



2008

Long-term changes on population dynamics and secondary production of *A. valida* and *M. palmata* (Amphipoda) in the Mondego estuary

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

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AGRADECIMENTOS

Ao meu orientador e amigo Professor Dr. Miguel Pardal pelo constante apoio, disponibilidade e acompanhamento demonstrado e sobretudo pelo sentido crítico que soube inculcar-me, neste meu ainda curto percurso de iniciação à investigação científica.

À Dra. Patrícia Cardoso por tudo o que fez para que a elaboração desta tese fosse possível. Quero expressar-lhe os meus mais sinceros agradecimentos pelo incansável incentivo e confiança que sempre demonstrou depositar em mim. Pela paciência, disponibilidade e pelos válidos conselhos e ideias enriquecedoras que em muito contribuíram para a concretização desta tese, o meu muito obrigado!

À Dra. Marina Dolbeth pela boa disposição e entusiasmo contagiante e pela grande ajuda prestada nos cálculos de produção secundária e na resolução dos mais variados problemas.

Ao IMAR – Instituto do Mar, pelo apoio técnico e logístico necessários à realização deste trabalho.

A toda a equipa do IMAR, em particular ao pessoal das triagens e das saídas de campo de bentos, pelo companheirismo, amizade e boa disposição, que proporcionando um ótimo ambiente de trabalho em muito contribuíram para a minha integração no seio do grupo e desenvolvimento desta tese.

A todos os meus amigos por, longe ou perto, permanecerem a meu lado e me incentivarem nesta caminhada, mesmo quando as frustrações e as adversidades insistem em demover-nos do que ambicionamos. Em especial, o meu obrigado ao Ricardo, Tânia, Lisa, Joana, Tozé, Patrocínia, João, Ana, Zé e tantos outros que me acompanharam nestes últimos anos.

Aos meus pais por continuarem a acreditar em mim e pela compreensão e apoio que sempre demonstraram nos momentos mais difíceis. A eles o meu muito obrigado, por me ajudarem continuamente a ser o que sou hoje!

RESUMO

Nas últimas décadas, o estuário do Mondego tem sofrido intensa perturbação ecológica devido ao crescente processo de eutrofização. As alterações biológicas mais visíveis foram a ocorrência de blooms macroalgais em paralelo com o declínio da área ocupada pelos bancos da macrófita *Zostera noltii*, que se traduziu num empobrecimento geral de todo o ecossistema em termos de abundância e biomassa macrofaunais e de riqueza específica. Devido à degradação da qualidade ambiental do estuário, foram implementadas, em 1998, várias medidas de gestão de forma a promover a recuperação dos bancos de *Zostera noltii*, diminuir a descarga de nutrientes e melhorar as condições hidrodinâmicas do sistema. Até ao início dos anos 90, espécies de anfípodes como *Ampithoe valida* e *Melita palmata* constituíam um grupo abundante de crustáceos, desempenhando um papel importante na estrutura trófica das comunidades macrobentónicas do estuário do Mondego. Durante o bloom macroalgal no verão de 1993 e em todos os períodos de maior disponibilidade de macroalgas verdes, foram observadas elevadas densidades de *A. valida* e *M. palmata*. O progressivo declínio da biomassa de macroalgas verdes, como resultado das medidas de recuperação implementadas no estuário, afectou negativamente ambas as espécies de anfípodes, especialmente *A. valida*. Os efeitos negativos foram particularmente visíveis ao nível da abundância, biomassa e produção secundária e quase implicaram o total desaparecimento de ambas as populações de anfípodes, em especial de *A. valida*. Ambas as espécies apresentaram comportamentos distintos ao longo do período em estudo, o que pode dever-se a diferentes estratégias alimentares e habitats preferenciais. A espécie *A. valida* parece demonstrar particular preferência por algas efémeras filamentosas e de consistência mole como as do género *Ulva* sp., devido ao seu elevado

conteúdo calórico, utilizando apenas os bancos de *Zostera noltii* como habitat de protecção contra potenciais predadores e acção das marés. Por outro lado, a população de *M. palmata* não sofreu um forte decréscimo na sua densidade e biomassa, o que pode indicar que a espécie não é, provavelmente, um consumidor primário de macroalgas verdes e pode fácil e rapidamente migrar para nichos ecológicos alternativos em busca de alimento para sobreviver.

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Ampithoe valida and *Melita palmata* (Amphipoda) in the Mondego estuary**

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ABSTRACT

The Mondego estuary (Portugal) suffered severe ecological stress over the last decades, due to increased eutrophication, recognized as a major threat worldwide. The most visible biological changes were the occurrence of macroalgal blooms and the concomitant decline in the area covered by *Zostera noltii* beds, which caused a general impoverishment of the whole ecosystem in terms of macrofaunal abundance, biomass and species richness. Since the conservation status of the estuary was compromised, several restoration measures were implemented in 1998 to promote the recovery of the seagrass beds, decrease nutrient loading and improve water dynamics. Until the early 1990s, amphipods like *Ampithoe valida* and *Melita palmata* appeared as a common and abundant group of crustaceans, playing an important role in the trophic dynamics of the macrobenthic communities of the Mondego estuary. Highest densities of *A. valida* and *M. palmata* were recorded during the macroalgal bloom in the summer of 1993 and in

all periods in which green macroalgae were available. Both species were negatively affected by the progressive decline of green macroalgal biomass promoted by the restoration measures implemented in the estuary, especially *A. valida*. The negative effects were particularly visible at abundance, biomass and growth production levels and implied the almost disappearance of amphipods population, mainly *A. valida*. Our data suggest that both species displayed distinct behaviours all over the time that could be related to different food strategies and habitat preferences. It seems that *A. valida* showed particular preference for ephemeral softer, filamentous or bladed algae (e.g. *Ulva* sp.) due to its high caloric content, using the *Z. noltii* beds only as a habitat for protection against predators or shelter from wave action. On the other hand, the gammaridean amphipod *M. palmata* did not suffer a strong decline of its population density/biomass, which can indicate that this species is not probably, a primary consumer of green macroalgae and may readily shift to alternative ecological niches searching food to ensure its survival.

Keywords: Eutrophication; Macroalgal blooms; *Zostera noltii* beds; Ecological restoration; *Ampithoe valida*; *Melita palmata*; Food preferences

1. INTRODUCTION

Estuaries represent partially enclosed transition zones where freshwater from inland rivers mixes with salt water from oceans, creating one of the most productive and ecologically rich ecosystems on Earth (Bergstrom et al., 2004). The benefits to society supported by estuarine environments and shallow coastal zones are recognized

worldwide since they provide essential biological functions (e.g. decomposition, nutrient cycling and flux regulation of water, particles and pollutants) and important services such as unique habitat for a wide diversity of marine life, food resources for migratory and resident species, shoreline protection, commercial fishing and recreational purposes (Kennish, 2002; Bergstrom et al., 2004; Dolbeth et al., 2007). Due to their shallow depth, estuaries are particularly vulnerable to environmental parameters and more often are susceptible to anthropogenic disturbances other than the natural ones, which add an extra stress to the system. (Marques et al., 2007).

In many coastal areas all over the world, anthropogenic activities are affecting the ecological functions of estuarine environments resulting in changes in the structure and dynamics of biotic communities and habitat modification. The primary causes of environmental deterioration of estuaries are adjustments of natural water flow and organic pollution, result of urban and industrial wastewater discharges, aquaculture and agricultural run-off. The main effect of this influx is a massive nutrient enrichment (N and P) of estuarine waters and adjacent coastal areas, which often give rise to eutrophication, recognized as a major problem, worldwide (Kennish, 2002; Pardal et al. 2004; Lillebø et al., 2005; Dolbeth et al., 2007). Eutrophication is considered a process by which productivity of a water body increases as a result of changes in its nutritional status and dominance patterns among marine macroalgae (Kraufvelin et al., 2006; Wang, 2006). When extreme, eutrophication can produce negative impacts through developing hypoxic conditions and possible blooms of undesirable taxa. Under such severe stress, the macrofaunal benthic assemblages can similarly present dramatic changes, with domination by capitellid polychaetes and other opportunistic species (Posey et al., 2006; Cardoso et al., 2007). One of the direct symptoms of the increase in nutrient availability in a given water body is the extreme growth and proliferation of

opportunistic green macroalgae, which can cover extensive areas of estuarine intertidal zones, in replacement of rooted plant communities dominated by slow-growing species, as the perennial macrophyte *Zostera noltii*. (Pardal et al., 2000).

Throughout the world, seagrass beds represent an ecologically important feature of shallow coastal areas by providing not only food, shelter and physical heterogeneity, but also contributing to the increase of coastal waters productivity, recycling nutrients, decreasing water turbulence and stabilising sediments through their rhizome systems (Jonge et al., 2000; Carvalho et al., 2006). Therefore, benthic invertebrate communities among seagrass beds are frequently more productive and display higher abundance compared to adjacent non-vegetated areas (Carvalho et al., 2006; Dolbeth et al., 2007).

Like many other coastal systems, the Mondego estuary (Portugal) has suffered intense environmental stress for several years which has mainly been caused by eutrophication processes (Lillebø et al., 2005). As a consequence, *Zostera noltii* beds, which represent the original more pristine conditions of the estuary (Dolbeth et al., 2007) and the richest habitat with regard to productivity and biodiversity (Pardal et al., 2000), has almost disappeared reducing in area extent from 15 ha in the early 1980s to a small patch of 0.02 ha in 1997 (Dolbeth et al., 2003; Pardal et al., 2004; Lillebø et al., 2005). Presumably, competition, related to different strategies to uptake nutrients and shading effects of macroalgae on macrophytes, was in the basis of replacement of seagrass by green opportunistic macroalgae (Rafaelli et al., 1991; Pardal et al., 2000). In parallel with the drastic decline of the *Zostera noltii* beds, the whole ecosystem became impoverished in terms of macrofaunal abundance, biomass and species richness with a concomitant decline of secondary production (Dolbeth et al., 2007; Lillebø et al., 2007).

In 1998, several restoration measures have been taken in order to restrain the advanced state of environmental degradation and to restore the original seagrass beds,

decrease nutrient loading and improve water dynamics in the Mondego estuary (Cardoso et al., 2008). The implementation of this restoration plan reflects the worldwide increasing concern to minimize adverse effects triggered by global human disturbance on estuarine, coastal and marine ecosystems.

According to the Society for Ecological Restoration (SER), *ecological restoration is the process of assisting the recovery and management of ecological integrity* (van Diggelen et al., 2001). The interest for ecological restoration in coastal areas, mainly those subjected to aquaculture and agricultural practices, urbanisation and tourism, has increased because of a large historical loss and habitats modification and therefore adverse ecological impacts. (Elliott et al., 2007). The general aim of restoration projects is to help a habitat return to a previously existing natural condition after being in a degraded or disrupted one (Kennish, 2000; Cardoso, 2005; Verdelhos et al., 2005), which is usually interpreted as being in poor ecological health (Elliott et al., 2007). Ecological restoration is becoming a crucial component of the conservation of biodiversity (Hobbs & Norton, 1996). Restoration requires explicit human intervention into degraded ecosystems to achieve a desired target, in opposition to conservation, which involves managing human activities to reduce their influence on nature (Peterson & Lipcius, 2003; Cardoso, 2005). Despite, both approaches, conservation and restoration, seem to be necessary to ensure integrity of natural ecosystems.

The success of restoration programmes depends at least in identifying and understanding the processes which have driven the observed ecological changes. There is also a need to monitor key system variables, evaluate progress of restoration and adjust procedures whether necessary (Hobbs & Norton, 1996, Cardoso, 2005; Verdelhos et al., 2005). If restoration is truly successful, then, the community established will be similar in species composition, population density and size and biomass structure to

that, previously, present in natural undisturbed conditions (reference site) (Elliott et al., 2007). The success of the restoration programme implemented in the Mondego estuary is evaluated in the present study through changes in the amphipods population, over a 12-year period.

Considering the species richness, abundance and its importance as a trophic resource for fish and bird populations, crustaceans constitute one of the central invertebrate groups of organisms within seagrass beds (Carvalho et al., 2006). Amphipods are probably the best represented and diverse group of crustaceans and appear to be a potentially strong ecological indicator for assessing seagrass degradation (Sánchez-Jerez et al., 2000; Carvalho et al., 2006). Species like *Ampithoe valida* (Smith, 1873) and *Melita palmata* (Montagu, 1804) are grazers that live on rocky substrata and on muddy bottoms, frequently in the presence of green macroalgae or macrophytes (e. g. *Zostera noltii*) in salinities higher than 29 (Pardal et al., 2000).

The species *A. valida* belongs to the family Ampithoidae and is considered a sedentary herbivore that builds mucous tubes on the plants they inhabit, thus somewhat limiting his foraging range or mobility (Cruz-Rivera et al., 2000). The gammaridean amphipod *M. palmata* is included in the family Melitidae and constitutes an invasive cosmopolitan species commonly found in European estuaries in connection with aggregates of the polychaete *Ficopomatus enigmaticus* (Obenat et al., 2006), besides associations with macroalgae and/or macrophytes.

Both species are broadly distributed worldwide, but even with this wide distribution, abundance and potentially important contribution to food webs in marine and estuarine communities, there are few studies and published information about population dynamics of the two species (Pardal et al., 2000; Obenat et al., 2006), especially under particular conditions, such as eutrophication. Between the mid-1980s

and the early 1990s, amphipods appeared as an abundant group in the Mondego estuary, most probably playing an important role in the structure and trophic dynamics of the benthic communities, by consuming micro and macroalgae and probably other small size invertebrates, and equally serving as prey for several organisms, such as commercially important fish species and migratory birds (Pardal, 1998; Pardal et al., 2000).

The present study aims to investigate the population dynamics and growth production of *Ampithoe valida* and *Melita palmata* during a long-term data series (12 years), comparing two distinctive periods: (1) pre-management and (2) post-management. It is also our interest to contribute to a better understanding about the ecology of these species, since the information available on the literature is very scarce.

2. MATERIALS AND METHODS

2.1. Study site

The Mondego estuary is a warm-temperate intertidal system, located in the central western coast of Portugal (40°08'N, 8°50'W). It covers an area of approximately 3.4 km² (Teixeira et al., 2007) and comprises two contrasting arms, northern and southern, separated by the Murraceira Island (Fig. 1).

The northern arm is deeper (4-8 m during high tide, tidal range 1-3 m), highly hydrodynamic and constitutes the principal navigation channel and the location of the Figueira da Foz harbour. The southern arm is shallower (2-4 m during high tide, tidal range 1-3 m) and is characterized by large areas of exposed intertidal flats during low

tide. Until 1998, this arm was almost silted up in the upstream sections, which obstructed the Mondego river outflow. So, water circulation in this system was mostly dependent on the tides and on the freshwater input from a small tributary, the Pranto River, with a flow controlled by a sluice which was regulated according to water needs of rice fields in the Mondego Valley. This freshwater discharge from agricultural areas represented an important source of nutrients into the southern arm (Cardoso et al., 2004, 2005; Lillebø et al., 2005; Verdelhos et al., 2005).

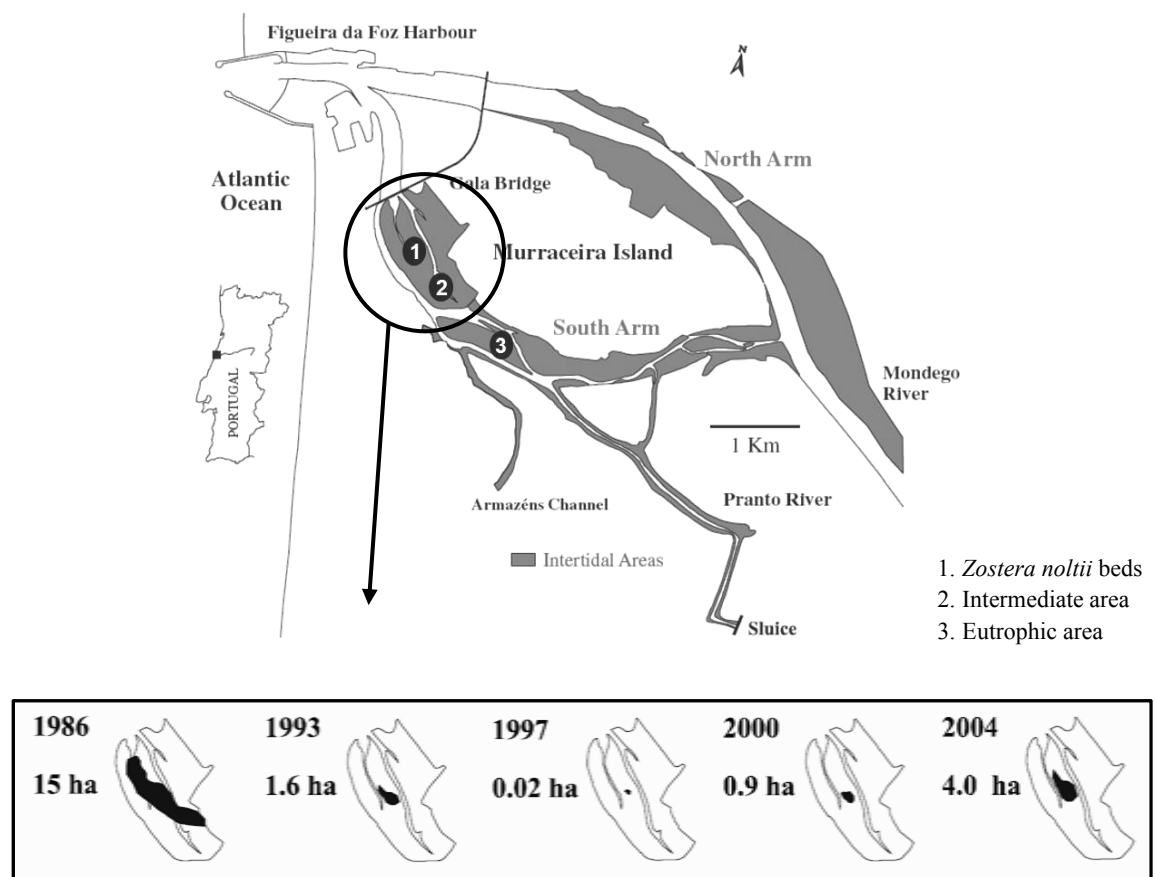


Figure 1. Location of the sampling stations and variation in the area covered by *Z. noltii* in the south arm of the Mondego estuary. Mapping of benthic vegetation is based in field observations, aerial photographs and GIS methodology (ArcView GIS version 8.2).

Since the 1930s, the Mondego estuary has suffered severe changes, particularly imposed by the construction of harbour facilities and consequent dredging activities on the northern arm that caused physical disturbance of the bottoms (Lillebø et al., 2005; Teixeira et al., 2007). Recently, more interventions were done along the river course and in the Mondego Valley to improve irrigation efficiency of the agricultural areas. The regularization of margins (to reduce flooding of the fields) and the construction of channels and sluices to regulate the water level inside the fields and intensification of land use are an example of that (Cardoso et al., 2007). These anthropogenic activities combined with specific physical characteristics (water residence time, hydrodynamics and depth) and climate conditions have contributed to an increase of environmental pressure on the estuary, leading to the emergence of the first symptoms of eutrophication, essentially in the south arm (Dolbeth et al., 2003; Cardoso et al., 2004; Pardal et al., 2004; Teixeira et al., 2007). The downstream areas of the south arm still remain relatively unchanged exhibiting sand muddy bottoms covered by *Spartina maritima* marshes and *Zostera noltii* beds, but in the inner parts, the seagrass community has completely disappeared and blooms of opportunistic green macroalgae *Ulva* spp. were frequent. The excessive nutrient release into the system coupled with low hydrodynamics and high salinity were in the basis of the occurrence of macroalgal (*Ulva* spp.) blooms (Pardal et al., 2000; Martins et al., 2005; Dolbeth et al., 2007).

In order to restore a previously existing stable state condition of the estuary and minimize the effects caused by the eutrophication process, namely the abruptly decline in area extent and biomass of the seagrass *Zostera noltii*, it was introduced in 1998 a management programme. The measures implemented included: (1) the improvement of water circulation by enlarging the upstream connection between the two arms; (2) the discharge of Pranto's freshwater by another sluice located furthermore upstream in the

Mondego River, which combined with the controlled use of fertilisers in agriculture fields decreased nutrient loading; (3) the physical protection of seagrass beds through the use of wooden stakes to prevent disturbance caused by fishermen who were digging in the sediment looking for bait; (4) several forums to inform local people for the ecological and economical importance of the *Zostera noltii* beds (Cardoso et al.2005, 2007; Lillebø et al., 2005; Verdelhos et al., 2005).

2.2. Sampling programme

Samples were taken during low tide in three study areas representing different environments along a spatial eutrophication gradient in the southern arm: (1) the *Zostera noltii* beds, corresponding to a non-eutrophic area, (2) the intermediate eutrophic area and (3) the eutrophic area (Fig 1).

The *Zostera noltii* beds, located towards the marine end of the estuary, are characterized by muddy sediments and covered with the macrophyte *Z. noltii*. Placed just upstream of the previous sampling station, the intermediate eutrophic area does not present seagrass cover, although some roots of *Zostera noltii* remain in the sediment. The eutrophic area, in the inner part of the estuary, does not support rooted macrophytes for a period larger than 25 years neither green macroalgal blooms (*Ulva* sp.) since the restoration measures were applied (Ferreira et al., 2007).

Sampling of amphipods was carried out from February 1993 to September 1995 and from February 1999 to December 2005. Samples were collected fortnightly in the first 18 months and monthly during the rest of the study period. On each sampling occasion, 6-10 sediment cores (141 cm² surface area) were randomly taken to a depth of 20 cm, by using a manual corer. Samples were washed in estuarine water

through a 500 μm mesh at each sampling station and then placed into plastic bottles and preserved in 4% buffered formalin. Environmental parameters as temperature, salinity, dissolved oxygen and pH were measured in situ in low water pools at each sampling occasion. Water samples were collected for subsequent analysis of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorous (DIP).

2.3. Laboratory procedures

Amphipods were identified to the species level, counted and measured. Due to the difficulties in accurately measuring total body length (T_L) in such small organisms, typically comma-shaped, was measured the cephalic length (C_L) as an alternative and more accurate length. It was considered that cephalic length corresponds to the distance measured between the extremity of the rostrum and the base of the head. An equation for C_L - T_L conversion ($T_L = - 0.1355 + 9.423^{C_L}$, $n = 262$, $r = 0.965$) and a single regression equation (W [weight] = $0.00085 \times C_L^{3631}$, $n = 95$, $r = 0.983$) were used, both based on previous estimations (Pardal et al., 2000). *A. valida* population was divided in age classes (juveniles < 2.5 mm (T_L); adults > 2.5 mm (T_L)), according to Pardal et al. 2000.

Amphipods biomasses were calculated as ash free dry weight (AFDW) after oven drying at 60 °C for 72 h and combustion at 450 °C for 8 h. This procedure was also used to quantify the seagrass and macroalgal biomasses (Pardal et al., 2000).

2.4. Production

Production was estimated by using size frequency method modified by Benke (1979) (Eq. 1).

$$P = a \left[\sum_{j=1}^{a-1} (\bar{N}_j - \bar{N}_{j+1}) \times \sqrt{(\bar{w}_j \times \bar{w}_{j+1})} \right] \frac{365}{CPI}$$

(Eq. 1)

where: \bar{N}_j , mean density in size class j (ind m⁻²); \bar{w}_j , mean individual weight in size class j (g AFDW); CPI , cohort production interval, i.e. life span (days); $j+1$, consecutive size classes ($j = 1, 2, \dots, a$).

Whenever data was not sufficient to apply the size frequency method, the sum of the increases in biomass from one sampling date to the next was done (Eq. 2).

$$P = \sum_{t=0}^{t=n} \left(\frac{N_t + N_{t+1}}{2} \right) \times (\bar{w}_{t+1} - \bar{w}_t)$$

(Eq. 2)

where: N_t , number of individuals from one sampling date (ind m⁻²); \bar{w}_t , mean individual weight at each sample date (g AFDW); t and $t+1$, consecutive sampling dates ($t=1, 2, \dots, n$).

For this procedure, the same equation of the increment summation method was applied, but without the recognition of cohorts. In spite of being less rigorous and accurate than size frequency method modified by Benke (1979) (Dolbeth et al. 2005),

this procedure was used to compute the secondary production due to low densities and biomasses presented by the two species in several years of the study period. The annual mean population biomass (\bar{B}) was also calculated and the P/\bar{B} ratios determined.

2.5. Data analysis

Differences regarding seagrass biomass between pre- and post-management periods were analysed with a two-sample t test by using the MINITAB 12.2 software package. The same statistical methodology was applied to determine differences in population density of *A. valida* and *M. palmata* before and after restoration measures. Changes in nutrients concentrations and macroalgal biomasses were assessed using the non-parametric Wilcoxon two-sample test. All data were previously checked for normality using Kolmogorov-Smirnov test and homogeneity of variances using the Levene's test (Zar, 1996). Data not meeting these criteria were transformed appropriately (Zar, 1996) and checked again for normality and homocedasticity.

3. RESULTS

3.1. Salinity and nutrients concentrations

The mean water temperature in the Mondego estuary showed a clear seasonal variation, with higher values during summer and lower temperatures along winter months. During summer, when precipitation was less frequent and water temperature reached values close to 30°C, salinity presented maximum values. On the contrary,

during periods of intense rainfall, which were more common during winter, the freshwater volume of the Pranto River naturally increased forcing to the opening of its sluice, which controls the water level in rice fields located upstream. As a result, salinity declined dramatically (sometimes to values lower than 5) suggesting that seasonal and inter-annual variation of salinity in the south arm of the Mondego estuary is strongly influenced by precipitation (see Lillebø et al., 2005) (Fig. 2). Results also suggest a tendency to an increase of salinity after the implementation of management measures, probably due to several drought periods that affected the region.

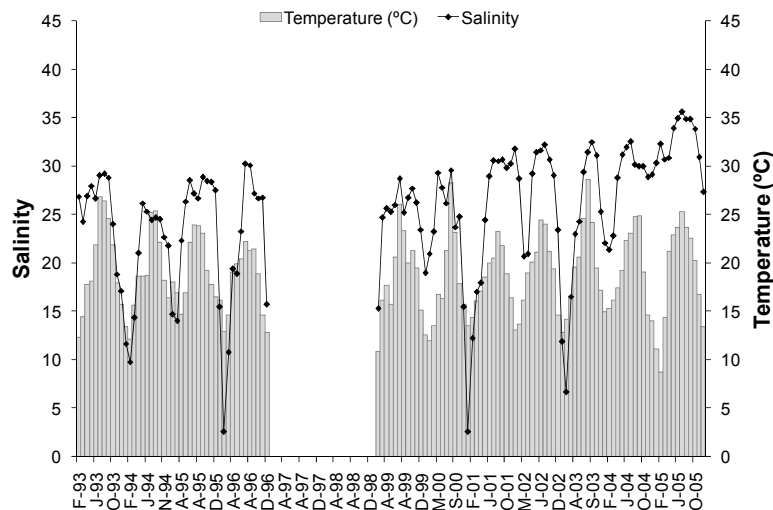


Figure 2. Long term variation of temperature and salinity in the Mondego estuary.

Regarding nutrients variation in the water column, there was a noteworthy reduction of DIN concentrations, essentially in the form of ammonia (Lillebø et al., 2005). Therefore, significant differences were found between the pre- and post-management periods for the two study sites (*Zostera noltii* beds, Wilcoxon two-sample test, $W = 4174.5$, $P < 0.05$; eutrophic area, Wilcoxon two-sample test, $W = 3997.5$, $P < 0.05$) (Fig. 3A). On the other hand, inorganic phosphorous concentrations (DIP) did not show the same tendency, showing an increase of its concentration through time (Fig.

3B). DIP levels exhibited significant differences between the two distinct periods (*Zostera noltii* beds, Wilcoxon two-sample test, $W = 2422.0$, $P < 0.05$; eutrophic area, Wilcoxon two-sample test, $W = 1724.5$, $P < 0.05$). Additionally, the variation of the N/P ratio showed the same pattern described for DIN concentrations, partly confirming the success of the management plan implemented in 1998 (Fig. 3C).

3.2. Seagrass and macroalgal biomasses

Significantly different scenarios were observed for seagrass biomass in the pre- and post-management periods (two sample t-test, $t_{45} = 12.59$, $P < 0.05$). Between 1993 and 1997, the *Z. noltii* beds suffered an area reduction from 1.6 to 0.02 ha (Fig. 1), as well as an abrupt decline in biomass from 253.3 to 5.7 g. AFDW m⁻², following eutrophication. After management, the scenario changed, with signs of gradual recovery of the seagrass biomass until the winter of 2001/2002 when an extreme drought occurred (Cardoso et al. 2008). In addition, during subsequent years, other stochastic weather events like the heat wave during the summer of 2003 seemed to have retarded the recovery of *Z. noltii*. Green macroalgal biomass was scarce throughout the 12-year period, whilst *Gracilaria* sp. increased (Fig. 4A).

In the intermediate area there were significant differences in macroalgal biomass before and after the management project (Wilcoxon two-sample test, $W = 3748.0$, $P < 0.05$). It was observed a considerable decline of green macroalgal biomass while the opposite succeeded with *Gracilaria* sp., whose biomass substantially increased, especially in the last two years (Fig. 4B).

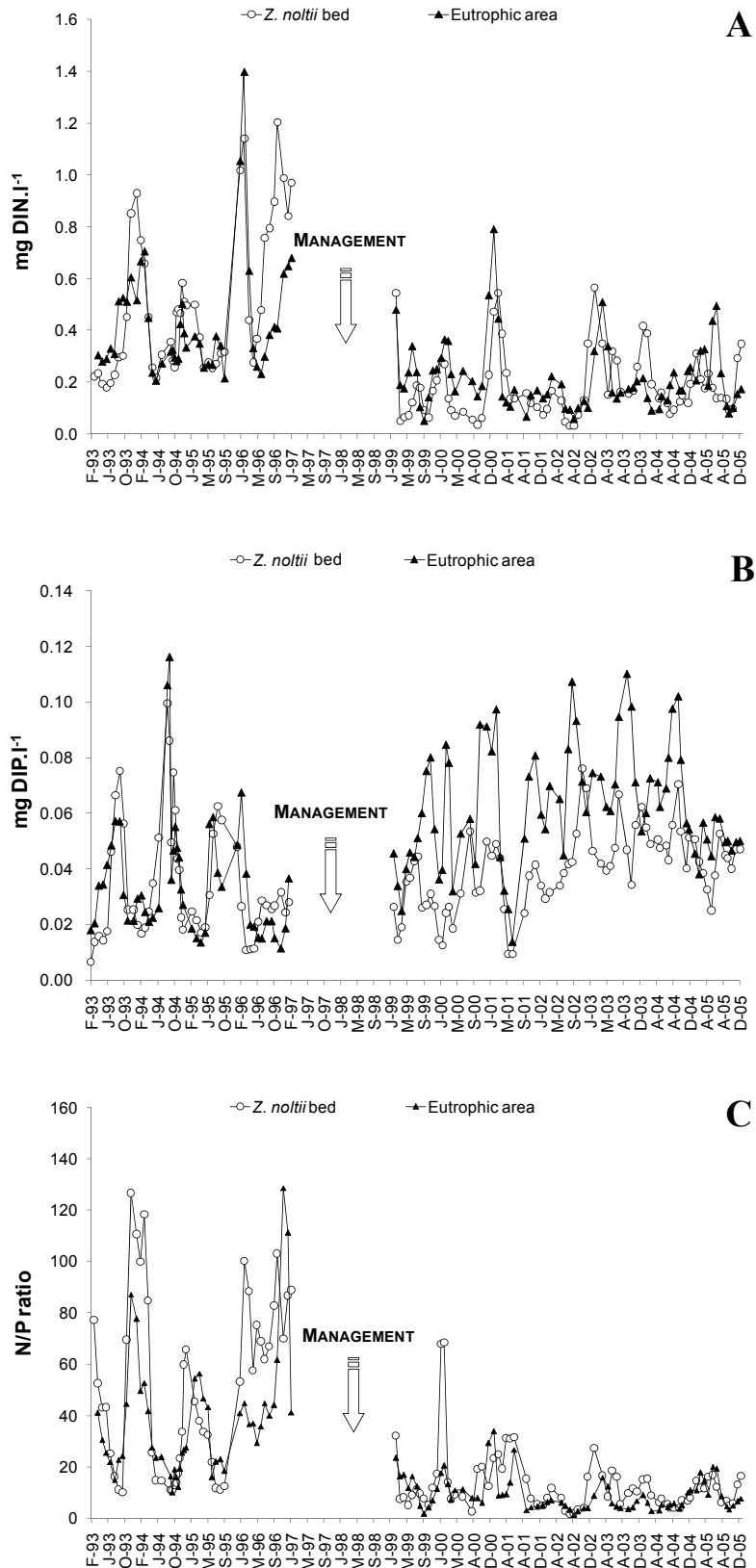


Figure 3. Long-term variation in nutrient concentrations. (A) Dissolved inorganic nitrogen (DIN); (B) Dissolved inorganic phosphorous (DIP); (C) N/P atomic ratio.

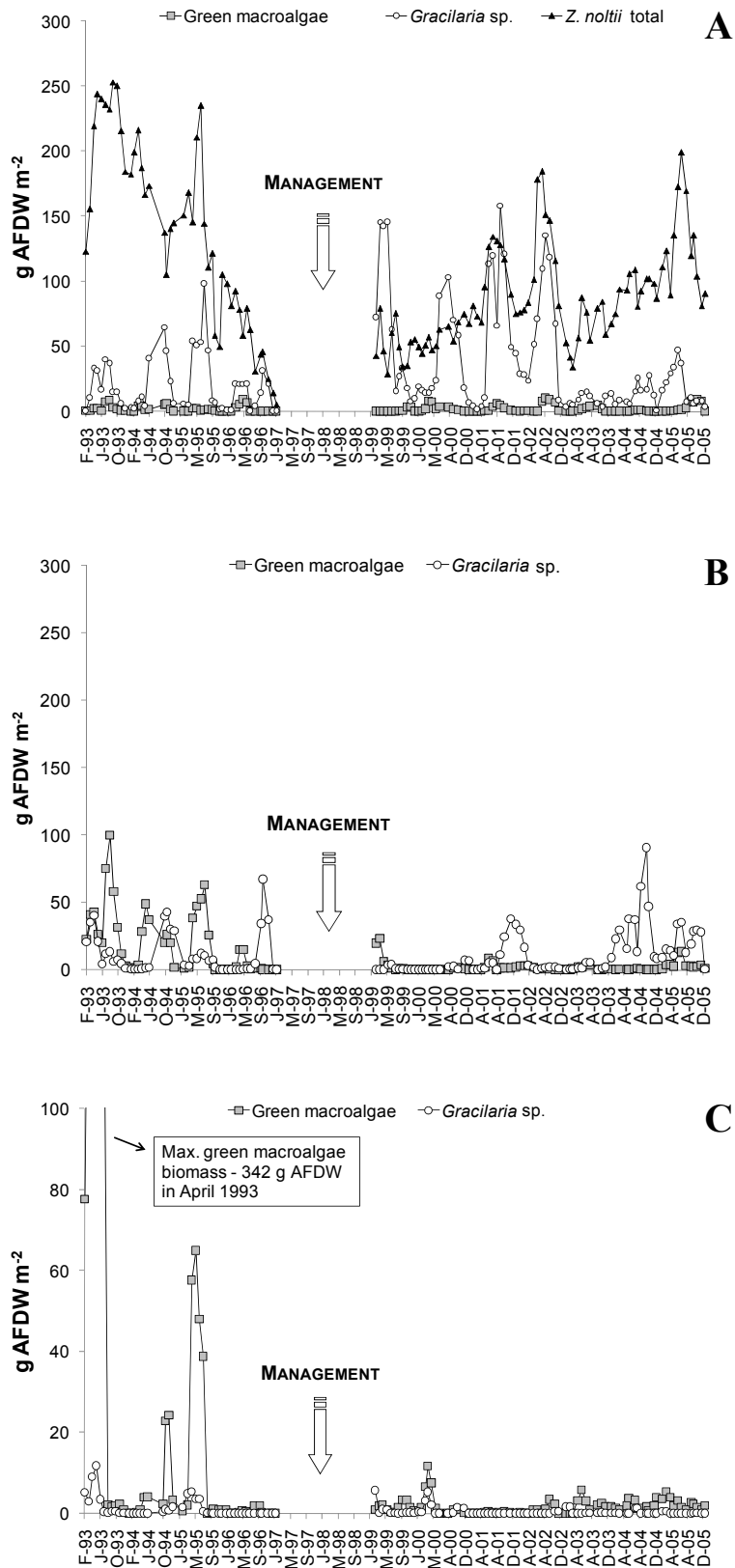


Figure 4. Variation of seagrass and macroalgal biomass from 1993 to 2005.

(A) *Zostera noltii* beds; (B) Intermediate area; (C) Eutrophic area.

The eutrophic area exhibited a clear spring algal bloom in 1993 reaching biomasses of 342 g.AFDW.m⁻² in April (Fig. 4C). In early summer an algal crash occurred, causing a severe impact on the population dynamics of amphipods (i.e. *A. valida* and *M. palmata*) (Fig. 5C). In 1994 no macroalgal bloom was recorded because it was a rainy year and salinity was kept low, therefore inhibiting green macroalgal growth. In the subsequent years, green macroalgal biomass decreased substantially following the introduction of restoration measures, in contrast to the previous period when these episodes were very common.

3.3. Macrofaunal abundance and biomass

3.3.1 *Ampithoe valida*

Ampithoe valida abundance and biomass presented considerable changes during the study period, but the pattern of variation was not the same along the eutrophication gradient. At the *Z. noltii* beds, were detected significant differences in population density before and after introduction of management measures (two sample t-test, $t_{106} = 6.10$, $P < 0.05$), being possible to verify a markedly decline in density and biomass throughout the 12-year period. *A. valida* was relatively abundant in the first three years, presenting maximum density values during autumn of 1993 and also during spring and summer of 1994 and 1995, respectively. After management, population density and biomass generally decreased and organisms almost disappeared, except during the spring of 2000, when a peak of density (887 ind.m⁻²) was observed (Fig. 5A).

In the intermediate area, abundance and biomass were always lower than in the seagrass beds. Population density of *A. valida* was significantly different between the

pre- and post-management periods (two sample t-test, $t_{99} = 10.56$, $P < 0.05$), being reduced after implementation of management measures (Fig. 5B). As well as in the *Z. noltii* beds, a considerable increase in density and biomass occurred in spring of 2000, but afterwards both decreased to residual values, maintaining a stable pattern until the end of 2005.

In the eutrophic area density and biomass were consistently higher during the algal bloom, with a peak in June of 1993 (1434 ind.m^{-2}), declining immediately after the algal crash until the end of the study. Significant differences in population density between the pre- and post-management periods were recorded (Wilcoxon two-sample test, $W = 3276.5$, $P < 0.05$). Nevertheless, *A. valida* population exhibited a slight increase in abundance and biomass whenever biomass of green macroalgal also increased, showing that population rapidly responds to any fluctuations in green macroalgal biomass. As examples, are some peaks observed in *A. valida* population during 2000 and between 2002 and 2005, which are consistent with the periods of higher green macroalgal biomasses (Fig. 5C).

Comparing the total population density with the adults' density, we can observe that the patterns are almost coincident indicating that the population of *A. valida* is mainly constituted by adults' individuals while juveniles only represent a small portion. This means that newborns are very rare, and the reproductive success of the species is threatened. For all sampling areas, significant differences were detected in adults' density between the pre- and post-management periods (*Zostera noltii* beds, two sample t-test, $t_{88} = 6.87$, $P < 0.05$; intermediate area, two sample t-test, $t_{58} = 5.26$, $P < 0.05$; eutrophic area, Wilcoxon two-sample test, $W = 2311.0$, $P < 0.05$), being observed a reduction in *A. valida* adults' density throughout the 12-year period (Fig. 6).

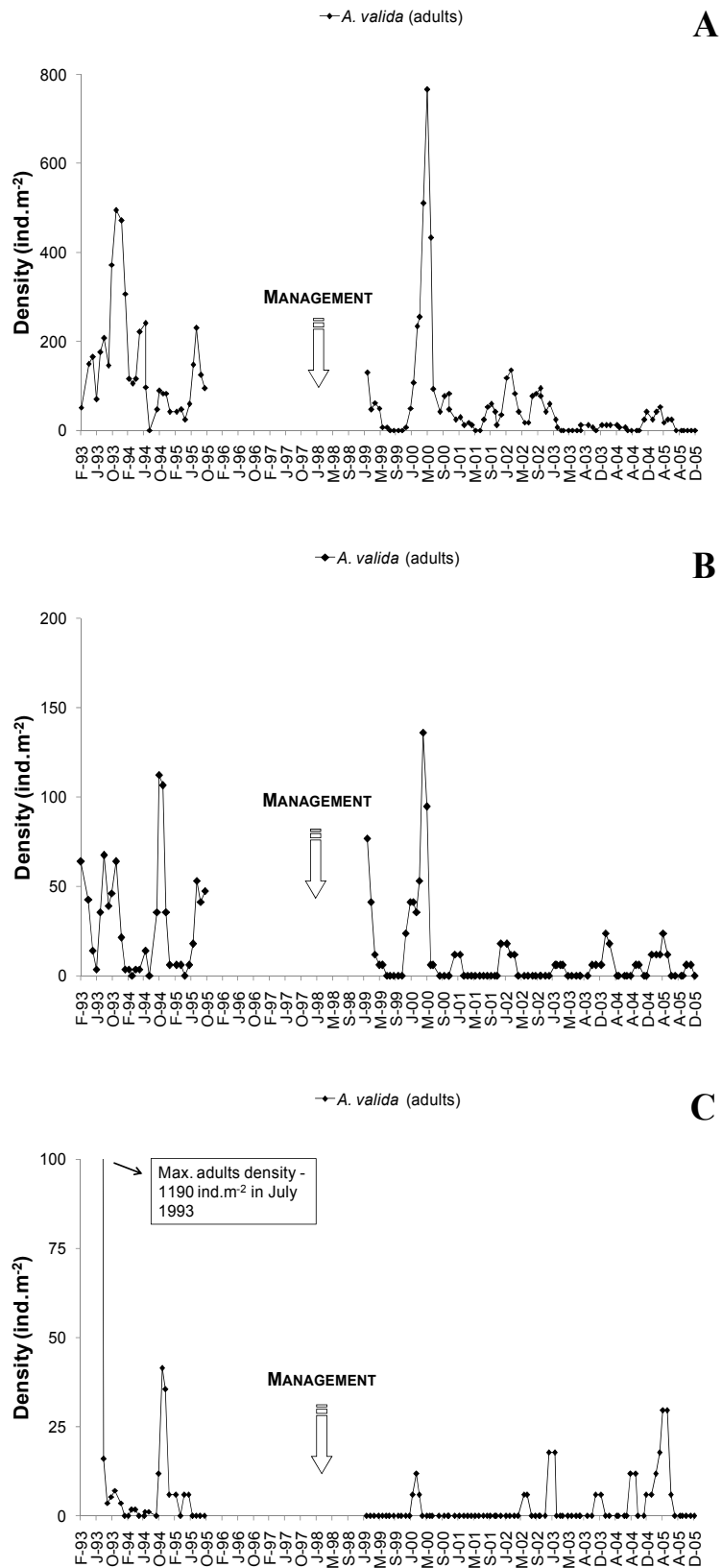


Figure 6. Variation of the adults' population of *Ampithoe valida* from 1993 to 2005.

(A) *Zostera noltii* beds; (B) Intermediate area; (C) Eutrophic area.

3.3.2. *Melita palmata*

The pattern of density and biomass of *M. palmata* is a little bit different from the one described for *A. valida*. Despite some oscillations in density pattern observed during the study period, the population did not suffer a huge decline after the management as *A. valida* did. Significant differences were found in population density in the *Zostera noltii* beds before and after management (two sample t-test, $t_{106} = 5.31$, $P < 0.05$). From 1993 to 1995, population density showed three peaks of abundance, the highest during fall of 1994 (maximum of 2730 ind.m⁻²). After implementation of management plan, density and biomass slightly decreased, although some peaks with similar magnitude to those registered in the summer of 1993 and 1995 were detected in dry periods (Fig. 7A).

Distinct scenarios were observed for the intermediate area between the pre- and post-management periods (Wilcoxon two-sample test, $W = 7847.0$, $P < 0.05$). Abundance and biomass of *M. palmata* after management were lower than in the pre-management period (Fig. 7B).

In the eutrophic area, no significant differences in population density (Wilcoxon two-sample test, $W = 1716.0$, $P > 0.05$) were established between the pre- and post-management periods. As described for *A. valida*, the algal bloom during the summer of 1993 caused an increase in density and biomass of *M. palmata*, with a maximum value in July (1252 ind.m⁻² / 0.3684 g.m⁻²), followed by a considerable decline after the algal crash. In the subsequent years, density and biomass remained low, but always higher than for *A. valida* (Fig. 7C).

3.4. Production

3.4.1. *Ampithoe valida*

Taking into account the whole study period, growth production (P) and mean population biomass (\bar{B}) exhibited higher values at the *Zostera noltii* beds than in the other sampling areas. Nevertheless, this difference does not reflect the entire reality. In fact, if we consider data on monthly production, we could recognize that, for a short period of time, during the influence of the algal bloom, P and \bar{B} were higher at the eutrophic area than in the *Zostera noltii* beds. From 1993 to 1995, a dramatic decline of P and \bar{B} occurred for all the three sampling stations, especially in the eutrophic area, where production and biomass were higher at the beginning of the study and decreased to residual values or even zero in 1994 and 1995, respectively. After the implementation of management measures, P and \bar{B} suffered a reduction and were always lower than in the previous period, for all study areas, except in 2000 when a production peak, only comparable to the values registered in 1994, was detected for the intermediate area and for the seagrass beds. The higher P/ \bar{B} ratio observed in 1993 for the eutrophic area and the *Zostera noltii* beds reflects the instability caused by the macroalgal bloom in these areas (Table 1).

Table 1. Growth production estimations of *Ampithoe valida* for the three sampling areas from 1993 to 2005. Legend: (-) no data available; () growth production values obtained by simple increment summation method.

	SEAGRASS BEDS			INTERMEDIATE AREA			EUTROPHIC AREA		
	P	\bar{B}	P/ \bar{B}	P	\bar{B}	P/ \bar{B}	P	\bar{B}	P/ \bar{B}
1993	0.701	0.095	7.377	0.052	0.017	3.024	0.958	0.134	7.164
1994	0.332	0.067	4.987	0.035	0.015	2.257	0.001	0.002	0.319
1995	0.128	0.033	3.903	-	-	-	0	0	-
1996	-	-	-	-	-	-	0	0	-
1997	-	-	-	-	-	-	-	-	-
1998	-	-	-	-	-	-	-	-	-
1999	0.016	0.010	1.706	<0.007	0.015	0.003	0	0	-
2000	0.335	0.070	4.815	0.033	0.016	2.019	0	0	-
2001	0.029	0.015	2.007	<u>0.007</u>	0.001	5.856	0	0	-
2002	0.036	0.021	1.688	0	0	-	<u>0.005</u>	0.001	5.652
2003	<u>0.010</u>	0.002	4.567	<u>0.009</u>	0.002	6.150	0	0	-
2004	<u>0.029</u>	0.004	6.833	<u>0.029</u>	0.005	6.290	<u><0.005</u>	<0.001	5.946
2005	<u>0.012</u>	0.017	1.900	<u>0.021</u>	0.004	4.846	0	0	-

3.4.2. *Melita palmata*

The annual production estimation for *M. palmata* at the three sampling areas showed that P and \bar{B} were generally higher at the *Z. noltii* beds for the whole study period. However, some exceptions must be mentioned. During the algal bloom of 1993 it was observed that P, \bar{B} and P/ \bar{B} ratio at the eutrophic area clearly exceeded the values registered in the seagrass beds, being rapidly reduced afterwards. Generally, during the post-management period, growth production and mean population biomass decreased and remained low for all sampling stations (Table 2).

Table 2. Growth production estimations of *Melita palmata* for the three sampling areas from 1993 to 2005. Legend: (-) no data available; ($_$) growth production values obtained by simple increment summation method.

	SEAGRASS BEDS			INTERMEDIATE AREA			EUTROPHIC AREA		
	P	\bar{B}	P/\bar{B}	P	\bar{B}	P/\bar{B}	P	\bar{B}	P/\bar{B}
1993	0.725	0.064	11.267	0.364	0.032	11.427	1.158	0.090	12.864
1994	2.221	0.351	6.323	3.077	0.357	8.608	0.035	0.013	2.584
1995	1.045	0.106	9.907	2.198	0.138	7.919	0.108	0.028	3.891
1996	-	-	-	-	-	-	-	-	-
1997	-	-	-	-	-	-	-	-	-
1998	-	-	-	-	-	-	-	-	-
1999	0	0	-	0	0	-	-	-	-
2000	0.053	0.146	0.362	<u>0.002</u>	0.003	0.794	0.017	0.005	3.371
2001	0.020	0.087	0.228	0.005	0.003	2.046	0	0	-
2002	0.136	0.372	0.366	0.003	0.006	0.581	<u>0.030</u>	0.014	2.123
2003	<u>0.017</u>	0.006	3.085	<u>0.009</u>	0.002	3.976	0.009	0.003	2.987
2004	<u>0.007</u>	0.002	4.624	0	0	-	<u>0.028</u>	0.004	6.320
2005	0.168	0.228	0.740	<u>0.003</u>	0.001	4.155	0.002	0.00	5.461

4. DISCUSSION

4.1. Salinity, nutrients concentrations and plants biomass

The seasonal and inter-annual variation of salinity in the south arm of the Mondego estuary is strongly influenced by precipitation, of which annual patterns vary according to the warm-temperate climate of the Mondego region, as well as temperature annual variation. The winters of 1995/1996 and 2000/2001 were particularly rainy comparatively to others. During those periods, an extensive opening of the Pranto River sluice contributed to the strong decline of salinity (Ferreira et al., 2007). On the contrary, the highest salinities were recorded during the warmer/dry periods, when the

freshwater discharges were lower and the run-off episodes almost negligible. This indicates that a major penetration of seawater occurred during spring and summer, while the lower salinities observed during autumn and winter resulted from the increase and predominance of freshwater inputs (Lillebø et al., 2005).

The nutrient-enriched waters, coupled with high residence time (5-7 days) and low transparency constituted the main sources of environmental stress in the Mondego estuary before implementation of management measures. All these factors led to a gradual decline in biomass and area covered by the macrophyte *Zostera noltii*. In contrast, opportunistic green macroalgae, through successful competition strategies to uptake nutrients, became the dominant primary producers causing severe implications to the ecological balance of the entire ecosystem, such as the impoverishment of macrofaunal benthic communities in terms of abundance, biomass and species richness (Pardal et al., 2000; Cardoso et al., 2004; Dolbeth et al., 2007). During the period comprised between 1993 and 1997, green macroalgal biomass exhibited several oscillations mainly dependent on temperature, salinity and hydrodynamic conditions. Huge spring algal blooms followed by sudden algal crashes were frequent in this period, which naturally caused instability in the macrobenthic community (Cardoso et al., 2004; Pardal et al., 2004; Verdelhos et al., 2005).

After 1998, the first signs of gradual recovery of the environmental quality of estuarine system started to emerge in response to the management measures previously introduced. The actions applied promoted, among other important changes, an improvement of water quality by reducing turbidity (from average particulate organic matter values of 0.02 mg.l⁻¹ before management to 0.003 mg.l⁻¹ after management) and increasing freshwater circulation that favoured nutrient dilution (DIN concentrations were effectively reduced after 1998) and decreased residence time to just 1 day

(Cardoso et al., 2005). As a consequence, green macroalgal biomass declined as well as the risk of spring algal blooms. In parallel, the *Zostera noltii* beds presented progressive signs of recovery, essentially visible in the increase of its biomass and area extent until the end of 2001. From this data on, an additional and unpredictable stressor retarded the total recovery of seagrass beds. Besides organic loading effects, the Mondego estuary suffered consecutive extreme weather events (floods, droughts and heat waves), which had a strong negative impact in the recovery process of the whole ecosystem (Cardoso et al., 2008).

4.2. *A. valida* and *M. palmata* abundances and biomasses

As a result of the macroalgal annual dynamics, both amphipod species showed clearly changes in population density and biomass along the eutrophication gradient. The abundance and biomass of both species in the eutrophic area were strongly influenced by the macroalgal bloom occurred during 1993, which provided favourable conditions for the presence of high densities of both species. During this episode, the population density and biomass for both species were much higher at the eutrophic area than in the *Z. noltii* beds that might be related to habitat protection against potential predators and food resources, since they can feed directly on the algae (Pardal et al., 2000). Afterwards, the scenario has completely changed with population's density in the inner parts of the estuary suffering a sudden and drastic decline due to the algal crash followed by extreme periods of anoxia in the summer of 1993, which acted as a catastrophic event (Marques et al., 1997; Pardal et al., 2000). *A. valida* is a well known sedentary herbivore that rarely leaves its tube except under adverse conditions. Individuals are able to move and swim rapidly for short periods in response to stressing

conditions (Nicotri, 1980). Our results suggest that *A. valida* population dynamics and spatial distribution may vary as a function of environmental changes through adaptive behavioural mechanisms (Pardal, et al., 2000). According to this, a possible movement of individuals inside the estuary to avoid severe conditions of anoxia (Pardal et al., 2000) caused by the algal crash, constitutes the most probable explanation for the increase in population density and biomass observed in the seagrass beds. As no macroalgal blooms were recorded at the eutrophic area in the subsequent years, there were no macroalgal habitats or food resources available to support amphipods population and therefore *A. valida* and *M. palmata* individuals seemed to occur preferentially in the *Z.noltii* beds and in the intermediate area.

Despite both species showed a quite similar response to the macroalgal bloom occurred during spring of 1993, the general behaviour displayed by the two species was clearly different when considered the whole study period. While abundance and biomass of *A. valida* strongly decreased, the population of *M. palmata* slightly declined and seemed to describe a more stable variation than *A. valida* throughout the study, which could be related to differences in food strategies and habitat preferences between both species.

Herbivore food preferences are of considerable interest to ecologists since they determine the quantity and quality of food ingested by a grazer and consequently affect its physiological condition and fitness (Nicotri, 1980). Numerous studies have shown that preference may be influenced by factors not exclusively related to food nutritional value, such as the concentration and composition of secondary metabolites and toughness of the food source (Poore et al., 2006). Additionally, there are other important habitat characteristics (protection against predators or shelter from wave action) that might influence herbivore choice as much as the food value of the plant (Nicotri, 1980)

or its chemical and physical properties. In general, the habitats of crustacean mesograzers are closely tied to their selection of diets, but the plant species that provides the best protection is perhaps not the one with the highest nutritive values (Kraufvelin et al., 2006). Understanding the factors involved in this selective behaviour is crucial for predicting the impact of herbivores on plant assemblages and the evolution of herbivore behaviour (Poore et al., 2006).

Attractiveness is one of the commonly recognized components of food preference and is related to the selection of a potential prey item. The amphipod *A. valida* is particularly attracted to ephemeral softer, filamentous or bladed algae (*Porphyra* sp. > *Ulva* sp. > *Gracilaria* sp.). This implies that macroalgae may be more important as a food source than as a habitat (Nicotri, 1980), contrarily to the genus *Zostera* sp. that is almost the last preferred food among 16 species of marine plants studied in attractiveness rank, which could indicate that these animals use the seagrass, essentially, as preferential habitat (Pardal et al. 2000; Poore et al. 2008). It seems that *A. valida* preference for these specific algal food types is directly related to its high caloric content. Furthermore, a significantly positive correlation between attractiveness and amphipods growth was found, suggesting that *A. valida* preferences are more related to the food value of the algae (Nicotri, 1980).

According to this, it becomes easy to understand the possible reason why population density and biomass of *A. valida* has decreased throughout the 12-year period. As a consequence of the disappearance of its preferential food source, observed by the decline in green macroalgal biomass, especially after the implementation of management measures, *A. valida* population also decreased. However, it was not probably just the absence of attractive food that determined its decline. The reduction in biomass and area covered by *Z. noltii*, caused by eutrophication, implied that less

suitable habitats were available for establishment of *A. valida* population, contributing in this way to its decline. In the present, the area covered by the macrophyte *Z. noltii* is still far from the area extent occupied in the early 1990s, which could also represent a constraint to the recovery of *A. valida* population.

Moreover, *A. valida* is not a specialist and it associates with a wide variety of macroalgae across all the three divisions (Chlorophyceae, Phaeophyceae and Rhodophyceae) as well as seagrasses at different sites around the world (Nicotri, 1980; Cruz-Rivera et al., 2000; Poore et al., 2008). The plasticity to colonize diverse habitats evidenced by this species, led us hypothesize about a possible mobilization of individuals to an alternative habitat besides the *Z. noltii* beds. According to a study evaluating the effects of diet mixing on consumer fitness of marine amphipods, *A. valida* occurred at high densities on *Fucus vesiculosus*, despite it was generally a poor food. The close association between *A. valida* and *Fucus vesiculosus* could be explained if the amphipod gains protection against predators, or achieves other indirect advantages, by living on and consuming a suboptimal food (Cruz-Rivera et al., 2000).

The ecology of melitids is less well known than the amphipods and the lack of reliable information about them represents an additional difficulty to a better understanding about its behaviour all over the time. In opposition to *A. valida*, the population density and biomass of *M. palmata* did not suffer a great impact with the progressive decline of green macroalgal biomass. As the population density and biomass remained more or less stable, even after the management measures, we may infer that the absence of green macroalgae did not constitute a limiting factor for the survival of the species. In other words, it means that *M. palmata* was not primarily consuming the green algae, but using it as a habitat only. Even if green algae is not available, individuals may readily shift to alternative ecological niches (A. Poore,

personal communication) and eventually adopt a different food strategy to survive, becoming detritivore organisms and feeding for example on periphyton.

Contrarily to other key species of the Mondego estuary, such as *Hydrobia ulvae* and *Scrobicularia plana*, that were positively affected by the management measures implemented, displaying a great increase in their abundance and biomass after 1998 (Verdelhos et al. 2005, Cardoso et al. 2008), both amphipods, in general, showed a negative response to the changes introduced in the estuarine system due to a significant decline in green macroalgal biomass, their preferential food.

4.3. Production

For both species, growth production estimations and mean population biomasses were consistently higher during the algal bloom in the eutrophic area. In the next years, with the absence of algal blooms and the decline of green macroalgae, growth production and mean population biomass decreased and were never able to recover at all the three sampling areas.

5. CONCLUSIONS

As a general conclusion, *A. valida* and *M. palmata* were negatively affected by the reduction of green macroalgal biomass promoted by the management measures implemented in 1998 in the estuary. The negative effects were particularly visible at abundance, biomass and growth production levels, especially for the *A. valida* population.

Interestingly, both species (especially *A. valida*) seemed to have an opposite response to the management measures comparatively to other species (e.g. *Hydrobia ulvae*, *Scrobicularia plana*) that responded positively to the changes implemented in the system, presenting higher abundance and biomass after 1998 (Verdelhos et al. 2005, Cardoso et al. 2008).

Despite green macroalgae constitute a good food resource for the amphipods population, when present in excess may have a strong negative impact by contributing to the destruction of its preferential habitat (seagrass beds) and eventually implying its total disappearance (Pardal et al., 2000). Thus, is important to preserve the *Z. noltii* beds, providing suitable habitat and protection from potential predators, and also maintain the proliferation of controlled amounts of green macroalgae in order to satisfy food needs of both amphipods' species and to ensure the ecological stability of the estuarine system.

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