediments, contribute to nutrient cycling, and create suitable habitats for other organisms that use their shells (Holopainen, 1979; Vaughn & Hakenkamp, 2001). Probably, the major reason for the low number of studies focusing on this family is the small size of these animals, making difficult the reconnaissance of their relevance in freshwater ecosystems. In contrast, the number of research studies that focus on freshwater bivalves of the families Margaritiferidae and Unionidae has increased in the last years. These freshwater mussel species have been decreasing in an exceptional rate and, principally in North America, they are now object of special conservational efforts (Lydeard *et al.*, 2004; Strayer *et al.*, 2004). In contrast, bivalves from the Sphaeriidae family have not attained this conservational status even while they may be facing a considerable extinction pressure. In this context, there is a strong need to study such organisms so that scientific information may contribute to reverse the decline presently shown by some species.

The clam *Pisidium amnicum* (Müller, 1774) is common throughout northern Europe and is a characteristic freshwater species. The southern limit of its geographic distribution seems to be the River Minho watershed [possible exceptions are populations from North Africa described by Kuiper (1972) and cited in Araujo *et al.* (1999)]. Despite its ubiquity, few studies on *P. amnicum* ecology exist and, therefore, the information available is scarce. Furthermore, in some ecosystems, this bivalve is suffering the effects of the interaction with non-indigenous invasive species (NIS). For example, *P. amnicum* had a great spatial distribution and abundance in the River Minho TFWs until the introduction of the NIS *Corbicula fluminea* (Müller, 1774) in 1989 (Araujo *et al.*, 1993; 1999). After the introduction of this NIS, however, its distribution suffered an extensive reduction.

Considering the relevance of *P. amnicum* in the River Minho TFWs and that this is likely the southern limit of its European distribution, it is very important to understand its role in the ecosystem. Therefore, the central objective of this study was to estimate the abundance, biomass, growth and production (growth and elimination) of *P. amnicum*.

Material and methods

Study area and sampling analysis

The characterization of the River Minho hydrological basin, with a particular emphasis to the molluscan fauna existent in the freshwater tidal area is described in Sousa *et al.* (2005, 2007c, 2008, in press).

The sampling strategy and analysis follow exactly the same procedure described in chapter 6.1., including the sites location.

Growth and production estimations

Growth and production estimates follow exactly the same procedure already described in chapter 6.1.

Data analysis

Principal Component Analysis (PCA) was carried out for ordination of sampling sites based on the abiotic factors measured using the CANOCO package (ter Braak & Smilauer, 2002). Abiotic factors were transformed using the log(X+1) as part of the analysis.

A length–biomass regression was used to estimate *P. amnicum* AFDW.

Results

Abiotic characterization

The abiotic characterization is described in chapter 6.1.

Abundance and biomass

P. amnicum abundance and biomass is shown on a monthly basis at the 3 sites in Figures 6.3.1. and 6.3.2., respectively. Mean abundance was 208, 234 and 384 ind./m^2 for the sites 1, 2 and 3, respectively. Minimum abundance was observed in site 1 in February 2005 and February and August 2006 with 24 ind./m^2, while the maximal abundance was 1144 ind./m^2 observed at site 1 in June of 2005. Mean biomass was 0.756, 1.310 and 2.255 g AFDW/m^2 for sites 1, 2 and 3, respectively. The minimum biomass observed was for site 1 in February of 2005 with 0.057 g AFDW/m^2 and a maximum of 5.466 g AFDW/m^2 in site 3 in February of 2005.

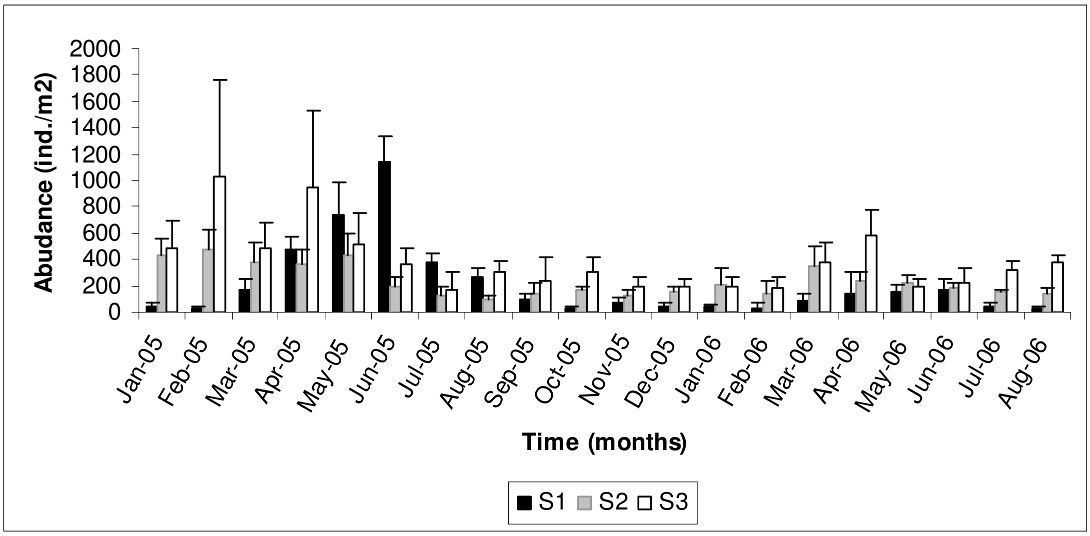


Fig. 6.3.1. Monthly variation of *P. amnicum* mean abundance (ind./m^2) in the 3 sites from January 2005 to August 2006 (the confidence bands represent the standard deviation).

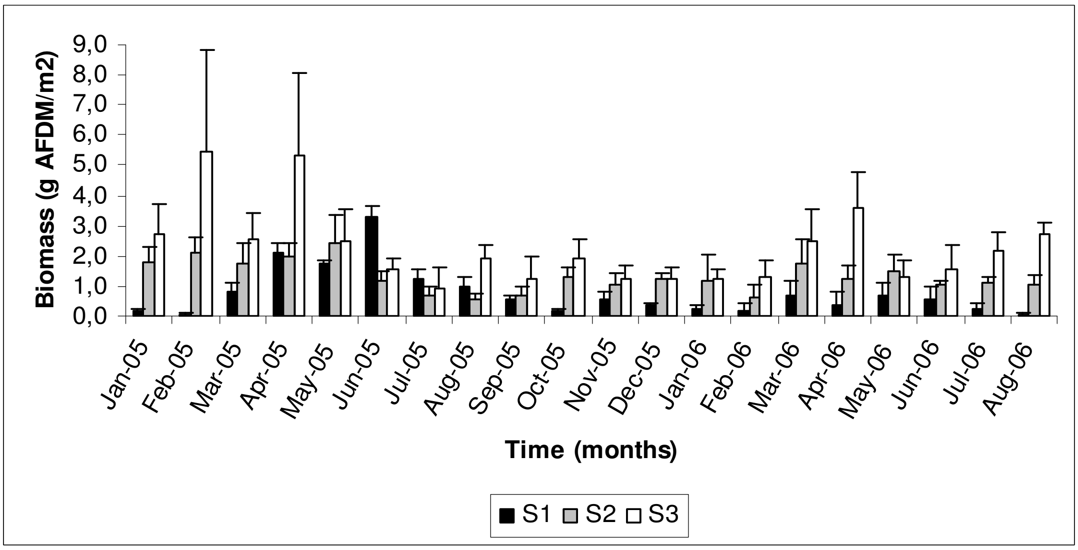


Fig. 6.3.2. Monthly variation of *P. amnicum* mean biomass (g AFDW/m^2) in the 3 sites from January 2005 to August 2006 (the confidence bands represent the standard deviation).

P. amnicum abundance and biomass were spatially heterogeneous, which is potentially a reflection of the different abiotic conditions. In addition, this bivalve also showed clear patterns of seasonal abundance and biomass variations. Maximum abundance and biomass were observed in spring and summer. Juveniles mostly appeared in March/April in both years suggesting that birth occurs in February/March. This recruitment period seems to be responsible for the higher abundance gathered in the spring and summer months. During this time massive mortality of the oldest individuals (8-10 mm long) was observed. It should also be noted that recruitment was significantly higher in 2005 relative to 2006.

Growth and production

Size-frequency distributions were analysed for recognisable cohorts (Fig. 6.3.3.). In the 20 months of sampling, the presence of 14 different cohorts was observed and the biggest clam had a length of 10.23 mm.

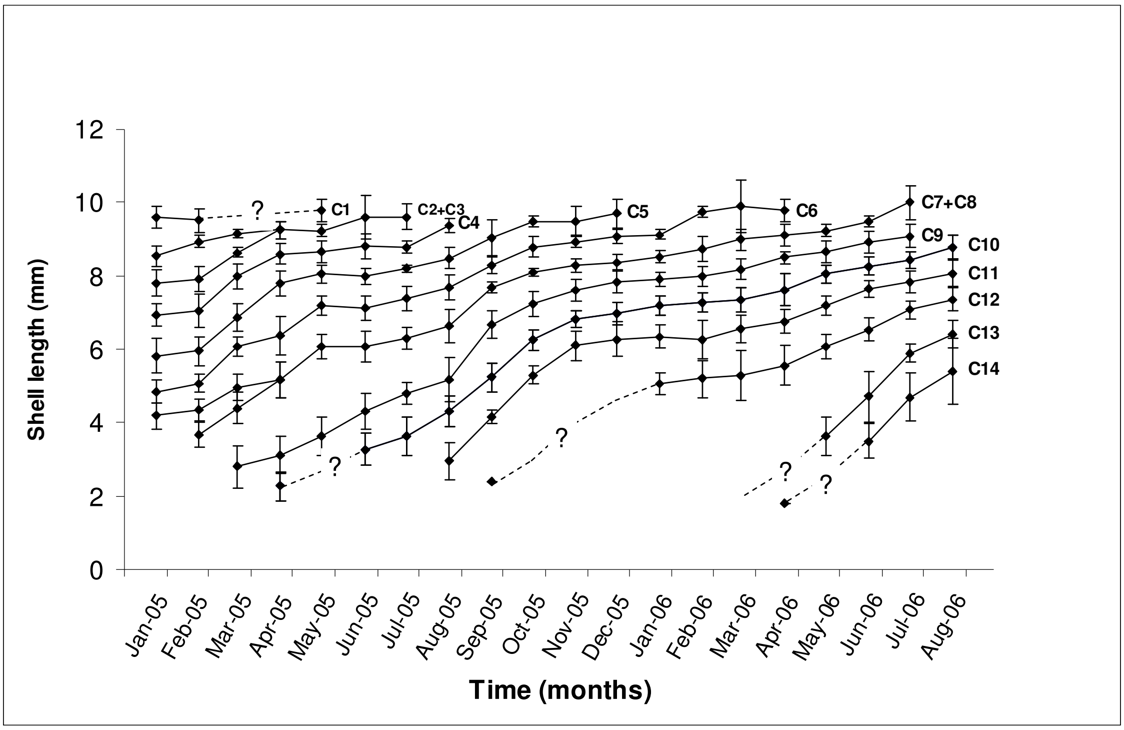
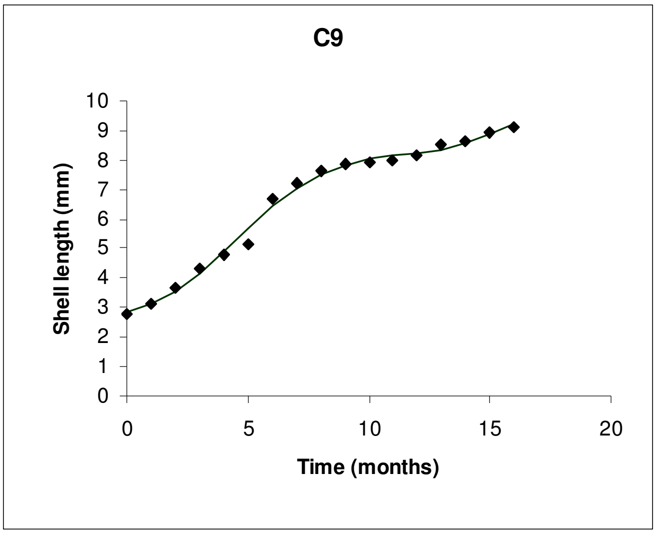
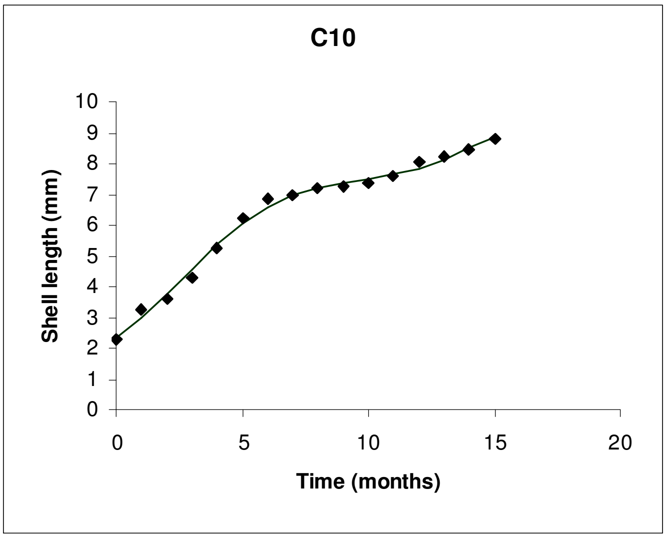


Fig. 6.3.3. Estimated growth of cohorts (mean shell length) from January 2005 to August 2006 (the confidence bands represent the standard deviation). Broken lines indicate probable evolutions.

Growth rates were estimated using the mean size of the recognizable cohorts identified. Cessation of growth was not observed in this population, however, growth rates varied with clam age and were highest during early phases of development. Life span was estimated to be 24 months. A mathematical model to estimate growth patterns (adjusted for seasonal differences in growth) was used to determine the recognizable cohorts 9 (Fig. 6.3.4.a) and 10 (Fig. 6.3.4.b). Equations used to generate these cohorts are described in Table 6.3.1.



a)



b)

Fig. 6.3.4. Graphic adjustment of the growth curves of cohorts 9 (a) and 10 (b).

Table 6.3.1. Application of a growth mathematical model (estimated with seasonal adjustment) to cohorts C9 and C10 data.

Cohort	L_{oo}	T_0	K	C	Ts	SS	r^2
C9	13.09	-0.37	0.75	0.70	0.44	0.50	0.99
C10	12.50	-0.37	0.78	0.64	0.30	0.40	0.99

The relationship between total length and biomass (B) was given by the following equation:

B (g AFDW) = 0.0001*Shell length (mm)$^{2.0684}$ (n = 288; R^2 = 0.97)

Monthly growth production ranged from 0.029 to 0.561 with a mean value of 0.158 g AFDW/m²/month (Fig. 6.3.5.). The higher growth production was obtained in spring months and was related with the presence of high abundance of juveniles. However, a clear difference in the growth production values between 2005 and 2006 can be observed, with lower values in 2006. Indeed, the growth production from January to August of 2005 was almost 3 times higher than the corresponding period of 2006. Monthly elimination production ranged from 0.044 to 2.455 with a mean value of 0.509 g AFDW/m²/month (Fig. 6.3.5.).

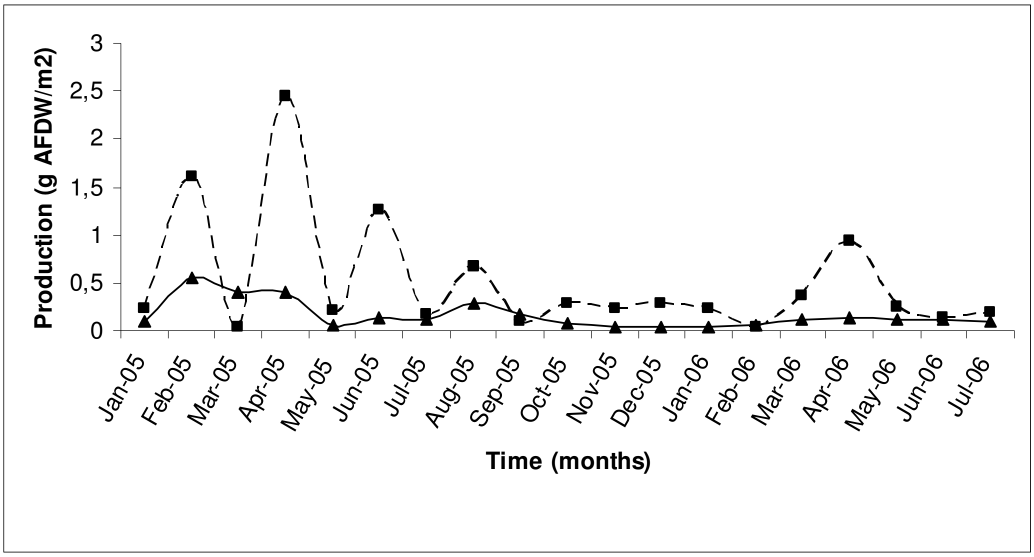


Fig. 6.3.5. Monthly variation of *P. amnicum* growth (▲) and elimination (■) production from January 2005 to August 2006.

For the elimination production, the higher values were obtained in the spring months. It should be noted that the highest value of 2005 (April) was related to the disappearance of the oldest (and larger) individuals during this period. Clear relationships were established between biomass and annual growth and elimination production estimated for each cohort (Fig. 6.3.6.).

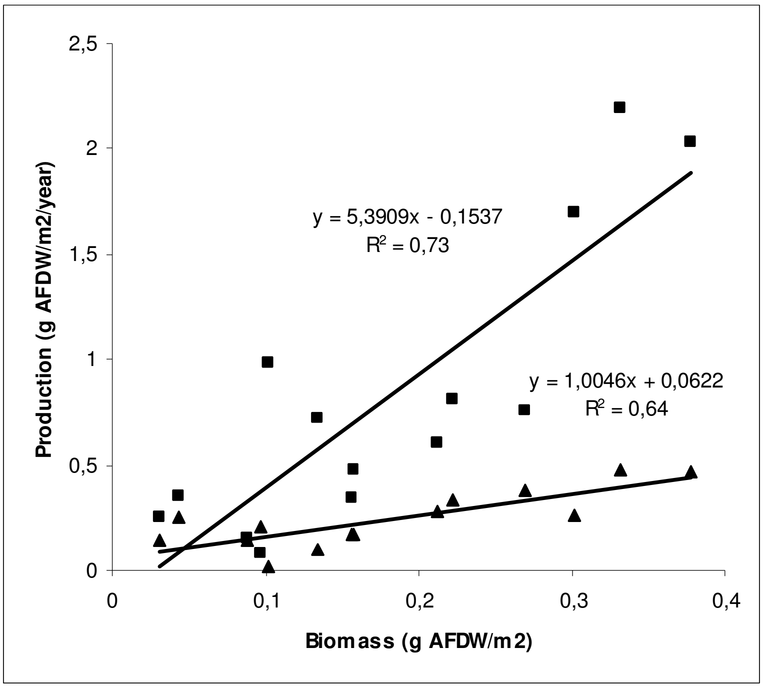


Fig. 6.3.6. Relationships between biomass and annual growth (▲) and elimination (■) production estimated for each *P. amnicum* cohort.

The annual 2005 growth production of *P. amnicum* estimated was 2.339 g AFDW/m²/year, and the mean 2005 annual biomass was 1.594 g AFDW/m², resulting in a $P/\bar{B}$ ratio of 1.47/year and a turnover time of 248.7 days. The annual 2005 elimination production estimated was 7.541 g AFDW/m²/year, resulting in an $E/\bar{B}$ ratio of 4.73/year.

Discussion

Abiotic characterization

The discussion about the abiotic characterization is in chapter 6.1.

Biotic characterisation and ecological and conservational significance

The bivalve *P. amnicum* was present throughout the study period at the 3 sites and marked differences were observed in its abundance and biomass in both space and in time. Nevertheless, this bivalve has much lower abundance and biomass in downstream estuarine areas not surveyed in this study (Sousa *et al.*, 2005). Indeed, the choice of the 3 sites location was based on previous studies that found high abundance of *P. amnicum* in these 3 sites (Sousa *et al.*, 2005). Due to its patchy distribution, the overall mean abundance and biomass for all the TFWs is likely much reduced compared to values observed at these three study sites.

Observed spatial variations may be related to different abiotic or biotic conditions at the 3 sites. Indeed, specimens from the *Pisidium* genus are known to have great spatial distributional variations even in a very small scale (Holopainen, 1979). In addition to the differences in granulometry and nutrient concentrations found in the 3 sites, *P. amnicum* abundance and biomass may respond to the different nutritional resources of the sediment and water column, flow rates, competition, presence of submerged vegetation, predation, and other factors. Anyway a stepwise multiple regression model showed that organic matter content and conductivity are important factors explaining the variation in *P. amnicum* abundance inside the River Minho estuary (chapter 6.2.).

Maximum abundance and biomass of *P. amnicum* were observed in spring and early summer due to the contribution of small juveniles that appeared after the recruitment period. In a study conducted in the same estuary in 1990 and 1991 (Araujo *et al.*, 1999), *P. amnicum* was found to exhibit a reproductive cycle with births and juvenile recruitment occurring in April-May, when water temperatures ranged from 15 to 20ºC. In our study, recruitment occurred almost two months earlier (February-March) than that found by Araujo *et al.* (1999). This situation may be related to increasing temperatures in the early spring months that have been recorded over the last decade, as suggested by the comparison of water temperature between the two studies showing higher values in March and April of 2005 and 2006 than in the corresponding months of 1991. Increases in temperature may be responsible for the earlier onset of recruitment found in our study. A similar situation was described for several species in temperate regions (Bradshaw & Holzapfel, 2006 and references therein). Additionally, in other European published studies on *P. amnicum* ecology (Danneel & Hinz, 1976; Bass 1979; Holopainen, 1979; Araujo *et al.*, 1999) newborns always appear in the spring with temporal differences depending on the latitude. Southern populations have earlier recruitment periods and the temperature seems to be an important factor responsible for larvae release in this bivalve (Araujo *et al.*, 1999).

Despite the seasonal variation in abundance and biomass characteristic of most aquatic benthic species from the temperate regions, a clear difference in the abundance gathered in the years 2005 and 2006 was found. Even considering a possible effect of some missing data in 2006, it is evident that the number of recruits is considerably lower in 2006 than in 2005. One factor that likely contributed to the lower recruitment observed in the second year of this study was the extreme high temperatures associated with the heatwave of 2005. Indeed, data from this and other studies conducted in the River Minho TFWs clearly show that benthic species were strongly affected by the abiotic conditions of the 2005 summer (Sousa *et al.*, 2007c, 2008). As a consequence of severe drought circumstances in 2005, the abiotic conditions experienced by organisms were hostile.

Increases in temperature and low river discharge resulted in decreases in the oxygen and redox potential which provoked the massive mortality of benthic species. Massive mortalities occurred, firstly in *C. fluminea* and after that in all the benthic species, including *P. amnicum* (Sousa *et al.*, 2007c, 2008). This occurrence was especially significant in sites with fine sediments and high organic matter content (for example, site 3 of this study). These stressful environmental conditions were not only responsible for high mortalities in 2005, but were likely also the cause of reduced reproduction in the *P. amnicum* in subsequent years. Indeed, the 2007 *P. amnicum* spatial distribution and abundance was even lower, with an almost complete disappearance of this bivalve in this estuarine area (chapter 6.2.). The effects of the heatwave resulted in an abundance and biomass decrease of the *C. fluminea* population but this species rapidly recover in the next year. In contrast, the heatwave caused ecological problems for the other molluscan species including freshwater mussels such as *Psilunio littoralis*, *Anodonta anatina*, *Unio pictorum*, species from the *Pisidium* genus and several gastropod species (Sousa *et al.*, 2007c).

Data from earlier studies performed in this estuary show a clear reduction in the *P. amnicum* spatial distribution in the last two decades, principally in the TFWs located downstream of site 1. However, this qualitative data lacks specific information on the abundance and biomass before the introduction of *C. fluminea* (the first record of this species in the River Minho was 1989). Notably, several studies conducted in the beginning of the 20th century until the 1990s show that *P. amnicum* was a common inhabitant throughout the TFWs and not restricted to the upper estuarine areas as it is today (Nobre, 1941; Araujo *et al.*, 1999). Despite the lack of quantitative data supporting the hypotheses that the introduction of *C. fluminea* was the responsible for the disappearance of *P. amnicum* (*e.g.* by direct competition) in the downstream areas of the River Minho TFWs, evidence sustains the idea that the observed reduction in the distribution of the indigenous species throughout the estuary coincides with the simultaneous expansion of the NIS. Indeed, competition by invasive species is not suppose to guide a indigenous competitor to extinction but in several cases, including molluscs, a enormous decrease in abundance and biomass have been described (Byers, 2000). In addition, the huge abundance and biomass attained by *C. fluminea* were responsible for several changes in ecosystem functioning (for a review see Sousa *et al.*, 2008). These changes may have also contributed to the decline in population size of several indigenous species, including *P. amnicum* (Sousa *et al.*, 2005, 2007c, 2008, in press).

Growth was continuous throughout the life cycle with a clear acceleration in the first months, which were coincident with the spring and summer months. This is typical for a benthic species in a temperate region. Certainly, benthic species from these latitudes have superior growth rates in spring and summer that correlate with favourable abiotic conditions and availability of food resources. *P. amnicum* lives in the superficial bottom sediments and obtains nutritional resources by filtering food from the sediment and the interstitial water and certainly take advantage of favourable ecological conditions existent in the spring and summer months (Holopainen, 1979). The life span of this bivalve was estimated as about 24 months and this finding is in accordance with Holopainen (1979) who suggests that *P. amnicum* live several years with one parturition time per year. In contrast, Araujo *et al.* (1999), with data also collected in the River Minho estuary in 1990 and 1991, suggests that *P. amnicum* had a life span of 15 months with a unique reproductive cycle. However this study only had 12 months and possibly the authors underestimate the *P. amnicum* life span.

In relation to growth production, higher values found in spring and summer are probably related to increased temperature and metabolism, and also with the availability of more food resources. The high elimination production estimated in this study could be related with elevated mortality rates which were associated with high mortalities of older (and larger) individuals occurred after reproductive efforts (spring months) and high mortalities that occurred in response to the extreme conditions during the 2005 heatwave and the winter months. In addition, close relationships were established between biomass and growth and elimination production estimated for each recognized cohort. These results could be used in future studies in this ecosystem and with some cautions could be also applied to other *P. amnicum* populations.

Values of growth and elimination productions estimated in this study could not be directly compared with other results, since we were not able to found any estimation of *P. amnicum* production in the literature. Anyway, the 2005 annual growth production to biomass ratio estimated in this study is higher than the already found for the species *P. casertanum* and *P. conventus* in Finland with values of 1.3 and 1.0/year, respectively (Holopainen, 1979).

Conclusion

Lack of quantitative ecological studies done with species from the Sphaeriidae family, including *P. amnicum*, does not allow a full comparison of our data with North European populations. However, this study provides critical information on *P. amnicum* growth and elimination production and about the ecological role of this species in the aquatic food web. Additionally, as a result of several human impacts (*e.g.* habitat loss, introduction of NIS, pollution, climate change and flow regularizations) this species is under considerable threat. In the particular case of the River Minho TFWs, the impacts imposed by the introduction of the NIS *C. fluminea* seem to have caused a considerable reduction in the spatial distribution of *P. amnicum* population in the last twenty years. Furthermore, the synergistic effects of the extreme heatwave of 2005 seem to have amplified the rate of population decline and until now the population did not recover from this additional threat. Since the patches colonised by this species are retreating enormously this may have consequences for the secondary production of this ecosystem. While the introduction of *C. fluminea* increased the overall secondary production (see chapter 6.1.), the replacement of *P. amnicum* by a NIS has substantially altered the trophic structure with possible consequences across the entire estuarine community. Therefore, this situation deserves higher attention since TFWs are extremely important habitats from the environmental and trophic dynamics point of view and future conservational measures that could be applied have to confer a special emphasis to population dynamics.

6.4. River Minho tidal freshwater wetlands: faunal biodiversity at stake?

Submitted: Sousa R., Dias S., Guilhermino L. & Antunes C. submitted. River Minho tidal freshwater wetlands: faunal biodiversity at stake? Aquatic Biology.

Abstract

Tidal freshwater wetlands (TFWs) support characteristic species composition and perform fundamental environmental and ecological functions. Despite this fact, the number of studies conducted in this type of habitat is scarce and very fragmented. In this paper, an overview of the River Minho TFWs' diversity and their conservation state is provided, including temporal comparisons documenting fish and macrozoobenthic (principally molluscs) declines. We also discuss how this study can be an example of the actual ecological and conservational conditions of TFWs and the alarming actual rates of biodiversity losses. Probably, other TFWs areas with comparable characteristics are under a similar unexpected risk and, therefore, they should also be considered for conservation purposes. Finally, the principal threats to this estuarine ecosystem are discussed and some practices that should be implemented to reverse this situation are indicated.

Introduction

Estuaries are critical transition zones that link land, freshwater habitats, and the sea (Day *et al.*, 1989; Herman *et al.*, 1999). These areas are usually recognised by their high productivity and the presence of habitats with potential conservational interest. Simultaneously, a considerable number of estuarine ecosystems represent some of the most human-degraded habitat types on Earth (Mclusky, 1999; Levin *et al.*, 2001). These areas were originally selected for human settlement due to proximity of fresh water sources, prevalence of fertile soils for agriculture, high fish, crustacean and shellfish productivity, and good accessibility via rivers to upstream regions (Edgar *et al.*, 2000). As they expand, these human agglomerations were responsible for an increasing number of industries, growth of ports and massive urban developments. As expected, this concentration of human activities has caused considerable environmental alterations and compromised the good condition of the natural estuarine communities.

Recently, the health of coastal and estuarine ecosystems entered the agendas of environmental managers and politicians. Several practices have been undertaken in an attempt to reduce the pressure on these systems allowing the return to natural conditions or at least to improve the present situation. Certainly, the pristine conditions will never be achieved but a great improvement is still possible. The European Union Directive 2000/60/EC (Water Framework Directive) is a good illustration of the efforts and financial investments that are now being conducted in order to reverse the situation (Borja *et al.*, 2004). In fact, there were improvements in the water quality of several aquatic ecosystems after the adoption of restoration practices (Eertman *et al.*, 2002; Van den Bergh *et al.*, 2005; Borja *et al.*, 2006). However, this progress may not be sufficient to enable the return of the original biological community since several new impacts are currently responsible for significant environmental and ecological alterations in estuarine communities (*e.g.* biological invasions, climate change). These new challenges will mark the political and scientific agenda in the near future (Ricciardi & MacIsaac, 2000; Sala *et al.*, 2000).

One of the most neglected habitats inside estuarine ecosystems in terms of study is constituted by tidal freshwater wetlands (TFWs). These limnetic areas have been subjected to few multidisciplinary studies when compared, for example, with brackish areas (Rundle *et al.*, 1998; Attrill & Rundle, 2002; Findlay *et al.*, 2006). However, TFWs are fundamental ecosystems comprising essential habitats for several species with a high commercial and/or conservational importance. Furthermore, TFWs are the connection between true freshwater ecosystems and the adjacent marine areas and are subjected to a mosaic of different abiotic conditions responsible for great heterogeneities, allowing their colonisation by a diverse fauna. In addition, these ecosystems are recognised as essential passages for commercial and conservational important migratory fish species; they offer nursery conditions or refuges from predators for juvenile fish, molluscs and crustaceans; they provide essential feeding and drinking grounds for an important number of birds; and the presence of submerged and riparian vegetation create several ideal habitats for a high number of both vertebrate and invertebrate species (Levin *et al.*, 2001; Durell *et al.*, 2005; West *et al.*, 2005; Findlay *et al.*, 2006).

In this paper, data from the River Minho TFWs are provided and discussed as an example of biodiversity loss. Special attention was given to significant temporal differences observed in the abundance, biomass and diversity of animal species (principally molluscs and fishes) and several hypotheses that may have been contributing to the present situation are discussed.

Material and methods

Study area

The River Minho originates in Serra da Meira, in the province of Lugo (Spain) and drains into the Atlantic Ocean. It is more than 300km long, of which the last 70km comprises its international section (the natural boarder between Portugal and Spain). Its hydrological basin has an area of 17080km^2, 95% of which located in Spain and only 5% in Portugal.

The River Minho estuary is located in the NW of the Iberian Peninsula and has a maximum width of just over 2km. This mesotidal estuary is partially mixed, however, during the period of high floods it tends to evolve towards a salt wedge estuary (Sousa *et al.*, 2005). The influence of spring tides extends approximately 40km upstream, and the TFWs are located in the upper 30km. These TFWs includes biotypes with mobile and rocky substrata, favourable to the occurrence of various types of organisms. Rocky substrata are, essentially, represented by river walls and rocky landscapes. Biotypes with mobile substrata occur in intertidal and subtidal areas and comprise areas close to the riverbanks, extensive sand and mud flats, inlets which form small bays and as marsh existing in the margins and on the various islands. The River Minho estuary is part of a Natura 2000 site which includes all the international section of the river. In the last years several studies, especially focused on macroinvertebrates and fish species, were carried out in this estuary to characterize the main environmental and ecological processes (Sousa *et al.*, 2005, 2007c, 2008, in press).

Sampling and Data analysis

The literature containing abiotic and biotic data for the area under study was compiled as thoroughly as possible.

The current abiotic characterization of the River Minho TFWs was made using two different strategies: samples gathered in 16 sites (Fig. 5.1.1.) along the total area of the River Minho TFWs in October of 2004, 2005, 2006 and 2007 and monthly samples taken in the sites 9, 11 and 12 (Fig. 5.1.1.) from January to December 2005. At each site, the water parameters temperature (T), conductivity (CND), total dissolved solids (TDS), redox potential (ORP), salinity (S), dissolved oxygen (DO) and pH were registered close to the bottom with a multiparameter probe YSI 6820. Water samples were also collected at intermediate depth to determine the concentration of nitrites, nitrates, ammonia, phosphates and water hardness. The concentrations of these parameters were determined by colorimetric methods using the Palintest 270 standard photometer 7000. The sediment granulometry, which was divided in six size classes [very coarse sand (VCS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS) and silt+clay (SC)] and the organic matter (OM) content of sediment were determined using the methodology described in Sousa *et al.* (2007b). In addition, the monthly River Minho inflow measured at Foz do Mouro hydrometric station (01G/02H) (INAG, www.inag.pt) was used to determine the degree of annual river inflow, using the available data from 1990 onwards.

Qualitative data available for the distribution of mollusc species collected at the beginning of the 20th century (Nobre, 1941), qualitative and quantitative data about the macrozoobenthic estuarine community distribution collected in 1977 and 1978 (Baños, 1978) and 1989 (Maze *et al.*, 1993) and qualitative data collected in 1990 and 1991 dealing with molluscan fauna (Araujo *et al.*, 1993; 1999) were used as baseline information about the assemblages colonising the River Minho TFWs and offer a general benchmark for the macrozoobenthic community that existed along the 20th century. These earlier studies were compared with data gathered in 2004, 2005, 2006 and 2007 in the same 16 sites (Fig. 5.1.1.) indicated for the abiotic characterization (Sousa *et al.*, 2005, 2007c, 2008, in press).

Some qualitative data on fish species were obtained from Antunes & Weber (1996), Antunes & Rodrigues (2004), Tola & Infiesta (2002) and also from unpublished studies carried out by the authors. Quantitative information on fish species abundance is scarce. Fisheries within the estuary include a vast part of TFWs and so were employed as indicative of temporal variations. Fisheries data were obtained from the official Portuguese authorities' statistics, and were used assuming a constant error in fisherman catch declarations along the years. It was not possible to calculate effective fishing effort since a single license concerns different target species and catches values correspond to a total fishing season per species and so, yearly total catch values are presented. According to the data available (from the early 1980s onwards) the number of registered licensed boats has slightly decreased but in an irregular trend, with a mean of 469 boats (minimum of 391 and maximum of 562).

Results

Abiotic Characterization

Unfortunately, the earlier studies done in the River Minho TFWs have an irregular and incomplete abiotic characterization. So, the characterization of the River Minho TFWs is restricted to recent studies (Sousa *et al.*, 2005, 2007c, 2008, in press) and a general description is in Appendices 6.2.1. and 6.4.1. The sites sampled are distributed along a physical and chemical gradient, from the lower to the upper estuarine areas. Downstream sites are characterized by higher values of conductivity, salinity and total dissolved solids compared with the upstream sites, which is consistent with the more pronounced influence of adjacent marine conditions. There is a great spatial variation in the granulometry and organic matter content of the sediments along the TFWs. In relation to concentrations of nitrates, nitrites, ammonia and phosphates, the values reported can be considered lower when compared to other estuarine areas. However, the values may increase in some areas, in particular in the vicinity of agricultural fields and in one tributary (River Louro) with higher organic pollution.

The location of the TFWs within the estuary depends on the balance between water volume and velocity of the incoming tide and the discharge of the out-flowing river. Given the high seasonal oscillation in the river discharge (INAG, www.inag.pt) the limits of the TFWs are highly variable: during high discharge, the tidal limit may be much further downstream than during low discharge.

The available data for pollutants showed low concentrations. For example, previous studies reported total concentrations of 17 different polybrominated diphenyl ethers congeners in sediments in four different sites located along the international river section (one site located in the adjacent coastal area) ranging from 0.3 to 4.8 ng g^{-1} (Lacorte *et al.*, 2003). Recent data also showed low heavy metals concentration (Cd, Cr, Cu, Ni, Pb and Zn) measured in the sediments and *Corbicula fluminea* (Müller, 1774) tissues in 6 different sites (sites 1, 4, 9, 11, 12 and 13 in Fig. 5.1.1.) along the TFWs (Sousa *et al.*, unpublished).

Macrozoobenthic decline

The comparison of earlier (Nobre, 1941; Baños, 1978; Araujo *et al.*, 1993, 1999) with recent (Sousa *et al.*, 2005, 2007c, 2008, in press) macrozoobenthic studies showed differences principally in the molluscan composition. In Table 6.4.1., the molluscan community that could be easily found 20 years ago is shown and compared with data collected in several surveys performed recently. It should be noted that the effort of sampling was much higher in the studies performed in the last four years than in the earlier ones.

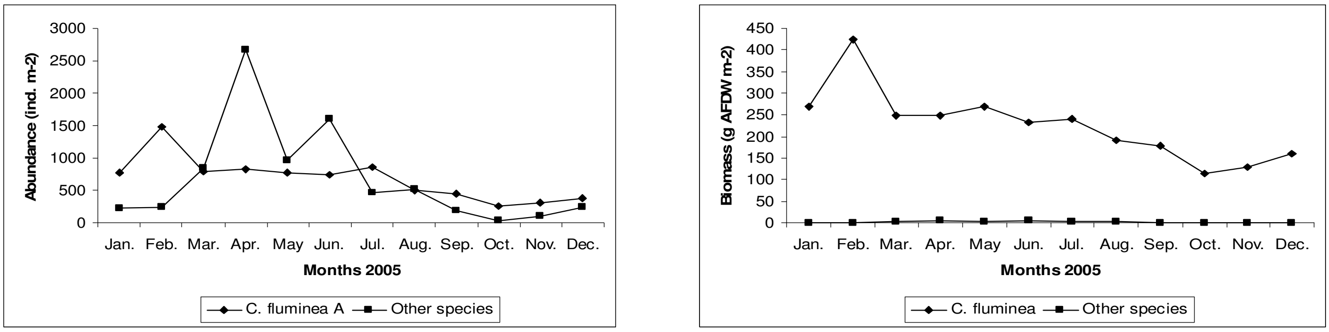
In summary, the earlier TFWs molluscan community supported a rich bivalve fauna consisting of several large species of freshwater mussels from the Margaritiferidae and Unionidae families; clams from the Sphaeriidae family and Gastropod species. Freshwater mussel species such as *Unio pictorum* (Linnaeus, 1758), *Anodonta cygnea* (Linnaeus, 1758), *Anodonta anatina* (Linnaeus, 1758), *Psilunio littoralis* (Lamarck, 1801) and *Margaritifera margaritifera* (Linnaeus, 1758) (never found in the TFWs in the last years, but still present in a near upstream tributary) are nowadays very rare and the abundance and biomass are now a small remnant of those recorded years ago. Other bivalve species such as *Pisidium amnicum*, *Pisidium subtruncatum* (Malm, 1855), *Pisidium casertanum* (Poli, 1791), *Pisidium henslowanum* (Sheppard, 1823)*, Pisidium milium* (Held, 1836) and *Musculium lacustre* (Müller, 1774) (the last 3 species were never found in the River Minho TFWs in the last years) were described by several authors in earlier studies (Nobre, 1941; Baños, 1978; Araujo *et al.*, 1993, 1999).

Table 6.4.1. Molluscs species (X – present) described for the Minho estuary TFW in earlier (Nobre, 1941; Baños, 1978; Maze *et al.*, 1993; Araujo *et al.*, 1993, 1999) and recent (Sousa *et al.*, 2005, 2007c, 2008, in press) studies.

	Freshwater Molluscs	**Earlier studies**	**Recent studies**
1	*Margaritifera margaritifera* (Linnaeus, 1758)	X	
2	*Psilunio littoralis* (Lamarck, 1801)	X	X
3	*Unio pictorum* (Linnaeus, 1758)	X	X
4	*Anadonta anatina* (Linnaeus, 1758)	X	X
5	*Anodonta cygnea* (Linnaeus, 1758)	X	X*
6	*Corbicula fluminea* (Müller, 1774)	X	X
7	*Pisidium amnicum* (Müller, 1774)	X	X
8	*Pisidium casertanum* (Poli, 1791)	X	X
9	*Pisidium henslowanum* (Sheppard, 1823)	X	
10	*Pisidium milium* (Held, 1836)	X	
11	*Pisidium subtruncatum* (Malm, 1855)	X	X
12	*Musculium lacustre* (Müller, 1774)	X	
13	*Ancylus fluviatilis* (Müller, 1774)	X	X
14	*Bithynia tentaculata* (Linnaeus, 1758)	X	X
15	*Gyraulus laevis* (Alder, 1838)	X	X
16	*Gyraulus albus* (Müller, 1774)	X	
17	*Lymnaea peregra* (Müller, 1774)	X	X
18	*Physella acuta* (Draparnaud, 1805)	X	X
19	*Potamopyrgus antipodarum* (Gray, 1843)	X	X
20	*Valvata piscinalis* (Müller, 1774)	X	X
21	*Succinea putris* (Linnaeus, 1758)	X	X

*Only two individuals found in the last 4 years. However some doubts persisted about the specific filiation of these two specimens since the identification was only based in morphological characteristics (possible taxonomical confusion with *A. anatina*).

Additionally, the gastropod species *Gyraulus albus* (Müller, 1774) was never found in the last 4 years. Nowadays the TFWs molluscan community is completely dominated by the presence of the non-indigenous invasive species (NIS) *C. fluminea*. This species dominates the benthic biomass of the estuarine area accounting for more than 95% of it (Sousa *et al.*, 2005, 2007c, 2008, in press). This alteration in the molluscan community occurred in 1989 when this NIS was introduced (Araujo *et al.*, 1993). Molluscan data collected recently (Sousa *et al.*, 2007c) shows a significant decline in the diversity, abundance and biomass during the 2005 summer (Fig. 6.4.1.).



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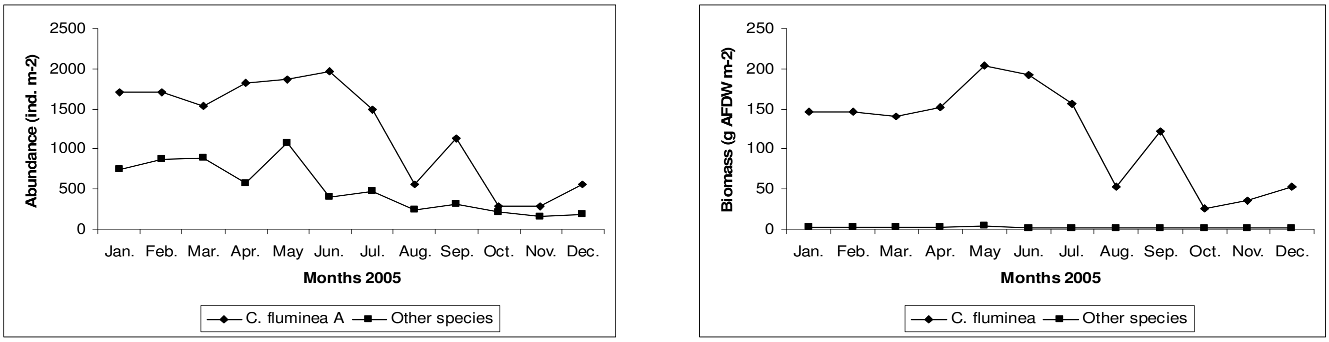

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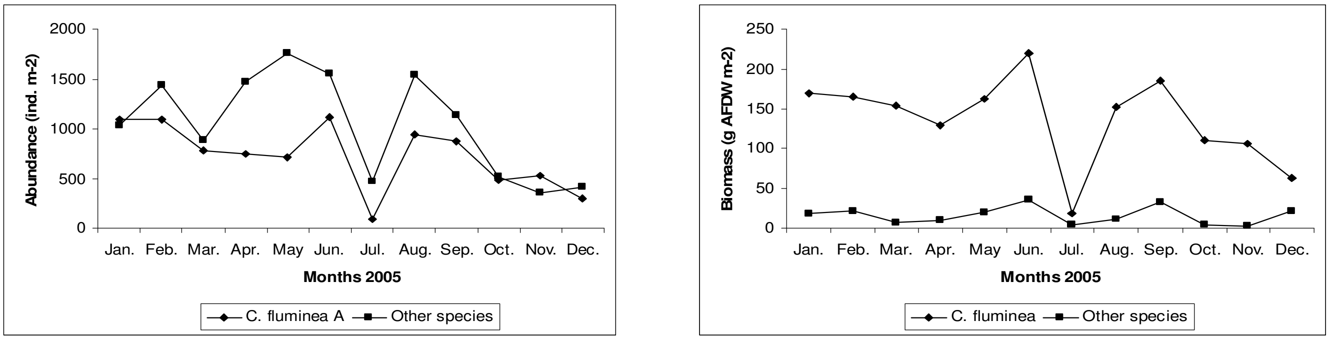

Site 12 Site 12

Fig. 6.4.1. Molluscs declines (abundance and biomass) after the 2005 summer heatwave (data obtained in the sites 9, 11 and 12 - Fig. 5.1.1.). Data is divided in *C. fluminea* and all the other molluscs' species. Further information about this study is in Sousa *et al.* (2007c).

The abiotic conditions of 2005 were responsible for massive mortalities of benthic species (firstly in *C. fluminea* followed by all the benthic assemblage). Indeed, 2005 was a very dry year in the Iberian Peninsula and a considerable reduction of the River Minho water flow was recorded in the summer. In that summer several abiotic alterations occurred in the upper estuarine limnetic areas: increase of the water temperature, and decrease in water flow, redox potential and dissolved oxygen (Sousa *et al.*, 2007c, 2008). This occurrence was especially dramatic for the molluscan species and the recruitment in the subsequent years was not sufficient to recover the prior abundance and diversity (Table 6.4.2.). However, our data also show that the NIS *C. fluminea* rapidly recovered from this impact but up to now the other molluscan species did not returned to the earlier numbers and distribution (Table 6.4.2.).

Table 6.4.2. Molluscan data (number of species, abundance and biomass) from sampling surveys performed in 16 sites (see Fig. 5.1.1. for the location) in October 2004, 2005, 2006 and 2007.

	2004	**2005**	**2006**	**2007**
Number of species	10	13	9	7
Total Abundance (ind./m^2)	1412	1049	1082	1303
C. fluminea Abundance (ind./m^2)	1256	956	1062	1283
Total Biomass (g AFDW/m^2)	98.4	100.7	122.7	161.6
C. fluminea Biomass (g AFDW/m^2)	95.2	99.1	121.4	161.0

In other macrozoobenthic groups, differences are not so significant. Crustacean and insect species present in the River Minho TFWs are nearly the same when earlier and recent studies are compared. However, data available from previous studies are almost exclusively composed by qualitative data. Species of transition areas, between fresh water and more saline waters are still present nowadays and in some places with high abundance [*e.g. Corophium multisetosum* (Stock, 1952), *Gammarus chevreuxi* (Sexton, 1913)]. Nevertheless, a clear reduction of the distribution and abundance of *Gammarus pulex* (Linnaeus, 1758)*,* a true freshwater species, can be noticed from the comparison between recent data (Sousa *et al.*, in press) and results from 1977 and 1978 surveys (Baños, 1978). Additionally, the native crayfish *Austropotamobius pallipes* also disappeared and was replaced by the NIS *Procambarus clarkii* (Girard, 1852).

Fish decline

The ichthyofauna of the River Minho estuary has been the subject of discontinuous studies in the last 25 years (*e.g.* Antunes, 1994; Antunes & Weber, 1996). TFWs are significantly used by economically important fish species such as *Anguilla anguilla* (Linnaeus, 1758) and *Platichthys flesus* (Linnaeus, 1758) in key periods of their life cycles. In a recent study (Cabral *et al.*, 2007) it is pointed out that the population of the epibenthic species *P. flesus* present in the River Minho estuary has its main nursery ground in the TFWs. In this area, the species *Salmo trutta fario* (Linnaeus, 1758) and *Chondrostoma polylepis* (Steindachner, 1866), appreciated as game fish, may also be found. Although without commercial value, other ecological important species such as *Gasterosteus aculeatus* (Linnaeus, 1758), *Rutilus arcasii* (Steindachner, 1866), *Leuciscus carolitertii* (Doadrio, 1988) and *Cobitis paludica* (De Buen, 1930) inhabit such area.

When trying to compare the actual fish species composition with those that could be found in TFWs conditions existing decades ago, the lack of quantitative data in earliest surveys prevents detailed comparisons. Although there are no recent records of *Lampetra fluviatilis* (Linnaeus, 1758), *Lampetra planeri* (Bloch, 1784) and *Acipenser sturio* (Linnaeus, 1758) in the River Minho, Almaça & Elvira (2000) and Tola & Infesta (2002) refer those species as historically present in this river. On the other hand, some fish species were introduced for various purposes, mainly in the last years, and are now easily found in the TFWs (Table 6.4.3).

Diadromous species such as the anadromous *Salmo salar*, *Salmo trutta trutta* (Linnaeus, 1758), *Petromyzon marinus* (Linnaeus, 1758), *Alosa alosa* (Linnaeus, 1758) and *Alosa fallax* (Lacepède, 1803) and the above mentioned catadromous *P. flesus* and *A. anguilla* (exploited both as glass and yellow eels), form the list of the most economically important species for traditional fishery in the River Minho estuary. Even taking into consideration the error inherent to fisheries statistics (Antunes & Weber, 1996) the decline in fish catches is evident (Fig. 6.4.2.). The exception seems to be fisheries data for *P. marinus*. However, this species fishery comprises a wider catching area and the method for data collection is reported to have changed over time.

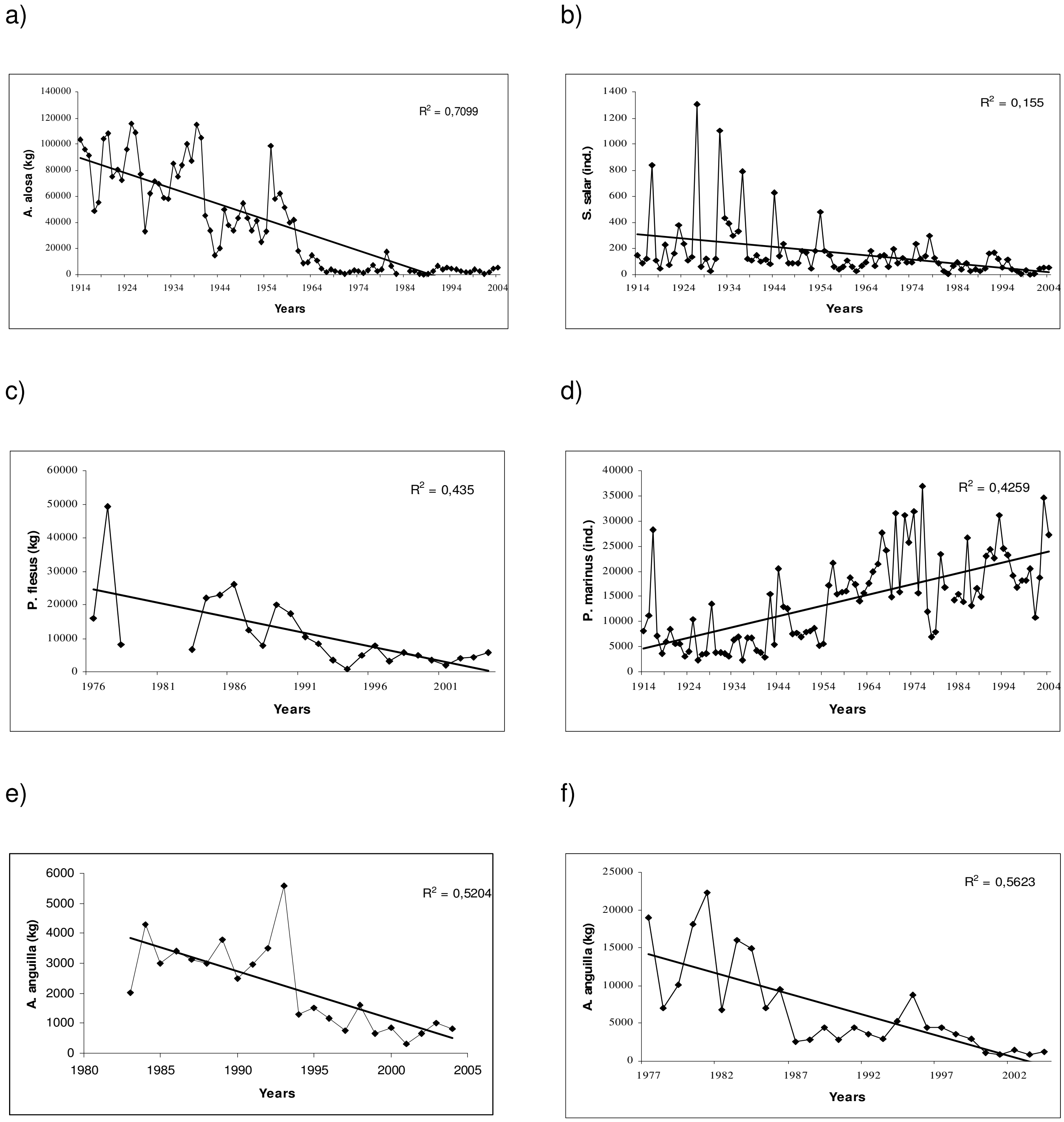

Fig. 6.4.2. Fisheries trends in the River Minho (data based on official Portuguese authorities' statistics): a) *Alosa alosa* (kilograms), b) *Salmo salar* (number of individuals), c) *Platichthys flesus* (kilograms), d) *Petromyzon marinus* (number of individuals), e) *Anguilla anguilla* - as yellow eel (kilograms) and f) *Anguilla anguilla* - as glass eel (kilograms). Highly significant relationships (P-value < 0.01) were obtained for all species.

Non-indigenous invasive species (NIS)

For the River Minho estuary, the presence of 18 nonindigenous animal species have been recorded (Table 6.4.3.) and the introduction sources had clearly a direct human hand (*e.g.* fish introduction for sports fisheries, and the use of macroinvertebrates as fish bait). Of those 18 nonindigenous species 15 are well established and have self-sustaining populations and only the mitten crab *Eriocheir sinensis* (Milne Edwards, 1853) and the Eurasian minnow *Phoxinus phoxinus* (Linnaeus, 1758) appear to have failed the establishment in this estuarine area. Additionally, *Lepomis gibbosus* (Linnaeus, 1758) was recorded for the first time in April 2007 and the successful establishment of this NIS has to be studied.

The major problems concerning NIS are related to the enormous abundance and biomass attained by *C. fluminea*. The distribution, abundance, biomass and ecology of *C. fluminea* has been investigated recently and detailed data is in Sousa *et al.* (2005, 2007a and c, 2008, in press). This NIS is distributed all over the TFWs colonising all type of soft sediments. For the years 2004, 2005, 2006 and 2007, the mean abundance and biomass was more than 1000 ind./m^2 and 100 g AFDW/m^2, respectively. The gastropods *Physella acuta* (Draparnaud, 1805) and *Potamopyrgus antipodarum* (Gray, 1843) have also a great spatial distribution in the River Minho TFWs but their abundance and biomass is a small fraction of the already measured for *C. fluminea* (Sousa *et al.*, 2005, 2007c). The crustacean species *P. clarkii* is also a common inhabitant. This NIS is also very abundant, being widely distributed in the TFWs and in several small tributaries. Possibly, this species was responsible for the disappearance of the native crayfish *A. pallipes* in the Minho estuary TFWs (*e.g.* due to interspecific competition and the infection with crayfish plague). Nine fish species were introduced in the last years and some of them can attain large abundance and biomass such as *Cyprinus carpio* (Linnaeus, 1758), *Micropterus salmoides* (Lacepède, 1802) and *Tinca tinca* (Linnaeus, 1758). Finally three species of birds [*Estrilda astrild* (Linnaeus, 1758), *Estrilda troglodytes* (Lichtenstein, 1823) and *Myopsitta monachus* (Boddaert, 1783)] and the mammal *Mustela vison* are also described as recent colonizers. However, the abundance, biomass and distribution of these populations needs to be determined and the information available is scarce.

Table 6.4.3. Non-indigenous invasive species (NIS) recorded in the River Minho estuary. The origin of the species and their category based in the definitions of Carlton (1992) is given.

	NIS	Origin	Category
1	*Corbicula fluminea* (Müller, 1774)	Asia	Established
2	*Physella acuta* (Draparnaud, 1805)	North America*	Established
3	*Potamopyrgus antipodarum* (Gray, 1843)	New Zealand	Established
4	*Eriocheir sinensis* (Milne Edwards, 1853)	Asia	Established not certain
5	*Procambarus clarkii* (Girard, 1852)	North America	Established
6	*Carassius auratus* (Linnaeus, 1758)	Euroasia	Established
7	*Cyprinus carpio* (Linnaeus, 1758)	Asia	Established
8	*Gambusia holbrooki* (Girard, 1859)	North America	Established
9	*Gobio gobio* (Linnaeus, 1758)	Euroasia	Established
10	*Lepomis gibbosus* (Linnaeus, 1758)	North America	Established?**
11	*Micropterus salmoides* (Lacepède, 1802)	North America	Established
12	*Onchorhynchus mykiss* (Walbaum, 1792)	North America	Established
13	*Phoxinus phoxinus* (Linnaeus, 1758)	Euroasia	Established not certain
14	*Tinca tinca* (Linnaeus, 1758)	Euroasia	Established
15	*Myopsitta monachus* (Boddaert, 1783)	South America	Established
16	*Estrilda astrild* (Linnaeus, 1758)	Africa	Established
17	*Estrilda troglodytes* (Lichtenstein, 1823)	Africa	Established
18	*Mustela vison* (Schreber, 1777)	North America	Established

*There is also the possibility of a Mediterranean origin for this NIS.
** First record in April 2007.

Discussion

Abiotic characterization

The River Minho estuary has been considered as a low chemical contaminated estuary and the present levels seem not to be a serious cause of concern. For this reason it has been used as a reference site in several ecotoxicological studies performed in the NW coast of Portugal (Cairrão *et al.*, 2004; Moreira *et al.*, 2005, 2006; Elumalai *et al.*, 2006; Quintaneiro *et al.*, 2006; Monteiro *et al.*, 2007). The main sources of environmental contaminants are fluvial traffic (*e.g.* small fishery and tourist boats, ferry boats connecting Portugal to Spain), agricultural fields, some industries mainly in River Minho tributaries, and urban effluents of relatively small towns and villages discharged into the river without previous treatment.

The construction of dams and potential impacts imposed by climate change can be responsible for the decrease in the river discharge, which may alter the River Minho TFWs limits. This decrease in the river discharge can modify the abiotic conditions (*e.g.* increase in salinity and conductivity), principally in the downstream limit of the River Minho TFWs, and be responsible for alterations in the faunal assemblages. In addition, disturbing climatic events can also be responsible for huge abiotic changes causing problems to the faunal assemblages. For example, during the summer of 2005, considered a dry year, reduction in water flow and increase in water temperature coincided. Consequently, important alterations such as decreases in redox potential and dissolved oxygen occurred in the upstream estuarine areas with very fine sediments and rich in organic matter content. These abiotic changes were responsible for massive mortalities in the benthic assemblages (Sousa *et al.*, 2007c, 2008).

Biotic characterization

In recent years the number of extinctions in freshwater ecosystems has dramatically increased and the concern about this phenomenon is now emerging as a global ecological and conservational problem (Ricciardi & Rasmussen, 1999). A closer look at global conservational studies demonstrates that some faunistic groups are more susceptible to extinction than others. Indeed, a revision of the data available in the IUCN Red List of Threatened Species (www.redlist.org) points out the great conservational concern for mollusc and fish species. Our results also show that molluscs and fish species present in the River Minho TFWs are particularly threaten by extinction and this situation deserve more attention from the conservational, political and management groups in order to reverse their extinction pressure and to increase funding for conservational studies performed with these species. In the River Minho TFWs, freshwater mussel species are nowadays very rare and the abundance and biomass are now vestigial when compared to those recorded in earlier studies. Freshwater mussels are declining at unprecedented rates and are currently the core of several freshwater conservational studies done principally in North America (Lydeard *et al.*, 2004; Strayer *et al.*, 2004). This circumstance is now very problematic due to specific traits [*e.g.* extended life spans, delayed maturity, reduced power of dispersal, great juvenile mortalities, long turnover times, the larvae (glochidia) are obligate parasites of fish species and the settlement appears to be particularly affected by environmental changes] that render freshwater mussels especially vulnerable to habitat disturbance. These traits make it considerably difficult for the successful implementation of conservational measures taken in an advanced phase of the problem and therefore, require an urgent intervention. Of the five freshwater mussel species found in the River Minho hydrological basin, only *M. margaritifera* is subjected to multidisciplinary studies in Europe due to its conservational importance (Hastie *et al.*, 2000; Reis, 2003; Geist & Kuehn, 2005). A special protection and increased ecological information are also needed for bivalve species from the Sphaeriidae family and gastropods that are also subject to extirpation in the River Minho TFWs. Nowadays, the TFWs molluscan community is completely dominated by the NIS *C. fluminea*. Consequently, one of the most important aspects to be studied in the River Minho TFWs is the possible difference in the ecosystem functioning (*i.e.* how this NIS altered previous ecological and environmental processes) (Hakenkamp

& Palmer, 1999; Hakenkamp *et al.*, 2001; Vaughn & Hakenkamp, 2001). This global decline in molluscan diversity, abundance, biomass, distribution and the replacement of native species by NIS has been described in several freshwater (Lydeard *et al.*, 2004; Strayer *et al.*, 2004; Mouthon & Daufresne, 2006) and marine (Edgar & Samson, 2004; Edgar *et al.*, 2005) ecosystems. In relation to other macrozoobenthic groups, the differences are not so clear due to scarcity of studies and because the existent earlier data is only qualitative. Consequently, further discussion may be speculative.

As in many other European TFWs, the autochthonous fish community present in the River Minho seems to be declining. Besides more or less long uses of TFWs, these areas constitute a vital migratory corridor for diadromous species either migrating for feeding or spawning and these ecosystems are considered conduits of nutrients travelling in different directions (from rivers to ocean and vice-versa). This general decline has been attributed to several anthropogenic pressures that mainly prevented migration paths or access to suitable habitats, thus, affecting the species life cycle. Despite the source of disturbance, diadromous and freshwater species that at least in a stage of their life cycle depend on TFWs find altered habitats that, regardless of the water quality, do not maintain the morphological or hydrological characteristics required for their development, reproduction or even the survival of their prey. Although the total number of fish species may have increased in the River Minho TFWs due to introduced NIS, the number and abundance decrease of native species is evident. Considering fisheries data, catches seem to be generally declining over the last decades but values cannot be directly interpreted and some considerations are necessary. Although only *A. alosa* decrease has been clearly related to a specific impact in the River Minho basin such as dam construction (Taverny *et al.*, 2000), general decreasing trends have been described on a worldwide scale also for *S. salar* and *A. anguilla*. That may evidence a general deterioration on habitat suitability or overexploitation, among others, but the roots of those trends are not restricted to TFWs given the specificity of the life cycle of these migratory fish species.

Principal threats

Estuaries are usually recognized as heavily impacted ecosystems. These impacts are usually described as single pressures but in the majority of cases they have synergistically effects. In the River Minho TFWs, the main problems follow the general trend described for other ecosystems, namely: habitat loss, introduction of NIS, climate changes, impoundments and river regularizations, fishing activities and pollution.

Habitat loss is the most common threat to biodiversity (Rodrigues *et al.*, 2006). Estuaries are not an exception since they are constantly subject to changes due to natural and human mediated processes. Loss in area and physical and chemical change in the River

Minho TFWs include alteration in the habitats due to construction of ditches, channels, roads, railways, houses or other type of construction (several times in flood areas). The existence of dams or other type of impoundments upstream the River Minho TFWs has also inevitable repercussions that may include fragmentation of once continuous ecosystems.

The introduction of NIS is one of the most important threats to aquatic ecosystems with potentially catastrophic ecological and economic impacts (Carlton & Geller, 1993; Cohen & Carlton, 1998; Kolar & Lodge, 2001; Grosholz, 2002). Given the characteristics of the species that have been invading this estuary, the ecosystem characteristics, the evolution of anthropogenic activities and the predicted global changes, more invasions are expected to occur in the near future. Furthermore, all the species that have been described for this estuarine area have freshwater characteristics and therefore, TFWs seems to be more susceptible to biological invasions than the brackish areas. For the macroinvertebrates, the major concern in these TFWs is the ecological and economic impacts provoked by *C. fluminea* (Sousa *et al.*, 2005, 2007c, 2008, in press) and *P. clarkii* (Geiger *et al.*, 2005; Cruz *et al.*, 2006). In relation to non-indigenous fish species, the main problems are the possible impacts of piscivorous species (*e.g. M. salmoides*) with potential top down effects in the estuarine food chain, and abundant and large species (*e.g. C. carpio* and *T. tinca*), preying upon already altered invertebrate assemblages and physically changing the habitat. The others faunal NIS have relatively low abundances and/or occupy trophic niches for which low pressure or competition exists and, therefore, their impacts seem to be less severe. However, the actual situation may change quickly depending on the community's readjustment to a new or an increased stress.

Given the present tendency for the increase of temperature, the occurrence of droughts and floods will tend to be much more pronounced in the future (Humphries & Baldwin, 2003). However, since the River Minho does not have a natural regime due to anthropogenic interventions, this situation can, in some cases, be mitigated by manipulations of the flow (McMahon & Finlayson, 2003). Possible climate changes in this area may be disastrous for several species well adapted to the present abiotic conditions. In this scenario, the species currently having their Southern distribution limit in the River Minho (*e.g. P. amnicum*) will be probably the first to disappear and the future estuarine community will have characteristics more close to those of southern estuaries. In the last decade, heatwaves have been recorded worldwide (Mouthon & Daufresne, 2006). The alterations caused by this phenomenon which may include an increase in seasonal and supra-seasonal drought conditions can have a dramatic impact in estuarine communities, particularly in sensitive areas such as TFWs. The impacts caused by the 2005 heatwave in the molluscan community of the River Minho TFWs are a good example of that and a similar situation has been described in other ecosystems (Mouthon & Daufresne, 2006).

Although not independent from natural conditions, the flow regime within the River Minho hydrological basin is subject to human control and the same is true for numerous worldwide hydrological basins (Lytle & Poff, 2004). The River Minho hydrological basin has more than 50 dams or other kind of impoundments which introduced great impacts on the natural river flow, created obstacles to migratory fish species and fragmented the habitats. The last 70km of the main river does not have any impoundment but suffer the consequences of the upstream regularizations. This situation is responsible for great daily flow oscillations that result in both abnormally high and low flows. In reality, the River Minho TFWs are not only subjected to the two normal tides but also to the daily discharge by the upstream Frieira dam. Because streams are basically linear systems, the altered flow and consequent changes in other abiotic conditions directly downstream from impoundments should display a gradual change in the ecosystem (Vaughn & Taylor, 1999). These impacts may have disastrous effects in benthic and pelagic communities introducing for example a novel stress factor by flushing downstream developing larvae or juveniles, or even adults that are not able to avoid displacement, and change the sediments composition.

Inland fisheries lost much of their significance in European and North American countries (Allan *et al.*, 2005). In Western Europe the number of professional fishermen was significantly reduced in the last decades but inland fisheries continue to have both social and financial significance. Additionally, these activities were replaced by sport fisheries which also have great economic importance and a significant potential expansion in the future. In the River Minho TFWs several fish species have medium (*S. trutta*, *A. fallax*, *P. flesus* and *A. anguilla* as yellow eel) or high (*S. salar, A. alosa, P. marinus* and *A. anguilla* as glass eel) economic value. Statements of the local population and official records of captures for some of these species indicate significant decreases in recent decades and the inland fisheries may have some responsibilities for that. Nowadays, inland fisheries have restrictive regulations but fishing gears confiscation allows us to say that illegal fisheries occurs achieving uncertain, but presumably high catches. Although drastic reductions in population size as well as local extinctions attributed to fisheries have been recorded for many species, documented cases of global extinctions in estuaries and coastal waters are extremely rare, being the result of overfishing sometimes combined with poor water quality (Blaber *et al.,* 2000). In the River Minho estuary, fishing pressure does not seem to act as a single, isolated factor leading to stocks impoverishment, but acting synergistically with other causes, including habitat disturbance.

Pollution can also contribute to the lost of biodiversity. It can cause the exclusion of sensitive species that are not able to survive or to adapt to certain levels of pollution, can alter abiotic factors to which some species have a low range of tolerance or can interfere in interspecific relationships (*e.g.* inducing mortality, decreasing the predatory or escape capabilities, or weakening defences against competitors, diseases and parasites). As a result of anthropogenic pressure, a considerable part of worldwide estuaries are now facing pollution problems due to industrial, agricultural and urban activities. Heavy metals, petrochemical products, pesticides, butyltin compounds and nutrients are common estuarine pollutants (Turner & Rabalais, 1994; Voorspoels *et al.*, 2004; Díaz *et al.*, 2007). In addition, the so-called "emergent pollutants of concern" (*e.g.* pharmaceuticals, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, polychlorinated naphthalenes, perfluorinated chemicals and brominated flame retardants) have been noticed in estuarine and coastal areas (de Boer *et al.*, 1998; de Wit, 2002; Eljarrat & Barceló, 2003; Cunha *et al.*, 2005). At present, pollution seems not to be a great threat in the River Minho TFWs and the deterioration of water quality never reached the dramatic situation described for other estuarine areas.

Future directions

The River Minho TFWs has great ecological and economic importance at least at the regional level. The main problems recognised as potential threats to this estuarine area are also identified as major problems in other ecosystems.

The introduction of NIS is one of the most important threats to this estuarine ecosystem. The arrival of other NIS is very difficult to predict and control but some practices could be implemented (*i.e.* education and information for fishermen and local populations, taking advantage of local infrastructure devoted to environmental education). In addition, good ecological assessment of the estuary could be a vehicle to detect the introductions in the first phases of development and mitigation practices could be applied with potentially increased success if completed in a short time after the introduction.

The major problems dealing with the fishing activities are related to the migratory species which have great economic importance. Local authorities, fishermen, and scientists all agree that several practices that are easily adopted could have great results and improve the actual situation. Among others, possible measures to take are to define more restricted areas for fishing, either in the river mouth in order to increase the upstream migration chances for diadromous fish species or in the margins of upstream areas where nursery grounds are located (*e.g. A. alosa*, *A. fallax*, *P. flesus, P. marinus*); redefine temporal periods for certain fisheries; define license numbers or catch limits; make fishery declarations compulsory, and provide, for instance, fish certification in return; and, obviously, enforce inspection.

Problems such as loss and fragmentation of habitat, global warming and river regularizations are aspects much more difficult to solve since some of them are global problems and are somehow impossible to revert, at least in the short term. Anyway, in relation to the loss and fragmentation of habitats, local authorities can play a major role. The construction near the margins should clearly be prohibited, given the vital functions taking place in these areas (*e.g.* mitigation of floods, nursery areas for several fish and macrozoobenthic species). The presence of dams and river regularizations are a very complicated aspect since this is an international river that is the natural border between Portugal and Spain for more than 70km. The most downstream dam, Frieira, is impossible to remove due to political and economic aspects. However, the great daily oscillations in the flow regime caused by this impoundment could be solved if the two countries had an effective water policy. Furthermore, solutions that should also be adopted in some of the adjacent watercourses include the removal of small inactive impoundments that still exist in the tributaries of the River Minho international section, increasing the availability of habitats (migratory fish species would certainly take advantage if these practices were adopted).

Finally, restoration of the degraded habitats can lead to re-establishment of some species, improved inland and coastal water quality and provide new areas for wildlife habitat and recreational activities (Palmer *et al.*, 2004; Bernhardt *et al.*, 2005). This kind of practice needs financial investment, but more important than that, huge planning efforts involving scientists, managers, politicians and local populations (Ewel *et al.*, 2001).

Conclusion

The problem of biodiversity loss in TFWs ecosystems is now a fact but this issue has received negligible attention from conservation, political and management groups. This disparity seems particularly dangerous given the actual ecological situation of these habitats and the biodiversity crisis in freshwater ecosystems. The actual rates of freshwater species extinction will have numerous consequences, with varying severity depending on the ecosystem. A progressive reduction of biodiversity means that fewer species are present to perform crucial functions and the consequences will be disastrous when species with disproportionately influence on biogeochemical cycles, energy fluxes and trophic dynamics are lost (Allan *et al.*, 2005).

In the River Minho TFWs the major conservational problems deals with migratory fish and molluscan species, which follow the trend already described in the IUCN Red List of Threatened Species. This estuarine area is within a protected area that belongs to the

Natura 2000 sites. However, the real protection is not effective since this area continues to suffer several impacts. Indeed, there is a need for an improved relationship between Portugal and Spain in order to effectively preserve this ecosystem. If some of the above described practices are adopted possibly the major threats can be effectively managed and this will be the most important tool used for biodiversity conservation in such important freshwater habitats. Rising knowledge about these areas is needed and this depends on a greater scientific investment that has to be conducted in a multidisciplinary way and also in an increase ability to disseminate information to the local population.

The long term advantages of the effective protection of the River Minho TFWs include shoreline protection, water quality improvement, and mitigation of flood impacts and can directly benefit activities like fisheries, tourism, agriculture and hunting, among others. Additionally, there is an aesthetic value that should be preserved and a moral obligation to prevent biodiversity loss in these ecosystems.

Chapter 7

Conclusion and future directions

Conclusion and future directions

General ecological theory suggests that invasion success depends on the species traits, recipient environment, interaction between species and the invaded habitat, and effort applied to the introduction. Given these principles the reasons behind the completely different invasive behaviour presented by *C. fluminea* in the Rivers Minho and Lima estuaries remains uncertain.

In relation to the characteristics of the recipient ecosystem, the two estuaries share some similarities. First, temperature, type of circulation (*e.g.* both estuaries are partially mixed but can change to a salt wedge estuary during great floods) and other abiotic characteristics (*e.g.* hardness, dissolved oxygen, total dissolved solids, pH) were similar. Second, both estuaries possess great sedimentological heterogeneities that result in a great diversity of patches that could be colonised by *C. fluminea*. Indeed, the soft bottom areas existent for colonization are vast and the type of sediment and the organic matter content available could, in theory, support large populations in both estuaries (but see below). On the other hand, the two estuaries also have dissimilarities, such as the availability of areas with freshwater conditions, which is much larger in the River Minho than in the River Lima estuary. In addition, the upstream areas of the River Minho estuary are rich in organic matter, which results in higher availability of food resources that could support higher abundance and biomass. The River Lima estuary has higher chemical contamination, principally in downstream areas but also in areas where the species is present, due to the existence of a pulp mill factory. Finally, the River Lima estuary has lower river discharge and therefore *C. fluminea* could be affected by higher salinities values in upstream areas, principally in the summer. Although recent data showed a small increase in the abundance and biomass, the River Lima population remains restricted to a very small area and until now has not dispersed upstream.

In relation to possible species traits that may interfere with the invasive success, the genetic study (chapter 5) showed that both populations shared a similar sequence of the mtCOI gene. Anyway, additive genetic variance is essential for evolutionary adaptation in response to environmental change and several studies have found high levels of additive genetic variance within source populations for traits that facilitate invasions (Lee, 2002 and references therein). Indeed, the observed time lag phase commonly preceding successful invasions could result from the need to accumulate adequate levels of additive genetic variance. This situation may have influenced the invasion dynamics of the River Lima population (with a more recent introduction). Only additional genetic studies using others methodologies can give insights into this problem and thus, further discussion will be speculative. Several biotic interactions (predation, parasitism, competition, facilitation processes) that can influence the abundance and distribution of this species were not fully explored in this project. However, the possible predators that could control the two populations are not significantly different in the two estuaries and possibly, in the River Minho, the number of species and abundance of these predators is higher. In relation to competition, at least for other molluscan species, the number of potential competitors is much higher in the River Minho than in the River Lima estuary. The possibility that parasites can control the two populations or the degree of facilitation processes in the two estuaries was beyond the scope of this dissertation and, therefore, I can not go further in these subjects.

The studies performed with the two populations were not sufficient to get a definitive answer about the factors contributing to the distinct invasive behaviour presented. Although the most traits for successful versus unsuccessful invasions were often context specific, it is my opinion that the abiotic characteristics in the River Minho TFWs may have extraordinary conditions for *C. fluminea* proliferation. The possible answer to the greater invasive behaviour in the River Minho estuary was the arrival of an invasive species to an almost empty niche with great resources available. This ecosystem has optimum conditions (*e.g.* extensive mud and sand flats, extensive areas subjected to freshwater conditions, high organic matter content) for the *C. fluminea* colonisation and for a rapid increase in abundance and biomass, almost until the carrying capacity of the system. In contrast, the Lima population with a much more recent introduction seems to be in the time lag phase. However, if the conditions became more favourable and/or the species successfully adapts to the environment, the population may rapidly expand their habitat and increase their abundance and biomass.

Because this research project gave maximum information about the ecology of this NIS, it opened several hypotheses that should be addressed in future studies. All the data were collected in only two Iberian estuaries, so further research should include a broader spatial scale, in order to comprise other invaded sites in North and South America and Europe, and also in the native range. For example, studies using other genetic markers (*e.g.* microsatelites, microarrays) are necessary to resolve several uncertainties about taxonomy, morphometry and pathways of dispersion inside the *Corbicula* genus. In addition, general models trying to find patterns of distribution along large scales and establishing relationships between *C. fluminea* abundance or biomass and abiotic factors will be very informative for future risk analysis. Further manipulative studies are also necessary addressing important ecological processes (*e.g.* ecosystem engineering, facilitation processes, competition, parasitism, predation, filtration rates, secondary production) that can be responsible for changes in the processes and functions inside the invaded ecosystems. *C. fluminea* is also a very interesting species from an ecotoxicological point of view and research using this organism as a sentinel species is possible.

C. fluminea is currently recognised as one of the most invasive species of freshwater ecosystems. The great abundance, biomass and secondary production attained by this species in the River Minho TFWs make it a fundamental component of this estuarine area. These values can be responsible for high modifications in the structure and function of this ecosystem and this situation deserves attention. On the other hand, possibly we are following the River Lima population almost from the beginning of its introduction (since 2002). If this species starts to disperse we will be in the possession of data that allowed the observation of the invasive process in real time, with important payoffs for ecological and evolutionary research studies.

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Appendices

Appendix 3.1.1. Physico-chemical data for the River Minho estuary. Temperature (T - ºC), total dissolved solids (TDS – mgl^{-1}), redox potential (ORP - mV), salinity (S - psu), dissolved oxygen (DO - mgl^{-1}) pH, nitrites (mgl^{-1}), nitrates (mgl^{-1}), ammonia (mgl^{-1}) and phosphates (mgl^{-1}) of water column and gravel (G - %), very coarse sand (VCS - %), coarse sand (CS - %), medium sand (MS - %), fine sand (FS - %), very fine sand (VFS - %), silt+clay (S+C - %) and organic matter (OM - %) of sediment at the twenty sampling sites.

Abiotic factors	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
T (ºC)	14.7	14.9	14.8	17.4	15.4	17.2	19.0	22.1	22.4	22.1	22.8	22.4	22.7	21.9	22.3	21.8	21.6	21.7	21.8	21.7
TDS (mgl^{-1})	34.52	33.84	33.98	28.52	32.80	28.80	20.21	1.63	2.18	3.29	0.30	0.08	0.07	0.07	0.08	0.24	0.06	0.07	0.06	0.06
ORP (mV)	77.60	71.40	77.10	60.70	78.60	72.60	73.30	57.60	66.40	49.20	52.20	65.10	26.60	40.50	36.50	25.20	42.70	41.00	35.70	35.00
S (psu)	34.53	34.27	34.25	28.49	32.30	28.60	19.37	1.50	1.80	2.53	0.22	0.06	0.05	0.05	0.06	0.18	0.05	0.05	0.05	0.04
DO (mgl^{-1})	7.04	7.38	7.41	7.43	7.30	7.88	8.45	8.03	7.92	7.82	10.43	11.52	11.57	11.82	10.95	1.82	11.94	12.46	12.71	13.04
pH	7.44	7.47	7.49	7.65	7.61	7.66	7.81	8.02	7.68	7.46	7.64	7.53	7.39	6.93	6.74	6.54	7.20	7.01	7.09	6.91
Nitrites (mgl^{-1})	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.04	0.02	0.04	0.05	0.06	0.08	0.05	0.04	0.15	0.03	0.03	0.05	0.04
Nitrates (mgl^{-1})	0.42	0.50	0.32	0.42	0.58	0.54	1.26	1.92	2.40	2.60	3.10	3.20	3.40	2.90	3.40	3.50	3.40	3.00	3.20	2.70
Ammonia (mgl^{-1})	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.06	0.06	0.11	0.16	0.18	0.17	7.43	0.08	0.08	0.08	0.06
Phosphates (mgl^{-1})	1.20	0.13	0.03	0.06	0.01	0.00	0.16	0.17	0.24	0.13	0.24	0.22	0.15	0.18	0.18	1.40	0.30	0.15	0.23	0.18
Hardness (mgl-1)	2500	2100	2080	1440	1400	500	500	135	185	115	40	15	25	20	15	30	20	35	10	35
G (%)	0.32	0.30	8.29	0.35	4.13	2.60	0.76	1.54	22.50	0.00	28.52	4.65	3.48	28.30	54.34	0.15	0.06	0.13	0.46	1.02
VCS (%)	1.47	0.91	12.49	1.85	12.74	6.62	2.35	3.78	17.61	1.30	28.45	29.19	10.08	16.70	2.21	0.36	0.04	0.81	0.39	9.12
CS (%)	30.86	4.69	32.01	17.15	41.51	14.59	23.24	38.31	28.48	3.81	24.97	57.53	45.78	37.91	2.75	2.31	1.55	6.33	2.64	47.18
MS (%)	66.71	10.67	40.72	43.95	38.63	64.00	67.72	46.35	27.05	15.35	16.42	7.48	40.45	10.05	3.97	12.38	7.37	7.37	7.74	36.48
FS (%)	0.60	22.78	6.22	21.20	2.83	11.43	5.73	6.27	3.29	16.14	1.04	0.74	0.18	2.52	5.49	33.21	21.91	27.77	12.48	4.55
VFS (%)	0.03	36.92	0.14	8.98	0.08	0.46	0.13	2.17	0.64	24.07	0.24	0.24	0.02	2.34	13.12	31.77	24.49	29.46	31.13	0.87
S+C (%)	0.01	23.72	0.13	6.51	0.08	0.31	0.07	1.59	0.43	39.34	0.36	0.18	0.01	2.17	18.13	19.83	44.58	28.13	45.16	0.79
OM (%)	0.96	7.91	1.64	2.59	0.95	1.49	1.26	1.79	0.95	14.46	0.72	0.63	0.73	2.67	6.62	6.49	17.21	11.77	16.30	2.14

Appendix 3.1.2. List of the identified species in each assemblage defined by MDS analysis. The mean (± standard deviation) abundance (ind./m^2) and biomass (g AFDW/m^2), total number of species (Total S) and Shannon-Wiener (H´log$_e$) and evenness (J´) indices are given.

Taxa	Assemblage A				Assemblage B				Assemblage C				Assemblage D				Assemblage E			
	Mean A	**± sd**	**Mean B**	**± sd**	**Mean A**	**± sd**	**Mean B**	**± sd**	**Mean A**	**± sd**	**Mean B**	**± sd**	**Mean A**	**± sd**	**Mean B**	**± sd**	**Mean A**	**± sd**	**Mean B**	**± sd**
Nemertea																				
n.i.									10.0	11.5	0.012	0.014								
Annelida																				
Polychaeta																				
Ampharete grubei (Malmgren, 1865)													5.0	12.8	0.002	0.004				
Alkmaria romijni (Horst, 1919)					8.3	15.9	0.003	0.006												
Capitella capitata (Fabricius, 1780)					46.7	81.1	0.016	0.026												
Eteone longa (Fabricius, 1780)					1.7	5.8	0.001	0.005												
Eunicidae n.i.																				
Eupolymnia nesidensis (Delle Chiaje, 1828)					5.0	12.4	0.003	0.006												
Glycera convoluta (Keferstein, 1862)					5.0	12.4	0.003	0.007												
Hediste diversicolor (Müller, 1776)	16.7	18.7	0.040	0.049	48.3	37.6	0.050	0.057					41.0	44.2	0.117	0.186				
Maldane sarsi (Malmgren, 1865)					6.7	15.6	0.002	0.005												
Nephtys hombergi (Savigny, 1818)	1.7	5.8	0.006	0.020	16.7	28.1	0.019	0.030												
Owenia fusiformis (Delle Chiaje, 1841)					3.3	11.5	0.001	0.003												
Pectinaria koreni (Malmgren, 1866)					6.7	9.8	0.003	0.005												
Pygospio elegans (Claparède, 1863)	5.0	9.0	0.002	0.004	3.3	7.8	0.003	0.006												
Scolelepis squamata (Müller, 1806)					13.3	29.9	0.003	0.006												
Spio filicornis (Müller, 1776)					21.7	32.4	0.005	0.006												
Spiophanes bombyx (Claparède, 1870)					5.0	12.4	0.002	0.005												
Streblospio benedicti (Webster, 1979)					53.3	91.2	0.012	0.018	15.0	19.1	0.007	0.009								
Oligochaeta																				
Lumbricidae n.i.																	41.9	86.7	0.450	0.969
Lumbriculidae n.i.																	1772.5	1836.6	0.788	0.854
Naididae n.i.									5.0	10.0	0.004	0.007	3.0	9.8	0.002	0.005	133.8	150.0	0.062	0.083
Tubificidae n.i.																	3.1	17.7	0.001	0.006
Hirudinea																				
sp1																	22.5	39.9	0.025	0.049
sp2																	4.4	18.1	0.006	0.023
sp3																	0.6	3.5	0.002	0.010

Mollusca																				
Bivalvia																				
Anodonta anatina (Linnaeus, 1758)																	1.3	4.9	0.325	1.352
Cerastoderma edule (Linnaeus, 1758)	3.3	7.8	0.007	0.018	1.7	5.8	0.002	0.006												
Cerastoderma glaucum (Poiret, 1789)					10.0	16.0	0.013	0.022												
Corbicula fluminea (Müller, 1774)													1838.0	965.2	170.653	118.116	1356.9	1415.1	160.998	171.08
Fabulina fabula (Gmelin, 1791)					3.3	7.8	0.006	0.014												
Mytilus galloprovincialis (Lamarck, 1819)	1.7	5.8	0.039	0.133																
Pisidium amnicum (Müller, 1774)																	127.5	142.8	0.882	1.144
Pisidium casertanum (Poli, 1791)																	3.8	9.4	0.002	0.004
Pisidium subtruncatum (Malm, 1855)																	1.3	4.9	0.001	0.003
Psilunio littoralis (Lamarck, 1801)																	1.9	5.9	1.207	3.852
Scrobicularia plana (da Costa, 1778)					50.0	63.5	0.054	0.071					1.0	4.5	0.026	0.116				
Spisula solida (Linnaeus, 1758)	1.7	5.8	0.053	0.184	1.7	5.8	0.046	0.158												
Unio pictorum (Linnaeus, 1758)																	1.9	5.9	0.449	1.442
Gastropoda																				
Ancylus fluviatilis (Müller, 1774)													1.0	4.5	0.001	0.003	4.4	12.2	0.010	0.029
Bithynia tentaculata (Linnaeus, 1758)																	18.8	32.5	0.550	1.016
Hydrobia ulvae (Pennant, 1777)					8.3	13.4	0.007	0.012												
Lymnaea peregra (Müller, 1774)													3.0	7.3	0.013	0.036	25.0	53.3	0.050	0.115
Physella acuta (Draparnaud, 1805)																	3.8	9.4	0.004	0.011
Potamopyrgus antipodarum (Gray, 1843)													118.0	152.2	0.054	0.082	99.4	204.8	0.067	0.127
Valvata piscinalis (Müller, 1774)																	8.8	12.4	0.028	0.048
Arthropoda																				
Crustacea																				
Asellus aquaticus (Linnaeus, 1758)																	0.6	3.5	0.000	0.001
Atyaephyra desmarestii (Millet, 1831)																	2.5	6.7	0.059	0.160
Bathyporeia pilosa (Lindström, 1855)	5.0	12.4	0.005	0.012	5.0	9.0	0.003	0.005												
Carcinus maenas (Linnaeus, 1758)					1.7	5.8	0.015	0.051												
Corophium multisetosum (Stock, 1952)													551.0	831.3	0.138	0.202				
Crangon crangon (Linnaeus, 1758)					6.7	13.0	0.125	0.241												
Cyathura carinata (Kroyer, 1847)					30.0	30.2	0.021	0.021					66.0	79.8	0.060	0.069				
Diogenes pugilator (Roux, 1829)	1.7	5.8	0.029	0.100																
Gammarus chevreuxi (Sexton, 1913)									60.0	81.6	0.034	0.040	371.0	649.5	0.130	0.235				
Gammarus pulex (Linnaeus, 1758)																	1.9	5.9	0.002	0.005
Gammarus sp													36.0	70.7	0.001	0.005				

Haustorius arenarius (Slabber, 1769)	10.0	18.1	0.045	0.097																
Melita palmata (Montagu, 1804)	1.7	5.8	0.002	0.006	1.7	5.8	0.002	0.006												
Praunus sp	1.7	5.8	0.004	0.016	0.0	0.0	0.000	0.000												
Saduriella losadai (Holthuis, 1964)									20.0	28.3	0.095	0.110	9.0	16.5	0.023	0.076				
Sphaeroma serratum (Fabricius, 1787)													3.0	9.8	0.001	0.004				
Insecta																				
Chironomidae n.i.													44.0	77.2	0.039	0.105	68.8	168.6	0.047	0.105
Leptoceridae n.i.													1.0	4.5	0.001	0.005				
Leptophlebiidae n.i.																	2.5	6.7	0.003	0.010
Insecta n.i.																	3.1	11.5	0.005	0.019
Vertebrata																				
Pisces																				
Ammodytes tobianus (Linnaeus, 1758)	1.7	5.8	0.093	0.322																
Anguilla anguilla (Linnaeus, 1758)																	1.3	4.9	0.419	1.968
Petromyzon marinus (Linnaeus, 1758)																	2.5	6.7	0.126	0.438
Total	51.7	24.8	0.325	0.500	365.0	214.4	0.416	0.318	110.0	108.9	0.151	0.141	3092.0	1816.3	171.262	118.140	3716.3	2504.1	166.568	171.76
Total number of species	12				26				5				17				28			
H'(loge)	2.09				2.70				1.27				1.31				1.36			
J'	0.84				0.83				0.79				0.46				0.41			

Appendix 3.2.1. Abiotic factors of water and sediments: temperature (T-ºC), conductivity (CND - µScm^{-1}), total dissolved solids (TDS – mgl^{-1}), redox potential (ORP - mV), salinity (S), dissolved oxygen (DO - mgl^{-1}) pH, nitrites (mgl^{-1}), nitrates (mgl^{-1}), ammonia (mgl^{-1}), phosphates (mgl^{-1}), very coarse sand (VCS-%), coarse sand (CS-%), medium sand (MS-%), fine sand (FS-%), very fine sand (VFS-%), silt+clay (S+C-%) and organic matter (OM - %) in the three sampling stations from January to December of 2005.

Station 1	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
T (ºC)	8.6	7.4	13.6	14.8	18.7	21.5	21.7	22.8	19.8	16.8	11.5	12.1
CND (µScm^{-1})	169.0	157.0	99.0	146.0	157.0	89.0	112.0	122.0	103.0	147.0	123.0	158.0
TDS (mgl^{-1})	0.16	0.15	0.08	0.12	0.12	0.07	0.08	0.08	0.07	0.12	0.11	0.15
ORP (mV)	180.9	74.5	165.8	192.4	103.6	112.2	128.8	9.5	70.3	118.7	84.3	128.3
S	0.11	0.11	0.06	0.09	0.08	0.06	0.06	0.06	0.05	0.08	0.08	0.08
DO (mgl^{-1})	4.6	5.4	5.0	5.6	4.8	5.8	4.9	8.7	7.7	5.5	10.8	7.1
pH	7.37	7.15	7.33	7.25	7.28	7.14	7.18	7.79	7.45	7.19	7.31	7.53
Nitrites (mgl^{-1})	0.18	0.17	0.13	0.13	0.08	0.04	0.01	0.09	0.08	0.09	0.11	0.01
Nitrates (mgl^{-1})	0.98	0.80	1.38	1.20	1.23	1.16	1.44	1.50	1.12	2.18	1.14	1.10
Ammonia (mgl^{-1})	2.32	2.44	1.23	1.30	0.87	0.62	0.31	0.21	0.19	0.47	0.13	1.27
Phosphates (mgl^{-1})	0.41	0.59	0.35	0.35	0.23	0.17	0.21	0.27	0.27	0.23	0.16	0.24
Hardness (mgl-1)	15.0	5.0	15.0	5.0	20.0	20.0	15.0	30.0	20.0	35.0	30.0	20.0
VCS (%)	0.2	0.1	0.3	0.2	0.3	0.1	0.2	0.3	0.2	0.6	0.5	0.4
CS (%)	0.8	0.6	0.7	1.2	2.2	0.7	0.7	1.0	0.8	1.7	1.6	0.7
MS (%)	8.9	8.9	11.7	6.9	6.6	5.8	5.8	12.0	15.3	14.8	18.3	14.2
FS (%)	45.0	35.0	36.3	43.7	38.5	26.9	32.8	31.1	41.4	41.1	41.8	41.3
VFS (%)	24.8	28.2	28.2	25.3	32.4	37.3	37.2	34.4	22.2	21.2	21.4	26.4
S+C (%)	20.2	27.1	22.8	22.7	20.0	29.2	23.4	21.2	20.1	20.6	16.4	17.0
OM (%)	7.6	7.7	7.7	7.4	6.4	10.7	7.7	7.0	6.2	6.2	6.0	6.3

Station 2	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
T (ºC)	7.3	6.7	14.1	15.0	18.9	21.2	21.5	23.1	19.9	16.8	11.9	9.4
CND (µScm^{-1})	68.0	66.0	69.0	71.0	76.0	73.0	105.0	115.0	99.0	87.0	93.0	71.0
TDS (mgl^{-1})	0.07	0.07	0.07	0.06	0.06	0.06	0.07	0.08	0.07	0.07	0.08	0.09
ORP (mV)	197.0	151.7	150.3	91.5	50.8	87.6	66.5	38.4	23.3	66.6	91.2	78.5
S	0.05	0.05	0.04	0.04	0.04	0.04	0.05	0.06	0.05	0.05	0.06	0.05
DO (mgl^{-1})	10.7	9.9	9.9	9.5	8.7	9.0	8.1	9.9	8.1	10.5	12.4	12.7
pH	7.40	7.84	7.73	7.53	7.61	7.52	7.45	7.81	7.53	7.77	7.85	7.86
Nitrites (mgl^{-1})	0.04	0.04	0.00	0.02	0.03	0.03	0.06	0.05	0.05	0.03	0.00	0.00
Nitrates (mgl^{-1})	0.94	1.10	1.02	0.98	0.92	1.04	2.18	1.14	1.20	0.78	0.62	1.08
Ammonia (mgl^{-1})	0.00	0.00	0.01	0.09	0.12	1.86	0.06	0.08	0.08	0.07	0.00	0.00
Phosphates (mgl^{-1})	0.04	0.02	0.09	0.06	0.04	0.05	0.16	0.04	0.18	0.19	0.03	1.05
Hardness (mgl-1)	20.0	10.0	15.0	30.0	25.0	20.0	60.0	30.0	35.0	25.0	70.0	25.0
VCS (%)	0.3	0.9	0.5	2.4	0.3	0.3	0.3	1.6	0.6	0.6	0.7	0.2
CS (%)	1.2	2.1	1.6	5.1	1.6	1.0	1.7	3.4	1.4	2.9	4.0	2.2
MS (%)	8.7	12.1	6.2	6.5	7.4	8.9	11.0	10.0	9.9	12.3	9.8	11.3
FS (%)	23.1	20.3	23.2	21.7	22.3	21.2	22.1	18.1	21.0	21.3	27.7	21.5
VFS (%)	26.1	23.4	31.4	27.8	29.8	31.9	27.2	32.4	30.2	28.3	32.0	35.5
S+C (%)	40.6	41.1	37.2	36.5	38.7	36.7	37.6	34.5	37.0	34.6	25.7	29.3
OM (%)	11.0	11.3	10.7	10.2	11.3	13.9	10.1	11.9	10.4	9.1	8.7	10.2

Station 3	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
T (ºC)	7.6	6.9	13.8	14.7	18.6	20.3	20.7	22.2	19.3	16.6	11.8	9.3
CND (µScm^{-1})	69.0	67.0	67.0	70.0	75.0	71.0	101.0	114.0	97.0	67.0	92.0	68.0
TDS (mgl^{-1})	0.07	0.07	0.07	0.06	0.06	0.06	0.07	0.08	0.07	0.05	0.08	0.08
ORP (mV)	181.5	272.4	119.0	147.9	98.6	98.7	78.7	16.5	74.0	60.4	95.7	76.4
S	0.05	0.05	0.04	0.04	0.04	0.04	0.05	0.06	0.05	0.04	0.06	0.05
DO (mgl^{-1})	10.6	10.2	10.4	10.3	9.1	8.2	8.0	9.6	7.3	10.5	12.3	12.6
pH	7.55	7.87	7.76	7.28	7.85	7.77	7.51	7.84	7.56	7.93	7.84	7.91
Nitrites (mgl^{-1})	0.01	0.10	0.01	0.01	0.01	0.00	0.02	0.04	0.08	0.04	0.00	0.00
Nitrates (mgl^{-1})	1.02	0.86	0.00	0.94	0.89	0.84	1.34	1.34	1.38	0.72	0.94	1.08
Ammonia (mgl^{-1})	0.00	0.03	0.05	0.03	0.04	0.06	0.07	0.05	0.04	0.03	0.00	0.00
Phosphates (mgl^{-1})	0.02	0.04	0.00	0.08	0.06	0.14	0.07	0.09	0.12	0.09	0.04	0.13
Hardness (mgl-1)	25.0	10.0	10.0	20.0	10.0	10.0	5.0	15.0	15.0	15.0	70.0	15.0
VCS (%)	0.3	0.8	2.2	0.2	0.9	0.3	0.2	0.6	0.6	2.8	2.9	2.5
CS (%)	2.8	2.3	1.6	1.2	2.3	2.6	1.0	2.2	2.5	3.0	4.1	5.3
MS (%)	11.7	11.2	13.8	15.1	8.3	8.5	9.9	7.4	4.0	6.7	6.6	8.1
FS (%)	15.7	16.1	16.7	14.5	13.4	16.3	12.7	13.9	8.9	13.4	13.6	14.3
VFS (%)	28.6	29.5	28.7	24.5	33.6	32.2	33.0	36.0	38.8	30.1	29.2	26.3
S+C (%)	40.9	40.1	37.0	44.4	41.5	40.1	43.3	39.9	45.1	44.1	43.6	43.5
OM (%)	14.6	15.7	15.4	15.6	17.7	18.4	17.1	19.3	18.8	16.7	16.2	17.0

Appendix 3.2.2. List of the taxa identified in the freshwater subtidal area of the River Minho estuary. For each taxa, the mean abundance (ind./m²) is given (x – absent).

	S1Jan.	**S2Jan.**	**S3Jan.**	**S1Feb.**	**S2Feb.**	**S3Feb.**	**S1Mar.**	**S2Mar.**	**S3Mar.**	**S1Apr.**	**S2Apr.**	**S3Apr.**	**S1May**	**S2May**	**S3May**	**S1Jun.**	**S2Jun.**	**S3Jun.**
Ancylus fluviatilis (Müller, 1774)	x	X	4	x	x	X	x	X	x	x	X	X	x	x	x	x	x	x
Anadonta anatina (Linnaeus, 1758)	x	X	x	x	x	4	x	X	x	x	X	X	x	x	4	x	x	4
Anodonta cygnea (Linnaeus, 1758)	x	X	x	x	x	x	x	X	x	x	X	X	x	x	x	x	x	x
Bithynia tentaculata (Linnaeus, 1758)	8	64	460	4	80	292	x	108	340	124	96	396	8	60	420	128	16	152
Corbicula fluminea (Müller, 1774)	780	1712	1092	1476	1708	1096	792	1536	784	832	1820	744	772	1864	712	744	1972	1116
Gyraulus laevis (Alder, 1838)	x	X	x	x	x	x	x	4	x	x	X	X	x	x	x	x	8	x
Lymnaea peregra (Müller, 1774)	12	40	16	4	32	32	x	32	12	16	16	X	x	136	628	20	36	824
Physella acuta (Draparnaud, 1805)	x	8	16	x	x	12	x	4	12	x	X	4	x	20	8	8	28	104
Pisidium amnicum (Müller, 1774)	44	424	484	24	476	1024	172	384	488	472	364	948	740	428	520	1144	196	364
Pisidium casertanum (Poli, 1791)	x	X	x	x	8	x	x	4	4	8	4	44	8	4	16	4	x	4
Pisidium subtruncatum (Malm, 1855)	x	X	x	x	x	x	x	X	x	x	X	X	x	x	8	4	x	4
Potamopyrgus antipodarum (Gray, 1843)	168	204	16	216	268	20	664	348	4	2044	88	52	212	432	120	300	116	76
Psilunio littoralis (Lamarck, 1801)	x	X	x	x	x	x	x	X	x	x	X	X	x	x	x	x	x	4
Unio pictorum (Linnaeus, 1758)	x	X	x	x	x	x	x	X	x	x	X	X	x	x	4	x	x	4
Valvata piscinalis (Müller, 1774)	x	12	36	x	8	48	8	4	20	4	X	28	x	4	36	x	8	8
Total	1012	2464	2124	1724	2580	2528	1636	2424	1664	3500	2388	2216	1740	2948	2476	2352	2380	2664

	S1Jul.	S2Jul.	S3Jul.	S1Aug.	S2Aug.	S3Aug.	S1Sep.	S2Sep.	S3Sep.	S1Oct.	S2Oct.	S3Oct.	S1Nov.	S2Nov.	S3Nov.	S1Dec.	S2Dec.	S3Dec.
Ancylus fluviatilis (Müller, 1774)	x	X	x	x	x	x	x	X	4	x	X	X	X	x	X	x	x	x
Anadonta anatina (Linnaeus, 1758)	x	X	x	x	x	x	x	X	8	x	X	X	X	x	X	x	x	x
Anodonta cygnea (Linnaeus, 1758)	x	X	x	x	x	x	x	X	x	x	X	X	X	x	X	x	x	4
Bithynia tentaculata (Linnaeus, 1758)	x	X	248	20	32	1096	32	28	712	x	12	128	4	8	104	8	16	144
Corbicula fluminea (Müller, 1774)	864	1500	92	508	556	940	440	1136	876	264	292	480	304	292	524	372	556	304
Gyraulus laevis (Alder, 1838)	x	X	x	x	x	x	x	X	x	x	X	X	X	x	X	x	x	x
Lymnaea peregra (Müller, 1774)	x	116	20	12	48	88	x	16	96	x	X	12	X	4	12	8	x	20
Physella acuta (Draparnaud, 1805)	x	52	8	4	4	24	4	4	16	x	X	X	X	x	X	4	x	4
Pisidium amnicum (Müller, 1774)	372	124	164	268	100	308	104	132	240	28	160	300	76	124	192	48	156	188
Pisidium casertanum (Poli, 1791)	x	X	4	8	x	x	x	X	x	x	12	X	X	x	4	4	x	x
Pisidium subtruncatum (Malm, 1855)	x	4	x	x	x	x	x	X	x	x	8	X	X	x	X	x	x	x
Potamopyrgus antipodarum (Gray, 1843)	100	180	8	208	64	4	44	128	x	12	8	X	28	16	X	164	20	44
Psilunio littoralis (Lamarck, 1801)	x	X	x	x	x	x	x	X	x	x	X	X	X	x	X	x	x	x
Unio pictorum (Linnaeus, 1758)	x	X	x	x	x	4	x	X	4	x	X	X	X	x	X	x	x	x
Valvata piscinalis (Müller, 1774)	x	X	16	4	x	16	4	8	60	x	20	72	4	x	44	4	4	12
Total	1336	1976	560	1032	804	2480	628	1452	2016	304	512	992	416	448	880	612	748	720

Appendix 3.3.3. List of the taxa identified in the freshwater subtidal area of the River Minho estuary. For each taxa, the mean biomass (g AFDW/m^2) is given (x – absent).

	S1Jan.	**S2Jan.**	**S3Jan.**	**S1Feb.**	**S2Feb.**	**S3Feb.**	**S1Mar.**	**S2Mar.**	**S3Mar.**	**S1Apr.**	**S2Apr.**	**S3Apr.**	**S1May**	**S2May**	**S3May**	**S1Jun.**	**S2Jun.**	**S3Jun.**
Ancylus fluviatilis (Müller, 1774)	x	x	0.010	x	x	X	x	X	x	x	x	x	x	x	X	x	x	x
Anadonta anatina (Linnaeus, 1758)	x	x	X	x	x	6.736	x	X	x	x	x	x	x	x	6.852	x	x	6.102
Anodonta cygnea (Linnaeus, 1758)	x	x	X	x	x	X	x	X	x	x	x	x	x	x	X	x	x	x
Bithynia tentaculata (Linnaeus, 1758)	0.169	0.558	15.934	0.029	0.256	9.135	x	0.652	4.357	1.097	0.469	5.014	0.118	1.051	4.282	2.106	0.157	3.576
Corbicula fluminea (Müller, 1774)	268.689	146.265	169.422	424.858	145.959	165.716	248.055	141.275	153.970	249.384	152.793	129.843	269.327	204.737	162.359	232.793	193.186	219.178
Gyraulus laevis (Alder, 1838)	x	x	X	x	x	X	x	0.004	x	x	x	x	x	x	X	x	0.008	x
Lymnaea peregra (Müller, 1774)	0.473	0.500	0.387	0.232	0.437	0.810	x	0.599	0.184	0.236	0.214	x	x	0.507	0.797	0.078	0.041	1.097
Physella acuta (Draparnaud, 1805)	x	0.014	0.030	x	x	0.024	x	0.016	0.027	x	x	0.058	x	0.067	0.029	0.016	0.053	0.111
Pisidium amnicum (Müller, 1774)	0.163	1.793	2.703	0.057	2.106	5.466	0.790	1.752	2.516	2.086	1.962	5.310	1.754	2.422	2.507	3.297	1.155	1.580
Pisidium casertanum (Poli, 1791)	x	x	x	x	0.012	X	x	0.005	0.006	0.010	0.006	0.074	0.008	0.007	0.028	0.008	x	0.006
Pisidium subtruncatum (Malm, 1855)	x	x	x	x	x	X	x	X	x	x	x	x	x	x	0.013	0.007	x	0.008
Potamopyrgus antipodarum (Gray, 1843)	0.212	0.256	0.014	0.240	0.219	0.013	0.628	0.292	0.008	1.299	0.126	0.060	0.164	0.409	0.099	0.210	0.102	0.053
Psilunio littoralis (Lamarck, 1801)	x	x	x	x	x	X	x	X	x	x	x	x	x	x	X	x	x	19.897
Unio pictorum (Linnaeus, 1758)	x	x	x	x	x	X	x	X	x	x	x	x	x	x	5.950	x	x	3.728
Valvata piscinalis (Müller, 1774)	x	0.031	0.048	x	0.026	0.058	0.020	0.013	0.046	0.018	x	0.084	x	0.008	0.098	x	0.030	0.014
Total	269.706	149.417	188.549	425.417	149.014	187.958	249.492	144.606	161.114	254.129	155.570	140.443	271.371	209.207	183.014	238.515	194.732	255.351

	S1Jul.	S2Jul.	S3Jul.	S1Aug.	S2Aug.	S3Aug.	S1Sep.	S2Sep.	S3Sep.	S1Oct.	S2Oct.	S3Oct.	S1Nov.	S2Nov.	S3Nov.	S1Dec.	S2Dec.	S3Dec.
Ancylus fluviatilis (Müller, 1774)	x	x	x	x	x	X	x	x	0.009	x	x	x	x	x	x	x	x	x
Anadonta anatina (Linnaeus, 1758)	x	x	x	x	x	X	x	x	15.016	x	x	x	x	x	x	x	x	x
Anodonta cygnea (Linnaeus, 1758)	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	16.742
Bithynia tentaculata (Linnaeus, 1758)	x	x	2.880	0.061	0.169	5.128	0.132	0.124	10.601	x	0.121	1.903	0.235	0.299	1.548	0.047	0.495	3.023
Corbicula fluminea (Müller, 1774)	241.322	157.232	19.312	191.879	53.785	152.774	178.519	122.784	185.567	115.022	25.464	111.234	130.318	35.771	106.850	160.204	53.688	63.907
Gyraulus laevis (Alder, 1838)	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x
Lymnaea peregra (Müller, 1774)	x	0.185	0.104	0.090	0.231	0.329	x	0.142	0.286	x	x	0.174	x	0.245	0.369	0.390	x	0.302
Physella acuta (Draparnaud, 1805)	x	0.146	0.068	0.008	0.013	0.113	0.061	0.072	0.052	x	x	x	x	x	x	0.020	x	0.014
Pisidium amnicum (Müller, 1774)	1.238	0.663	0.947	1.010	0.576	1.906	0.535	0.677	1.272	0.158	1.331	1.929	0.569	1.079	1.244	0.343	1.220	1.271
Pisidium casertanum (Poli, 1791)	x	x	0.008	0.016	x	X	x	x	x	x	0.018	x	x	x	0.007	0.008	x	x
Pisidium subtruncatum (Malm, 1855)	x	0.007	x	x	x	X	x	x	x	x	0.012	x	x	x	x	x	x	x
Potamopyrgus antipodarum (Gray, 1843)	0.130	0.156	0.015	0.162	0.106	0.022	0.084	0.146	x	0.012	0.009	x	0.022	0.009	x	0.170	0.031	0.046
Psilunio littoralis (Lamarck, 1801)	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x
Unio pictorum (Linnaeus, 1758)	x	x	x	x	x	3.542	x	x	6.130	x	x	x	x	x	x	x	x	x
Valvata piscinalis (Müller, 1774)	x	x	0.073	0.022	x	0.045	0.031	0.066	0.218	x	0.166	0.374	0.026	x	0.136	0.026	0.019	0.040
Total	242.689	158.389	23.407	193.248	54.881	163.859	179.362	124.010	219.151	115.192	27.122	115.614	131.169	37.403	110.154	161.207	55.453	85.345

Appendix 4.1. Sequences available in GenBank used in this study.

Species*	**Origin**	**Haplotype***	**Accesion number**	**Reference**
C. fluminea	New Jersey, USA (NJ)		U47647	Baldwin *et al*., 1996
C. fluminea	Korea (Kr)		AF196269	Siripattrawan *et al*., 2000
C. fluminea	Thailand (Th)		AF196270	Siripattrawan *et al*., 2000
C. leana	Japan (Jp)		AF196268	Siripattrawan *et al*., 2000
C. japonica	Japan		AF196271	Siripattrawan *et al*., 2000
C. sandai	Japan		AF196272	Siripattrawan *et al*., 2000
C. sandai	Japan		AF196273	Siripattrawan *et al*., 2000
C. australis	Australia (Au)		AF196274	Siripattrawan *et al*., 2000
C. madagascariensis	Madagáscar (Md)		AF196275	Siripattrawan *et al*., 2000
Corbicula sp.	Michigan, USA (Mi)	Form A	AF196280	Siripattrawan *et al*., 2000
Corbicula sp.	North Carolina, USA (NC)	Form A	AF196281	Siripattrawan *et al*., 2000
Corbicula sp.	Utah, USA (Ut)	Form B	AF196278	Siripattrawan *et al*., 2000
Corbicula sp.	New Mexico, USA (NM)	Form B	AF196279	Siripattrawan *et al*., 2000
C. fluminea	France (Fr)	I	AF269090	Renard *et al*., 2000
			AF269092	
			AF269093	
C. fluminea	The Netherlands (Ne)	I	AF269091	Renard *et al*., 2000
C. fluminea	France	II	AF269094	Renard *et al*., 2000
Corbicula sp.	France	IV	AF269095	Renard *et al*., 2000
C. fluminalis	The Netherlands	V	AF269096	Renard *et al*., 2000
			AF269097	
C. fluminalis	France	V	AF269098	Renard *et al*., 2000
C. fluminea			AF120666	Giribet *et al*., 2002
C. japonica	Korea	KR1	AF367440	Park *et al*., 2002
C. japonica	Korea	KR2	AF367441	Park *et al*., 2002
Corbicula sp.	China (Ch)	FW2	AF457989	Park & Kim, 2003
Corbicula sp.	China	FW3	AF457990	Park & Kim, 2003
Corbicula sp.	Taiwan (Ta)	FW8	AF457991	Park & Kim, 2003
C. javanica	Indonesia (In)	FW9	AF457993	Park & Kim, 2003

Corbicula sp.	Korea	FW10	AF457992	Park & Kim, 2003
Corbicula sp.	China	FW11	AF457994	Park & Kim, 2003
Corbicula sp.	China	FW12	AF457995	Park & Kim, 2003
Corbicula sp.	China	FW13	AF457999	Park & Kim, 2003
Corbicula sp.	Vietnam (Vt)	FW15	AF468017	Park & Kim, 2003
Corbicula sp.	Vietnam	FW16	AF468018	Park & Kim, 2003
C. fluminalis	China	A	AF457996	Park & Kim, 2003
C. fluminalis	China	B	AF457997	Park & Kim, 2003
C. fluminalis	China	C	AF457998	Park & Kim, 2003
Corbicula sp.	Michigan, USA	Form A	AF519495	Lee *et al.*, 2005
Corbicula sp.	North Carolina, USA	Form A	AF519496	Lee *et al.*, 2005
Corbicula sp.	California, USA (Ca)	Form A	AF519497	Lee *et al.*, 2005
Corbicula sp.	Florida, USA (Fl)	Form A	AF519498	Lee *et al.*, 2005
Corbicula sp.	Cuba (Cu)	Form A	AF519499	Lee *et al.*, 2005
Corbicula sp.	Mexico (Me)	Form A	AF519500	Lee *et al.*, 2005
			AF519501	
			AF519502	
Corbicula sp.	Panama (Pa)	Form A	AF519503	Lee *et al.*, 2005
Corbicula sp.	Ecuador (Ec)	Form A	AF519504	Lee *et al.*, 2005
			AF519505	
Corbicula sp.	Peru (Pe)	Form A	AF519506	Lee *et al.*, 2005
Corbicula sp.	Argentina (Ar)	Form A	AF519507	Lee *et al.*, 2005
Corbicula sp.	Utah, USA	Form B	AF519509	Lee *et al.*, 2005
Corbicula sp.	New Mexico, USA	Form B	AF519510	Lee *et al.*, 2005
			AF519511	
Corbicula sp.	Argentina	Form C	AF519508	Lee *et al.*, 2005
			AF519512	
C. fluminea			AY874525	
C. fluminea	Texas, USA (Tx)		AY943243	
M. mercenaria	China	Y1	DQ399403	

(*) described by the authors

Appendix 5.1.1. Measured abiotic characteristics in each station, for the two sampled years. Temperature (T - ºC), conductivity (CND - µScm^{-1}), total dissolved solids (TDS - mgl^{-1}), redox potential (ORP - mV), salinity (S - psu), dissolved oxygen (DO – mgl^{-1}) pH, chlorophyll (Chl - µgl^{-1}), nitrites (mgl^{-1}), nitrates (mgl^{-1}), ammonia (mgl^{-1}), phosphates (mgl^{-1}) and hardness (mgl^{-1}) of water column and organic matter (OM - %), very coarse sand (VCS - %), coarse sand (CS - %), medium sand (MS - %), fine sand (FS - %), very fine sand (VFS - %) and Silt+Clay (S+C - %) of the sediment. The distance to the ocean (DST - km) is also given. Stations correspond to Fig. 5.1.1. Map and run SW to NE.

Abiotic variables/station	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**
Year 2004																
T (ºC)	17.4	17.3	17.1	17.0	17.6	17.3	17.7	17.9	17.9	17.5	17.3	16.3	16.4	16.3	16.4	15.8
CND (µS cm^{-1})	479.0	296.0	277.0	134.0	141.0	443.0	88.0	91.0	95.0	74.0	93.0	70.0	69.0	70.0	70.0	65.0
TDS (mgl^{-1})	0.38	0.24	0.22	0.11	0.11	0.34	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06
ORP (mV)	202.7	193.8	195.0	193.4	195.2	272.8	262.4	239.8	174.8	154.4	169.5	198.1	231.2	241.1	263.3	257.4
S (psu)	0.80	0.77	0.56	0.34	0.26	0.25	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04
DO (mgl^{-1})	10.8	11.1	10.9	10.8	9.9	8.6	9.2	9.1	9.3	10.7	10.7	10.6	10.3	9.9	9.9	9.5
pH	8.2	8.0	7.9	7.9	7.9	7.4	7.5	7.5	7.6	7.6	7.4	7.7	7.6	7.5	7.7	7.6
Chl (µgl^{-1})	5.0	5.2	4.9	5.3	5.1	5.2	5.6	5.3	4.8	4.3	5.6	5.5	5.4	5.3	5.4	5.1
Nitrites (mgl^{-1})	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.04
Nitrates (mgl^{-1})	0.80	1.04	0.60	0.52	0.63	1.14	0.94	0.78	0.96	0.81	1.02	1.12	1.08	0.64	0.84	1.22
Ammonia (mgl^{-1})	0.09	0.05	0.00	0.04	0.03	0.15	0.05	0.05	0.18	0.04	0.13	0.00	0.00	0.00	0.00	0.00
Phosphates (mgl^{-1})	0.07	0.19	0.04	0.06	0.07	0.00	0.13	0.00	0.08	0.00	0.05	0.07	0.06	0.03	0.07	0.29
Hardness (mgl^{-1})	55.0	40.0	35.0	35.0	20.0	10.0	10.0	20.0	40.0	20.0	20.0	15.0	20.0	10.0	5.0	5.0
OM (%)	1.3	10.7	0.5	0.6	0.9	2.6	3.0	2.3	8.7	10.9	10.4	14.0	0.8	0.4	1.4	0.5
VCS (%)	5.4	2.1	9.5	10.0	35.5	0.5	0.9	0.5	1.2	0.5	2.2	2.1	66.0	12.0	67.2	91.4
CS (%)	51.0	13.1	39.2	78.7	42.4	2.6	1.7	1.5	3.7	6.1	14.5	4.9	20.9	75.6	21.9	5.6
MS (%)	38.5	16.2	49.3	10.4	20.4	20.6	9.6	12.9	21.9	23.7	31.8	7.1	8.6	12.2	4.5	2.2
FS (%)	3.3	21.7	1.8	0.4	0.8	43.5	50.9	72.9	32.8	24.2	23.4	23.9	3.6	0.2	4.7	0.4
VFS (%)	1.1	20.7	0.1	0.3	0.5	27.9	29.6	10.4	18.4	25.0	13.6	34.5	0.8	0.0	1.4	0.2
S+C (%)	0.9	26.3	0.1	0.3	0.5	4.8	7.2	1.8	22.0	20.5	14.6	27.6	0.3	0.0	0.3	0.1
DST (km)	8	10	11	14	17	18	25	27	30	32	34	35	36	37.5	39	42

Year 2005																
T (ºC)	16.9	16.8	16.8	16.9	16.9	16.9	16.9	16.9	16.8	17.0	16.8	16.6	16.7	16.6	16.5	16.2
CND (μS cm^{-1})	1459.0	1715.0	247.0	138.0	109.0	104.0	101.0	97.0	147.0	106.0	87.0	67.0	72.0	62.0	60.0	50.0
TDS (mgl^{-1})	1.12	1.32	0.19	0.11	0.08	0.08	0.08	0.07	0.12	0.07	0.07	0.05	0.05	0.05	0.05	0.04
ORP (mV)	420.0	46.8	42.2	60.0	68.9	70.9	82.9	60.0	118.7	70.0	66.6	60.4	98.7	126.4	104.3	103.0
S (psu)	0.92	1.00	0.35	0.15	0.06	0.06	0.06	0.05	0.08	0.05	0.05	0.04	0.04	0.03	0.03	0.03
DO (mgl^{-1})	11.3	8.1	10.5	10.9	10.5	9.4	9.3	8.7	5.5	9.9	10.5	10.5	10.9	9.4	9.1	9.1
pH	8.4	8.3	8.0	8.1	8.0	7.4	7.4	7.4	7.4	8.0	8.1	8.0	8.0	8.2	8.2	8.2
Nitrites (mgl^{-1})	0.11	0.11	0.13	0.04	0.03	0.05	0.01	0.00	0.09	0.03	0.03	0.04	0.02	0.01	0.04	0.00
Nitrates (mgl^{-1})	1.22	1.42	1.42	1.38	1.24	1.22	2.18	1.34	2.18	1.88	0.78	0.72	0.72	0.72	0.56	0.92
Ammonia (mgl^{-1})	0.06	0.05	0.05	0.05	0.05	0.07	0.11	0.07	0.47	0.09	0.07	0.03	0.00	0.03	0.07	0.03
Phosphates (mgl^{-1})	0.16	0.22	0.17	0.13	0.10	0.16	0.13	0.09	0.23	0.22	0.19	0.09	0.06	0.10	0.02	0.10
Hardness (mgl^{-1})	60.0	35.0	35.0	30.0	15.0	5.0	10.0	15.0	35.0	25.0	25.0	15.0	45.0	15.0	0.0	0.0
OM (%)	1.6	11.9	0.9	0.6	0.9	1.1	3.8	12.6	6.2	12.3	9.1	16.7	1.6	0.8	2.5	0.6
VCS (%)	6.8	0.1	3.7	4.1	19.1	2.5	4.2	0.2	0.6	0.2	0.6	2.8	35.5	20.4	83.2	88.1
CS (%)	33.6	8.9	37.5	68.7	46.6	21.0	3.1	2.0	1.7	1.7	2.9	3.0	47.4	47.5	5.2	8.6
MS (%)	49.2	15.7	56.7	26.6	31.2	36.3	12.9	10.6	14.8	13.4	12.3	6.7	11.5	27.1	3.7	2.4
FS (%)	7.9	18.2	1.6	0.4	3.0	35.1	42.2	19.0	41.1	12.2	21.3	13.4	3.7	3.7	4.1	0.4
VFS (%)	1.5	22.5	0.2	0.1	0.1	4.0	25.2	31.1	21.2	30.2	28.3	30.1	1.5	0.9	2.2	0.1
S+C (%)	1.1	34.6	0.4	0.1	0.1	1.1	12.5	37.1	20.6	42.3	34.6	44.1	0.4	0.3	1.6	0.4
DST (km)	8	10	11	14	17	18	25	27	30	32	34	35	36	37.5	39	42

Appendix 5.2.1. Physical and chemical parameters of water and sediments at the nine sampling stations in the years 2004 and 2005: temperature (T - ºC), salinity (S - psu), dissolved oxygen (DO - mgl^{-1}), pH, gravel (G - %), very coarse sand (VCS - %), coarse sand (CS - %), medium sand (MS - %), fine sand (FS - %), very fine sand (VFS - %) and silt+clay (S+C - %) and organic matter (OM - %).

Station	**T (ºC)**	**S (psu)**	**DO(mgl^{-1})**	**pH**	**G (%)**	**VCS (%)**	**CS (%)**	**MS (%)**	**FS (%)**	**VFS (%)**	**S+C (%)**	**OM (%)**
S1/04	20.78	0.71	7.91	7.81	43.39	25.82	15.31	10.07	3.91	1.28	0.23	1.98
S2/04	20.13	0.74	8.11	8.72	10.14	22.67	25.92	14.08	13.11	10.70	3.39	6.05
S3/04	20.86	0.67	7.68	8.84	8.35	17.38	27.84	22.18	10.37	9.79	4.08	5.01
S4/04	20.38	0.70	8.42	8.73	6.38	15.33	20.40	23.83	15.19	9.58	9.30	6.71
S5/04	20.21	0.68	7.74	8.00	35.68	21.59	19.75	18.43	4.13	0.38	0.04	1.49
S6/04	20.53	1.20	7.89	7.98	32.08	33.75	23.15	8.73	1.63	0.53	0.12	0.92
S7/04	20.75	1.14	7.58	8.12	52.89	21.49	17.80	6.38	1.26	0.15	0.03	0.69
S8/04	20.58	0.65	8.01	8.26	37.51	24.39	22.99	13.08	1.78	0.22	0.04	0.71
S9/04	20.49	0.41	7.95	8.19	45.29	22.39	19.17	10.32	2.42	0.33	0.08	0.99
S1/05	22.65	3.14	8.01	7.63	43.82	26.81	14.82	9.75	3.79	0.79	0.22	1.90
S2/05	22.44	1.79	7.89	8.14	10.98	25.07	23.07	14.33	14.00	10.42	2.12	5.87
S3/05	22.86	2.69	7.52	8.84	9.52	17.86	30.91	22.85	9.50	7.01	2.35	4.76
S4/05	22.54	1.95	7.89	8.81	5.21	15.45	21.77	23.92	16.80	9.36	7.49	6.52
S5/05	22.38	1.80	8.42	8.73	33.00	22.30	21.03	20.26	3.01	0.37	0.04	1.71
S6/05	21.53	10.52	7.75	7.13	33.41	30.86	24.43	9.61	1.09	0.49	0.11	0.80
S7/05	22.75	12.06	7.74	7.29	48.40	22.82	18.24	9.51	0.81	0.19	0.04	0.74
S8/05	22.49	1.58	7.89	7.63	34.55	26.30	23.56	12.76	2.47	0.29	0.07	0.76
S9/05	21.18	0.57	7.65	7.70	41.29	23.60	22.42	10.63	1.42	0.50	0.14	1.11

Appendix 6.2.1. Mean values of the abiotic factors measured in the 16 sites along the four years of sampling. Standard deviation is given in brackets. Temperature (T), conductivity (CND), total dissolved solids (TDS), redox potential (ORP), salinity (S), dissolved oxygen (DO), pH, nitrites, nitrates, ammonia, phosphates and hardness of water column and organic matter (OM), very coarse sand (VCS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS) and silt+clay (S+C) of the sediment.

Abiotic factors	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
T (ºC)	17.23	(0.26)	17.31	(0.33)	17.18	(0.31)	17.13	(0.24)	17.25	(0.28)	17.16	(0.17)	17.25	(0.37)	17.30	(0.42)
CND (µScm^{-1})	703.00	(540.24)	635.50	(728.89)	209.50	(65.39)	121.00	(17.40)	114.75	(17.67)	188.75	(169.50)	98.25	(6.85)	99.25	(6.85)
TDS (mgl^{-1})	0.52	(0.42)	0.47	(0.57)	0.15	(0.06)	0.09	(0.02)	0.08	(0.02)	0.14	(0.13)	0.07	(0.01)	0.08	(0.01)
ORP (mV)	255.53	(109.67)	163.01	(77.98)	168.69	(85.36)	174.95	(78.33)	143.25	(53.75)	180.27	(83.31)	183.58	(74.81)	148.23	(74.00)
S (psu)	0.54	(0.39)	0.51	(0.45)	0.26	(0.24)	0.15	(0.14)	0.11	(0.10)	0.10	(0.10)	0.05	(0.01)	0.05	(0.00)
DO (mgl^{-1})	10.50	(0.70)	9.72	(1.24)	9.99	(1.05)	10.13	(0.92)	10.14	(0.38)	9.56	(0.70)	9.49	(0.43)	9.46	(0.74)
pH	7.99	(0.50)	7.94	(0.40)	7.82	(0.24)	7.82	(0.33)	7.81	(0.27)	7.48	(0.12)	7.49	(0.10)	7.46	(0.08)
Nitrites (mgl^{-1})	0.05	(0.05)	0.05	(0.05)	0.05	(0.06)	0.05	(0.05)	0.03	(0.03)	0.02	(0.02)	0.02	(0.02)	0.01	(0.01)
Nitrates (mgl^{-1})	0.98	(0.18)	1.50	(0.76)	1.19	(0.53)	1.17	(0.52)	1.40	(0.74)	1.37	(0.43)	1.85	(0.62)	1.29	(0.37)
Ammonia (mgl^{-1})	0.08	(0.04)	0.06	(0.02)	0.06	(0.06)	0.06	(0.02)	0.66	(1.23)	0.10	(0.04)	0.06	(0.04)	0.06	(0.01)
Phosphates (mgl^{-1})	0.29	(0.38)	0.55	(0.74)	0.27	(0.35)	0.24	(0.33)	0.23	(0.32)	0.36	(0.56)	0.21	(0.20)	0.13	(0.20)
Hardness	46.25	(13.77)	40.00	(7.07)	32.50	(8.66)	30.00	(4.08)	23.75	(8.54)	17.50	(11.90)	22.50	(15.00)	26.25	(16.01)
OM (%)	1.62	(0.33)	11.28	(0.71)	0.77	(0.19)	0.58	(0.03)	1.26	(0.42)	1.38	(0.84)	2.79	(0.80)	8.45	(5.04)
VCS (%)	6.50	(2.46)	1.09	(0.79)	5.88	(2.85)	20.31	(17.34)	16.16	(14.82)	5.48	(7.39)	23.97	(26.20)	0.24	(0.17)
CS (%)	41.71	(7.75)	9.15	(2.79)	45.49	(8.35)	66.13	(12.57)	28.21	(19.29)	28.31	(23.37)	11.34	(10.65)	1.74	(0.34)
MS (%)	44.86	(6.28)	15.05	(1.10)	47.30	(7.71)	12.86	(9.30)	35.60	(12.58)	32.67	(12.91)	11.57	(1.60)	10.23	(2.53)
FS (%)	4.75	(2.14)	18.56	(2.28)	1.14	(0.64)	0.39	(0.05)	17.44	(18.16)	22.74	(20.04)	28.05	(21.74)	40.35	(26.29)
VFS (%)	1.18	(0.27)	21.34	(0.80)	0.08	(0.06)	0.15	(0.08)	1.92	(2.23)	9.03	(12.74)	17.63	(11.57)	24.09	(9.76)
S+C (%)	1.00	(0.41)	34.80	(6.30)	0.13	(0.18)	0.16	(0.08)	0.67	(0.53)	1.77	(2.09)	7.43	(3.75)	23.35	(17.08)

Abiotic factors	**9**		**10**		**11**		**12**		**13**		**14**		**15**		**16**	
T (ºC)	17.02	(0.66)	17.19	(0.23)	17.06	(0.23)	16.78	(0.39)	16.82	(0.32)	16.72	(0.36)	16.68	(0.28)	16.40	(0.53)
CND (μScm^{-1})	183.75	(126.63)	93.50	(16.82)	93.00	(9.93)	82.00	(17.839	83.25	(17.29)	78.75	(15.73)	78.00	(16.08)	72.25	(18.12)
TDS (mgl^{-1})	0.14	(0.11)	0.07	(0.00)	0.07	(0.00)	0.06	(0.01)	0.06	(0.01)	0.07	(0.01)	0.06	(0.01)	0.06	(0.01)
ORP (mV)	117.85	(65.68)	107.80	(50.15)	132.03	(53.48)	156.21	(64.51)	166.07	(60.58)	192.87	(48.31)	213.98	(73.85)	183.52	(64.03)
S (psu)	0.10	(0.07)	0.05	(0.00)	0.05	(0.00)	0.05	(0.01)	0.05	(0.01)	0.05	(0.01)	0.04	(0.01)	0.04	(0.01)
DO (mgl^{-1})	6.72	(2.33)	9.96	(0.53)	10.39	(0.32)	10.29	(0.42)	10.34	(0.47)	10.19	(1.12)	10.13	(0.88)	10.01	(1.15)
pH	7.41	(0.13)	7.77	(0.24)	7.66	(0.30)	7.71	(0.25)	7.62	(0.29)	7.59	(0.39)	7.65	(0.39)	7.61	(0.44)
Nitrites (mgl^{-1})	0.05	(0.03)	0.02	(0.01)	0.02	(0.01)	0.03	(0.02)	0.02	(0.01)	0.02	(0.01)	0.04	(0.02)	0.03	(0.02)
Nitrates (mgl^{-1})	1.45	(0.54)	1.10	(0.55)	0.78	(0.35)	0.89	(0.43)	0.89	(0.30)	0.66	(0.31)	1.08	(0.72)	0.99	(0.22)
Ammonia (mgl^{-1})	1.47	(2.13)	0.06	(0.03)	0.07	(0.04)	0.15	(0.27)	0.34	(0.67)	0.02	(0.03)	0.06	(0.05)	0.02	(0.02)
Phosphates (mgl^{-1})	0.49	(0.50)	0.18	(0.15)	0.20	(0.20)	0.18	(0.18)	0.17	(0.20)	0.19	(0.16)	0.26	(0.39)	0.22	(0.13)
Hardness	33.75	(17.02)	25.00	(10.80)	23.75	(12.50)	16.25	(10.31)	31.25	(11.09)	16.25	(4.79)	12.50	(11.90)	12.50	(21.79)
OM (%)	5.91	(2.05)	12.35	(1.11)	8.86	(1.45)	16.08	(1.42)	2.96	(2.32)	0.62	(0.17)	2.05	(0.53)	0.97	(0.47)
VCS (%)	0.91	(0.38)	0.44	(0.31)	1.86	(1.70)	3.14	(1.25)	33.13	(25.24)	27.64	(22.36)	51.89	(34.30)	81.76	(13.45)
CS (%)	2.33	(0.95)	3.12	(2.37)	7.24	(5.52)	4.24	(1.50)	27.65	(14.15)	50.74	(21.20)	16.14	(7.97)	7.44	(4.87)
MS (%)	18.20	(3.88)	12.29	(8.33)	18.28	(9.24)	10.11	(6.57)	16.73	(11.34)	19.79	(7.96)	20.97	(22.63)	6.19	(5.97)
FS (%)	42.19	(6.94)	21.44	(7.09)	24.98	(6.03)	18.42	(5.84)	12.51	(12.03)	1.38	(1.56)	7.32	(6.07)	3.90	(4.19)
VFS (%)	18.62	(3.48)	26.84	(2.63)	22.14	(7.33)	30.02	(3.68)	6.16	(5.82)	0.32	(0.42)	2.22	(0.71)	0.47	(0.38)
S+C (%)	17.75	(4.18)	35.85	(11.85)	25.51	(10.62)	34.08	(11.94)	3.83	(4.14)	0.13	(0.12)	1.47	(0.89)	0.24	(0.14)

Appendix 6.4.1. Mean values of the abiotic factors measured monthly from January to December 2005 in sites 9, 11 and 12. Standard deviation is given in brackets. Temperature (T), conductivity (CND), total dissolved solids (TDS), redox potential (ORP), salinity (S), dissolved oxygen (DO), pH, nitrites, nitrates, ammonia, phosphates and hardness of water column and organic matter (OM), very coarse sand (VCS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS) and silt+clay (S+C) of the sediment.

Abiotic factors		**Site 9**			**Site 11**			**Site 12**	
	Mean	**Maximum**	**Minimum**	**Mean**	**Maximum**	**Minimum**	**Mean**	**Maximum**	**Minimum**
T (ºC)	15.8	22.8	7.4	15.5	23.1	6.7	15.1	22.2	6.9
CND (μScm^{-1})	131.8	169.0	89.0	82.8	115.0	66.0	79.8	114.0	67.0
TDS (mgl^{-1})	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
ORP (mV)	114.1	192.4	9.5	91.1	197.0	23.3	110.0	272.4	16.5
S (psu)	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.0
DO (mgl^{-1})	6.3	10.8	4.6	9.9	12.7	8.1	9.9	12.6	7.3
pH	7.3	7.8	7.1	7.7	7.9	7.4	7.7	7.9	7.3
Nitrites (mgl^{-1})	0.1	0.2	0.0	0.0	0.1	0.0	0.0	0.1	0.0
Nitrates (mgl^{-1})	1.3	2.2	0.8	1.1	2.2	0.6	0.9	1.4	0.0
Ammonia (mgl^{-1})	0.9	2.4	0.1	0.2	1.9	0.0	0.0	0.1	0.0
Phosphates (mgl^{-1})	0.3	0.6	0.2	0.2	1.1	0.0	0.1	0.1	0.0
Hardness (mgl^{-1})	19.2	35.0	5.0	30.4	70.0	10.0	18.3	70.0	5.0
VCS (%)	0.3	0.6	0.1	0.7	2.4	0.2	1.2	2.9	0.2
CS (%)	1.1	2.2	0.6	2.4	5.1	1.0	2.6	5.3	1.0
MS (%)	10.8	18.3	5.8	9.5	12.3	6.2	9.3	15.1	4.0
FS (%)	37.9	45.0	26.9	22.0	27.7	18.1	14.1	16.7	8.9
VFS (%)	28.2	37.3	21.2	29.7	35.5	23.4	30.9	38.8	24.5
S+C (%)	21.7	29.2	16.4	35.8	41.1	25.7	42.0	45.1	37.0
OM (%)	7.2	10.7	6.0	10.7	13.9	8.7	16.9	19.3	14.6