Macroinvertebrate response to different species of macroalgal mats and the role of disturbance history

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Abstract

Over the last 20 years, the Mondego estuary, Portugal has experienced excessive growth of macroalgae especially in the inner parts of the system, with several algal species implicated. In this study, we compare the effects of morphologically different species, the red alga \textit{Gracilaria verrucosa} and the green macroalga \textit{Enteromorpha intestinalis} on macrobenthic assemblages, by a field experiment whereby the biomass of algae was manipulated and the resultant changes in macrofauna abundance evaluated. The experiments were carried out in different areas (a relatively undisturbed sea grass bed and an upstream eutrophic area) experiencing different degrees of overall enrichment.

Measurements of sediment redox potential revealed a rapid anoxia with a significant increase in algal biomass after 4 weeks. The effects of macroalgae were different at the two sites, being more marked in the eutrophic area. In addition, the effects of \textit{Gracilaria} and \textit{Enteromorpha} were significantly different, with \textit{Enteromorpha} having a greater detrimental effect for most of the macrofauna, in particular \textit{Cyathura carinata}, \textit{Scrobicularia plana}, \textit{Cerastoderma edule} and \textit{Alkmaria romijni}. However, three of the most abundant invertebrates (\textit{Hydrobia ulvae}, \textit{Hediste diversicolor} and \textit{Capitella capitata}) showed significant increases in abundance in weed affected compared to weed-free plots. \textit{Gracilaria} had less of an impact on macrobenthic assemblages leading to a more enriched community. Between-site differences in overall impact were related to their previous disturbance history.

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1. Introduction

Eutrophication of coastal waters as a result of anthropogenic activities is now widely recognized as a major, worldwide pollution threat (Vadas and Beal, 1987; Beukema, 1991; Fletcher, 1996; Norkko and Bonsdorff, 1996a,b; Beukema and Cadée, 1997; Valiela et al., 1997; Raffaelli et al., 1998; Lillebø et al., 1999; Pardal et al., 2000; Cloern, 2001; Sfriso et al., 2001; Cardoso et al., 2002). One of the direct symptoms of enrichment in estuaries is the extreme growth of opportunistic green macroalgae, often associated with increases in nutrition load (Raffaelli et al., 1989, 1998; Norkko and Bonsdorff, 1996a,b; Martins et al., 2001; Sfriso et al., 2001).

In intertidal areas, these algae can form dense mats, which negatively impact on the underlying macrofaunal assemblages, although at low biomasses there may be enhanced effects (Hull, 1987; Raffaelli et al., 1998). At low densities and for restricted periods of time, a patchy cover of algae increases habitat complexity and may facilitate local recruitment (Norkko and Bonsdorff, 1996a,b; Raffaelli et al., 1998). However, at high biomasses, macroalgal mats have been shown to negatively affect macrofaunal communities (Soulsby et al., 1982; Hull, 1987; Everett, 1994; Norkko and Bonsdorff, 1996a,b; Norkko et al., 2000). The majority of studies on the effects of macroalgal mats on intertidal macrofauna has focused only on green algae, mainly Enteromorpha (Raffaelli et al., 1998; Bolam et al., 2000) while few, if any, studies have concentrated on the effects of red macroalgal species.

In the Mondego estuary, Portugal, which has undergone significant eutrophication over the last two decades (Marques et al., 1997; Lillebø et al., 1999; Lopes et al., 2000; Pardal et al., 2000; Martins et al., 2001; Cardoso et al., 2002; Dolbeth et al., 2003), seasonal macroalgal blooms have been observed, particularly in the inner areas of the southern arm of the estuary from late winter to early summer when algae often collapse. These blooms are characterized by two quite different macroalgal taxa, the red Gracilaria verrucosa and the green Enteromorpha intestinalis, which have different morphological and ecological characteristics (Cabioc’h et al., 1992), and distinctly different macrofaunal responses might be expected (Raffaelli, 2000; Bolam and Fernandes, 2002). Here, we compare the effects of these two algae on the intertidal community in two different areas, an undisturbed sea grass bed and an eutrophic mudflat—to explore the effect of disturbance history on the outcome of such experiments.

2. Materials and methods

2.1. Study site and experimental design

The Mondego estuary is a warm temperate system located on the Atlantic coast of Portugal (40°08N, 8°50W). It comprises two arms, northern and southern, separated by an alluvium-formed island (Murraceira Island) (Fig. 1). The northern arm is deeper (4–8 m during high tide, tidal range 1–3 m) than the southern arm (2–4 m during high tide, tidal range 1–3 m) and is almost silted up in the upper zones, resulting in the freshwater outflow being mainly via the northern arm. Circulation in the southern arm is mostly
dependent on the tides and on the freshwater input from the Pranto River, a small tributary. The discharge from this tributary is controlled by a sluice and is regulated according to the water needs of rice fields in the Mondego Valley (Flindt et al., 1997; Lillebø et al., 1999).

Two different areas were selected as experimental sites along a well-documented gradient (Marques et al., 1993a,b, 1997; Lillebø et al., 1999; Pardal et al., 2000; Martins et al., 2001; Cardoso et al., 2002; Dolbeth et al., 2003) in the southern arm of the Mondego estuary: (a) sea grass (*Zostera noltii*) meadows, corresponding to a non-eutrophic area, and (b) the eutrophic area upstream (Fig. 1). The *Z. noltii* meadows are located downstream and are characterized by a mud flat covered by sea grass and where the red macroalga (*G. verrucosa*) is abundant, especially in late summer (50–100 g AFDW m$^{-2}$) (Lillebø et al., 1999; Pardal et al., 2000). The eutrophic area is located upstream in the inner part of the estuary. This sandy-mud area is characterized by the absence of rooted macrophytes (for more than 15 years) but covered seasonally by green macroalgae, especially *E. intestinalis* (Pardal et al., 2000; Martins et al., 2001; Cardoso et al., 2002; Dolbeth et al., 2003). These two areas are characterized by different macrobenthic communities as a result of their distinct biological and physicochemical features (e.g. presence/absence of sea grasses, pH, oxygen, salinity, total inorganic nitrogen) (Cardoso et al., 2002).

Within each of these experimental areas, six blocks, each consisting of eight treatment plots (plot size = 0.25 × 0.25 m), were set up in mid-September 2002. For each of the two
species of alga, three levels of algal biomass were added to the plots: 0.3 kg wet weight m$^{-2}$ (low biomass), 1.0 kg wet weight m$^{-2}$ (intermediate biomass) and 3.0 kg wet weight m$^{-2}$ (high biomass). The biomasses used span the range found at the sites (see Fig. 2 and Cardoso et al., 2002) and are consistent with those used by other workers (Hull, 1987; Raffaelli, 1999, 2000; Raffaelli et al., 1998).

Algae were maintained in position under sheets of wire mesh anchored with corkscrewed wire at each corner, penetrating several centimetres into the sediment. Additionally, a weed control (treatment left clear of algae but covered with wire mesh) and a mesh control (treatment clear of both algae and mesh) were established within each block. A mesh control was used to reveal any artefacts associated with the mesh while the weed control was used to assess weed effects on invertebrate assemblages by comparing this treatment with the other algal treatments. Thus, each block contained a plot of low, intermediate and high algal biomass for the two algal taxa, a mesh control and a weed control. The blocks were dispersed over the site to provide a randomized block design.

Algal material used in the experiment was previously collected from sites on the estuary where the species occur naturally and washed carefully to remove any associated fauna.

The experiment was set up at low tide and ran for 4 weeks and redox potential assessed regularly. After 4 weeks, the redox potential under the algal treatments became markedly negative and the experiment was terminated. This experimental period is

![Fig. 2. Variation of algal biomass in the Mondego estuary from January 1993 to September 1995.](image-url)
somewhat shorter than that of other experiments (Hull, 1987; Raffaelli, 1999, 2000; Bolam and Fernandes, 2002), due to higher temperatures recorded at these sites, which led to a rapid algal decomposition and anoxia. For this reason, we decided to finish the experiment before an algal crash could happen. After this period, plots were sampled for macrofauna, taking a single core (containing material at the surface) from each plot using a 13-cm diameter corer to a depth of 10 cm. Each sample was taken from the centre of the plot to avoid possible edge effects. Samples were washed in estuarine water over a 500 μm mesh and the fauna retained preserved in 4% buffered formalin. Later, animals were separated and kept in 70% ethanol. Macrofauna was identified to the lowest possible taxon and counted. Sediment redox potential values were measured at the end of the experiment using an Eh electrode (Crison pH/mv-506). Recordings were made at 4-cm depth.

2.2. Data analysis

Two-factor ANOVA (sites × algal treatments) was carried out followed by a multiple comparison test, the Tukey test, if significant, in order to determine where differences between treatments lay. All data were previously checked for normality using the Kolmogorov–Smirnov test and for 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.

Between-treatment and between-site comparisons were also made at the community level using nonmetric Multi-Dimensional Scaling (MDS) ordination (Clarke and Warwick, 2001; Clarke and Gorley, 2001). The MDS plot was derived from the similarity matrix based species abundance data in each replicate plot for all the treatments. Raw counts were square root transformed to scale down the effects of very abundant species (Clarke and Warwick, 2001). To validate our interpretation of the MDS, we performed the ANOSIM test (analysis of similarities), built on a simple nonparametric permutation procedure, applied to the similarity matrix underlying the ordination of the samples (treatments) (Clarke and Warwick, 2001).

3. Results

3.1. Macrofauna

After 4 weeks of algal cover, there were effects of both algal species at both experimental sites. However, the effects were dependent on both the algal taxon and whether the site had a history of disturbance. The abundance of the several species suffered modifications, some of them increased while others decreased in the presence of weed (Enteromorpha and Gracilaria). Furthermore, there was no change in the total number of species: from the 18 species of the eutrophic area and 14 of the Z. noltii meadows, just 7 were sufficiently abundant for statistical analysis.

No significant effects of the mesh on species abundance were detected. For clarity, we review the effects of the two algal taxa at each site and then compare the sites.
3.1.1. Effects of algae in the disturbed area

Overall, *E. intestinalis* had a greater impact than *G. verrucosa* for all biomass levels on most of the macroinvertebrate species, namely *Cyathura carinata*, *Scrobicularia plana*, *Cerastoderma edule*, *Hediste diversicolor*, *Alkmaria romijni* and *Capitella capitata* (Fig. 3, Table 1). There were dose-dependent negative effects of both types of algae on *C. carinata*, while for the bivalves, *S. plana* and *C. edule*, there was a slight increase in numbers in the presence of low weed biomass then a marked decline at high weed biomass.

For the polychaete *H. diversicolor*, there was a general trend of an increase in abundance of this species with increasing weed biomass in the *Enteromorpha* plots.

*A. romijni* tended to slightly decline with increasing biomasses of *Enteromorpha* but there was no effect of *Gracilaria*.

*C. capitata* increased at high biomasses of *Enteromorpha*, but the response was the opposite for *Gracilaria*.

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**Fig. 3.** Mean species abundance (±95% confidence intervals) after 4 weeks of experiment in the two experimental areas.
3.1.2. Effects of algae in the sea grass meadows

In contrast to the eutrophic area, the effects of green and red macroalgae on benthic macrofauna were considerably less in the sea grass meadows except for *Hydrobia ulvae* (Fig. 3) in the presence of *Enteromorpha*. As expected, *H. ulvae* exhibited a positive response to green macroalgae, showing an increase in abundance with increasing weed biomass. For most of the other species (e.g. *C. carinata*, *C. edule*, *A. romijni*) there were no effects possibly due to their low abundance in the cores. For the polychaete *H. diversicolor* there was slight tendency for an increase in density with increasing *Enteromorpha* biomass.
3.1.3. Two-way analysis of variance

There were no significant algal treatment effects for *H. ulvae* (*P* = 0.347), but a difference between sites (*P* = 0.000) and a significant interaction (*P* = 0.003), almost certainly due to the different direction of the algal effects at the two sites (in the sea grass meadows, *Hydrobia* increases with algal biomass, while at the eutrophic area, abundance decreases).

*C. carinata* was significantly affected by site and algal treatments (*P* = 0.000 for both factors) and there was a significant interaction (*P* = 0.000) term, probably because there were effects of algal treatments at the eutrophic area while in the sea grass meadows almost no effect was recorded.

For other species (e.g. *S. plana*, *C. edule*, *H. diversicolor* and *C. capitata*), there were both between-biomass and between-site differences but no interaction between the two factors (Table 1).

3.1.4. Redox potential

Sediment redox potential differed consistently between the two experimental areas (Fig. 4), being significantly lower in the eutrophic area consistent with the notion that this site is more disturbed than the sea grass bed. In the eutrophic area, redox potential became more negative with increasing algal biomass and weed treatment plots were significantly more reduced than the control plots (ANOVA, *F*_23 = 3.10, *P* = 0.029). However, Tukey tests
failed to reveal any significant differences between the Enteromorpha and Gracilaria plots. In contrast, the sediments in the sea grass meadows did not show significant differences between the treatments.

3.1.5. Multivariate analysis of the macrofaunal assemblages

The macrobenthic communities of the two study areas are clearly separated in the MDS plot (Fig. 5). For the eutrophic area, macrobenthic assemblages within the weed controls are detached from the ones corresponding to the treatments with highest biomass of...
Enteromorpha and Gracilaria (Ent 3.0 and Gr 3.0), with the intermediate treatments occupying an intermediate position (Fig. 5). On the other hand, for the Zostera meadows, differences between treatments are less evident.

Significant differences were detected by ANOSIM between the two sites. Zostera meadows were significantly different from the eutrophic area at the 5% level ($R = 0.786$; $P = 0.001$). ANOSIM test was also used to detect any differences between treatments in the two areas. The significant results are expressed in Table 2.

### 4. Discussion

The results of the present study demonstrate that the response of the macrobenthic species to macroalgae is different in the two study areas. Previous studies have indicated that the sediment environment in the two areas is quite different (Pardal et al., 2000; Cardoso et al., 2002) and this is confirmed by our redox results. These indicate that the sea grass meadows have a less hostile redox environment than the upstream disturbed area, which is probably less resistant to additional impacts. It is perhaps not surprising that the effects of the algae are more marked in the disturbed site (characterized by bare sediments).

Within each area, the responses of the benthic macrofauna to algal mats were algal-species dependent, with some species being negatively affected and others being enhanced by the algae. On the other hand, for some species (e.g. *A. romijni*, *H. ulvae*) differences between the weed control and the mesh control were observed, however, no significant effects of the mesh on species abundance were recorded.

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**Table 2**

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<th>Groups</th>
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<th>Significant level</th>
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<td>$P = 0.037$</td>
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</table>
For *H. ulvae*, one of the dominant taxa in the Mondego estuary (see Lillebø et al., 1999; Cardoso et al., 2002), there were positive effects of the green macroalgae in the *Z. noltii* meadows. This is consistent with other studies, which show that *Hydrobia* is an opportunistic species, utilising intertidal algal mats both as food resource and refuge (Soulsby et al., 1982; Norkko and Bonsdorff, 1996a,b; Norkko et al., 2000; Schanz et al., 2002). In contrast, the effects of this alga on *Hydrobia*, in the eutrophic area, were insignificant. This could be due to the short period of the experiment, which associated to the fragile population structure in this area (since during most of the time, only juveniles are present while adults only appear during periods with macroalgal blooms—see Cardoso et al., 2002) may not have allowed *Hydrobia* to respond in the same way as in the *Z. noltii* meadows. *Gracilaria* had no detectable effects on *Hydrobia*, probably because snails do not seem to use this type of algae as a food resource because of its stiff consistency, while its gross morphology possibly affords *Hydrobia* less protection than *Enteromorpha*.

The isopod *C. carinata* was negatively affected by both kinds of algae. The most likely explanation for this is the physical barrier created by mats of macroalgae that will interfere negatively with its feeding mechanism at the sediment–water interface (*Cyathura* is a deposit feeder and a predator). This response is similar to that observed for the amphipod *Corophium volutator* in other estuarine systems (Hull, 1987; Raffaelli, 1999, 2000). In the sea grass meadows, *Cyathura* is too rare to permit the detection of such effects.

For the bivalves, *S. plana* and *C. edule*, despite the low abundances found, there was a positive effect at low algal biomasses and a decline at high biomasses. This was much more evident within the *Enteromorpha* plots, possibly because *Gracilaria* does not create such a rigid barrier between infauna and the oxygenated water column and hence generate such a hostile sediment environment. The effects of *Enteromorpha* are similar to those in previous surveys carried out by Everett (1994), Bolam et al. (2000), Bolam and Fernandes (2002) and Lewis et al. (2003) but dissimilar to those reported by Hull (1987) and Raffaelli (1999). In the latter studies, the positive effects were due to the presence of a large number of juveniles, which was not the case here.

*H. diversicolor*, overall, seems to benefit from green algal cover since *Enteromorpha*, gives protection and constitutes a favourable food-source. Nevertheless for this endofaunal species, occurred a small decline in the eutrophic area at the highest biomasses, probably due to a new factor of stress induced by the large amount of weed that interferes with the sediment properties on an already disturbed area. This is consistent with the findings from other studies that tested lower weed biomasses (e.g. Norkko and Bonsdorff, 1996a). In contrast, *A. romijni* was negatively impacted by green macroalgae. This polychaete is a surface deposit feeder and the physical barrier created by macroalgae will probably interfere with its feeding behaviour.

*Capitella* is the only species that increased within the high biomass plots, although less so for *Gracilaria*. *Capitella* is an opportunistic detritivore species, usually associated with organically enriched sediments (Pearson and Rosenberg, 1978; Soulsby et al., 1982; Raffaelli, 1999, 2000; Bolam et al., 2000). Due to higher tissue thickness and lower surface/volume ratio, *Gracilaria* decomposition rate is much slower than the *Enteromorpha* one (sometimes only a few hours at temperatures above 25 °C), contributing less for food resources. Therefore, this species might be expected to do better in areas affected by green macroalgae than those covered by red macroalgae.
The present study has shown that the morphological and ecological features of the algal species involved in blooms are an important determinant of algal–invertebrate interactions. The effects of *Enteromorpha* on the macrobenthic communities are much greater than those for *Gracilaria* in the present study. In the presence of *Enteromorpha*, the community tends to be impoverished, dominated mainly by small size opportunistic species, like *A. romijni*, *H. ulvae* and *C. capitata*, while in the presence of *Gracilaria* the negative effects are usually less (Marques et al., 2003; Cardoso et al., in press). In addition, the prior disturbance history of a site appears to be important in determining the extent of the impact of such blooms.

The present results and the one by Cardoso et al. (in press) confirmed the knowledge that, in the long term, sustained eutrophication together with spring macroalgal blooms may lead to complete replacement of sea grass habitat by unvegetated coarser sediments. The macrofaunal assemblages tend to be dominated by opportunistic invertebrate taxa with total macrobenthic biomass and species richness tending to decrease. Recovery from this situation may not only require reduction in nutrient loadings to the estuary, but also active sea grass restoration programmes to reverse the positive feedback processes involved in the decrease of the environmental quality of eutrophic systems.

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