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Food webs in intertidal ecosystems: a stable isotope approach and trophic network analysis

Alexandra Baptista Vicente Baeta

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BAPTISTA VICENTE BAETA Alexandra

FOOD WEBS IN INTERTIDAL ECOSYSTEMS

A STABLE ISOTOPE APPROACH AND TROPHIC NETWORK ANALYSIS

-

RESEAUX TROPHIQUES DES ECOSYSTEMES INTERTIDAU

ETUDE PAR LES ISOTOPES STABLES ET L'ANALYSE DES RESEAUX

Soutenue le 21 septembre 2010

Composition du jury :

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FOOD WEBS IN INTERTIDAL ECOSYSTEMS
A STABLE ISOTOPE APPROACH AND TROPHIC NETWORK ANALYSIS

Doctoral dissertation in Biology (Scientific area of Ecology)
presented to the University of Coimbra

Dissertação apresentada à Universidade de Coimbra para
obtenção do grau de Doutor em Biologia (especialidade
Ecologia)

ALEXANDRA SOFIA BAPTISTA VICENTE BAETA

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To Vi, with much love

*I would not give a fig for simplicity on this side of complexity, but
I'd give my life for the simplicity on the other side of complexity*
Oliver Wendell Holmes, Jr.

There's a Universe inside these waters
Patty Marshall-Stace

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This thesis is based on the following manuscripts, which constitute the different chapters:

Baeta A, Valiela I, Rossi F, Pinto R, Richard P, Niquil N, Marques JC (2009) Eutrophication and trophic structure in response to the presence of the eelgrass *Zostera noltii*. *Mar Biol* 156:2107–2120;

Baeta A, Pinto R, Valiela I, Richard P, Niquil N, Marques JC (2009) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the Mondego estuary food web: Seasonal variation in producers and consumers. *Mar Environ Resear* 67:109–116;

Baeta A, Rossi F, Marques JC. Habitat-related diet of macrofauna consumers in intertidal areas. Submitted to *Estuar Coast Shelf Sci*;

Baeta A, Niquil N, Marques JC, Patrício J. Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine benthic food webs. Submitted to *Ecol Modell*.

Résumé

Cette thèse de Doctorat examine le réseau trophique estuarien lié aux herbiers à zostères, *Zostera noltii* et quantifie le rôle trophique des consommateurs de la macrofaune benthique, dans deux zones intertidales de l'estuaire du Mondego (Figure 1), à différentes périodes de 1993 à 2008. Elle s'intéresse spécifiquement (i) à l'incorporation de l'azote issu des activités humaines, en considérant l'assimilation d'Azote comme un indicateur de l'eutrophication et (ii) au rôle des zostères dans le réseau trophique benthique. Six modèles à l'état stable ont été développés afin d'analyser les effets, (i) d'un enrichissement en nutriments, (ii) des mesures de mitigation, et (iii) d'une inondation centennale, sur les propriétés du réseau trophique benthique estuarien.

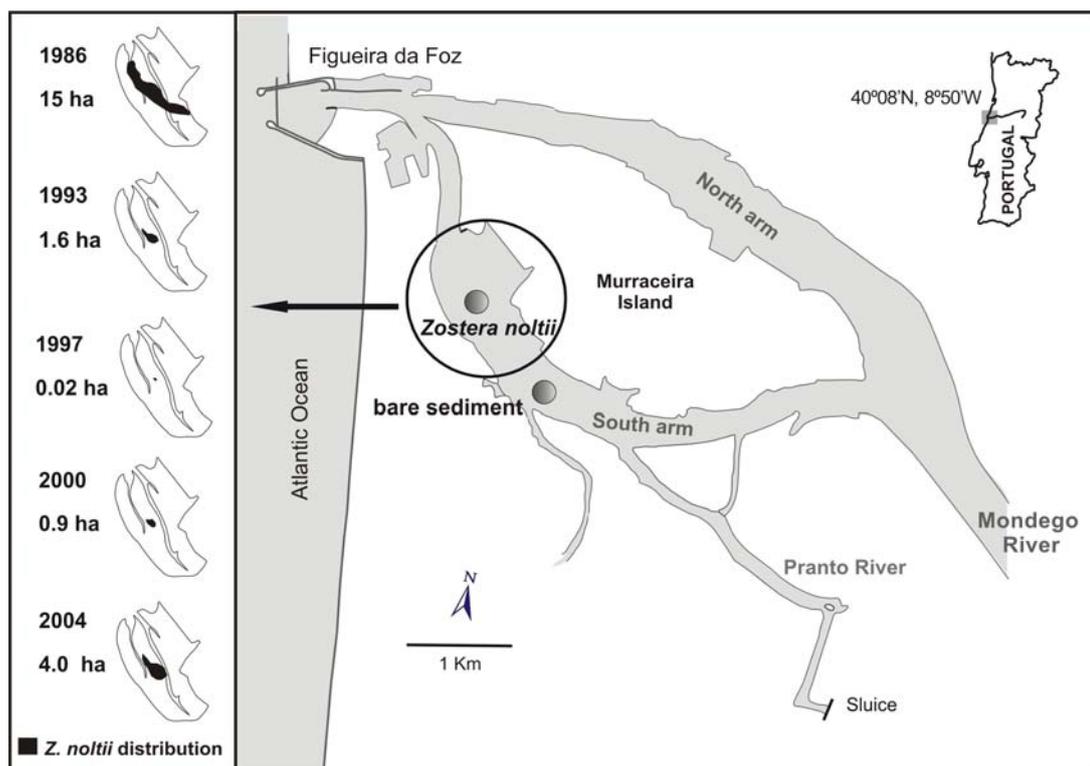


Figure 1. Carte de l'estuaire du Mondego, montrant les 2 sites d'échantillonnage (cercles gris) : un site en herbier à Zostères (*Zostera noltii*) et un site à sédiment nu (bare sediment). Changement de surface couverte par *Zostera noltii* dans le bras sud de l'estuaire du Mondego. La cartographie de la végétation benthique est basée sur des observations de terrain, des photographies aériennes et une application d'un système d'information géographique (Arc View GIS version 8.2).

Il est possible que la zostère, *Z. noltii*, utilise l'azote issue des activités humaines, car la concentration en azote de l'eau dans l'estuaire et des sources utilisées par les producteurs primaires sont similaires entre la zone de sédiment nu et celle occupée par *Z. noltii*. Aucune différence dans le niveau d'eutrophication n'est observée entre les sites (2005/2006) (Figure 2). Les fortes valeurs en signature isotopique de l'azote des producteurs primaires pourraient indiquer que les sources d'azote viennent des activités humaines (par exemple des rejets d'eau usée et de l'agriculture).

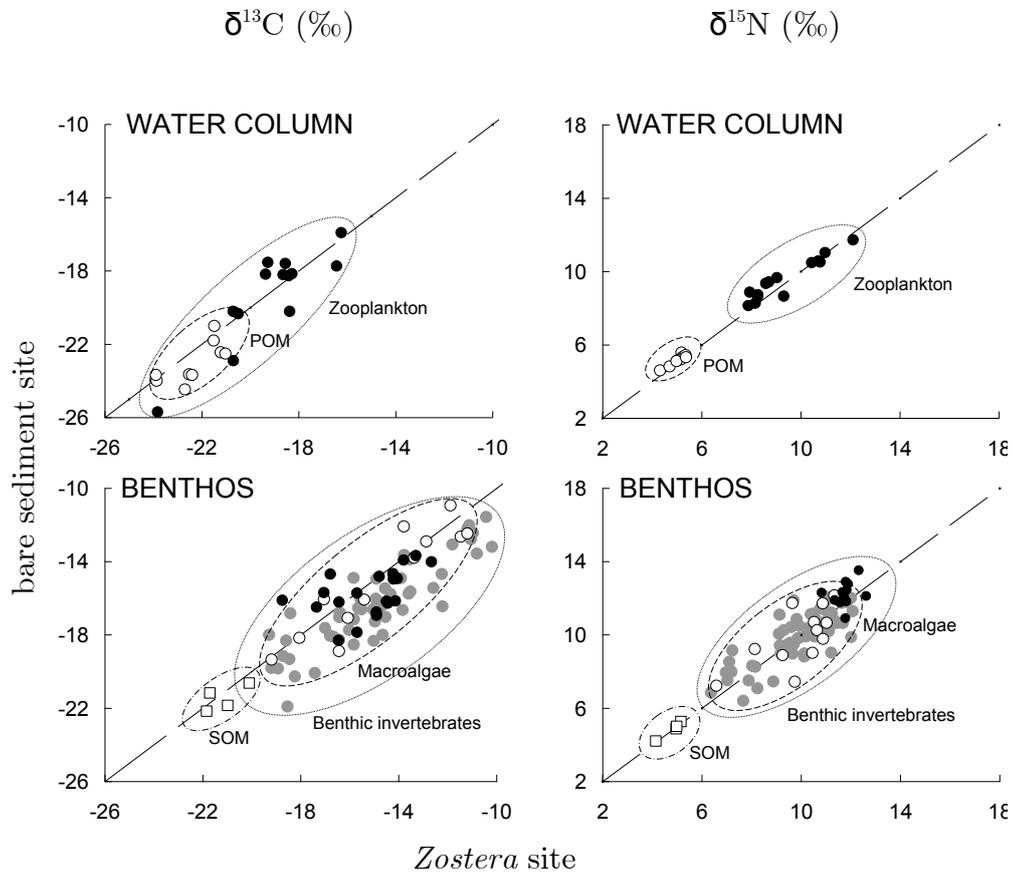


Figure 2. Comparaison des ratios isotopiques stables du C (gauche) et du N (droite) dans la colonne d'eau (figures du haut) [(○) matière organique particulaire; (●) zooplancton] et le benthos (figures du bas) [(●) consommateurs primaires; (●) consommateurs secondaires; (○) macroalgues; (□) matière organique en suspension] collectés dans les deux sites (site à zostères en abscisses et site à sédiment nu en ordonnées) de l'estuaire du Mondego. La ligne en pointillés indique la bissectrice 1 :1.

La présence de *Z. noltii* ne change pas beaucoup la structure du réseau trophique planctonique, soutenu en partie par la matière organique particulaire et supporte principalement des poissons comme prédateurs (Figure 3). Globalement, le réseau trophique n'utilise pas de nourriture issue de la zostère. Les filtreurs utilisent la matière organique particulaire issue de la remise en suspension du sédiment, tandis que les valeurs de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ des dépositivores montrent que les microalgues benthiques jouent parfois un rôle clé comme source de nourriture.

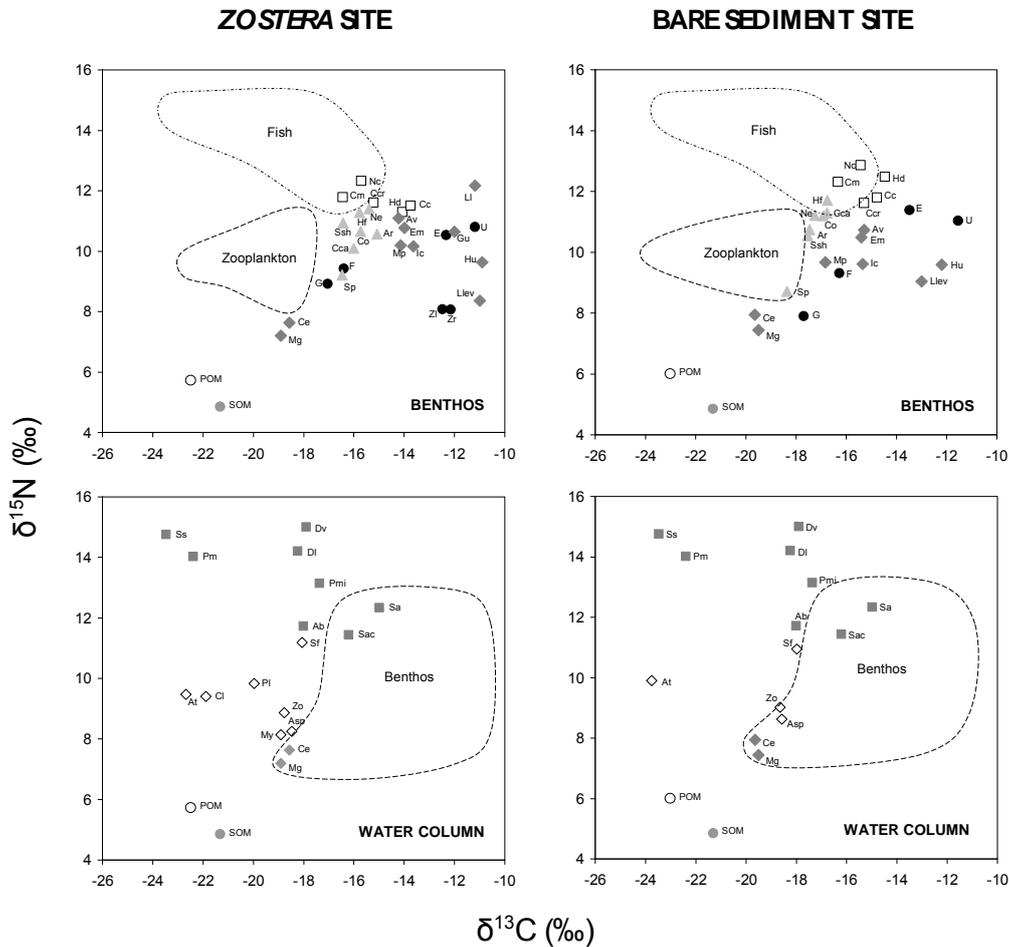


Figure 3. Ratios $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ des réseaux trophiques benthiques et pélagiques de l'estuaire du Mondego, dans l'herbier à zostères (à gauche) et dans le site du sédiment nu (droite). Benthos: (●) producteurs primaires ; (▲) détritivores ; (◆) brouteurs/détritivores; (□) omnivores. Colonne d'eau : (■) poissons ; (◇) zooplancton. La moyenne présentée représente les échantillons collectés de novembre 2005 à juillet 2006. Les abréviations des espèces ou des groupes d'espèces sont explicitées en Table 2 du Chapitre 1.

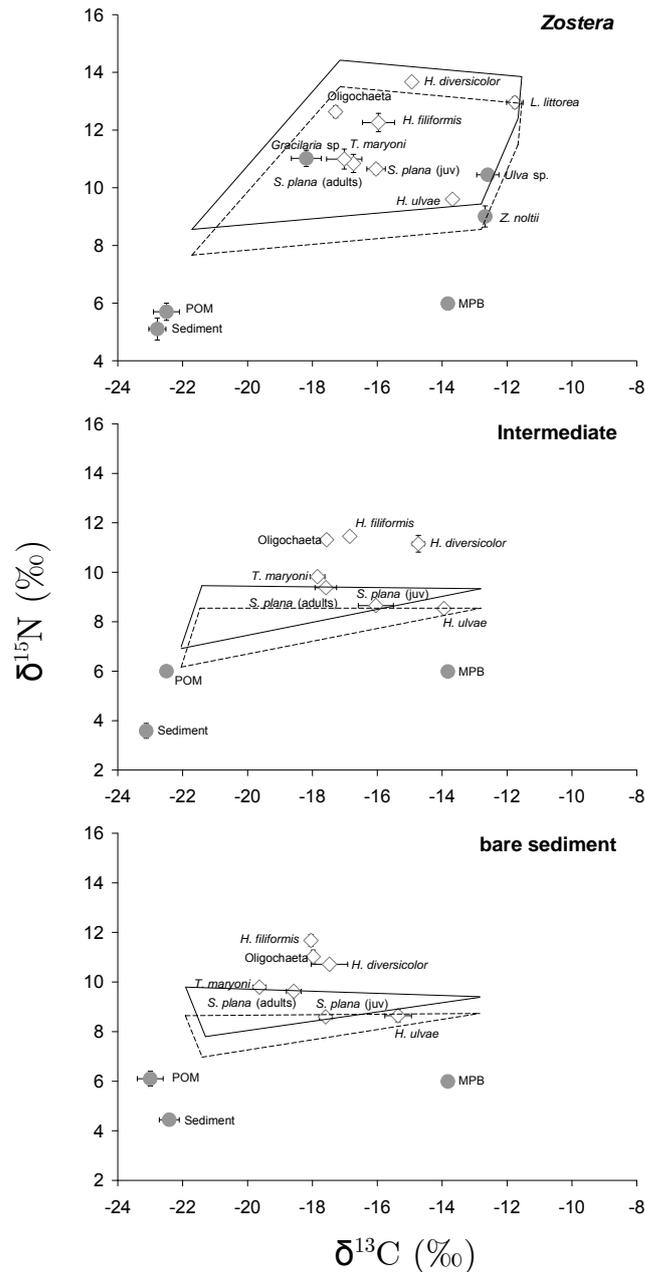


Fig. 4. Graphique des ratios des isotopes stables $\delta^{13}C$ et $\delta^{15}N$ dans les deux sites d'étude : herbier à zostères (*Zostera*, en haut) et sédiment nu (bare sediment, en bas), ainsi que dans un site intermédiaire occupé par un herbier peu dense (Intermediate, au milieu), entre juin et juillet 2008, pour les espèces montrant des différences entre les sites et pour les sources. Sont reportées, les valeurs moyennes (les erreurs standards de la moyenne et le nombre de réplicats sont explicités en Table 1 du Chapitre 3). Les cercles gris indiquent les sources et les losanges blancs les consommateurs. Les polyèdres représentent les projections de la signature des sources corrigées par la fragmentation (voir le Matériel et méthodes du Chapitre 3). La ligne continue indique une correction en accord avec la fragmentation souvent utilisée de +1 et +3.4‰ pour $\delta^{13}C$ et $\delta^{15}N$, respectivement (DeNiro and Epstein 1978, Wada et al. 1991, Post 2002). Le polyèdre en pointillés indique une fragmentation de +1 and +2.5‰ pour $\delta^{13}C$ et $\delta^{15}N$, respectivement, rencontrée chez d'autres auteurs (Vander Zanden and Rasmussen 2001).

Les autres consommateurs montrent une grande variabilité de signature isotopique, ce qui suggère qu'ils peuvent changer de régime alimentaire en fonction des changements de l'environnement (Figure 4). Ces changements de régime alimentaire sont liés à la présence des herbiers, puisque les différences entre habitats observées chez certains consommateurs sont liées à la proximité de l'habitat, herbier à zostères. Ces différences de régime se rencontrent surtout entre habitats bien séparés, et sont moins marqués entre l'herbier à zostère et la zone adjacente. Parfois, ces différences entre habitats existent pour différents stades de développement (en particulier chez *Scrobicularia plana*), affectant par conséquent la prise de nourriture des juvéniles et probablement le recrutement.

Les $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ des producteurs et consommateurs de l'estuaire du Mondego montrent très peu de variation saisonnière, malgré une saisonnalité météorologique marquée, ainsi qu'une forte variation saisonnière des paramètres de la colonne d'eau (apports en sels nutritifs et concentration en chlorophylle *a*). Seules les macrophytes et deux brouteurs (*Idotea chelipes* et *Lekanesphaera levii*) montrent des signatures isotopiques de l'azote très hautes en juillet 2006, pendant une période de forte température et de sécheresse. Les forts ratios isotopiques de l'azote rencontrés chez les macrophytes peuvent être liés à des changements saisonniers des processus biogéochimiques, tels que la dénitrification. Pour les deux isotopes présentant de fortes valeurs de $\delta^{15}\text{N}$, il pourrait s'agir d'un taux de renouvellement plus rapide des populations d'isopodes.

Les perturbations liées à l'activité humaines ou naturelles, telles que celles liées aux enrichissements en sels nutritifs, aux modifications d'habitats, ou encore aux inondations, se traduisent par des changements dans la composition spécifique et dans les abondances des espèces présentes. Elles se traduisent ainsi par des changements dans la structure du réseau trophique. Trois modèles à l'état stable ont été développés pour chacun des deux sites d'étude : l'herbier à zostères et la zone de sédiments nus, afin de décrire les changements de propriétés du réseau trophique benthique dans la zone intertidale de l'estuaire du Mondego. Du fait de la forte complexité de sa communauté, l'herbier à zostères présente toujours un plus grand nombre de compartiments et de niveaux trophiques et une plus forte activité totale (somme de l'ensemble des flux formant le réseau trophique). Ces 6 modèles de réseaux trophiques (un par site et par période décrite) sont illustrés en Figure 5.

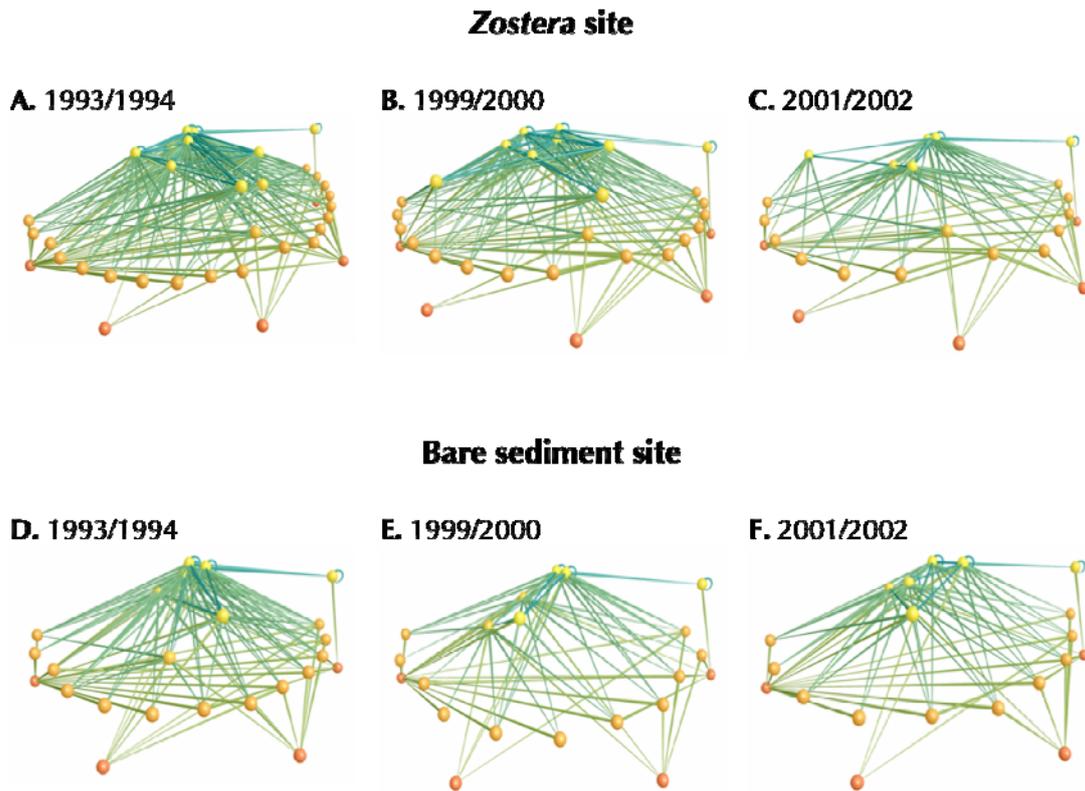


Figure 5. Représentation 3D des 6 réseaux trophiques de l'estuaire du Mondego. Figures de A à C: *Zostera* site = herbier à zostères en 1993/1994, 1999/2000 et 2001/2002 respectivement. Figures de D à F: Bare sediment site = zone de sédiment nu en 1993/1994, 1999/2000 et 2001/2002 respectivement. Images produites à l'aide du logiciel FoodWeb3D écrit par R.J. Williams, Pacific Ecoinformatics and Computational Ecology Laboratory. Les points de différentes couleurs représentent les différents groupes fonctionnels selon les niveaux trophiques : rouge = producteurs primaires, orange = consommateurs primaires et jaune = consommateurs secondaires. Les lignes plus ou moins foncées représentent les liens trophiques entre ces groupes fonctionnels.

Durant la période d'enrichissement en sels nutritifs, les deux zones modélisées présentent des fortes valeurs d'exportation et de flux alimentant le compartiment des détritux. Les différences entre les périodes modélisées, se retrouvant pour les deux sites, sont principalement des baisses d'activité liées à une chute de biomasse des producteurs primaires. Les flux de consommation, respiration et de formation de détritux sont principalement dominés par les brouteurs *Hydrobia ulvae* et *Scrobicularia plana*, dans les sites d'herbiers à zostères et de sédiments nus, respectivement (Figure 6). Dans ces deux sites, les mesures mises en place afin d'améliorer la qualité du milieu, se sont traduites par une augmentation de la biomasse, de la consommation, de la respiration et de la formation de détritux des espèces *S. plana* et *Hediste diversicolor* et une chute de ces variables chez *H. ulvae*. La population de cette dernière remonte cependant suite à l'épisode de forte crue.

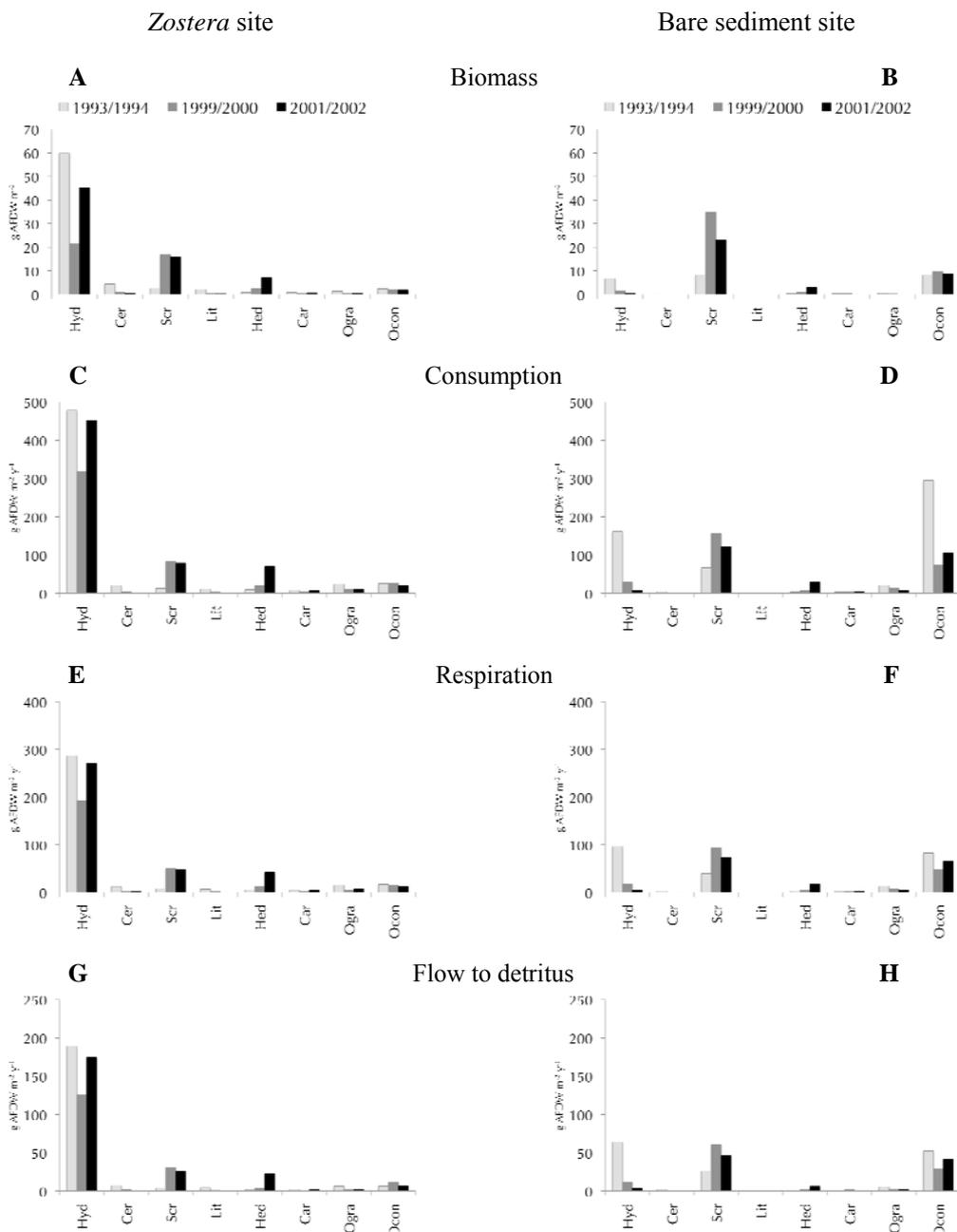


Figure 6. Biomasses (A et B), consommations (C et D), respiration (E et F) et flux vers les détritits (G et H) pour *Hydrobia ulvae* (Hyd), *Cerastoderma edule* (Cer), *Scrobicularia plana* (Scr), *Littorina spp.* (Lit), *Hediste diversicolor* (Hed), *Carcinus maenas* (Car), tous les autres brouteurs (Ogra) et les autres consommateurs (Ocon) au site d'herbier à zostères = Zostera site et au site de sédiments nus = bare sediment site, respectivement, au cours des trois périodes étudiées (1993/1994, 1999/2000 et 2001/2002).

Les modèles à l'état stable montrent ainsi que la structure trophique de la communauté benthique de l'estuaire du Mondego est affectée différemment par chacun des évènements particuliers étudiés. Il est intéressant de noter pour finir que, dans notre système d'étude, une forte activité (somme de l'ensemble des flux présentant une valeur forte) du réseau trophique semble être associée à des conditions correspondant à un système en bonne santé.

Summary

This dissertation examines the estuarine food web related to the eelgrass *Zostera noltii* and quantifies the ecological trophic role of benthic macrofauna consumers, in intertidal areas of the Mondego estuary, covering different time periods (from 1993 to 2008). It specifically focus (i) on the incorporation of the nitrogen derived from human activities, considering the N uptake as an eutrophication index, and (ii) on the role of the eelgrass in the benthic food web. In addition, six mass-balanced models were developed to assess the effects of (i) a period of nutrient enrichment, (ii) the implementation of mitigation measures, and (iii) a centenary flood, on the benthic estuarine food web properties.

The eelgrass *Z. noltii* may rely on the nitrogen derived from human activities, since the concentration of nitrogen in the overlying water and the sources of nitrogen used by primary producers were very similar between bare sediment and sediment occupied by *Z. noltii*. No differences in the level of eutrophication were found between sites (2005/2006). The high nitrogen isotopic signatures of primary producers could indicate that the sources of nitrogen were from human activities (e.g. sewage discharges, agriculture).

Z. noltii did not change considerably the trophic structure of the planktonic food web which was in part sustained by particulate organic matter and supported most predator fish. Overall, the benthic food web did not use food derived from the eelgrass. Suspension feeders used particulate and resuspended sediment organic matter; whereas the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the deposit feeders indicated that sometimes benthic microalgae played a key role as food source. Other consumers also showed a large variability in their isotopic signature suggesting they could shift diet following some environmental changes. These diet shifts were related to the presence of seagrass, since diet differences among habitats were observed in some consumers, following the proximity to the *Z. noltii* habitat. These differences in diet occurred particularly between well-separated habitats, being less clear between the eelgrass meadow and the area adjacent to it. Sometimes these habitat-related differences may occur at different stages of the animal development (*Scrobicularia plana*), thereby affecting juvenile food uptake and, probably, recruitment.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of producers and consumers in the Mondego estuary showed very little seasonal variation, despite a marked seasonality in weather and water column related parameters (nutrient supply and chlorophyll *a* concentrations). Only macrophytes and two grazers (*Idotea chelipes* and *Lekanesphaera levii*) showed high nitrogen isotopic signatures in July 2006, during a period of high temperatures and drought condition. Increased nitrogen isotope ratios in the macrophytes may have resulted from seasonal changes in biogeochemical processes, such as denitrification, while for two isopods, higher ^{15}N values might be the result of the fast turnover rate of isopod populations, since the other groups might not feed directly on fresh macroalgae.

Human-mediated and natural disturbances such as nutrient enrichment, habitat modification, and flood events resulted in shifts in species composition and abundance that were translated into changes in the food web structure. Three mass-balanced models were developed for each of two sites (*Z. noltii* meadows and bare sediment area) to assess changes in benthic food web properties in the Mondego estuary intertidal area. *Z. noltii*, due to its complex community, presented higher number of compartments and higher level of system activity. During the period of nutrient enrichment, both areas showed higher exports and flows to detritus. The differences at the two sites in the three time periods in the breakdown of throughput were mainly due to differences in the biomass of the primary producers. Consumption, respiration and flow to detritus were dominated by the grazers *Hydrobia ulvae* and *S. plana* at the *Z. noltii* and bare sediment sites, respectively. At both sites, after recovery measures were implemented there was an increase in *S. plana* and *Hediste diversicolor* biomass, consumption, respiration and flows to detritus, and a decrease in *H. ulvae* biomass and associated flows, which increased again after the flood event. The mass-balanced models showed that the trophic structure of the benthic communities in Mondego estuary was affected differently by each disturbance event. Interestingly, in our study a high system throughput seems to be associated with higher stress levels, which contradicts the idea that higher system activity is always a sign of healthier conditions.

Resumo

Esta dissertação examina a rede trófica estuarina relacionada com a macrófita *Zostera noltii* e quantifica o papel trófico dos consumidores pertencentes à macrofauna bentónica, em zonas intertidais do estuário do Mondego, cobrindo diferentes períodos de tempo (de 1993 a 2008). Centra-se particularmente (i) no estudo dos processos relacionados com a incorporação de azoto proveniente de actividades humanas, considerando esta absorção de N como um índice de eutrofização, e (ii) no papel da macrófita na teia trófica bentónica. Para além disso, foram desenvolvidos seis modelos de massa balanceados de forma a poder avaliar os efeitos (i) de um período de enriquecimento orgânico, (ii) da implementação de medidas de mitigação após um período de eutrofização, e (iii) uma cheia centenária, nas propriedades exibidas pelas redes tróficas bentónicas do estuário.

Provavelmente, a macrófita *Z. noltii* utiliza e assimila azoto proveniente de actividades humanas, uma vez que a concentração de azoto presente na coluna de água e as fontes de azoto utilizadas pelos produtores primários foram muito similares quer no local caracterizado por sedimento sem vegetação, quer no sedimento coberto por *Z. noltii*. Em 2005/2006, não foram encontradas diferenças no nível de eutrofização entre os dois locais. As elevadas assinaturas isotópicas de azoto encontradas na análise dos produtores primários sugerem que as fontes de azoto são de facto provenientes de actividades humanas (e.g., descargas de esgotos, agricultura).

A presença de *Z. noltii* não alterou significativamente a estrutura da rede trófica plantónica que foi em parte sustentada por matéria orgânica particulada, tendo suportado a maioria dos peixes predadores. De uma forma geral, a rede trófica não utilizou a macrófita como fonte de alimento. Os organismos filtradores utilizaram matéria orgânica particulada e em suspensão, ao passo que os detritívoros (deposit feeders), segundo os valores de $\delta^{13}\text{C}$ e de $\delta^{15}\text{N}$ terão utilizado as microalgas bentónicas como fonte de alimento fundamental. Outros consumidores mostraram igualmente uma grande variabilidade na sua assinatura isotópica sugerindo que podem alterar a sua dieta na sequência de alterações ambientais. O nosso estudo indicou que estas alterações na dieta estariam relacionadas com a presença da macrófita, uma vez que foram encontradas diferenças na dieta de alguns consumidores dependendo dos habitats, diferenças essas relacionadas com a proximidade desse habitat à

Z. noltii. Mais, estas diferenças na dieta verificam-se principalmente entre habitats mais distanciados entre si, sendo menos claras entre os bancos de macrófita e o habitat adjacente. Por vezes estas diferenças relacionadas com o habitat pareceram ocorrer em diferentes fases do desenvolvimento do animal (*Scrobicularia plana*), afectando assim a ingestão de alimento nos juvenis e, provavelmente, o recrutamento.

O $\delta^{13}\text{C}$ e o $\delta^{15}\text{N}$ dos produtores e consumidores no estuário do Mondego mostraram poucas variações sazonais, apesar de uma acentuada sazonalidade climática e em parâmetros associados com a coluna de água (disponibilidade de nutrientes e concentração de clorofila *a*). Apenas as macrófitas e dois herbívoros (*Idotea chelipes* e *Lekanesphaera levii*) apresentaram elevados valores isotópicos de azoto em Julho de 2006, valores estes coincidentes com um período onde se registaram temperaturas elevadas e condições de seca. O aumento dos ratios de isótopos de azoto nas macrófitas poderá ter resultado de variações sazonais de determinados processos biogeoquímicos, tais como a denitrificação. No caso dos dois isópodes, elevados valores de ^{15}N poderão ser o resultado de uma elevada taxa de “turnover” das populações destes herbívoros, uma vez que os restantes grupos, ao contrário destas duas espécies, possivelmente não se alimentam directamente de algas frescas.

No nosso estudo, verificámos que perturbações antropogénicas ou naturais como o enriquecimento orgânico, alterações no habitat ou eventos climáticos extremos (e.g. cheias), tiveram como consequência alterações na composição e abundância das espécies tendo, por seu turno, dado origem a modificações na estrutura trófica das comunidades. Foram construídos três modelos de massa balanceados para cada um de dois locais (bancos de *Z. noltii* e sedimento vasoso sem cobertura vegetal) com o objectivo de analisar as alterações nas propriedades das redes tróficas das comunidades intertidais do estuário do Mondego. A rede trófica das comunidades bentónicas dos bancos de *Z. noltii*, graças à sua complexidade, apresentou um maior número de compartimentos e um nível de actividade do sistema mais elevado. Durante o período de enriquecimento orgânico (1993/1994), ambas as áreas, evidenciaram uma percentagem de exportações e de fluxos para os detritos mais elevada. As diferenças observadas na repartição do nível de actividade, nos dois locais e nos três períodos de estudo, deveram-se essencialmente a diferenças na biomassa dos produtores primários. O consumo, respiração e fluxo para os detritos foram dominados pelos herbívoros *Hydrobia ulvae* e *S. plana* nos bancos de *Z. noltii* e na área de sedimento sem cobertura vegetal, respectivamente. Em ambos os locais, depois da implementação das medidas de mitigação, observou-se um aumento da biomassa, consumo, respiração e fluxos

para detritos de *S. plana* e *Hediste diversicolor* e uma diminuição clara da biomassa de *H. ulvae* e fluxos associados, aumentando novamente depois da ocorrência da cheia centenária. Os modelos de massa balanceados mostraram claramente que a estrutura trófica da comunidade bentónica do estuário do Mondego foi afectada diferenciadamente por cada tipo de perturbação estudada. Digno de registo é o facto de, no nosso estudo, um elevado nível de actividade (total system throughput) parece estar associado a níveis superiores de stress, contrariando a ideia de que elevada actividade representa melhor qualidade ambiental.

General Introduction

Food webs

“Perhaps the most fundamental property of life is its ability to use energy and materials to maintain and reproduce itself, in turn providing energy and materials to support more life. This generation and consumption of biomass enabled the evolution of biological diversity and concomitant trophic structure among ecosystems.”

Dunne et. al. (2008)

Naturalists have long noted that the distribution, abundance, and behaviour of organisms are influenced by interactions with other species (MacArthur 1955). Within ecosystems, species interact in various ways (e.g. predator-prey, plant-seed disperser, host-parasite, plant-pollinator). Types and strengths of interaction change through time and space, following the characteristics of the individuals inhabiting an habitat and the environmental changes shape species behaviour, population and community dynamics (Polis 1996). The strength of trophic relationships (e.g. predation and availability of resources) may regulate feeding habits, reproduction and recruitment, as well as population abundance and biomass. Not only the strength, but also the nature of these relationships determines the flux of energy and nutrients, which crucially regulate the recycling of carbon and nutrients as well as associated ecosystem goods and services (Eggers and Jones 2000; Jordán 2003).

How organisms are connected following their trophic links is named food webs. The concept and study of food web dates back to Elton’s classical text on Animal Ecology (Elton 1927). His research revealed the complex interconnection of species in food web networks. Lindeman (1942) arranged species into food chains that consisted of discrete trophic levels. Plants were the primary producers (trophic level 1), herbivores were the primary consumers (trophic level 2), carnivores that feed on the herbivores were the secondary consumers (trophic level 3), and so on. The basic assumption of food chain studies is that these trophic levels can be treated as discrete populations. Perhaps the most serious shortcoming of the food chain concept is the failure to incorporate the complexity

and omnivory that are well known and inherent to many ecosystems (Polis 1991), as it is recognized by many field-oriented ecologists that species in nature rarely conform to discrete trophic levels. Indeed, later the work of MacArthur (1955) shifted the focus from the linearity of food chains to the complexity of food webs and the implications of this complexity for energy flow and ecosystem stability. In the late 1970s and early 1980s the interest in food webs increased and scientists started studying food webs through mathematical models and field studies. The theory on energy flow and matter became preponderant in these studies (Odum 1985). Communities became described as sets of nodes (species) connected by a suite of edges (trophic relations) of different intensity to underline the amount of energy transferred from one node to the following. Food webs defined the trophic connections and ecological networks included also the magnitude of these interactions, in terms of amount of matter (or energy) that is exchanged in time (Pahl-Wostl 1993).

At present, the major goal for ecology is to understand how communities and ecosystems will change following pollution, habitat destruction, overexploitation, invasion, and climate change. Such changes have triggered and accelerated the decrease in biodiversity and modified the structure and functioning of aquatic ecosystems, thereby jeopardising the maintenance of goods and services provided to humans (e.g. Margalef 1968; Odum 1969, 1985; Pauly et al. 2002; Lotze et al. 2006; Coll et al. 2008).

A vision of community and ecosystem changes related to trophic interactions may allow a more holistic understanding of how communities and functioning may change in response to global change (e.g. Christensen and Pauly 1993; Dunne et al. 2002, 2008; Bascompte et al. 2005). Under this framework, food web studies should take into account the dynamic nature of the trophic relationships, which vary following species behaviours and population dynamics as well as for spatial and temporal variability of the habitat considered. Such an approach may contribute to the preservation and management of ecosystems in view of global change.

Estuaries

Among the most important environments of the coastal zone are estuaries which constitute transition zones where freshwater from land drainage mixes with seawater, creating some of the most biologically productive areas on Earth (Levin et al. 2001).

The intertidal habitat

Intertidal areas are on the limit between the marine and terrestrial biosphere. During the tidal cycle they are exposed either to the atmosphere or to the overlying water. For this reason, intertidal habitats are unique in the biosphere, and, although quite limited in surface as compared to ocean, they are of great ecological importance. The communities inhabiting these areas therefore must be able to cope with this continuous change from an aquatic to a semi-terrestrial environment (Levin et al. 2001).

The intertidal seagrass ecosystem

Seagrasses sustain highly productive ecosystems, increase biodiversity and provide numerous important ecological services to estuarine environment, including nutrient cycling and sediment stabilization (Larkum et al. 2007). Seagrasses provide refuge from predators and increase food resources, thus enhancing reproduction, recruitment and survival of several consumers (Duarte 2002). They also contribute to carbon and nutrient sequestration and storage, by sequestering inorganic carbon and nutrients and storing them as biomass due to their long life-cycle and low edibility (Duarte 2005). The biomass produced is then partly slowly decomposed and recycled by the food web associated to the meadows and partly exported to adjacent areas, often beaches poor of nutrients and carbon (Cebrian 1999; Holmer et al. 2002; Heck et al. 2008).

The important ecological roles of seagrass habitats have been identified and found to represent a high value of ecosystem services to the planet (Costanza et al. 1997; Orth et al. 2006; Waycott et al. 2009).

Stable isotopes in food web research

Trophic interactions have been often studied by analysing the stomach content of consumers. However, this technique has the disadvantage of inferring dietary composition of animals based on ingestion rather than assimilation (Sheppard and Hardwood 2005), therefore data derived from this technique may show significant deviations from the actual dietary composition of an organism (e.g. Taylor 1986; Gearing 1991).

Stable isotopes analyses may represent a valid technique to understand the fraction of ingested prey incorporated. The stable isotope approach, particularly those of nitrogen and carbon, provides a number of potential advantages over dietary methods, and has enhanced our understanding of trophic structure and dynamics of ecological communities, as well as ontogenetic shifts in consumer diet.

Stable isotopes offer three potential advantages in terms of food web analysis; firstly, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of animal tissue represent the integration of carbon and nitrogen over a prolonged period; secondly, they are based on assimilation rather than ingestion; and third they can be measured from comparatively small samples. In addition to time-integrated trophic information, isotope signatures have the potential to simultaneously capture complex interactions, including trophic omnivory, and to track energy or mass flow through ecological communities (Vander Zanden and Rasmussen 1999, 2001; Post 2002).

This technique has been used in the archaeological and geological disciplines for many years (e.g., Craig 1954; DeNiro and Epstein 1978, 1981) and proposed in ecological studies few years later (Peterson and Fry 1987). Since then, its use in food web studies had risen exponentially (Fry 2006). This technique is based on the chemical elements appearing in nature in different isotope forms, being the lightest form generally more common than the heaviest form. Common elements utilized include hydrogen (H), oxygen (O), carbon (C), nitrogen (N), and sulphur (S). Studies of trophic ecology have considered primarily C and N for their diffusion in biological tissues (Archambeau et al. 1998; Fry 2006).

The 95% of vital tissues are composed of carbon 12 and nitrogen 14, the number specifying the atomic weight. The rest is composed of carbon 13 and nitrogen 15. The measurements of isotope composition of each tissue are given as ratio of the fraction of the rare element (e.g. ^{13}C and ^{15}N) to the common one (e.g. ^{12}C or ^{14}N). This ratio is quantified as deviation from the isotope composition of a reference material and expressed in parts per thousand. For example the carbon $\delta^{13}\text{C}$ (‰) of a sample is:

$$\delta^{13}\text{C} = \frac{\frac{^{13}\text{C}}{^{12}\text{C}}_{\text{sample}} - \frac{^{13}\text{C}}{^{12}\text{C}}_{\text{reference}}}{\frac{^{13}\text{C}}{^{12}\text{C}}_{\text{reference}}} \cdot 1000 \quad (1.1)$$

The reference material for carbon is carbonate Vienna Pee Dee Belemnite which has a isotope ratio of $^{13}\text{C}/^{12}\text{C}$ of 0.0112372.

The isotopic structure of the prey is roughly assumed by the predator, based on the assumption “you are what you eat” (Eggers and Jones 2000). Indeed, predator metabolism

operate a selection between the isotopic forms, preferentially respiring the light C isotope (^{12}C) and excreting the light N isotope (^{14}N). As a result consumers are generally enriched with heavier isotopes in relation to their food, since lighter isotopes are preferentially used in metabolism (DeNiro and Epstein 1978, 1981). This enrichment, classically called trophic shift or fractionation, usually has been considered to be predictable within a group of consumers and considering the same body tissue, generally the muscle one. Thus, nitrogen isotope ratios ($\delta^{15}\text{N}$) can be used to estimate trophic position because $\delta^{15}\text{N}$ of a consumer is typically enriched by 3-4‰ relative to its diet (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987); in contrast, the ratio of carbon isotopes ($\delta^{13}\text{C}$) can indicate feeding and carbon flow pathways because there is little fractionation from prey to predator (0-1‰), and different energy sources can have distinct ^{13}C signatures. This estimate is an average value over a wide variety of taxa and several investigations have recently listed species-specific differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation values from the literature over the past 20 years (Vander Zanden and Rasmussen 2001; Vanderkluft and Ponsard 2003; McCutchan et al. 2003). They showed, for example, differences between carnivores and herbivores, or invertebrates and vertebrates, for $\delta^{15}\text{N}$ as well as $\delta^{13}\text{C}$ to a lesser extent (Vander Zanden and Rasmussen 2001).

Stable isotopes have helped demonstrating the dynamic nature of trophic interactions and existence of a discrepancy between the theory and the field observation. For instance, in estuarine communities, Rossi et al. (2004) observed changes during development of the bivalve *Macoma balthica* by correlating the $\delta^{13}\text{C}$ signatures to the size of the bivalve, whereas van Oevelen et al. (2006) showed the low contribution of bacterial carbon to most intertidal benthic fauna, by using ^{13}C -enrichment experiments.

Another promising approach involves the use of stable isotopes to quantify the contribution of different food sources in an organism's diet (Phillips and Gregg 2003). This method to interpret stable isotope data is by means of a linear mixing model and can be used to assess diet composition in mass-balance food web models (e.g. Bozec et al. 2005; Dame et al. 2008), as well as be integrated in a linear inverse model (e.g. Eldridge et al. 2005; van Oevelen et al. 2006, 2010). Mixing models use mass balance equations and the distinct isotopic signatures of various sources to determine their relative contribution to the mixed signature in an end product. It is therefore surprising that stable isotope data have not been frequently used in food web models. The great advantage is that they exclude diet combinations that are in conflict with the isotope data, and this exclusion strongly narrows the flow ranges in the food web. A clear illustration is provided by a recent study of an

estuarine intertidal food web, where $\delta^{13}\text{C}$ data distinguished diet contributions from benthic microalgae (heavy $\delta^{13}\text{C}$) from those of phytoplankton and detritus (lighter $\delta^{13}\text{C}$), decreasing significantly the uncertainty range of many flows (van Oevelen et al. 2006).

Trophic network analysis

Understanding how ecosystems react to and recover from perturbations is a fundamental goal of ecology (Cottingham and Schindler 2000). The increasing interest towards ecosystem status and performance, and the need to approach complex environmental problems, stimulate the application of tools for whole-system assessment. The most common method for quantifying system level events is simulation modelling that implies five main steps: (a) identifying relevant taxa; (b) defining the significant interactions among those taxa; (c) modelling such interactions; (d) calibrating and validating the model; (e) making predictions.

A trophic network is a representation of the significant taxa (or nodes) comprising the ecosystem. It answers two questions: (a) who eats whom?, and (b) at what rate? Systems are depicted as directed graphs with compartments (taxa) as boxes, vertices or points connected by arrowhead arcs portraying trophic relations (exiting the prey items and entering the predator). In addition, being ecological networks open systems exchanging material and energy with their surroundings, exogenous transfers are classified as: (a) input (e.g. primary production, immigration or inbound advection of material or energy) into the receiving compartment; (b) export (e.g. emigration, harvesting by humans, and advection out of the system), matter or energy exiting from a compartment; (c) respiration (e.g. energy dissipated into heat or material degraded into its lowest-energy form as denitrification to N_2), leaving compartments. Trophic links in these networks stand for energy flows (e.g. $\text{kcal m}^{-2} \text{yr}^{-1}$) or nutrient transfers of different currencies (e.g. dry weight, $\text{gDWm}^{-2}\text{yr}^{-1}$; carbon, $\text{gCm}^{-2}\text{yr}^{-1}$; nitrogen, $\text{mgNm}^{-2}\text{day}^{-1}$).

Trophic networks provide a topological picture of the interactions of species in a community, and can inform our understanding of ecosystem responses to perturbations (Dell et al. 2005). Due to their temporal and biological complexity, it is difficult to understand the structure of food webs and trophic interactions by direct observation (Pimm 1982). There are many urgent reasons to improve our understanding of how estuarine ecosystems respond to environmental perturbations. Global climate change (Kennedy et al.

2002), localized and widespread eutrophication (Micheli 1999), overexploitation of fisheries resources (Coll et al. 2008), and invasive species (Clark et al. 2001) are some examples of the types of perturbations estuarine ecosystems are subjected to, in an increasingly populous world. Accordingly, with the advent of quantitative ecosystem modelling tools (e.g. Christensen and Pauly 1992), food web analysis is leading towards a better understanding of food web structure and the design of better management strategies for conservation. Nevertheless, our quantitative understanding of complex food webs (higher connectance) with multiple sources is still very limited, which is part due to the large number of possible pathways and the difficulty to distinguish among them, what makes complicated to reconstruct the trophic links (Moore et al. 2004).

General objectives and thesis outline

The material presented in this thesis is intended to expand our understanding of food webs structure and functioning, in *Zostera* and bare sediment habitats. The overall aim of this thesis is to study the estuarine food web related to the eelgrass *Zostera noltii* and quantify the ecological trophic role of consumers inhabiting the benthos. The working hypotheses are that (i) the presence of seagrass in intertidal ecosystems modifies the diet of benthic consumers and the transfer of nutrients in the food web, and (ii) the effects of human-mediated and natural disturbances on the benthic food web properties are different in areas with and without the eelgrass.

The thesis core is structured into four chapters, comprising four papers, each of which has been published during the PhD or is at various stages of the publication process in peer-reviewed international journals (impact factor > 1). Essentially, there are four main questions to be answered, that constitute the four papers:

- » Do *Zostera* rely on the nitrogen derived from human activities? Is the N incorporation an indication of eutrophication? How benthic consumers use *Zostera* biomass or other primary producers as main food sources?
- » Is there seasonal variation in N and C stable isotopic ratios of producers and consumers within the food web of the Mondego estuary?

- » Do benthic consumers vary their diet according to the habitat? Are the contributions of the potential basal sources to the benthic consumers different according to the seagrass habitat?
- » What were the effects of (i) a period of nutrient enrichment of the system, (ii) the implementation of mitigation measures, and (iii) a centenary flood on the benthic food web properties in *Zostera* meadows and bare sediment areas?

A summarising discussion with main conclusions is presented, providing a general overview and integration of the four chapters shortly described below.

Chapter 1 – This chapter investigates if the presence of the eelgrass *Z. noltii* decreases the nitrogen concentration in the overlying water, affects the sources of nitrogen sequestered by primary producers and changes the benthic and pelagic food web structure. In addition, the importance of these food webs in providing food to fish is examined. Accordingly, nutrient concentrations in the water column and $\delta^{15}\text{N}$ in primary producers as indicators of anthropogenic inputs of nutrients is examined, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the tissues of plants and consumers is measured to establish food web structures.

Corresponding manuscript: Baeta A, Valiela I, Rossi F, Pinto R, Richard P, Niquil N, Marques JC (2009) Eutrophication and trophic structure in response to the presence of the eelgrass *Zostera noltii*. Mar Biol 156:2107–2120

Chapter 2 – In this chapter, assessments of temporal variation in stable carbon and nitrogen ratios are used to examine seasonal trends of the water column and benthic food webs in the Mondego estuary. In addition, the changes in ratios in organisms are compared to the seasonal changes in temperature, precipitation, dissolved nutrients, and phytoplankton chlorophyll in the Mondego intertidal ecosystem.

Corresponding manuscript: Baeta A, Pinto R, Valiela I, Richard P, Niquil N, Marques JC (2009) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the Mondego estuary food web: Seasonal variation in producers and consumers. Mar Environ Resear 67:109–116;

Chapter 3 – This chapter explores the variability in the diet of macrofauna consumers following the presence of the eelgrass *Z. noltii*, by analysing the isotopic signatures of invertebrate consumers and their food sources in two bare-sediment habitats and in eelgrass meadows. The contributions of the potential basal food sources to the

consumers from the three sites are calculated with an isotope mixing model using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The ontogenetic changes in diet of the tellinid *Scrobicularia plana* are also analysed.

Corresponding manuscript: Baeta A, Rossi F, Marques JC. Habitat-related diet of macrofauna consumers in intertidal areas. Submitted to Estuar Coast Shelf Sci;

Chapter 4 – In this chapter it is described the steps followed to develop mass balanced models regarding two areas (a *Zostera* and bare sediment sites), and three time periods (1993/94, a period of nutrient enrichment of the system; 1999/00, a post-mitigation measures period; 2001/2002, post-mitigation measures and post-natural disturbances) using the Ecopath with Ecosim software package. The main purpose is to assess spatial and temporal differences in benthic food web properties between sites to evaluate how changes in species composition and biomass have affected food-web properties.

Corresponding manuscript: Baeta A, Niquil N, Marques JC, Patrício J. Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine benthic food webs. Submitted to Ecol Modell.

Chapter 1

Eutrophication and trophic structure in response to the presence
of the eelgrass *Zostera noltii*

Abstract

In estuaries, eelgrass meadows contribute to fundamental ecosystem functions of estuaries, providing food to several predators and buffering the negative effects of eutrophication. We asked whether the presence of the eelgrass *Zostera noltii* decreased the nitrogen concentration in the overlying water, affected the sources of nitrogen sequestered by primary producers and changed the benthic and pelagic food web structures. We also studied the importance of these food webs in providing food to fish. We compared bare sediment to sediment covered by a *Z. noltii* meadow, and examined nutrient concentrations in the water column and $\delta^{15}\text{N}$ in primary producers as indicators of anthropogenic inputs of nutrients. We then measured both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the tissues of plants and consumers to establish food web structures. There were no differences in the concentrations and sources of nitrogen between sites. Rather, $\delta^{15}\text{N}$ values indicated anthropogenic inputs of N (e.g. sewage discharges, agriculture) in

both sites. There were no major differences in the structure of the planktonic food web, which was in part sustained by particulate organic matter and supported most predator fish, and in the structure of the benthic food web. Nonetheless, there were differences in the sources of food for omnivore consumers and for the detritivore *Scrobicularia plana*. Overall the benthic food web did not use food derived from the eelgrass or macroalgae deposited on the substratum. Suspension feeders used particulate and sediment organic matter, whereas the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the other consumers indicated a likely contribution of benthic microalgae. Furthermore, in both habitats we found large variability in the isotope signatures of benthic macrofauna consumers, which did not allow distinguishing clearly different trophic groups and indicated a high level of omnivory and a mixed diet opportunistically making use of the availability of food in the surroundings.

Keywords: Mondego estuary; $\delta^{13}\text{C}$; $\delta^{15}\text{N}$; Coastal eutrophication; Seasonality; Estuarine species

Introduction

Nutrient enrichment due to human activities may increase eutrophication of aquatic habitats and severely impact the identity and diversity of primary producers and consumers with consequent alterations in the food-web structure, ecosystem productivity and nutrient recycling. Estuaries, which are critical habitats for nutrient recycling and ecosystem productivity, often receive large inputs of nutrients derived from human-activities and agricultural discharges, followed by severe eutrophication events (Valiela et al. 1997; Cloern 2001; Bode et al. 2006; Lotze et al. 2006). In these habitats, one of the most relevant aspects of eutrophication is the proliferation of fast-growing macroalgae, which

may replace slow-growing macrophytes and significantly decrease areal extent of seagrass meadows and the ecological value of the entire estuary (Short and Wyllie-Echeverria 1996; Howarth 1988; Bricker et al. 1999; Valiela 2006).

In estuaries, seagrass meadows as compared to bare sediment may largely contribute to the estuarine functioning, by sustaining longer and more complex food webs, which may affect ecosystem productivity and stability under environmental change. Seagrasses increase sedimentation of fine sediment particles and thus enhance quantity and quality of food for many macrofauna invertebrates, which are the fundamental trophic link between basal resources and predators such as fish and seabirds. Seagrass meadows also represent a nursery for fish of economic value and a refuge from predators (Duarte 2002). Furthermore, seagrasses, particularly *Z. noltii*, may contribute to carbon and nutrient sequestration and storage for longer periods than macroalgae, thereby keeping the water overlying the meadow of higher quality than the water overlying bare sediment and mitigating eutrophication effects (Cebrian 1999).

Natural abundance of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of detritus, primary producers and consumers are extensively used to assess trophic structure of communities (Tieszen et al. 1983; Peterson and Fry 1987; Kwak and Zedler 1997; McClelland et al. 1997; Riera et al. 2000; Abreu et al. 2006; Bode et al. 2006). Carbon stable isotope data are typically used in ecological research to determine which primary producer components are the ultimate carbon source assimilated by higher trophic level consumers (Fry and Sherr 1984; Peterson and Fry 1987). Consumers typically have $\delta^{13}\text{C}$ values similar (generally within 1‰) to their food source (DeNiro and Epstein 1978; Wada et al. 1991), yielding slightly more enriched values. Nitrogen stable isotopes are more often used to study trophic levels in communities (Wada et al. 1991). Organisms tend to fractionate nitrogen positively by 3-4‰ (Wada et al. 1991; Post 2002) at each trophic level. Thus, higher trophic levels will have higher $\delta^{15}\text{N}$ values due to the discriminatory retention of the heavy isotope over the light one (Post 2002). Furthermore, high levels of $\delta^{15}\text{N}$ in primary producers can be used as an alternative method to establish the level of human eutrophication since anthropogenic sources of nitrogen are generally enriched in the heavy isotope compared to natural sources (Cole et al. 2006).

In this study we investigated whether nitrogen concentrations of the water column, sources of nitrogen for primary producers, and trophic relationships between primary producers, consumers and predators of the benthic and the water column food web differed in a site where *Z. noltii* was abundant and a site characterised by bare sediment.

Materials and methods

Study site

The Mondego estuary, on the western coast of Portugal is a relatively small (1600 ha), warm-temperate, polyhaline, intertidal system located on the Atlantic coast of Portugal, consisting of two arms, north and south (Fig. 1). The southern arm is characterised by intertidal mudflats (almost 75% of the area) exposed during low tide. The tidal range varies between 0.35 and 3.3 m depending on the site and tide coefficient, while water residence time varies between 2 (northern arm) and 3 days (southern arm).

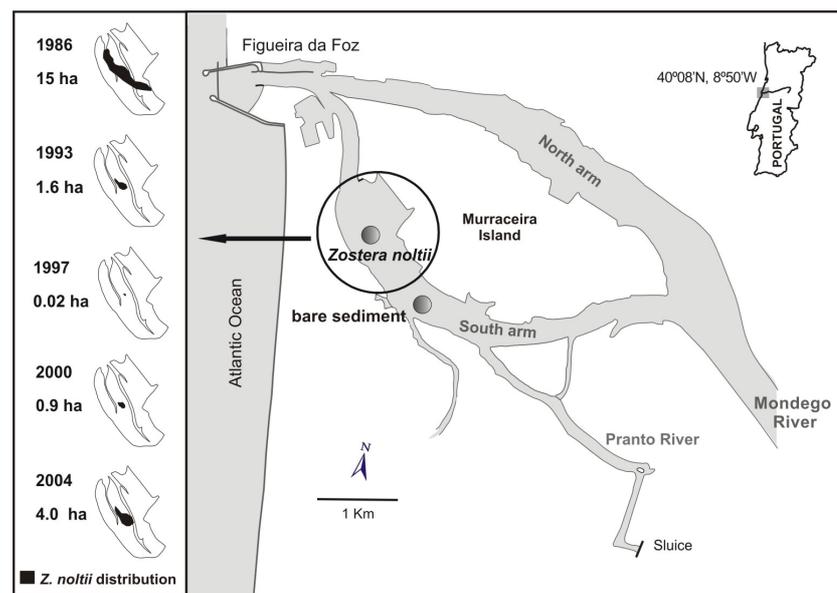


Fig. 1. Mondego estuary map showing sampling sites: *Zostera* and bare sediment sites (grey circles). Change in the area covered by *Zostera noltii* in the south arm of the Mondego estuary. Mapping of benthic vegetation is based on field observations, aerial photographs and GIS methodology (Arc View GIS version 8.2).

In the early 1990s, the communication between the two arms of the estuary became totally interrupted in the upstream area. The combined effect of an increased water residence time and of nutrient concentrations became major driving forces behind the occurrence of seasonal *Ulva* spp. blooms and a concomitant severe reduction of the area occupied by *Z. noltii* beds, and also of *Z. noltii* biomass in the areas where it still remained (Marques et al.

1997), as a function of competition with macroalgae (Marques et al. 2003). The shift in benthic primary producers has changed trophic structure of benthos (Marques et al. 1997, 2003; Pardal et al. 2000, 2004; Cardoso et al. 2004a, 2005; Patrício and Marques 2006; Dolbeth et al. 2007).

Since 1998 the *Z. noltii* has been recovering and *Ulva* spp. blooms reducing (Lillebø et al. 2007), due to the decreased nutrient discharges from the river and the partial re-opening of the communication between the north and south arms (Neto 2004).

Sample collection and preparation

To assess the relative nutrient enrichment at the two sites, we measured concentrations of nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), and chlorophyll *a* in the water column. We also compared the $\delta^{15}\text{N}$ in primary producers collected in benthos and the bulk organic matter in the sediment (sedimentary organic matter, SOM) and in the water column (particulate organic matter, POM) between the two sites as a measure of the eutrophication level.

To examine and compare the food web structure between the two study sites, we measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in primary producers, bulk sediment and consumers. We sampled particulate organic matter (POM), sedimentary organic matter (SOM), meiofauna, seagrass, macroalgae, macrobenthos and zooplankton at each of the two sites. Fish were collected at the bare sediment site. Given their high mobility, we retained collection in both sites irrelevant for the purpose of this study. Samples for nutrient analyses and POM were collected monthly, from November 2005 to July 2006. Plants and animals were collected in November 2005 and February, May, and July 2006. At each site and sampling date, 5 samples were taken from an area of about 4×4 m and pooled before analyses. Samples taken at each time in each site are considered here as replicates since there were no temporal differences (Baeta et al. 2009). Water for nutrient concentration (700ml) was filtered through a precombusted 0.45 μm pore size, 4.7-cm diameter glass fiber filters (Whatman GFF filter). In addition, 700 ml of water was passed through a Whatman GFF filter to measure Chl *a* concentration in the collected material (Parsons et al. 1985). All filters and water samples were stored on ice until arrival at the laboratory, when they were transferred to the freezer (-18°C). POM was obtained by filtering 0.5-1 l of seawater, from a depth of 0.5 m below the surface, onto precombusted (450°C, 4h) Whatman GFF filters with a low pressure vacuum pump.

Sediment samples from the upper 1 cm were collected with an acrylic corer (31 mm of diameter). For the meiofauna, sediment samples were collected, and the top 3 cm of each sediment core was then passed through 500 μm and 38 μm sieves. Meiofauna were examined from the 38 μm fraction, and 50 to 300 individuals were collected for isotope analyses. Seagrass leaves and roots, and macroalgae were collected by hand and gently cleaned of epiphytic material. Macroinvertebrates were also taken manually from each site, and held in filtered sea water for 24 h to allow their guts to clear.

Zooplankton was collected at each site by towing a Bongo net (0.5 m diameter, 200 μm mesh size) against the current for 20 min. The zooplankton samples for isotope analysis were composites of 20 to 200 individuals. Resident (*Atherina boyeri*, *Pomatoschistus microps*, *Pomatoschistus minutus*, *Syngnathus abaster*, *Syngnathus acus*) and transient (*Dicentrarchus labrax*, *Diplodus vulgaris*, *Solea solea*) fish species were collected using a 2 m beam trawl, with a 5 mm stretched mesh size on the cod end. The trawls were carried out during the night, at low water spring tides.

All samples were rinsed with Milli-Q water, before freeze-drying. Filters with POM were also rinsed with a few milliliters of ammonium formate to remove the salts that may damage the combustion columns of the IRMS elemental analyzer. Despite ammonium formate may alter the $\delta^{15}\text{N}$, no trace of ammonium formate is left in the filter because it sublimates when the filters are freeze-dried overnight (P. Richard, personal communication). When dry, samples were ground (filters with POM were kept whole) into homogenous powder using mortar and pestle, weighted, and loaded into tin capsules. For the macrofauna, we removed the shell from molluscs and skeleton from crustaceans. For the zooplankton, we analysed the entire animals, given their small size. For fish, the muscle of the dorsal region was analysed.

Samples were analysed using an EA-IRMS (Isoprime, Micromass, UK). Isotopic values were expressed in the δ unit notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and N_2 in air for $\delta^{15}\text{N}$) following the formula: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 103$, where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The precision of the measurement was 0.2‰ for both carbon and nitrogen.

Results

Eutrophication level

There were no significant differences in NO_3^- , NH_4^+ , PO_4^{3-} and chlorophyll *a* concentrations and in the total nitrogen to phosphorus ratio ($\text{DIN}/\text{PO}_4^{3-}$) between the two sites (Table 1). Nitrate concentrations ranged from 0.06 to 0.96 mg L^{-1} (Fig. 2). Concentrations of NH_4^+ and PO_4^{3-} were usually much lower than those of NO_3^- (Fig. 2a). N/P was generally above the 16:1 Redfield mass ratio during colder months, and below that in warmer months. This suggests that in winter P supply might be the limiting nutrient, while in summer, N might limit producer growth nutrient (Fig. 2b). Chlorophyll *a* concentrations ranged between 1.45 and 8.92 mg m^{-3} , and peaked in spring, perhaps from drawing down nitrate concentration during the warmer months (Fig. 2c).

Table 1 Results of paired *t* tests of differences between the two sites for nutrient and chlorophyll concentrations in the Mondego estuary. n.s. means no significant differences ($p > 0.05$).

	<i>t</i>	df
NO_3^-	0,068 n.s.	8
NH_4^+	1.385 n.s.	8
PO_4^{3-}	1.867 n.s.	8
Chlorophyll <i>a</i>	0,701 n.s.	8
$\text{DIN}/\text{PO}_4^{3-}$	0,464 n.s.	8

The $\delta^{15}\text{N}$ of primary producers ranged between 9.7 and 13.2‰ in the bare sediment site and between 9.7 and 13.2 in the *Zostera* site (Table 2). There were clearly no differences in $\delta^{15}\text{N}$ between the *Zostera* and the bare sediment site for green ($F_{1,10} = 1.95$, $P = 0.20$), red ($F_{1,5} = 0.60$, $P = 0.48$) or brown algae ($F_{1,4} = 0.02$, $P = 0.98$).

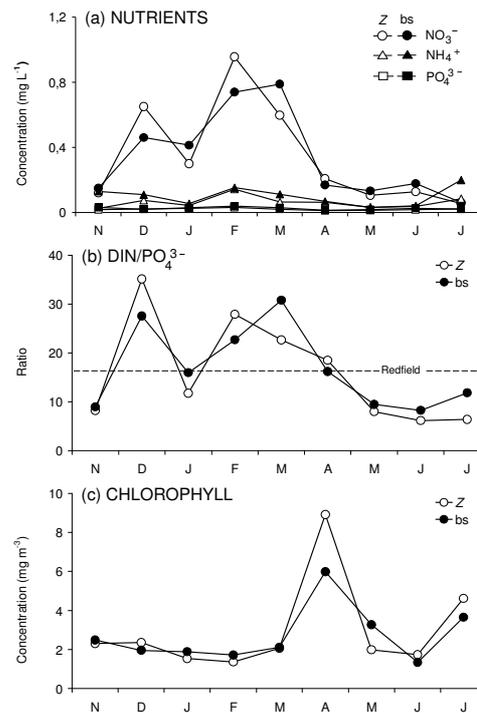


Fig. 2. Nitrate, ammonium, and phosphate (a), DIN/PO₄³⁻ (b), and phytoplankton chlorophyll *a* (c) concentrations, at the *Zostera* site and bare sediment site, in the Mondego estuary, from November 2005 to July 2006.

Food web structure

A total of 45 groups/species were identified, including 5 species of primary producers, bulk POM, SOM and 38 consumer groups. Among these consumers, there were 21 species of macrofauna, 2 of meiofauna, 8 fish species, and 7 zooplankton taxa (Table 2). The dual-stable isotope plots (Fig. 3) showed that both the benthic and plankton food webs were similar between sites. There were, however, some differences for the position of the detritivore bivalve *Scrobicularia plana*, for the omnivore polychaetes *Nephtys cirrosa* and *Hediste diversicolor*, the omnivore crustaceans *Cyathura carinata*, *Crangon crangon* and *Carcinus maenas*. *S. plana* was closer to the suspension feeder bivalves *Cerastoderma edule*, and *Mytilus galloprovincialis*, whereas the remaining species, especially *H. diversicolor*, were more enriched in ¹⁵N at the bare sediment than the *Zostera* site. Accordingly, there were significant differences between the two sites in the δ¹⁵N values of omnivores ($F_{1,10} = 13.85$, $P = 0.003$).

At both sites, POM and SOM had the most depleted values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Macroalgae and *Z. noltii* had a relatively variable range of values, similar to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of macrofauna consumers (Table 2, Fig. 3). Among the macrofauna consumers, the isotopic signatures of the suspension feeders *M. galloprovincialis* and *C. edule* were very close to the planktonic food web and were 1-3 ‰ enriched compared to POM (1-2 ‰ in ^{13}C and 2-3 ‰ in ^{15}N , Fig. 3). The grazer macrofauna gastropod *Hydrobia ulvae* had the highest $\delta^{13}\text{C}$ values among all other macrofauna consumers and very low $\delta^{15}\text{N}$ values. Only the macrofauna suspension feeder *C. edule* and *M. galloprovincialis* showed smaller values than *H. ulvae*. The highest $\delta^{15}\text{N}$ values were found for the predator *Glycera tridactyla* and the omnivore *N. cirrosa*, especially in the bare sediment (Table 2, Fig. 3).

The zooplankton consumers occupied a relatively narrow area, as compared to the macrofauna consumers. Those zooplankton species that are considered phyto- and microzooplanktivores were at an intermediate position between POM and the zooplankton predator *Sagitta friderici*. Eventually, the predator fish *D. labrax*, *D. vulgaris* and *P. minutus*, were 3-4 ‰ enriched in ^{15}N and 1-2 ‰ in ^{13}C as compared to intermediate plankton consumers. The juveniles of the predator fish *S. solea* and *P. microps* were instead very depleted in ^{13}C , but not in ^{15}N , whereas the predator seahorse *S. abaster* and *S. acus* had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values close to those of macrofauna omnivore.

Table 2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean \pm SE) of primary producers and consumers collected from the *Zostera* site and the bare sediment site in the Mondego estuary. Trophic groups and feeding habit of consumers based on literature data (*b* benthivore; *z* zooplanktivore).

Group/species	Abreviaton	<i>Zostera</i> site			bare sediment site			Trophic group	Feeding habit	References
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>N</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>N</i>			
		Mean \pm SE	Mean \pm SE		Mean \pm SE	Mean \pm SE				
Macrophytes										
<i>Enteromorpha</i> sp.	E	-12.3 \pm 0.5	12.4 \pm 1.7	4	-13.5 \pm 0.5	13.2 \pm 1.8	4			
<i>Fucus</i> sp.	F	-16.4 \pm 0.5	9.6 \pm 0.6	4	-16.3 \pm 0.9	9.7 \pm 0.5	4			
<i>Gracilaria</i> sp.	G	-17.0 \pm 0.9	9.7 \pm 1.2	4	-17.7 \pm 1.2	9.7 \pm 1.8	4			
<i>Ulva</i> sp.	U	-11.2 \pm 0.4	12.0 \pm 1.2	4	-11.6 \pm 0.6	12.5 \pm 1.5	4			
<i>Zostera noltii</i> (leaves)	Zl	-12.5 \pm 0.1	9.5 \pm 1.5	4						
<i>Zostera noltii</i> (roots)	Zr	-12.2 \pm 0.2	11.3 \pm 3.3	4						
Particulate organic matter	POM	-22.5 \pm 0.4	5.7 \pm 0.3	9	-23.0 \pm 0.4	6.0 \pm 0.3	9			
Sediment	SOM	-21.2 \pm 0.4	4.8 \pm 0.2	4	-21.5 \pm 0.4	4.9 \pm 0.2	4			
Amphipoda										
<i>Amphithoe valida</i>	Av	-14.2 \pm 0.6	11.1 \pm 0.2	4	-15.3 \pm 0.2	10.7 \pm 0.2	4	Grazer	Surface-deposit feeder	Pardal et al. (2000)
<i>Echinogammarus marinus</i>	Em	-14.0 \pm 0.6	10.8 \pm 0.3	4	-15.4 \pm 0.7	10.5 \pm 0.6	4	Grazer/omnivore	Surface-deposit feeder	Dick et al. (2005)
<i>Melita palmata</i>	Mp	-14.2 \pm 0.6	10.2 \pm 0.1	4	-16.8 \pm 0.8	9.7 \pm 0.2	4	Grazer	Surface-deposit feeder	Pardal et al. (2000)
Bivalvia										
<i>Cerastoderma edule</i>	Ce	-18.6 \pm 0.1	7.6 \pm 0.3	4	-19.6 \pm 0.3	7.9 \pm 0.5	4	Grazer/detritivore	Suspension feeder	Garcia-Arbera & Rallo (2002)
<i>Mytilus galloprovincialis</i>	Mg	-18.9 \pm 0.2	7.2 \pm 0.3	4	-19.5 \pm 0.8	7.4 \pm 0.5	4	Grazer/detritivore	Suspension feeder	Eklöf et al. (2005)
<i>Scrobicularia plana</i>	Sp	-16.5 \pm 0.5	9.2 \pm 0.2	4	-18.4 \pm 0.6	8.7 \pm 0.4	4	Detritivore	Deposit/Suspension feeder	Verdelhos et al. (2005)
Decapoda										
<i>Carcinus maenas</i>	Cm	-16.4 \pm 0.6	11.8 \pm 0.1	4	-16.3 \pm 0.3	12.3 \pm 0.2	4	Omnivore	Surface-deposit feeder	Baeta et al. (2006)
<i>Crangon crangon</i>	Ccr	-15.2 \pm 0.6	11.6 \pm 0.1	4	-15.3 \pm 0.4	11.6 \pm 0.2	4	Omnivore	Surface-deposit feeder	Oh et al. (2001)
Gastropoda										
<i>Gibbula umbilicalis</i>	Gu	-12.0 \pm 0.8	10.7 \pm 0.7	3				Grazer/detritivore	Surface-deposit feeder	Bode et al. (2006); Pigeot et al. (2006)
<i>Hydrobia ulvae</i>	Hu	-10.9 \pm 0.2	9.6 \pm 0.1	4	-12.2 \pm 0.3	9.6 \pm 0.1	4	Grazer/detritivore	Surface-deposit feeder	Cardoso et al. (2004b)
<i>Littorina littorea</i>	Ll	-11.2 \pm 0.4	12.2 \pm 0.1	4				Grazer/detritivore	Surface-deposit feeder	Bode et al. (2006); Pigeot et al. (2006)
Isopoda										
<i>Cyathura carinata</i>	Cc	-13.7 \pm 0.4	11.5 \pm 0.2	4	-14.7 \pm 0.6	11.8 \pm 0.3	4	Omnivore	Surface-deposit feeder	Ferreira et al. (2004)
<i>Idotea chelipes</i>	Ic	-13.6 \pm 0.4	10.0 \pm 0.8	4	-15.4 \pm 0.6	9.7 \pm 0.6	4	Grazer	Surface-deposit feeder	Bamber (2004)
<i>Lekanesphaera levii</i>	Llev	-11.0 \pm 0.3	8.6 \pm 0.7	4	-13.0 \pm 0.3	8.8 \pm 0.6	4	Detritivore/grazer	Surface-deposit feeder	Mancinelli et al. (2005)

Table 2 continued

Group/species	Abreviaton	<i>Zostera</i> site			bare sediment site			Trophic group	Feeding habit	References
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>N</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>N</i>			
		Mean \pm SE	Mean \pm SE		Mean \pm SE	Mean \pm SE				
Meiofauna										
Nematoda	Ne	-15.4 \pm 0.4	11.4 \pm 0.5	4	-17.3 \pm 0.5	11.2 \pm 0.5	4	Detritivore/carnivore	Deposit feeder	Danovaro & Gambi (2002)
Copepoda Harpaticoida	Co	-15.7 \pm 0.6	10.7 \pm 0.4	4	-16.9 \pm 0.3	11.2 \pm 0.4	4	Detritivore	Deposit feeder	Danovaro & Gambi (2002)
Polychaeta										
<i>Alkmaria romijni</i>	Ar	-15.1 \pm 0.7	10.6 \pm 0.1	3	-17.5 \pm 0.6	10.7 \pm 0.3	4	Detritivore	Deposit/suspension feeder	Bamber (2004)
<i>Capitella capitata</i>	Cca	-16.0 \pm 0.3	10.1 \pm 0.5	3	-16.7 \pm 0.4	11.3 \pm 0.1	4	Detritivore	Deposit feeder	Gaston & Nasci (1988)
<i>Glycera tridactyla</i>	Gt	-14.2 \pm 0.3	13.6 \pm 0.3	4	-14.7	12.9	1	Carnivore		Garcia-Arbera & Rallo (2002)
<i>Hediste diversicolor</i>	Hd	-14.1 \pm 0.3	11.3 \pm 0.3	4	-14.5 \pm 0.4	12.5 \pm 0.2	4	Omnivore	Deposit feeder	Garcia-Arbera & Rallo (2002)
<i>Heteromastus filiformis</i>	Hf	-15.7 \pm 0.1	11.3 \pm 0.2	4	-16.6 \pm 0.2	11.6 \pm 0.1	4	Detritivore	Deposit feeder	Oug et al. (1998)
<i>Nephtys cirrosa</i>	Nc	-15.7 \pm 1.4	12.3 \pm 0.2	3	-15.4 \pm 0.3	12.9 \pm 0.4	3	Omnivore	Deposit feeder	Hartmann-Schröder (1996)
<i>Streblospio shrubsolii</i>	Ssh	-16.4 \pm 1.1	10.9 \pm 0.7	3	-17.5 \pm 0.7	10.5 \pm 0.1	3	Detritivore	Deposit/suspension feeder	Mazik & Elliot (2000)
Fishes										
<i>Atherina boyeri</i>	Ab				-18.0 \pm 0.6	11.7 \pm 0.7	2	Predator (<i>z/b</i>)		Vizzini & Mazzola (2005)
<i>Dicentrarchus labrax</i> (juv)	DI				-18.2 \pm 0.6	14.2 \pm 0.6	2	Predator (<i>z/b</i>)		Martinho (2005)
<i>Diplodus vulgaris</i> (juv)	Dv				-17.9 \pm 0.5	15.0 \pm 0.3	4	Predator (<i>z/b</i>)		Cabral & Costa (2001)
<i>Pomatoschistus microps</i> (juv)	Pm				-22.4 \pm 0.3	14.0 \pm 0.7	2	Predator (<i>b</i>)		Leitão et al. (2006)
<i>P. minutus</i>	Pmi				-17.4 \pm 0.4	13.1 \pm 0.3	4	Predator (<i>b</i>)		Leitão et al. (2006)
<i>Syngnathus abaster</i>	Sa				-15.0 \pm 0.9	12.3 \pm 0.6	2	Predator (<i>b</i>)		Vizzini & Mazzola (2003)
<i>S. acus</i>	Sac				-16.2 \pm 0.0	11.4 \pm 0.9	2	Predator (<i>z/b</i>)		Vizzini & Mazzola (2003)
<i>Solea solea</i> (juv)	Ss				-23.5 \pm 0.4	14.8 \pm 0.6	4	Predator (<i>z/b</i>)		Martinho (2005)
Zooplankton										
<i>Acartia tonsa</i>	At	-22.7 \pm 1.3	9.5 \pm 0.7	4	-23.7 \pm 1.3	9.9 \pm 0.5	4	Phyto-microzooplanktivore		Kleppel (1993)
<i>Acartia</i> sp.	Asp	-18.5 \pm 0.6	8.3 \pm 0.2	4	-18.6 \pm 0.3	8.6 \pm 0.3	4	Phyto-microzooplanktivore		Kleppel (1993)
Cladocera	Cl	-21.9 \pm 1.0	9.4 \pm 0.8	3				Microzooplanktivore		He et al. (2001)
Mysidacea (juv)	My	-18.9 \pm 0.7	8.1 \pm 0.4	4				Phytoplanktivore		Froneman (2001)
<i>Pomatoschistos</i> sp. (larvae)	Pl	-20.0 \pm 0.5	9.8 \pm 0.3	3				Microzooplanktivore		Mousseau et al. (1998)
<i>Sagitta iriderici</i>	Sf	-18.1 \pm 0.8	11.1 \pm 0.5	3	-18.2 \pm 0.6	11.0 \pm 0.4	3	Predator (<i>zf</i>)		Liang et al. (2003)
Zoeae (brachyura)	Zo	-18.7 \pm 0.3	8.9 \pm 0.3	3	-18.7 \pm 0.6	9.0 \pm 0.3	3	Omnivore		Thatje et al. (2003)

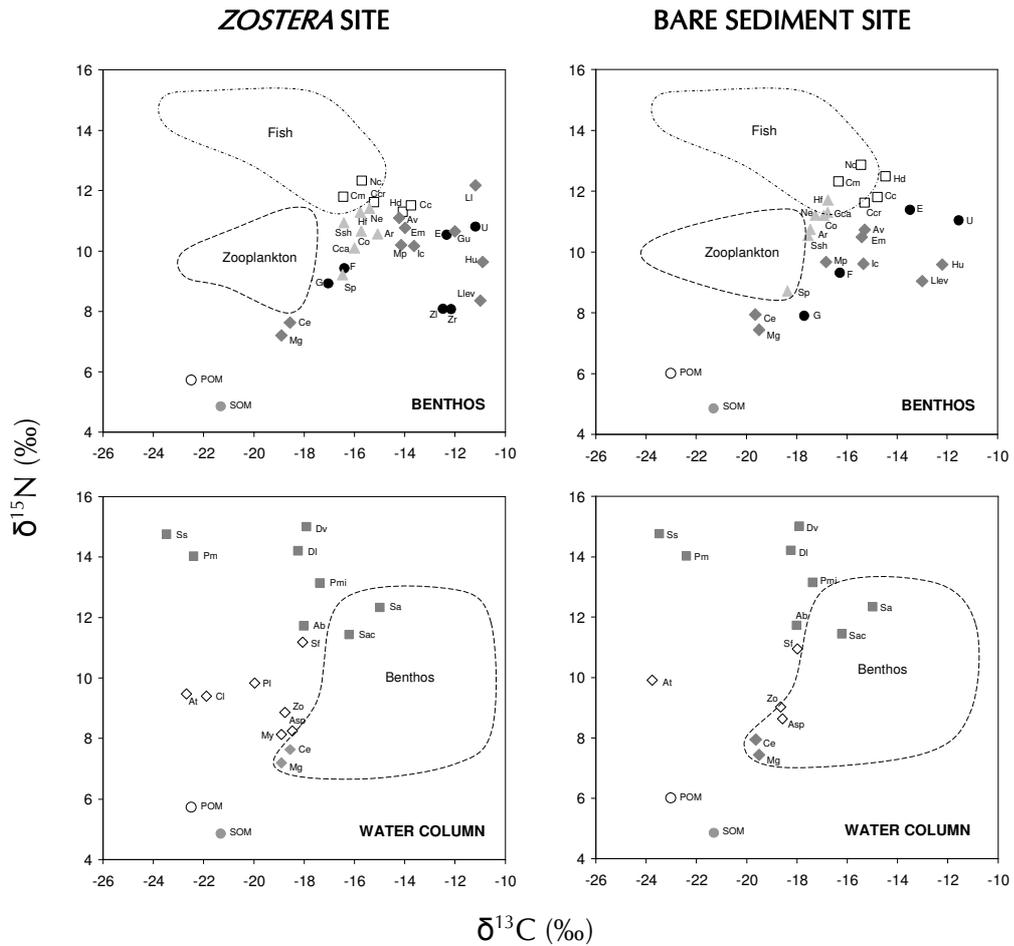


Fig. 3. $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ of the benthic and water column food webs, in the *Zostera* site (left) and bare sediment site (right), in the Mondego estuary. Benthos: (●) primary producers; (▲) detritivore; (◆) grazer/detritivore; (□) omnivore. Water column: (■) fish; (◇) zooplankton. Average is presented for biota collected from November 2005 to July 2006. The abbreviations of the species/groups are shown on Table 2.

To test statistically for differences between the trophic food web of *Zostera* vs. bare sediment, we first identified all the taxa in common between the two sites within different trophic groups (e.g. Macroalgae, benthic primary consumers such as detritivores and grazers, benthic secondary consumers such as omnivores and predators, and Zooplankton). Then, we correlated the natural abundance of stable isotopes measured in the *Zostera* site vs. the bare sediment (Fig. 4, Table 3). We expected significant correlation and values of the

slope close to 1 if there were no differences in the isotope signature of primary producers and consumers among the two sites. In all cases, except for $\delta^{15}\text{N}$ for the benthic secondary consumers, there was significant correlation (R values) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the two sites and the slope was close to 1 (Table 3). Heavier carbon isotopic signatures for benthic consumers in the *Zostera* site were found, compared to those from the site with no *Z. noltii*. $\delta^{13}\text{C}$ values of the bare sediment site macrobenthos were 1.2‰ depleted, relative to those of the *Zostera* site.

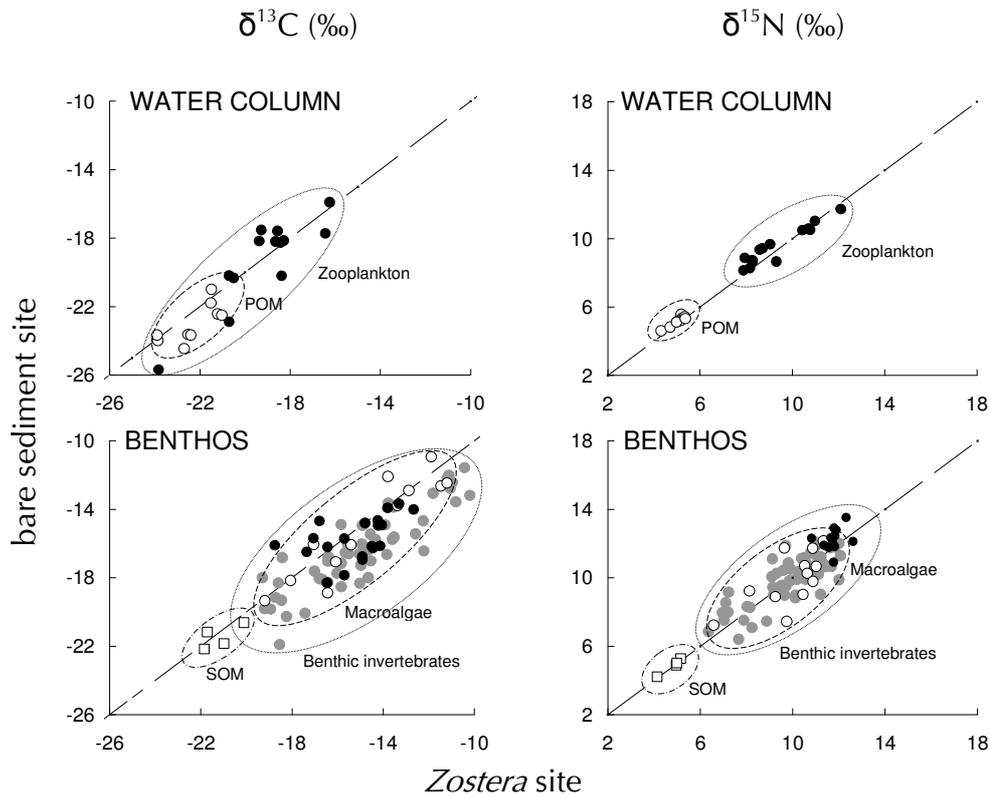


Fig. 4. Comparison of C (left) and N (right) stable isotope ratios, for the water column (top) [(○) POM; (●) zooplankton], and benthos (bottom) [(○) primary consumers; (●) secondary consumers; (○) macroalgae; (□) SOM], collected from the *Zostera* and bare sediment sites, in the Mondego estuary. Dashed line shows 1:1 correlation.

To further explore the relationship between the values of $\delta^{15}\text{N}$ and the trophic guild in which consumers are classified in the literature, we simplified published data into

“producer”, “primary consumer”, and “secondary consumer” and plotted the $\delta^{15}\text{N}$ values of each category versus the others in the *Zostera* (Fig. 5) and the bare sediment site (Fig. 6). Among macrofauna consumers, the different trophic groups were overlapped at both sites, whereas the separation among planktonic groups was clear (Fig. 5, 6). The range of variability of each trophic group was also quite large, varying between 1.5 to 4.5‰, indicating that trophic guilds are probably inadequate for invertebrate macrofauna (Figs. 5, 6).

Table 3 Correlation coefficient test results testing differences, in the companion of Fig. 3, between sites for the groups benthic and water column food webs collected in the Mondego estuary on C and N isotope ratios. ** = $P < 0.05$; *** = $P < 0.001$; n.l.r. = no linear relationship between the variables ($P > 0.05$).

Groups	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$			
	R	<i>P</i>	<i>N</i>	Slope	R	<i>P</i>	<i>N</i>	Slope
Macrophytes	0.920	***	12	1.001	0.680	**	12	0.790
Benthic primary consumers	0.880	***	50	0.820	0.824	***	50	0.820
Benthic secondary consumers	0.510	**	21	0.420	0.068	n.l.r.	21	0.091
POM	0.732	**	9	0.778	0.948	***	9	0.830
Zooplankton	0.923	***	14	1.110	0.927	***	14	0.776

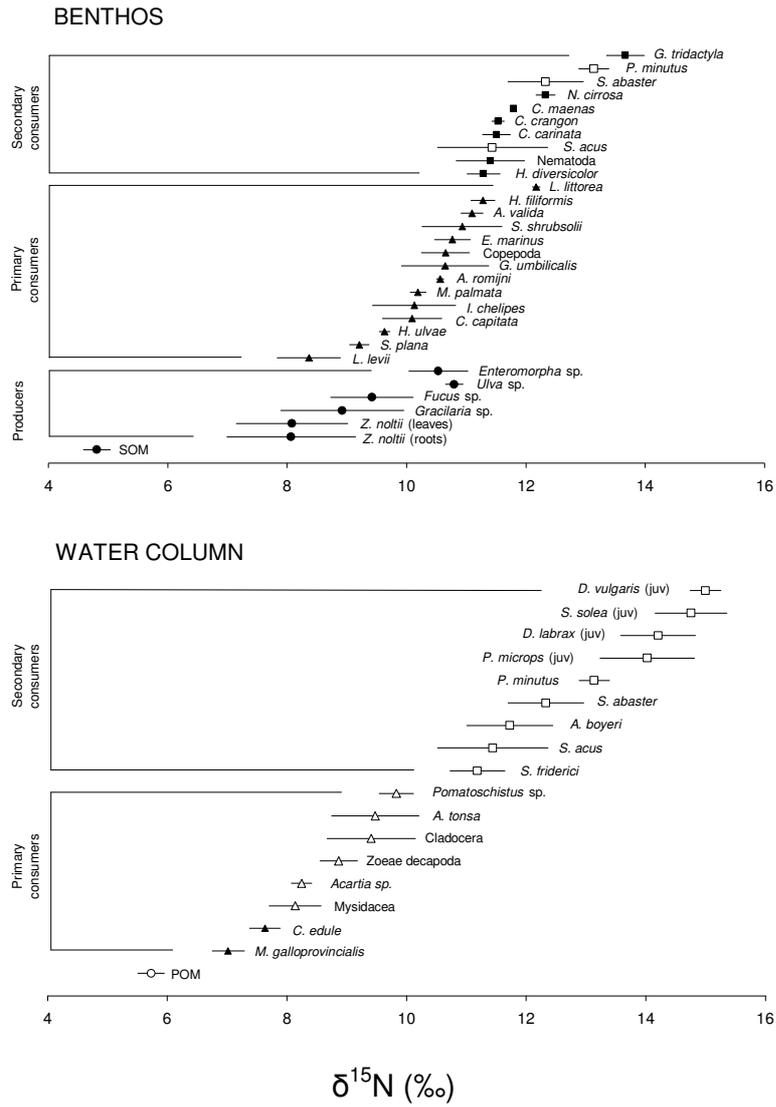


Fig. 5. Nitrogen stable isotope signatures of producers and consumers for the benthic (top) and water column (bottom) food webs from the *Zostera* site, in the Mondego estuary. Black symbols represent species/groups collected in the benthos [(●) producers; (▲) primary consumers; (■) secondary consumers]. White symbols represent species/groups collected in the water column [(○) producers; (△) primary consumers; (□) secondary consumers]. Average \pm standard error is presented for biota collected from November 2005 to July 2006.

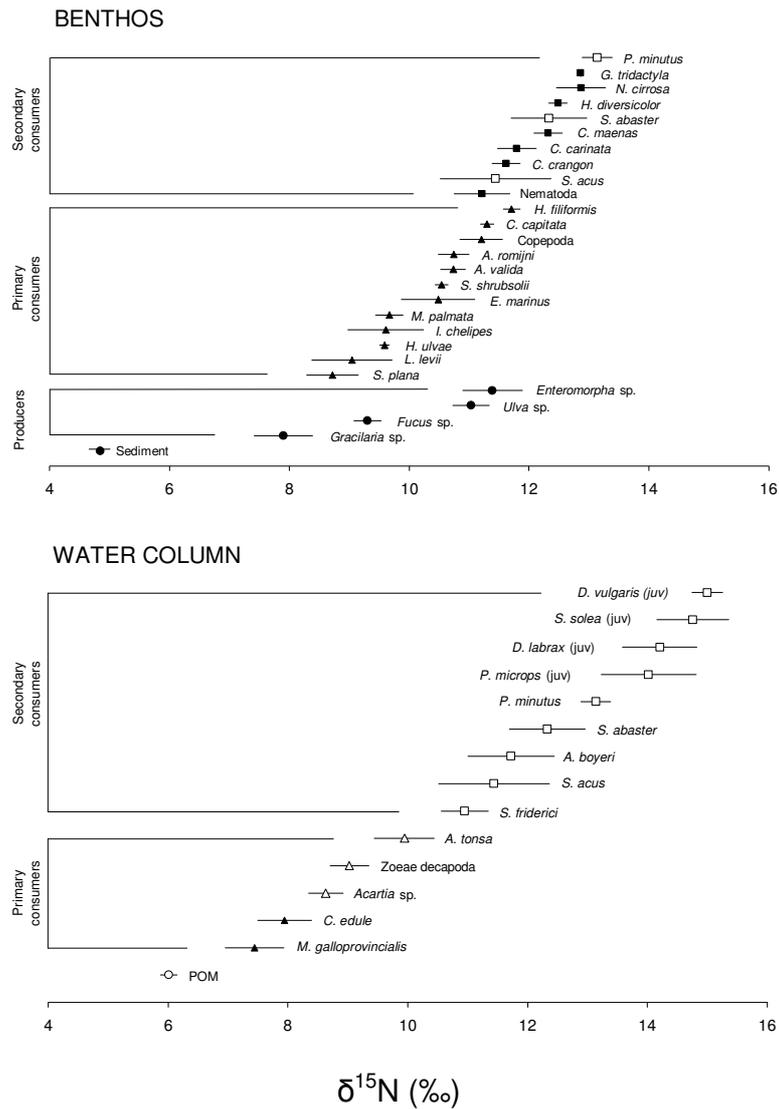


Fig. 6. Nitrogen stable isotope signatures of producers and consumers for the benthic (top) and water column (bottom) food webs from the bare sediment site, in the Mondego estuary. Black symbols represent species/groups collected in the benthos [(●) producers; (▲) primary consumers; (■) secondary consumers]. White symbols represent species/groups collected in the water column [(○) producers; (△) primary consumers; (□) secondary consumers]. Average \pm standard error is presented for biota collected from November 2005 to July 2006.

Discussion

Eutrophication and anthropogenic input of N

The concentration of nitrogen in the overlying water and the sources of nitrogen used by primary producers were very similar between the two sites, despite expected differences in the level of eutrophication between sites, due to presence of *Z. noltii* that may buffer effects of nutrient enrichment, by storing nutrients in its tissue and by supporting a more diverse food web which can better recycle nutrients (Cebrian 1999; Duarte 2002),

The high nitrogen isotopic signatures of primary producers could indicate that the sources of nitrogen were from human activities. Indeed, $\delta^{15}\text{N}$ values ranging from +10 to +20‰ in primary producers strongly indicate anthropogenic sources, whereas nitrate derived from atmospheric deposition produce values smaller than 6‰ (Kendall 1998). In our study, the $\delta^{15}\text{N}$ for *Z. noltii* leaves ($9.5\pm 3.5\text{‰}$), and green algae *Ulva* sp. ($12.5\pm 3.0\text{‰}$) were about 3-4 ‰ higher than values measured in Ria Formosa, Portugal (6.1‰; 8.8‰, respectively) (Machás et al. 2003), or in estuaries of the NW Iberian peninsula ($5.6\pm 0.4\text{‰}$; $8.4\pm 0.3\text{‰}$, respectively) (Bode et al. 2006). Seawater nitrogen signatures in the Atlantic coast have values ranging from 5.2‰ (Liu and Kaplan 1989) and 6.5‰ (Bode et al. 2003). Furthermore, by comparing the nutrient concentration in the water column to the values of 106 North American estuaries (Tomasky et al. submitted for publication), we found that the Mondego estuary was more enriched than 90% of American estuaries. Despite mitigation procedures implemented in the Mondego estuary in 1998, and 2002 (Lillebø et al. 2007), high nitrogen loads are thus still entering the system and the eelgrass habitat is not able to buffer these inputs of nutrients. The measures currently employed seem thus insufficient to ensure high environment quality. Probably larger eelgrass meadows could sequester part of these nutrients and more environmental protection might be needed to ensure a full recovery of the eelgrass in the system.

Food web structure

The presence of *Zostera* did not change considerably the trophic structure of both the sediment and the water column. There were, however, differences in the isotopic signatures of some benthic macrofauna consumers, such as the detritivore *S. plana* and the omnivores *N. cirrosa*, *H. diversicolor*, *C. carinata*, *C. crangon* and *C. maenas*. These differences may suggest the occurrence of a diet shift following the characteristics of the habitat where these

animals are. *Zostera* meadows has a more complex habitat structure created by the presence of rooted macrophytes, and can offer higher protection from predators and high levels of organic matter in the sediment (Duarte 2002). Differences in isotopic signatures of marine invertebrates often indicate a shift in feeding behaviour following species interactions, when for instance feeding choice depends on the availability of resources and the time spent handling the food under high predation risk (Stephens and Krebs 1986; Burrows and Hughes 1991, Rossi et al. 2004, Sommer et al. 2005). In our study, omnivore macrofauna showed to feed at lower levels of the food web at the *Zostera* than at the bare sediment site. Furthermore the detritivore *S. plana* showed a diet close to that of the suspension feeders in the bare sediment, whereas in the *Zostera* site, the diet was close to that of other detritivores. By providing refuge and increasing organic matter availability, *Z. noltii* might allow these macrofauna species to rely on organic matter, rather than on other consumers or on the particles suspended in the water column.

Nonetheless, the bulk organic matter seemed to not support the macrofauna consumers, including the detritivores and the omnivores that varied diet among habitats. In our study, these consumers had $\delta^{13}\text{C}$ and ^{15}N values ($\delta^{13}\text{C}$: between -11 and -18‰ and $\delta^{15}\text{N}$: higher than 8‰) too high to be feeding on SOM, if we consider the general fractionation values proposed in the literature of 1 and 3-4‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Minagawa and Wada 1984). Similarly, macroalgae or eelgrass did not seem to be the main sources of food for the macrofauna consumers. The organic matter available in the sediment (SOM) is made up of various terrestrial and marine sources, on which many macrofauna consumers may forage selectively. It may be possible that in both sites animals fed on a mix of different sources. The values of SOM in both sites indicated a mix of terrestrial organic matter, very depleted in ^{13}C (-30 to -23‰, Fry and Sherr 1984), microalgae (between -21 and -12‰ for $\delta^{13}\text{C}$ and between 5 and 8‰ for $\delta^{15}\text{N}$, France 1995; Currin et al. 1995; Riera et al. 1996, 1999; Herman et al. 2000; Moens et al. 2002; Page and Lastra 2003) and macroalgae (-18 to -11‰, in this study). Microalgae indeed represent a fundamental source of food for benthic macrofauna in many estuaries, especially on intertidal bare sediment (Herman et al. 2000; Middelburg et al. 2000; Rossi et al. 2009). In the bare site, sediment microalgae were more abundant than in the *Zostera* site and the isotopic values of microalgae measured in November 2008 ranged between -13 and -14‰ for $\delta^{13}\text{C}$ and 6 ‰ for $\delta^{15}\text{N}$ (Alexandra Baeta, personal observation). It may thus be possible that animals foraged on a mix of surface detritus, macroalgae and benthic microalgae, selecting for benthic microalgae when available.

Recently, empirical studies have found variable $\delta^{15}\text{N}$ values within trophic levels and demonstrated that the generally recognized average 3-4‰ $\delta^{15}\text{N}$ increase at each trophic level (e.g. Minagawa and Wada 1984) does not always apply to the marine system because food sources are temporally and spatially variable and because consumers vary their metabolism depending on the source of food ingested (Sommer et al. 2005; Vanderklift and Ponsard 2003). Furthermore, omnivory is a very common feature in estuarine habitats and it may further complicate the interpretation of isotopic signatures of consumers and their food sources (Svensson et al. 2007; Jaschinski et al. 2008). In our study, it is possible that ^{15}N enriched values of macrofauna species compared to the $\delta^{15}\text{N}$ of SOM or macroalgae were due to a different metabolism of these consumers with respect to their nitrogen sources. In addition, the high frequency of omnivory may explain why $\delta^{15}\text{N}$ values overlapped among the trophic groups in which macrofauna species are categorized. We indeed found that the $\delta^{15}\text{N}$ values for the herbivores and detritivores (primary consumers) of the benthos ranged from 7.8 to 12.2‰ and overlapped to those of the predators/omnivores (secondary consumers), which ranged from 10.8 to 14.2‰. This variability in trophic signatures may have important consequences for interpreting aquatic food webs because it may undermine the estimates of energy or mass flow through food webs, when such estimates are based on the assumption that trophic levels are discrete. Indeed, considering discrete trophic levels cannot capture the complex trophic interactions and the omnivory typical of natural environment, underestimating the flow of energy in the ecosystem (Vander Zanden and Rasmussen 2001; Post 2002; Vanderklift and Ponsard 2003; Bode et al. 2006).

Compared to the benthic food web, the food web of the water column was less complex. The values of $\delta^{13}\text{C}$ for the organic matter suspended in the water column (POM) were in agreement with the literature values of surface waters in the latitudinal range around 40° N (from -25 to -18‰, Goerick et al. 1994) and were within the range for estuarine and coastal marine phytoplankton from other temperate regions (see Gearing et al. 1984). Taking into account a fractionation of 3-4‰ for $\delta^{15}\text{N}$ and of 1‰ for $\delta^{13}\text{C}$, in both sites the link between the food web of benthos and the water column was clearly represented by the suspension feeders *M. galloprovincialis* and *C. edule*. The $\delta^{13}\text{C}$ of *C. edule* (-18‰) was similar to other studies that suggested *C. edule* has a diet based on plankton microalgae and organic matter (POM) mixed to benthic microalgae resuspended on the overlying water (Rossi et al. 2004). In addition, a part of the water column trophic web was supported by POM. The primary consumer *A. tonsa*, a micro-zooplankivore and other phyto-microzooplanktivores could forage on POM and transfer part of this carbon and nitrogen up

to the food web. Fish fed on a mixed diet that may include both allochthonous and autochthonous food sources, from both the benthos and the plankton. For instance, the juveniles of *S. solea* and *P. microps* were too depleted in ^{13}C to indicate they only foraged on plankton consumers. It may be possible they used other sources derived from the terrestrial food web, as found in the Rhone river (Darnaude 2005). Furthermore, the ^{13}C enrichment of other fish (*A. boyeri*, *P. minutus*, *D. labrax*, *D. vulgaris*) was up to 5‰ compared to the copepod *A. tonsa* and to the other phyto-microzooplanktivores. They were thus too enriched in ^{13}C to suggest a diet exclusive on these species. Rather, their diet could include benthic consumers and also other plankton consumers (*Acartia* sp. and zoeae).

In conclusion, in the Mondego estuary the presence of the eelgrass *Z. noltii* did not alter nutrient enrichment and the transfer of these nutrients and carbon in the food web, since we found very similar trophic structures between bare sediment and sediment occupied by *Z. noltii*. The benthic food web revealed more complex than the water column food web and evidenced high variability in the isotopic signatures, probably due to the variability of food sources and the physiological characteristics of the macrofauna. We suggest that macrofauna could rely on a complex mix of microalgae, macroalgae and detritus, actively selecting for the most nutritive food sources when available. Other studies are however needed to establish the trophic significance of the different food sources and, in particular, of benthic microalgae.

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Chapter 2

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the Mondego estuary food web: Seasonal variation in producers and consumers

Abstract

Assessments of temporal variation in stable carbon and nitrogen ratios were used to examine seasonal trends of the water column and benthic food webs in the Mondego estuary (Portugal). There was a marked seasonality in weather and water column conditions, including nutrient supply and chlorophyll concentrations. In spite of the pronounced environmental changes, we found little evidence of seasonal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of producers and consumers in the Mondego estuary, with a few notable exceptions. Nitrogen isotope ratios in macrophytes (*Zostera noltii*, *Ulva* sp., *Enteromorpha* sp., and *Gracilaria* sp.), and in

two grazers (*Idotea chelipes* and *Lekanesphaera levi*) increased during late summer, with the highest $\delta^{15}\text{N}$ values being measured in July, during a period of elevated temperatures and drought, which may have favored high rates of denitrification and heavier $\delta^{15}\text{N}$ values. The results suggest that stable-isotope values from macrophytes and selected grazers are useful as tracers of seasonal changes in nitrogen inputs into estuaries, and that those of consumers reflect other factors beyond seasonal variations in N and C sources.

Keywords: Mondego estuary; $\delta^{13}\text{C}$; $\delta^{15}\text{N}$; Coastal eutrophication; Seasonality; Estuarine species

Introduction

Increased anthropogenic delivery of nutrients to water bodies, both freshwater and estuarine, has caused detrimental changes in habitat, food web structure, and nutrient cycling (Valiela et al. 1997; Cole et al. 2004). The resulting eutrophication has many adverse effects within the estuaries (D'Avanzo et al. 1996; Hauxwell et al. 2003). Increased N loading can lead to the loss of important estuarine habitats such as seagrass meadows (Hauxwell et al. 2003). Eutrophic estuaries can also suffer from hypoxia and anoxia (Zimmerman and Canuel 2000), and phytoplankton and macroalgal blooms (Hauxwell et al. 2003).

To better understand management of water quality, it is important to know the sources, as well as the amount of inputs of the nutrient limiting production. In the Mondego estuary, as in most estuarine ecosystems, there is evidence that at least for macroalgal growth, nitrogen is the limiting factor (Teichberg et al. submitted). $\delta^{15}\text{N}$ has proven useful as a tracer of the major source of nitrogen entering coastal waters. Joint use of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ has further shown promise as a tool that helps to explain how the external N

sources, as well as the C sources, move up into estuarine food webs. Application of these isotopic ratios has largely remained an item of research rather than a management tool (Peterson and Fry 1987; Cole et al. 2004). The practical utility of stable-isotopic ratios to some degree depends on the relative sensitivity of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ to seasonal variation.

Stable-isotopic N ratios might, in addition, change with increased temperatures such as we might find seasonally, but also as might be forced by global atmospheric warming. Microbial processes such as denitrification are strongly affected by temperatures (Valiela 1995), and higher denitrification could result in notable fractionation of $\delta^{15}\text{N}$. This indirect linkage could furnish heavier N that is taken up by producers.

Some studies reported that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of producers showed seasonal variation (Riera and Richard 1996, 1997; Fourqurean et al. 1997; Kang et al. 1999; Adin and Riera 2003; Machás et al. 2003; Riera and Hubas 2003; Pruell et al. 2006), while others did not (McClelland and Valiela 1998; Cole et al. 2004). Similarly, some studies showed variation in consumers (Goering et al. 1990; Riera and Richard 1996, 1997; Buskey et al. 1999; Kang et al. 1999; Carman and Fry 2002; Kibirige et al. 2002; Moens et al. 2002; Adin and Riera 2003; Machás et al. 2003; Riera and Hubas 2003; Vizzini and Mazzola 2003, 2005), and others did not (Goering et al. 1990). Knowledge of seasonal variation in stable-isotopic ratios is important as a reflection of biogeochemical and ecological processes, as well as in regard to sampling schedules and expected variability for applied monitoring schedules.

In this paper, we examine the seasonal variation in N and C stable-isotopic ratios of producers and consumers within the food web of the Mondego estuary, and compare the changes in ratios in organisms to the seasonal changes in temperature, precipitation, dissolved nutrients, and in phytoplankton chlorophyll we measured in the Mondego ecosystem. This comparison aims to discern the degree to which seasonally varying driving factors might be manifest in the isotopic ratios of the food web, as well as identify the components of the food web that might be reasonably reliable indicators of changes in nutrient enrichment and warming.

Methods

Study site

The Mondego estuary is a relatively small (1600 ha), warm-temperate, polyhaline, intertidal system located on the Atlantic coast of Portugal, and consists of two arms, north and south

(Fig. 1). The southern arm is characterised by large areas of intertidal mudflats (almost 75% of the area) exposed during low tide. The system receives agricultural runoff from 15,000 ha of upstream cultivated land (mainly rice fields) and supports a substantial population, industrial activities, salt-works, and aquaculture farms, and is also the location of the Figueira da Foz city harbour, which constitutes a tourism centre. All these activities have imposed a strong anthropogenic impact. A mixture of inputs from sewage effluent, agricultural runoff, as well as releases from maricultural activity contributes to the nutrient loads entering the Mondego estuary.

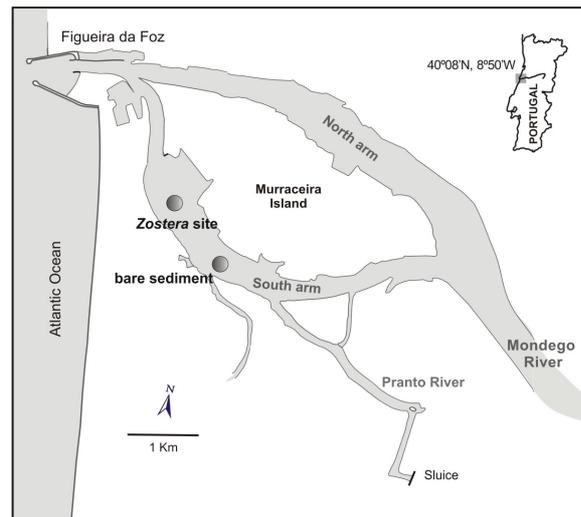


Fig. 1. Mondego estuary map showing sampling sites: *Zostera* and bare sediment sites (grey circles).

In the early 1990s, the southern arm was almost silted up in the upstream areas, causing the river discharge to flow essentially through the northern arm. Consequently, the water circulation in the southern arm became mostly dependent on the tides and on the small freshwater input from a tributary, the Pranto River, artificially controlled by a sluice (Marques et al. 2003). In 1990-1992, the communication between the two arms of the estuary became totally interrupted in the upstream area due to the completion of stonewalls in the northern arm banks. Following this interruption, the ecological conditions in the southern arm suffered a rapid deterioration. The combined effect of an increased water residence time and of nutrient concentrations became major driving forces behind the occurrence of seasonal blooms of *Ulva* sp. and a concomitant severe reduction of the area occupied by

Zostera noltii beds, previously the richest habitat in terms of productivity and biodiversity (Marques et al. 1997, 2003). The shift in benthic primary producers affected the structure and functioning of the biological communities, and through time such modifications started inducing the emergence of a new selected trophic structure, which has been analysed in abundant literature (e.g. Dolbeth et al. 2003; Cardoso et al. 2004 a, b; Patrício et al. 2004).

From 1998 to 2006 several interventions were carried out to ameliorate the condition of the system, namely, by improving water circulation, which was followed by a partial recovery of the area occupied by *Z. noltii* and the cessation of green *Ulva* sp. blooms (Lillebo et al. 2005, 2007).

Sample collection and preparation

To assess water quality of the Mondego waters, we collected water samples on a monthly basis at two sites (*Zostera* site and bare sediment site; Fig. 1), from November 2005 to July 2006. In a companion paper (Baeta et al. submitted) we established that there were no differences in nutrients or chlorophyll concentrations in samples taken from the two sites, and so here we treat the samples as replicates. In each sample, we measured concentrations of nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}), and the concentration of chlorophyll *a*. Samples were immediately filtered (Whatman GF/F glass-fibre filter) and stored frozen at -18°C until the analysis following standard methods described in Limnologisk Metodik (1992) for NH_4^+ and PO_4^{3-} , and in Strickland and Parsons (1972) for NO_3^- , and nitrite NO_2^- . The phytoplankton chlorophyll *a* determinations were performed by filtering 0.5-1.0 l of water through Whatman GF/F glass-fibre filters (Parsons et al., 1985). In the field and during transportation to the laboratory, samples were stored on ice and protected from light. Data on monthly precipitation and air temperature were derived from the nearby city of Coimbra (Instituto de Meteorologia, Coimbra forecast station).

We measured stable-isotopic values in components of the Mondego food web, including particulate organic matter (POM), sedimentary organic matter (SOM), meiofauna, seagrass, macroalgae, macrobenthos, zooplankton, and the fish in each of the two sites. To evaluate the seasonal variation in the isotopic values, we repeated the sampling in November (2005), and in February, May, and July (2006) at the two sites in the south arm of the estuary. Water samples for POM were collected monthly.

POM was obtained by filtering 0.5-1 l of seawater, from a depth of 0.5 m below the surface, onto precombusted (450°C , 4h) Whatman GF/F filters (0.45 μm pore size) with a

low pressure vacuum pump. Sediment samples from the upper 1 cm were collected with an acrylic corer (31 mm of diameter), and analysed for the isotopic composition. For the meiofauna, sediment samples were collected, and the top 3 cm of each sediment core was then passed through 500 μm and 38 μm sieves. Meiofauna were examined from the 38 μm fraction, and samples for isotopic analysis were composites of 50-300 individuals. Seagrass leaves and roots, and macroalgae were collected by hand and gently cleaned of epiphytic material. Macroinvertebrates were also taken manually from each site, and held in filtered sea water for 24 h to allow their guts to clear.

Zooplankton was collected by towing a Bongo net (0.5 m diameter, 200 μm mesh size) against the current for 20 min. The zooplankton samples for isotope analysis were composites of 20-200 individuals. Resident (*Atherina boyeri*, *Pomatoschistus microps*, *Pomatoschistus minutus*, *Syngnathus abaster*, *Syngnathus acus*) and transient (*Dicentrarchus labrax*, *Solea solea*) fish species were collected using a 2 m beam trawl, with 5 mm stretched mesh size on the cod end. The trawls were carried out during the night, at low water during spring tides, but only at the bare sediment site. These mobile taxa (fish) could easily manage the short distance between the two sites (Fig. 1), so it was not considered worthwhile to collect samples at the two sites.

All samples were rinsed with Milli-Q water (filters with POM were rinsed with ammonium formate to remove the salts), and then freeze-dried. When dry, samples were ground (filters with POM were kept whole) into a homogenous powder using mortar and pestle, and combined to make single composite samples of each species/group per site per sampling date. Samples were then weighed, and loaded into tin capsules. Whole organisms were used in all cases except for bivalves and decapods, the shells of which were removed, and for fish, only muscle of the dorsal region was analysed. No acidification was applied to the samples to avoid alterations in the isotopic signal (Mateo et al. 2008).

Samples were analysed using an EA-IRMS (Isoprime, Micromass, UK). Isotopic values were expressed in the δ unit notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and N_2 in air for $\delta^{15}\text{N}$) following the formula: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The analytical precision for the measurement was 0.2‰ for both carbon and nitrogen.

The data were analysed using ANOVA to test the null hypothesis that there were no significant differences in either the $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ composition of each group/species among seasons (autumn, winter, spring, and summer).

Results and discussion

Seasonal ambient conditions

Weather varied substantially during the sampling period (Fig. 2). Temperatures varied according to the season, with, on average, winter maxima around 15 °C rising to 25 °C in summer. In addition, during the period over which we sampled the estuary there were marked departures from average conditions. In particular, during our last sampling interval in July, very warm temperatures (near 40 °C) (Fig. 2, top) were brought about by a northern incursion of an African air mass. The sampling period was also the one during which Portugal suffered a lengthy drought, relative to average long-term precipitation (Fig. 2, bottom).

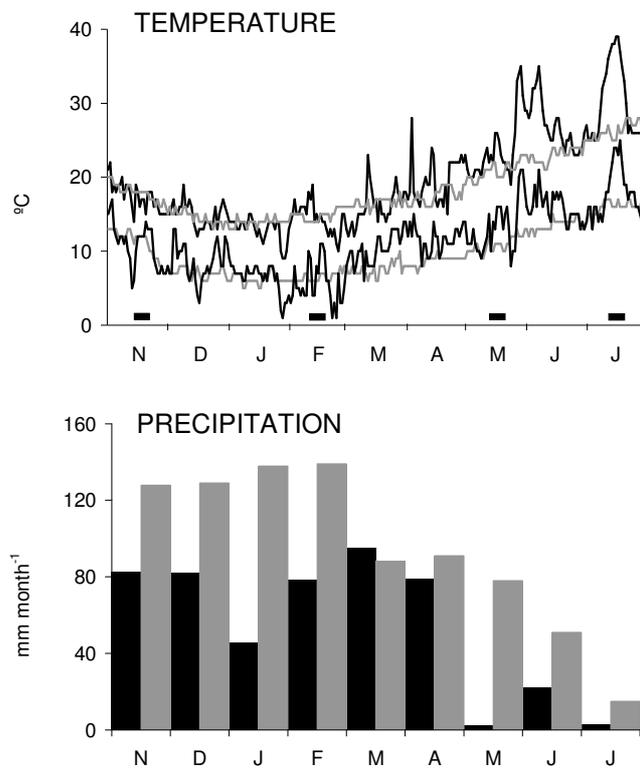


Fig. 2. Top: daily maximum and minimum air temperature, from November 2005 to July 2006 (black lines), and daily maximum and minimum air temperature means for 1961-1990 (grey lines). Black rectangles show when were the sampling periods. Bottom: precipitation values, from November 2005 to July 2006 (black bars), and precipitation means for 1961-1990 (grey bars).

There was a marked seasonality to conditions in the water column (Fig. 3). Nitrate concentrations were high during winter, and diminished about fourfold during the warmer months. Concentrations of NH_4^+ and PO_4^{3-} were usually much lower than those of NO_3^- (Fig. 3, top). N/P was generally above the 16:1 Redfield ratio during colder months and below it in warmer months. This suggests that throughout the winter months, P supply might have been the limiting nutrient, while during the summer, N might limit producer growth (Fig. 3, middle). Chlorophyll *a* concentrations peaked in spring, perhaps drawing down nitrate concentration during warmer months (Fig. 3, bottom).

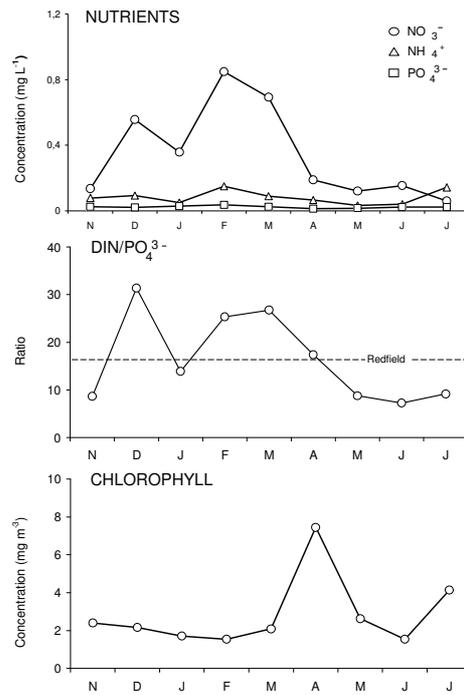


Fig. 3. Mean of nitrate, ammonium, and phosphate (top), $\text{DIN}/\text{PO}_4^{3-}$ (middle), and phytoplankton chlorophyll *a* (bottom) concentrations, from the two sampling sites, *Zostera* and bare sediment sites, in the Mondego estuary, from November 2005 to July 2006.

Seasonal changes in isotopic values

We collected 45 different taxa and analysed their stable carbon and nitrogen isotopic compositions. These taxa included 5 primary producers, POM, sediment, 21 macroinvertebrate species, 2 meiofauna groups, 8 fish species, and 7 zooplankton taxa (Table 1).

Table 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of primary producers and consumers collected from Mondego estuary. Data are sample size (N), and mean δ values (\pm SE), from November 2005 to July 2006.

Group/species	Abbreviation	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	N
		Mean \pm SE	Mean \pm SE	
Macrophytes				
<i>Enteromorpha</i> sp.	E	-12.9 \pm 0.4	12.7 \pm 1.2	8
<i>Fucus</i> sp.	F	-16.3 \pm 0.5	9.7 \pm 0.4	8
<i>Gracilaria</i> sp.	G	-17.4 \pm 0.7	9.7 \pm 1.0	8
<i>Ulva</i> sp.	U	-11.4 \pm 0.3	12.3 \pm 0.9	8
<i>Zostera noltii</i> (leaves)	Zl	-12.5 \pm 0.1	9.5 \pm 1.5	8
<i>Zostera noltii</i> (roots)	Zr	-12.2 \pm 0.2	11.3 \pm 3.3	8
Particulate organic matter	POM	-22.8 \pm 0.2	5.9 \pm 0.2	18
Sediment	SOM	-21.31 \pm 0.4	4.8 \pm 0.4	8
Amphipoda				
<i>Amphithoe valida</i>	Av	-14.8 \pm 0.3	10.9 \pm 0.2	8
<i>Echinogammarus marinus</i>	Em	-14.7 \pm 0.5	10.6 \pm 0.3	8
<i>Melita palmata</i>	Mp	-15.5 \pm 0.7	9.9 \pm 0.2	8
Bivalvia				
<i>Cerastoderma edule</i>	Ce	-19.1 \pm 0.2	7.8 \pm 0.3	8
<i>Mytilus galloprovincialis</i>	Mg	-19.2 \pm 0.4	7.2 \pm 0.3	8
<i>Scrobicularia plana</i>	Sp	-17.4 \pm 0.5	9.0 \pm 0.2	8
Decapoda				
<i>Carcinus maenas</i>	Cm	-16.4 \pm 0.3	12.1 \pm 0.2	8
<i>Crangon crangon</i>	Ccr	-15.3 \pm 0.3	11.6 \pm 0.1	8
Gastropoda				
<i>Gibbula umbilicalis</i>	Gu	-12.0 \pm 1.5	10.7 \pm 1.3	3
<i>Hydrobia ulvae</i>	Hu	-11.5 \pm 0.3	9.6 \pm 0.1	8
<i>Littorina litorea</i>	Ll	-11.2 \pm 0.4	12.2 \pm 0.1	4
Isopoda				
<i>Cyathura carinata</i>	Cc	-14.3 \pm 0.4	11.7 \pm 0.2	8
<i>Idotea chelipes</i>	Ic	-14.5 \pm 0.5	9.9 \pm 0.5	8
<i>Lekanesphaera levii</i>	Llev	-12.0 \pm 0.4	8.7 \pm 0.4	8
Meiofauna				
Nematoda	Ne	-16.3 \pm 0.5	11.3 \pm 0.3	8
Copepoda	Co	-16.3 \pm 0.4	10.9 \pm 0.3	8
Polychaeta				
<i>Alkmaria romijni</i>	Ar	-16.7 \pm 0.7	10.7 \pm 0.2	6
<i>Capitella capitata</i>	Cca	-16.4 \pm 0.3	10.8 \pm 0.3	7
<i>Glycera tridactyla</i>	Gt	-14.3 \pm 0.2	13.5 \pm 0.3	5
<i>Hediste diversicolor</i>	Hd	-14.2 \pm 0.2	11.8 \pm 0.3	7
<i>Heteromastus filiformis</i>	Hf	-16.2 \pm 0.2	11.5 \pm 0.2	7
<i>Nephtys cirrosa</i>	Nc	-15.6 \pm 0.7	12.6 \pm 0.3	6
<i>Streblospio shrubsolii</i>	Ssh	-16.9 \pm 0.7	10.8 \pm 0.4	5
Fishes				
<i>Atherina boyeri</i>	Ab	-18.0 \pm 0.6	11.7 \pm 0.7	2
<i>Dicentrarchus labrax</i> (juv)	Dl	-18.2 \pm 0.6	14.2 \pm 0.6	2
<i>Diplodus vulgaris</i> (juv)	Dv	-17.9 \pm 0.5	15.0 \pm 0.3	4
<i>Pomatoschistus microps</i> (juv)	Pm	-22.4 \pm 0.3	14.0 \pm 0.7	2
<i>P. minutus</i>	Pmi	-17.4 \pm 0.4	13.1 \pm 0.3	4
<i>Syngnathus abaster</i>	Sa	-15.0 \pm 0.9	12.3 \pm 0.6	2
<i>S. acus</i>	Sac	-16.2 \pm 0.0	11.4 \pm 0.9	2
<i>Solea solea</i> (juv)	Ss	-23.5 \pm 0.4	14.8 \pm 0.6	4
Zooplankton				
<i>Acartia tonsa</i>	At	-23.2 \pm 0.9	9.7 \pm 0.4	8
<i>Acartia</i> sp.	Asp	-18.5 \pm 0.5	8.4 \pm 0.2	8
Cladocera	Cl	-21.9 \pm 1.0	9.4 \pm 0.8	3
Mysidacea (juv)	My	-18.9 \pm 0.7	8.1 \pm 0.4	4
<i>Pomatochistos</i> sp. (larvae)	Pl	-20.0 \pm 0.5	9.8 \pm 0.3	3
<i>Sagitta friderici</i>	Sf	-18.2 \pm 0.6	11.1 \pm 0.3	6
Zoeae (brachyura)	Zo	-18.7 \pm 0.4	9.0 \pm 0.2	6

There was a consistent lack of seasonal pattern in the isotopic values in most compartments of the Mondego ecosystem (Figs. 4, 5). Thirty of the 37 compartments measured showed no evidence of seasonal changes (Table 2).

For $\delta^{13}\text{C}$ only the copepod *Acartia tonsa* showed a seasonal variation, becoming less negative in spring and summer. Buskey et al. (1999) showed that *A. tonsa* living over seagrass beds obtain a larger proportion of their carbon from seagrass than do nearby populations living over muddy bottoms without seagrass. In our study, during the most intense periods of the phytoplankton bloom, $\delta^{13}\text{C}$ values in *A. tonsa* became less negative, suggesting that more seagrass carbon might have been entering their diets. On the other hand, the much depleted carbon signatures for the most of the year could be due to a great contribution of terrestrial organic matter, since several studies have shown that terrestrial plants have the most depleted $\delta^{13}\text{C}$ signatures, around -26‰ (e.g. Vizzini and Mazzola 2003).

The $\delta^{15}\text{N}$ of producers and consumers consistently lacked significant seasonal variation across most of the growing season (Table 2), and only on one date in July did it become significantly higher ($p < 0.05$) in the $\delta^{15}\text{N}$ of producers. The stable-isotopic values of C and N in these producers and consumers therefore showed no seasonal variation. In contrast, the $\delta^{15}\text{N}$ of producers rapidly increased seemingly as a seasonal response to certain conditions.

High nitrogen isotopic signatures found in producers in July may have resulted from seasonal changes in biogeochemical processes, such as denitrification. Denitrification is temperature dependent and takes place under anaerobic conditions. This process may lead to a loss of isotopically light ^{14}N , which enriches the remaining DIN pool with ^{15}N . During the unusually warm event in July there was a strong sulfitic smell, which suggested widespread anoxia that could have favored high denitrification rates.

The enrichment of $\delta^{15}\text{N}$ in the producers increased significantly with warmer temperatures (Fig. 6), as might be expected if a temperature-dependent process such as denitrification was indeed involved. It is not surprising to find that macrophyte isotopic values show seasonal changes in N supply: isotopic values of macrophyte fronds change in a matter of hours to a few days (Teichberg et al. 2007) since their internal nitrogen pools turnover rather quickly. The importance of the results of Figure 6 is that if indeed global atmospheric warming increases water temperatures in estuaries such as the Mondego, we can expect a gradual increase in $\delta^{15}\text{N}$ in the producers. $\delta^{15}\text{N}$ values then could therefore be thought of as indirect indicators of warming.

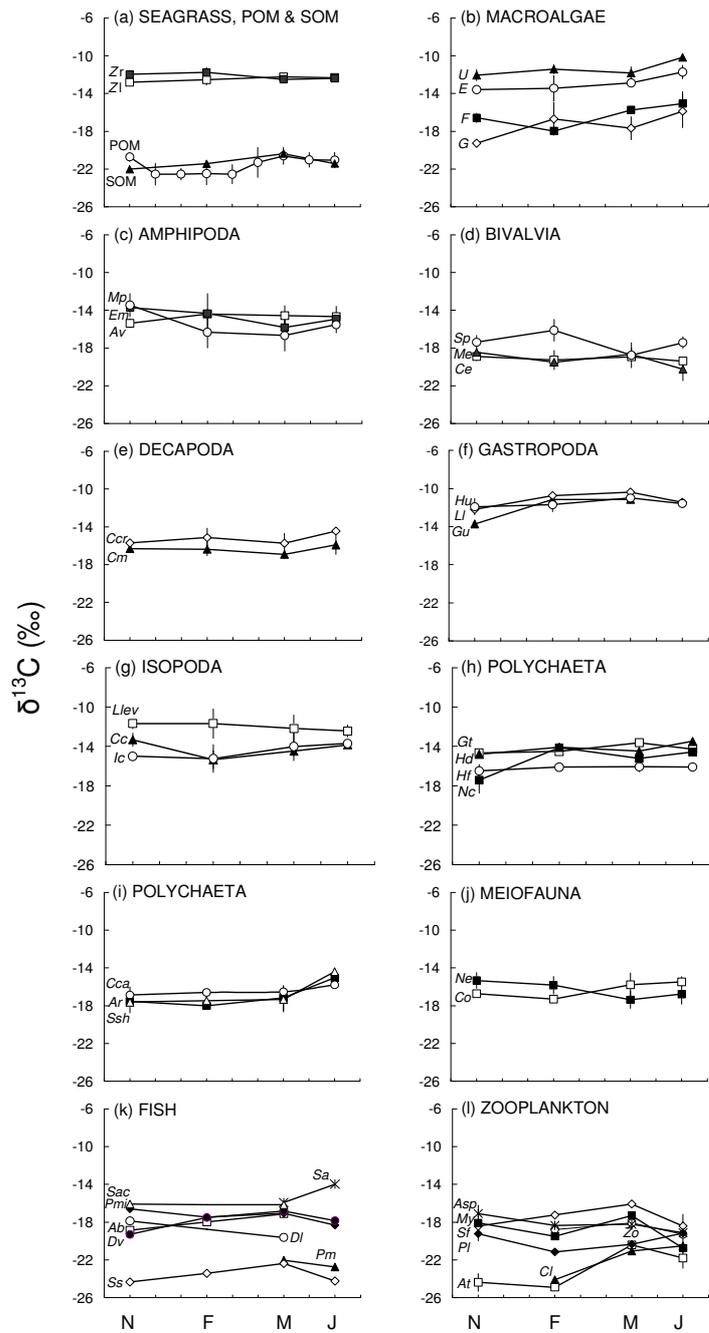


Fig. 4. C stable-isotopic values (mean \pm SE) for all the groups/species collected in the Mondego estuary, from November 2005 to July 2006. Abbreviations of species/groups are shown in table 1.

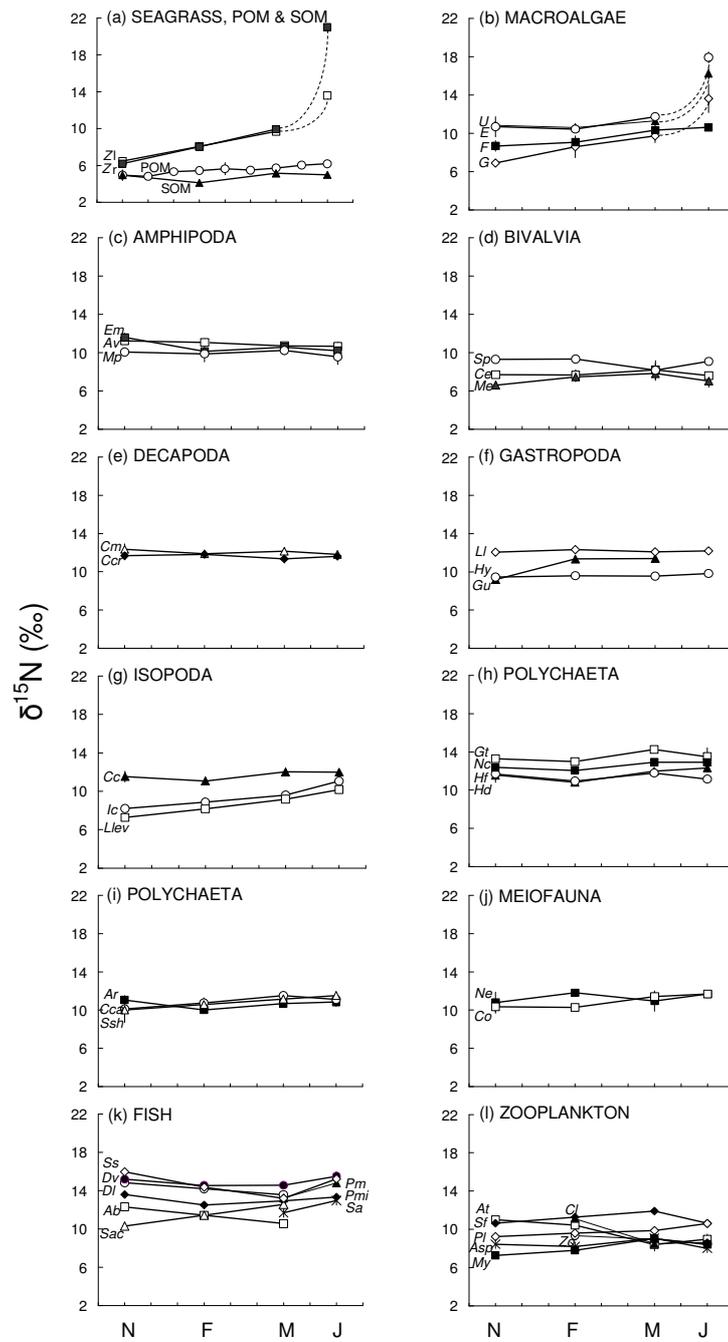


Fig. 5. N stable-isotopic values (mean \pm SE) for all the groups/species collected in the Mondego estuary, from November 2005 to July 2006. Abbreviations of species/groups are shown in table 1.

Table 2 ANOVA results testing seasonal differences for C and N isotope ratios of groups/species collected in the Mondego estuary. * = $p < 0.05$, ** = $p < 0.01$; the absence of * means no significant differences.

Group/Species	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	F	df	F	df
Macrophytes				
<i>Enteromorpha</i> sp.	0.983	3	30.678**	3
<i>Fucus</i> sp.	0.822	3	3.990	3
<i>Gracilaria</i> sp.	1.051	3	2.801	3
<i>Ulva</i> sp.	3.615	3	49.339**	3
<i>Zostera noltii</i> (leaves)	2.004	3	32.239**	3
<i>Zostera noltii</i> (roots)	1.229	3	32.247**	3
Particulate organic matter	1.926	3	2.046	3
Sediment	5.412	3	3.294	3
Amphipoda				
<i>Amphithoe valida</i>	0.218	3	0.77	3
<i>Echinogammarus marinus</i>	0.648	3	1.178	3
<i>Melita palmata</i>	1.074	3	4.091	3
Bivalvia				
<i>Cerastoderma edule</i>	0.159	3	0.204	3
<i>Mytilus galloprovincialis</i>	0.797	3	0.899	3
<i>Scrobicularia plana</i>	1.167	3	1.856	3
Decapoda				
<i>Carcinus maenas</i>	0.397	3	0.526	3
<i>Crangon crangon</i>	0.315	3	0.561	3
Gastropoda				
<i>Hydrobia ulvae</i>	0.347	3	1.348	3
Isopoda				
<i>Cyatura carinata</i>	2.063	3	1.797	3
<i>Idotea chelipes</i>	0.532	3	11.204*	3
<i>Lekanesphaera levii</i>	0.116	3	10.327*	3
Meiofauna				
Nematoda	0.903	3	0.435	3
Copepoda	0.853	3	4.471	3
Polychaeta				
<i>Alkmaria romijni</i>	1.203	2	1.774	2
<i>Capitella capitata</i>	0.659	3	0.669	3
<i>Glycera tridactyla</i>	0.77	3	0.359	3
<i>Hediste diversicolor</i>	4.305	3	0.645	3
<i>Heteromastus filiformis</i>	0.131	3	4.557	3
<i>Nephtys cirrosa</i>	1.943	3	0.552	3
<i>Streblospio shrubsolii</i>	2.966	2	1.746	2
Fishes				
<i>Diplodus vulgaris</i> (juv)	7.428	3	4.261	3
<i>P. minutus</i>	4.668	3	3.875	3
<i>Solea solea</i> (juv)	3.939	3	7.186	3
Zooplankton				
<i>Acartia tonsa</i>	15.897*	3	3.628	3
<i>Acartia</i> sp. (marine species)	5.837	3	4.245	3
<i>Sagitta friderici</i>	8.585	2	3.613	2
Mysidacea	4.016	3	1.320	3
Zoea (<i>C. maenas</i>)	0.787	2	14.653	2

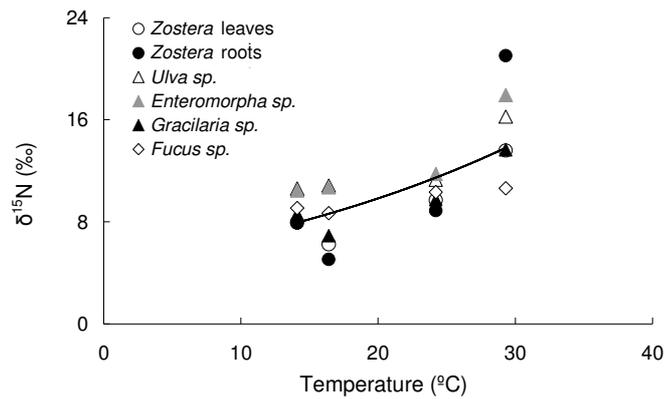


Fig. 6. N stable-isotopic ratio for primary producers (seagrass and macroalgae) collected in the Mondego estuary. N stable-isotopic data are plotted against the air temperature.

Nitrogen isotope ratios in two isopods also showed seasonal variation, increasing in July. Both *I. chelipes* and *L. levii* are grazers, feeding on macrophytes (Bamber 2004), so the increased nitrogen isotopic ratio in summer could be due to the enrichment found in the producers during this period, since complete turnover of these populations could occur in a matter of days if all individuals were equally mobile (Shafir and Field 1980). Accordingly, the lack of seasonal variation of $\delta^{15}\text{N}$ enrichment in most consumers of the Mondego food web might be related to a slower turnover of internal N pools in consumers (weeks/months) compared to pools in macrophytes (days), position of species in the food web and omnivores feeding behaviour, and also probably due to the fact that, excepting isopods, the other groups do not feed directly on fresh macrophytes. This suggests that consumer isotopic values constitute a more time-integrated reflection of nitrogen isotopic values, as reported by Vander Zanden et al. (1998).

The results of this study show that there were strong seasonal driving variables in the Mondego estuary. Increased temperature increased $\delta^{15}\text{N}$ of important producers; this might be an indirect result of microbial N transformations and suggests that producer $\delta^{15}\text{N}$ might become an indicator of larger climatic trends. The influence of this seasonal forcing, as manifest in stable-isotopic ratios of consumers within the Mondego food web, was surprisingly modest, with most species showing no significant seasonal trends. These results suggest that the seasonal variation in the various factors we measured (temperature, precipitation, nutrients, and chlorophyll) within the Mondego was not enough to change

isotopic signatures in consumers. This is convenient for monitoring purposes, as it frees sampling protocols from seasonal schedules.

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Chapter 3

Habitat-related diet of macrofauna consumers in intertidal
areas

Abstract

Seagrasses increase the availability and diversity of food sources, thereby changing trophic structure of their associated communities as well as the ecosystem goods and services regulated by the food web. We compared the benthic trophic structure of bare-sediment and the eelgrass *Zostera noltii* habitats, by measuring the natural abundances of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in primary producers, detritus and invertebrate consumers. We expected (i) differences in the isotopic signatures of consumers among habitats, following the presence of the eelgrass and (ii) changes in the contribution of primary producers and resources to the diet of the consumers. We also analysed the changes in the diet of the tellinid *Scrobicularia plana* during development and

expected differences according to the animal size and the habitat. There were differences among habitats in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the omnivore *Hediste diversicolor*, and the detritivores *Heteromastus filiformis*, *Hydrobia ulvae*, *Taryx marioni*, *S. plana*, and Oligochaetes. Overall, these species were ^{15}N enriched in the *Z. noltii* meadow, whereas they were ^{13}C depleted in the muddy area, as compared to both the eelgrass meadow and bare sediment characterised by patches of *Z. noltii*. *S. plana* was more ^{13}C depleted at increasing body size especially in this habitat, suggesting that diet shift may occur during development as a consequence of habitat-related changes.

Keywords: trophic ecology; food web structure; ontogenetic shifts; seagrass; stable isotopes

Introduction

In estuaries, benthic macroinvertebrates represent important trophic links between primary producers or detritus and predators such as fishes and birds (Herman et al. 1999). These macroinvertebrate communities are partially responsible for the extremely high production of estuarine areas (Rosenberg 2001) and contribute to ecosystem properties and services that help to improve water and sediment quality. For example, grazing of particulates by filter feeders from the water column reduces turbidity, thereby increasing light availability to the bottom and enhancing the growth of benthic seagrasses and benthic microalgae (Newell and Ott 1999). Identifying food sources for these animals can be extremely difficult because of the numbers of food sources that compose sediment detritus and for the capability of these animals to switch diet, following changes in the availability of food sources or biological interactions such as space competition or predation as well as their metabolic needs or physiological constraints related to their development (Levinton

1991; Taghon and Greene 1992; Bock and Miller 1997; Hentschel et al. 1998; Rossi et al. 2004).

In the marine and estuarine system, seagrasses provide heterogeneous and complex habitats that may sustain complex and diverse food webs, by providing refuge from predators and sequestering nutrients and detritus through enhanced deposition (Duarte 2002; Larkum et al. 2007). Furthermore, seagrasses greatly contribute to carbon and nutrient sequestration and storage, by fixing inorganic carbon and nutrients and holding back their recycling in the food web through the grazing and the detritus food web inside the meadows or in adjacent areas (Cebrian 1999; Duarte 2005; Holmer et al. 2002; Heck et al. 2008). By providing refuge and food, the presence of seagrasses in estuaries could modify food uptake by macrofauna consumers, thereby altering the carbon and nutrient recycling in this ecosystem.

In this study we quantified the variability in the diet of macrofauna consumers in a *Z. noltii* meadow and in two bare-sediment habitats, one characterised by the presence of small patches of *Z. noltii* and adjacent to the meadow, the other outside the influence of the eelgrass. By measuring the isotopic signatures we expected that (i) there would be differences in the isotopic signatures of consumers among habitats and (ii) increased numbers of food sources contributing to these consumer diets in the *Z. noltii* habitat. When possible, we also investigated relationships between diet and animal size to make hypotheses on the role of habitat characteristics in determining ontogenetic changes in diet.

Materials and methods

Field collections

During summer 2008, on June (25 of June 2008) and July (22 of July 2008), we sampled three intertidal habitats in the south arm of the Mondego estuary (Fig. 1): (1) the *Z. noltii* bed (hereafter Z), characterised by a flourish meadows; (2) an intermediate area, characterised by small patches of *Z. noltii*, which were absent during our sampling although residual roots can still be found in the sediment (hereafter I); and (3) a muddy-sediment areas, of bare sediment (hereafter B). For a detailed description of these areas see Marques et al. (2003). We sampled in summer because the highest growth rates occur in this period, following increasing temperatures and availability of food resources, including macroalgal-derived from spring blooms (Marques et al. 2003). During the first sampling

date, two sites (20×20 m plots) were randomly chosen in the central part of each habitat. At each site and date, three 50×50 cm plots were sampled. In each plot, 1 core (13.5-cm of diameter) was taken to the depth of 30-cm for macrofauna and plant samples. Three additional small cores were collected for sedimentary organic matter (SOM) around each large core. Then the sediment of these three small cores was pooled before analyses. Three small cores, rather than one large core were sampled to better estimate the average isotopic signature of the sediment at each plot. Pooling was done because it was necessary maintaining a reasonable number of replicates.

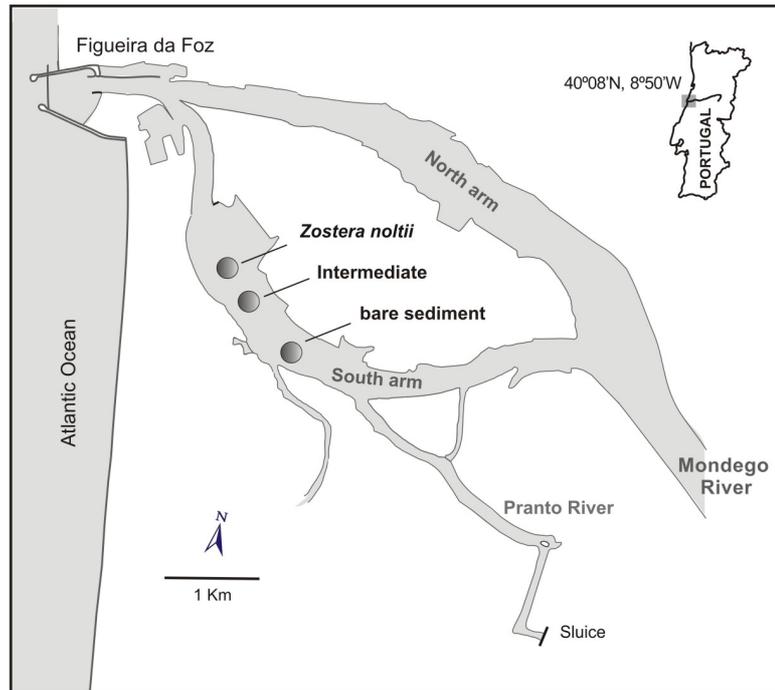


Fig. 1. Mondego estuary map showing sampling sites: *Zostera noltii*, intermediate and bare sediment sites (grey circles).

In the laboratory, animals were sorted alive within 3 days from collection and recognised to the species level. The material was then cleaned and freeze-dried. When possible, only the muscle was retained for the analyses. The entire animals were used, except the stomachs which were carefully removed under the dissecting microscope. The shells and any other skeletal parts were removed.

The bivalve *Scrobicularia plana* was abundant at any habitat and sampling dates and showed a well structured population, with both juveniles and adults of variable size. We therefore examined the relationships between variation of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ and the size of the animals. The size was estimated by measuring the shell length with a caliper to the precision of 0.1 mm; then the soft parts of the bivalves were removed from shells and freeze-dried. Animals from the same core that had the same size were pooled together for the isotope analyses. The animals were also grouped into two size-classes (< 10 mm and > 10 mm) according to their frequency-distribution.

Seagrass leaves and roots, and macroalgae collected in the large core were rinsed with filtered seawater to clean of epiphytic material and then freeze-dried and ground to fine powder. Sediment was freeze dried and then ground-powdered. All dried samples were stored frozen ($-20\text{ }^{\circ}\text{C}$) until isotopic analysis.

Samples to estimate isotopic composition of benthic diatoms (the dominant group of MPB in terms of biomass) were collected at low tide in November 2008, at the bare sediment site, by scraping the surface of the sediment carefully. The sediment containing the diatoms was put into flat trays to form a 1-2 cm thick layer. Three nylon screens (63 μm mesh) were put on top of the sediment, and sprayed with filtered water from the sampling site. The trays were illuminated for several hours, until dense brown mats appeared at the surface. The upper screen was removed and the diatoms were collected by spraying the screen into a glass. The material was observed at the microscope, to be secure that only benthic diatoms were removed from the sediment. Benthic diatoms were then collected with a pipette and freeze-dried.

Samples for particulate organic matter (POM) were collected monthly, from November 2005 to July 2006 at the Z and B sites (Baeta et al. 2009a).

Analytical technique

The carbon and nitrogen isotopic composition of the samples was determined using a Flash EA 1112 Series elemental analyser coupled on line via Finningan conflo II interface to a Thermo delta V S mass spectrometer. The carbon and nitrogen isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PDB limestone for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$); $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Precision in the overall preparation and analysis was better than 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Samples were acidified before analysis to insure removal of any carbonate residual. Acidification may introduce a bias in determining the natural abundance of ^{15}N (Mateo et al. 2008), therefore, we analysed a sub-sample of the most abundant animals before and after acidification to control for the effects of acidification on the nitrogen isotope composition. Based on 52 samples from 9 species, we found no differences between the samples non-acidified and acidified in $\delta^{15}\text{N}$ ($R = 0.89$), while a decrease after acidification in $\delta^{13}\text{C}$ ($R = 0.72$) measurements.

Analyses of data

To test for differences among habitats in the stable isotopic signatures of benthic consumers and producers ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), we used different models of analyses of variance (ANOVA), according to the distribution of the animals. Data were balanced for the ANOVA, when possible. Otherwise opportune corrections for the unbalanced number of replicates were done. The majority of the species was analysed only in July because in June they occurred at very low frequency and abundance. We thus tested for differences among habitats, a Nested design was used (factors: habitats and plots; plots were treated as random, and nested in habitats). *S. plana* juveniles (< 10 mm) occurred in both the sampling dates of June and July. We therefore used a 3-factor ANOVA, with time as random and orthogonal, habitats as fixed, and plots nested in habitats. Before analysis, the homogeneity of variances was evaluated by using Cochran's test (Winer et al. 1991). When significant differences among treatments and their interactions were found, Student–Newman–Keuls (SNK) tests were undertaken as a posteriori comparisons (Underwood 1997).

Estimation of food sources

We calculated the contribution of the potential basal food sources to the consumers *Hydrobia ulvae*, *Littorina littorea*, and *S. plana* (juveniles and adults) from the three sites with a isotope mixing model using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (ISOSOURCE software, <http://www.epa.gov/wed/pages/models/.htm>) (Phillips and Gregg 2001, 2003), to statistically constrain the relative proportions of various sources to consumers.

To apply the mixing model it is necessary to include in the model the fractionation that each isotope value undergoes during the digestion and assimilation process. Although fractionation is usually accepted to be relatively constant at each trophic level, herbivores

in general may show great variability (Vander Zanden and Rasmussen 2001). We therefore applied two different fractionation values to the herbivores (*H. ulvae* and *L. littorea*). We assumed the well-accepted positive fractionation of +1‰ for $\delta^{13}\text{C}$ and a mean trophic enrichment of +3.4‰ for $\delta^{15}\text{N}$ as a result of the assimilation of food (DeNiro and Epstein 1978; Wada et al. 1991; Post 2002). We then used for the gastropods a fractionation value of 2.5‰ for $\delta^{15}\text{N}$, in agreement of reported mean fractionation coefficients proposed by Vander Zanden and Rasmussen (2001) and Riera (1998). Vander Zanden and Rasmussen (2001) reported a mean value of 2.5 ± 2.5 ‰ for herbivores; and Riera (1998, 2010) pointed out a mean ^{15}N -enrichment of 2‰ between the microphytobenthos and *H. ulvae* in the Marenne-Oléron bay (France), since this species was identified to feed almost exclusively on microphytobenthos in this intertidal mudflat (Blanchard et al. 2000).

The data corrected for fractionation can be represented graphically with a variety of methods. Here, we have reported the real signatures of sources and consumers as points and we have superimposed on the graph the food sources isotopic signatures corrected for fractionation as points delimiting a polygon (Fig. 2). This graphical representation may also help to visually estimate the importance of each food source for each consumer. The consumer feeds on some of the collected food sources only when its isotopic signature is delimited by the polygon. The closer the isotopic signatures of the consumer are to one of the food source isotopic signatures delimiting the polygon, the larger is the contribution of that food source used by the consumer (Phillips et al. 2005).

Results and discussion

Description of food sources

The sediment of the *Zostera* habitat (Z) presented fresh macroalgal detritus, especially of the genera *Gracilaria* and *Enteromorpha*. Macroalgal detritus was not found in the other two habitats. The isotopic signatures of these macroalgae did not significantly vary between the time of sampling or the sites. OM, SOM, and MPB were the only food sources at I and B sites. No differences for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were found between June and July (time*plot; two-way ANOVA with sites and time as orthogonal, random factors) in the Z for the producers *Gracilaria* sp. (ANOVA, $F_{1,7} = 1.69$, $P = 0.24$ and $F_{1,7} = 4.82$, $P = 0.06$, respectively), *Ulva* sp. (ANOVA, $F_{1,4} = 0.08$, $P = 0.79$ and $F_{1,4} = 0.02$, $P = 0.90$, respectively), and *Z. noltii* (ANOVA, $F_{1,7} = 1.82$, $P = 0.22$ and $F_{1,7} = 7.20$, $P = 0.05$, respectively).

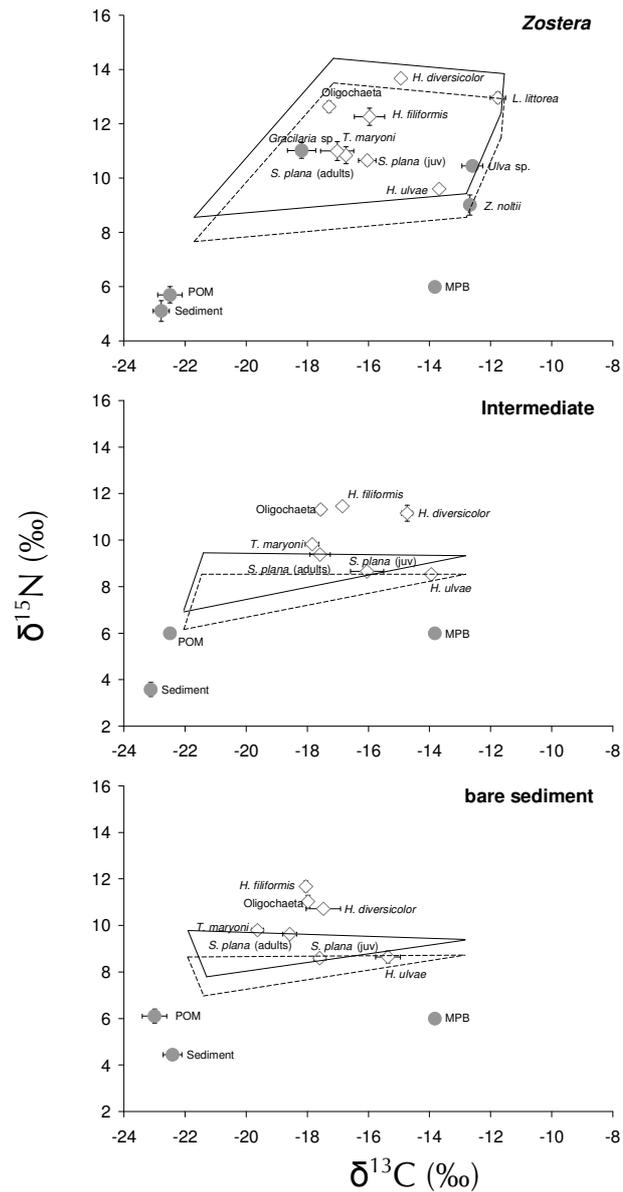


Fig. 2. Dual stable isotope plots for each sampling site (*Zostera*, Intermediate, bare sediment), during June and July 2008, for the species that showed differences among sites and basal sources. Mean values are reported (Standard errors and number of replicates are in Table 1). Grey circles indicate basal sources and open diamonds are consumers. The polygons represent the projections of basal sources signatures corrected for fractionation (see Material and methods). Continuous line indicates the correction according to the well-accepted fractionation of +1 and +3.4‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (DeNiro and Epstein 1978, Wada et al. 1991, Post 2002). The polygon indicated as dashed line indicate the fractionation of +1 and +2.5‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Vander Zanden and Rasmussen 2001).

Table 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean \pm SE) of basal sources and consumers collected from the *Zostera*, Intermediate and the bare sediment sites in the Mondego estuary.

Group/species	<i>Zostera</i> site			Intermediate site			bare sediment site		
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>N</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>N</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>N</i>
	Mean \pm SE	Mean \pm SE		Mean \pm SE	Mean \pm SE		Mean \pm SE	Mean \pm SE	
Macrophytes									
<i>Gracilaria</i> sp.	-18.2 \pm 0.5	11.0 \pm 0.3	11						
<i>Ulva</i> sp.	-12.6 \pm 0.3	10.5 \pm 0.2	8						
<i>Zostera noltii</i> (leaves)	-12.7 \pm 0.2	9.0 \pm 0.4	11						
Microphytobenthos (MPB)									
							-13.8 \pm 0.0	6.0 \pm 0.2	4
Sediment									
	-22.8 \pm 0.3	5.1 \pm 0.4	12	-23.1 \pm 0.2	3.7 \pm 0.3	12	-22.4 \pm 0.3	4.4 \pm 0.2	12
Bivalvia									
<i>Cerastoderma edule</i>	-18.6 \pm 0.7	9.4 \pm 0.9	3	-19.4 \pm 0.3	7.3 \pm 0.3	3	-19.6 \pm 0.6	7.2 \pm 0.3	7
<i>Scrobicularia plana</i> (adults)	-16.7 \pm 0.1	10.8 \pm 0.3	11	-17.6 \pm 0.3	9.6 \pm 0.1	2	-18.6 \pm 0.2	9.9 \pm 0.2	4
<i>S. plana</i> (juveniles)	-16.0 \pm 0.3	10.7 \pm 0.2	11	-16.1 \pm 0.5	8.7 \pm 0.1	11	-17.6 \pm 0.2	8.6 \pm 0.2	10
Decapoda									
<i>Carcinus maenas</i>	-13.9 \pm 0.2	13.2 \pm 0.2	9	-16.2 \pm 0.4	10.4 \pm 0.4	2			
Gastropoda									
<i>Hydrobia ulvae</i>	-13.7 \pm 0.2	9.6 \pm 0.2	6	-13.9 \pm 0.1	8.5 \pm 0.1	6	-15.4 \pm 0.4	8.6 \pm 0.3	6
<i>Littorina littorea</i>	-11.8 \pm 0.3	13.0 \pm 0.2	11						
Isopoda									
<i>Cyathura carinata</i>				-16.2 \pm 0.2	11.2 \pm 0.2	6	-17.5 \pm 0.2	11.5 \pm 0.2	8
Oligochaeta									
	-17.3 \pm 0.2	12.6 \pm 0.2	9	-17.6 \pm 0.1	11.3 \pm 0.2	8	-18.0 \pm 0.1	11.0 \pm 0.3	2
Polychaeta									
<i>Hediste diversicolor</i>	-14.9 \pm 0.1	13.7 \pm 0.1	4	-14.7 \pm 0.2	11.2 \pm 0.3	10	-17.5 \pm 0.6	10.7 \pm 0.1	9
<i>Heteromastus filiformis</i>	-16.0 \pm 0.5	12.3 \pm 0.3	9	-16.9 \pm 0.1	11.5 \pm 0.1	10	-18.1 \pm 0.1	11.7 \pm 0.2	5
<i>Tharyx marioni</i>	-17.0 \pm 0.5	11.0 \pm 0.4	3	-17.8 \pm 0.2	9.8 \pm 0.1	5	-19.6 \pm 0.2	9.8 \pm 0.2	8

The bulk sediment did not show any difference among habitats at any time, for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$: (Z: ANOVA, $F_{1,8} = 0.01$, $P = 0.94$ and $F_{1,8} = 0.42$, $P = 0.54$, respectively); (I: ANOVA, $F_{1,8} = 0.79$, $P = 0.40$ and $F_{1,8} = 0.09$, $P = 0.77$, respectively); (B: $F_{1,8} = 0.45$, $P = 0.52$ and $F_{1,8} = 0.83$, $P = 0.39$, respectively). Rather, it showed relatively constant values depleted in both ^{13}C and ^{15}N at any habitats, as compared to the macroalgae collected in Z (Table 1). Macroalgae were indeed enriched in ^{15}N , as compared to the other sources.

Variability in the isotopic composition of consumers

A total of 10 taxa of consumers were identified. Only the species that occurred in the three habitats were analysed. There were significant differences in the $\delta^{13}\text{C}$ composition of tissues among sites for the gastropod *H. ulvae*, the polychaets *Hediste diversicolor*, *Heteromastus*

filiformis and *Taryx marioni*, and for Oligochaeta (Table 2; Fig. 2). The $\delta^{13}\text{C}$ values of these species were enriched at the Z and I sites than at the B area (Sites: Z=I>B, SNK test $P < 0.05$; Fig. 2, Fig. 4). The juveniles of the bivalve *S. plana* showed differences in the interaction of habitat with time for $\delta^{13}\text{C}$ values. Specimens were ^{13}C enriched at the Z habitat in June and at the I site in July (Table 2, Fig. 2, SNK test for interaction term; $P < 0.05$). The co-specific adults were collected numerous only in July, when they showed differences among habitats, with enriched values in Z (Table 2, Fig. 2, SNK test for interaction term; $P < 0.05$).

There were also differences in $\delta^{15}\text{N}$ values for these species. However, overall these species were ^{15}N enriched in Z as compared to both I and B (Sites: Z>I=B, SNK test $P < 0.05$; Fig. 2, Fig. 4). The $\delta^{15}\text{N}$ signature of *S. plana* juveniles showed differences among habitats, with heavier values at Z than I and B (Table 3, Fig. 2). Instead, *S. plana* adults did not significantly change their $\delta^{15}\text{N}$ (Table 3).

Intraspecific variation in $\delta^{13}\text{C}$ of *Scrobicularia plana*

Carbon isotope signature values in the tissues of *S. plana* overall decreased with the size of the animals, especially in I (Fig. 3). At the Z and B sites the $\delta^{13}\text{C}$ from both juveniles and adults varied in a very similar narrow area (-14 to -17‰ at the *Zostera*, and -16 to -19‰ at the bare sediment sites) compared to those at the Intermediate (-12 to -18‰ and -16 to -18‰ for juveniles and adults, respectively). Juveniles showed more variability in their ^{13}C values than adults.

Mixing model

Together with the sediment, microphytobenthos, macroalgae, macrophytes, POM values were included based on data collected in 2006 (Baeta et al. 2009a). The dual-isotope plots (Fig. 2) showed that *H. ulvae*, *S. plana* (adults), *S. plana* (juveniles) were included or very close to the polygon drawn by the sources of food isotopic composition using the classical fractionation of 1 and 3.4 ‰ (dashed line in Fig. 2) at the three habitats. *H. ulvae* was included in the polygon drawn using the discrimination values from Zanden and Rasmussen (2001) at the three areas, as well as *L. littorea* at the Z.

The mixing models showed that MPB was the most important source in the diet of the gastropod *H. ulvae*, in all habitats, with higher contribution at the I area (> 82 %, Table 4). At the Z site, detritus from the macrophyte and macroalgae could have a contribution of 17 and 10 % of the gastropod diet. For *S. plana* (adults), at the Z site a mix of sources were

important in the bivalve diet (POM, SOM, *Ulva*, *Z. noltii*); at the I and B sites, MBP and POM represented more than 90 % of the bivalve diet (46 and 44 % at the Intermediate; 37 and 60 % at the bare sediment, respectively), with an higher mean dietary proportion of POM at the B site. For the juveniles of *S. plana*, at the Z a similar importance of sources of food to adults was found; however, at the I habitat the estimated mean dietary proportion of MPB was 64 %, while at the B the mixing model showed a major contribution of MBP and SOM (41 and 40 %, respectively). Green algae and *Z. noltii* were preferentially used as food sources by the grazer *L. littorea*; other coexisting sources were less important in the diet of the gastropod (Table 4).

Table 2 Summary of ANOVA results for $\delta^{13}\text{C}$ [*df* degrees of freedom; *MS* mean square; *F* Fischer's *F*; *P* significance level ($P \geq 0.05$, non significant difference)]. Only species with significant results are shown.

Source of variation	<i>H. diversicolor</i>				<i>H. filiformis</i>				<i>H. ulvae</i>				Oligochaeta				<i>S.plana</i> (juveniles)				<i>S.plana</i> (adults)				<i>T. marioni</i>			
	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Site	2	18.03	10.28	0.00	2	3.25	60.56	0.00	2	4.88	15.07	0.00	2	0.41	4.8	0.04	2	9.03	7.92	0.00	2	3.15	27.29	0.00	2	6.1	23.46	0.00
Time																	1	0.45	0.40	0.54								
Time×Site																	2	7.35	6.45	0.01								
Plot(Site)	3	0.69	0.40	0.76	3	0.16	2.98	0.09	3	8.09	2.76	0.10	3	0.06	0.69	0.59	3	1.22	1.07	0.38	3	0.29	2.47	0.16	3	0.19	0.74	0.56
Plot×Time																	3	0.51	0.45	0.72								
Residual	10	1.75			9	0.05			12	0.32			7	0.09			20	1.14			6	0.116			7	0.26		

Table 3 Summary of ANOVA results for $\delta^{15}\text{N}$ [*df* degrees of freedom; *MS* mean square; *F* Fischer's *F*; *P* significance level ($P \geq 0.05$, non significant difference)]. Only species with significant results are shown.

Source of variation	<i>H. diversicolor</i>				<i>H. filiformis</i>				<i>H. ulvae</i>				Oligochaeta				<i>S.plana</i> (juveniles)				<i>S.plana</i> (adults)				<i>T. marioni</i>			
	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Site	2	9.91	105.72	0.00	2	2.13	13.25	0.00	2	1.15	5.04	0.03	2	3.11	13.68	0.00	2	13.48	62.82	0.00	2	1.90	3.16	0.12	2	0.92	5.65	0.04
Time																	1	1.51	7.03	0.02								
Time×Site																	2	0.06	0.27	0.77								
Plot(Site)	3	0.61	6.52	0.01	3	0.35	2.19	0.16	3	0.47	2.08	0.16	3	0.23	1.01	0.44	3	0.42	1.95	0.15	3	0.37	0.61	0.63	3	0.09	0.56	0.66
Plot×Time																	3	0.21	0.97	0.43								
Residual	10	0.09			9	0.16			12	0.23			7	0.23			20	0.22			6	0.60			7	0.16		

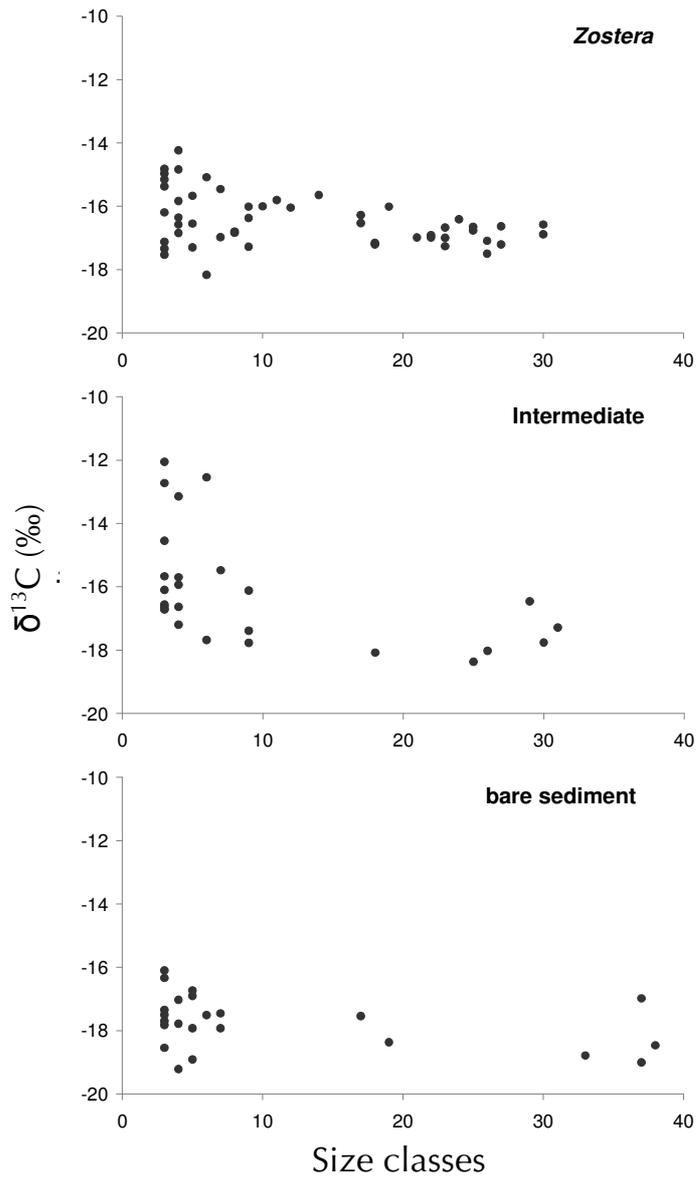


Fig. 3. Variation in *S. plana* tissue $\delta^{13}\text{C}$ with size classes (body size, mm) at each habitat (i.e. *Zostera*, Intermediate, bare sediment).

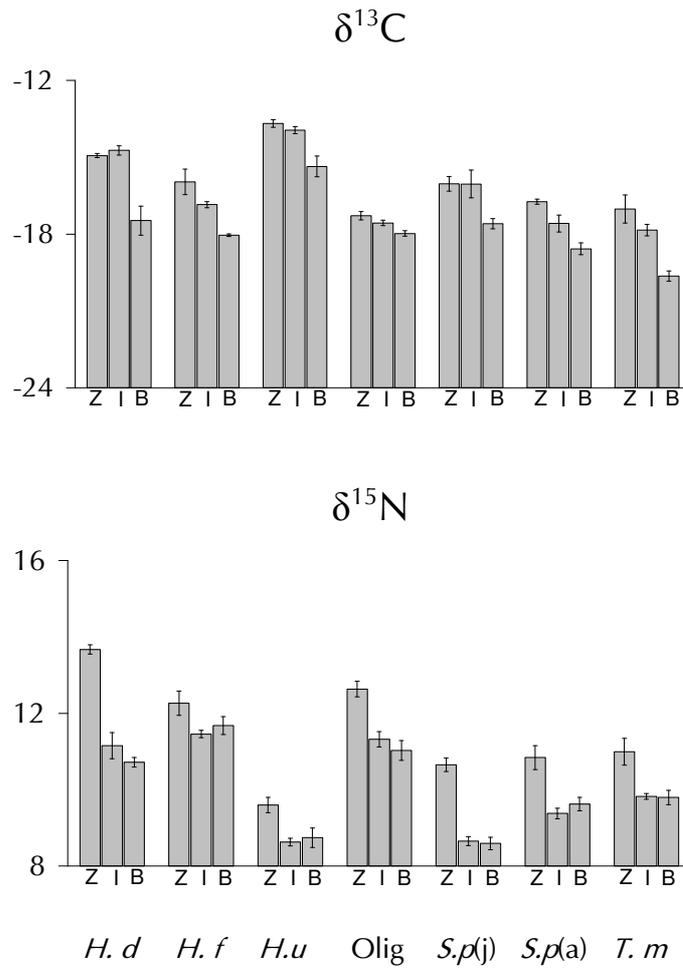


Fig. 4. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (\pm SE) in *H. diversicolor* (*H.d*), *H. filiformis* (*H.f*), *H. ulvae* (*H.u*), Oligochaeta (Olig), *S. plana* (juveniles), *S. plana* (adults), *T. marioni* (*T.m*) at each habitat (i.e. *Zostera*, Intermediate, bare sediment).

Table 4 Mean contributions (% together with minimum and maximum contributions in brackets) of each food source (ISOSOURCE) in the diet of *H. ulvae*, *S. plana* (adults and juveniles) and *L. littorea*, in the three study sites (increment of 1% and a tolerance of 0.05%). A: fractionation of +1 and +3.4‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively; B: fractionation of +1 and +2.5‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (see Materials and methods).

Sources of food		<i>Zostera</i>				Intermediate			bare sediment		
		<i>H. ulvae</i>	<i>S. plana</i> (adult)	<i>S. plana</i> (juvenile)	<i>L. littorea</i>	<i>H. ulvae</i>	<i>S. plana</i> (adult)	<i>S. plana</i> (juvenile)	<i>H. ulvae</i>	<i>S. plana</i> (adult)	<i>S. plana</i> (juvenile)
MPB	A	75 (52–95)	9 (0–40)	12 (0–50)	8 (0–33)	85 (83–87)	46 (40–51)	64 (59–70)	70 (64–75)	37 (32–42)	41 (32–50)
	B	56 (19–90)			2 (0–11)	87 (82–93)		71 (66–77)			
POM	A	5 (0–19)	29 (0–65)	24 (0–61)	1 (0–6)	1 (0–4)	44 (29–60)	14 (0–32)	5 (0–15)	60 (52–68)	19 (0–43)
	B	6 (0–22)			2 (0–7)	7 (0–18)		19 (6–33)			
SOM	A	7 (0–19)	22 (0–59)	21 (0–56)	1 (0–6)	14 (13–17)	10 (0–20)	22 (9–39)	25 (19–36)	3 (0–6)	40 (25–61)
	B	7 (0–22)			1 (0–6)	6 (0–17)		10 (0–20)			
<i>Gracilaria</i> sp.	A	1 (0–9)	2 (0–11)	3 (0–14)	2 (0–12)						
	B	4 (0–19)			1 (0–5)						
<i>Ulva</i> sp.	A	5 (0–19)	21 (0–50)	20 (0–51)	60 (0–94)						
	B	10 (0–39)			88 (67–100)						
<i>Z. noltii</i>	A	7 (0–29)	17 (0–55)	20 (0–62)	28 (0–100)						
	B	17 (0–59)			7 (0–33)						

Discussion

Habitat-related variability in food sources

Soft sediment macrofauna is well-known to rely on different food sources, sometimes at different trophic levels and show plastic feeding behaviour depending on the environmental conditions (Carlier et al. 2009; Riera 2010). Differences in isotopic signatures of marine invertebrates often indicate a shift in feeding behaviour following species interactions, when for instance feeding choice depends on the availability of resources and the time spent handling the food under high predation risk (Stephens and Krebs 1986; Burrows and Hughes 1991; Sommer et al. 2005). In intertidal systems, the high diversity of food sources makes it difficult to determine the most important trophic pathways, and generates an important organic matter pool which can be used differently by animals (Mann 1988).

This general observation is also confirmed in the Mondego estuary, where macrofauna seemed rely on a complex mix of microalgae, macroalgae and detritus in both bare sediments and eelgrass meadows (Baeta et al. 2009a). Our results showed that part of the variability observed in consumer food sources is habitat-related. Indeed, the most abundant species of macrofauna consumers showed variable isotopic signatures of both ^{13}C and ^{15}N . The benthic macrofauna consumers *H. ulvae*, *H. diversicolor*, *H. filiformis*, Oligochaeta, and *T. marioni* became depleted in ^{13}C in the bare sediment, but there were no differences between the Z and the I habitat. There were also some changes for ^{15}N , which however did not vary to the extent to indicate clear changes in the trophic position. Rather, it confirmed variations in the basal food sources among habitats. It is interesting to notice that enriched ^{15}N values for the omnivore *H. diversicolor*, the sub-surface-deposit feeders *H. filiformis*, Oligochaeta, and the surface-deposit feeder *T. marioni* were found at the Z beds, only. At the Z, enriched $\delta^{15}\text{N}$ signatures might reflect the other coexisting food sources, particularly eelgrass and *Ulva* sp. The differences in the diet of these consumers among habitats followed the proximity of the habitat to the *Z. noltii* meadow (Fig. 4).

This result does not agree with our expectation of differences between Z and other sites characterised by the lack of plant covers. Our hypothesis was supported by the fact that seagrass meadows increase habitat complexity and can offer protection from predators and increased amount and diversity of food sources (Duarte 2002). Indeed in the seagrass meadow we collected seaweed and seagrass detritus, which we did not find in the other

habitats. These sources of food seemed to play a minimal role in the diet of these animals. It is then possible that organic matter from bare sediment areas will deposit, since Z is located downstream.

Microphytobenthos seemed instead a major source of nutrition for some of the species, especially for the gastropod *H. ulvae* (Table 4). Benthic diatoms have been often described as the privileged food source for *H. ulvae* in intertidal flats (Morrisey 1988; Blanchard et al. 2000; Haubojs et al. 2005), and the major contribution of MPB to their diet is consistent with a relatively high presence of epipelagic diatoms in these sediments, with a decrease in MPB concentration from the BS to Z beds (6.0, 5.0, 4.2 gAFDWm⁻² for BS, I, and Z, respectively).

The generalist herbivore *L. littorea*, associated to the eelgrass meadows, has been reported to feed mainly on benthic diatoms at intertidal mud flats and (Riera et al. 2004, 2009), and on brown and green algae at sedimentary tidal coastal flats and rocky shores (Wilhelmssen and Reise 1994; Riera 2009). In addition, studies on seagrass beds suggest that *L. littorea* consumes epiphytes and detrital particles from the surface of eelgrass leaves, and not the eelgrass tissue (Stephenson et al. 1986). Our mixing model results show that this gastropod feeds mainly on green macroalgae, with a significant contribution of eelgrass or food derived from the eelgrass deposited on the substratum as a source of nutrition (if we assume an isotopic discrimination of +1 and +3.4‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively), and surprisingly the contribution of MPB in the diet is negligible. Thus, individuals may assimilate carbon selectively feeding on green macroalgae, abundant at this site during spring/summer periods (Alexandra Baeta, personal observation).

The depletion of ^{13}C in the B site is consistent with an increased use of the detritus found absorbed to the sediment grains. The organic matter available in the sediment (SOM) is made up of various terrestrial and marine sources, on which many macrofauna consumers may forage selectively. It may be possible that in addition with feeding on a mix of different sources among sites, animals may use different sources of carbon and nitrogen, or are feeding at different trophic levels.

Food sources for *S. plana* and intraspecific differences

The tellinid clam *S. plana* (da Costa, 1778) is abundant and very common on sandy and muddy coasts from the Norwegian Sea to Senegal including the Mediterranean (Tebble, 1976). In some countries like Portugal and Spain it is commercially exploited (Langston et

al., 2007). This species is facultative deposit feeder, able to take its food from the surface of the sediment and out of the water column (Riera et al. 1999; Compton et al. 2008, 2009). Knowledge of its diet composition and especially of its niche is crucial for understanding the benthic food web structure because individuals of different size classes can play a different functional role in the ecosystem, based on diet or habitat use (Olson 1996). However, to our knowledge this aspect is poorly investigated.

Diet composition of the bivalve *S. plana*, as reflected in their isotope ratio, varied among habitats, most likely due to the availability of food in the surroundings. Smaller individuals were ^{13}C enriched as compared to their co-specific adults or showed a more variable diet. ^{13}C enrichment reached values close to those of *H. ulvae*, indicating that microphytobenthos is more important during the first stages of recruitment, whereas later animals may filter more quantities of seston. Indeed, the mixing models showed that juveniles of *S. plana* are mainly deposit-feeders at the three study sites, since the mean contribution of POM as food source was lower than 24% for all the areas. This trend was particularly evident in the I site. Both Z and I habitats are characterized by muddy sediments with high organic matter contents ($6.3 \pm 1.5\%$, $5.8 \pm 1.3\%$, for Z and I, respectively), and higher water-flow velocity ($1.2\text{-}1.4 \text{ m s}^{-1}$), whereas B, sand flat, presents lower organic matter content ($3.7 \pm 1.0\%$), and lower water flows ($0.8\text{-}1.2 \text{ m s}^{-1}$). In addition, during low tide, water pools are frequent in these muddy sediments (Verdelhos et al. 2005). Accordingly, higher resuspension of benthic diatoms might be possible at the I site, and higher contribution of MBP in the diet of *S. plana* might be attributed to the uptake of microphytobenthos resuspended in the water column (seston) or increased grazing. At Z habitat all the sources contributed similarly to both adults and juveniles, except *Gracilaria* sp. to the diet. By providing refuge and increasing organic matter availability, *Z. noltii* might allow these macrofauna species to rely on organic matter, rather than on the particles suspended in the water column.

The tellinid *S. plana* has been described as a facultative deposit feeder, able to take its food both from the top layer the sediment and out of the water, eating benthic and pelagic algae (Hughes 1969), with the potential to select their diet based on particle quality via their feeding processes (Levinton et al. 1996; Ward and Shumway 2004; Ward et al. 1997). The deposit-feeding behaviour was also observed in a salt marsh by Riera et al. (1999), where *S. plana* preferentially used benthic diatoms (64.5%). Our results confirm the suspension-deposit feeding behaviour of *S. plana*, and show differential diet selection,

individuals switch from deposit to suspension feeding, along this estuarine gradient in the intertidal areas of the Mondego estuary.

Ontogenetic changes in *S. plana*

S. plana did not change diet from juveniles to adults. Rather, larger animals tended to include higher proportions of compounds typical of phytoplankton. Shifts in diet with size might result from physiological constraints of juveniles in deposit-feeder worms (Hentschel 1998). Small individuals have a small gut, which may be unable to process enough sediment to satisfy nutritional requirement for growth and metabolic functions. Sediment is nutrient poor, and the strategy adopted to increase diet quality might be to forage in a more macrophagous way, choosing more nutritive particles such as benthic diatoms. In *S. plana*, shift in diet and in feeding behaviour might be related to the need of processing enough quantities of seston to reach adequate nutritive value, since the suspension-feeder diet is poorer and therefore more food needs to be ingested. Suspension-feeding mode can be limited when animals are small. Cardoso et al. (2010) observed that *S. plana* enhance size dependent vertical distributions, with smaller individuals higher densities in the superficial layers, and less abundant but deeper in the sediment profile adults. This has been described by other authors, being depth a function of siphon size (Zwarts 1986; Zwarts and Wanink 1989). Accordingly, small individuals tend to live shallower than large animals, since they do not have a siphon long enough to suspension-feed, and adults burrow deep and deposit and suspension feed. Therefore, the shift in diet observed might be consistent with the size of the animals because of their vertical distribution in the sediment. Small animals burrow shallow more often and, thus, feed on the surface of the sediment, while large animals burrow deep and feed on the sediment or on the water column. A similar pattern was found in *Macoma balthica*, with smaller animals feeding on microphytobenthos more than large animals (Rossi et al. 2004). The occurrence of ontogenetic differences in diet in these tellinid clams reveal the need to consider different stages of development when considering contribution to the food webs, since these individuals of different size classes play a different functional role in the ecosystem.

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Chapter 4

Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine benthic food webs

Abstract

Human-mediated and natural disturbances such as nutrient enrichment, habitat modification, and flood events often result in significant shifts in species composition and abundance that translate into changes in the food web structure. Six mass-balanced models were developed using the “Ecopath with Ecosim” software package to assess changes in benthic food web properties in the Mondego estuarine ecosystem (Portugal). Field, laboratory and literature information were used to construct the models. The main study objective was to assess at 2 sites (a *Zostera* meadow and a bare sediment area) the effects of: 1) a period of anthropogenic enrichment, which led to excessive production of organic matter in the form of algal blooms (1993/94); 2) the implementation of mitigation measures, following a long period of eutrophication (1999/2000); and 3) a centenary flood (winter 2000/2001). Different numbers of compartments were identified at each site and in each time period. In general, the *Zostera* site, due to its complex community, showed a higher number of compartments and a higher level of system activity (i.e. sum of consumptions, respiration, flow to detritus, production, total system throughput, net primary production and system omnivory index). The differences at the two sites in the three time periods in the breakdown of throughput were mainly due to differences in the biomass of the primary producers (higher primary production at the *Zostera* site). Consumption, respiration and flow to detritus were dominated by the grazers *H. ulvae* and *S. plana* at the *Zostera* and bare sediment sites respectively. At both sites, after recovery measures were implemented there was an increase in *S. plana* and *H. diversicolor* biomass, consumption, respiration and flows to detritus, and a decrease in *H. ulvae* biomass and associated flows, which increased again after the flood event. The mass-balanced models showed that the trophic structure of the benthic communities in Mondego estuary was affected differently by each disturbance event. Interestingly, in our study a high system throughput seems to be associated with higher stress levels, which contradicts the idea that higher system activity is always a sign of healthier conditions.

Keywords: ecological model, food web, eutrophication, management, flood, Ecopath, Mondego estuary, Portugal

1. Introduction

Estuaries are among the most productive, diverse, and economically important ecosystems on earth (Hobbie 2000; Paerl 2006). With the high population densities and increasing socioeconomic demands typical of coastal areas, estuarine ecosystems are subjected to multiple anthropogenic stressors. These do not usually operate independently, but rather

interact to produce combined impacts on biodiversity and ecosystem functioning (Vinebrooke et al. 2004; Dolbeth et al. 2007; Cardoso et al. 2008).

Nutrient enrichment is one of the widespread stressors. Estuaries, which are critical habitats for nutrient recycling and ecosystem productivity, often receive large nutrient inputs derived from human activities and agricultural discharges, which are generally followed by severe eutrophication events (Valiela et al. 1997; Cloern 2001; Bode et al. 2006; Lotze et al. 2006). Eutrophication severely impacts the diversity of primary producers and consumers, which leads to alterations in the food web structure, ecosystem productivity and functioning. One of the most important aspects of this type of disturbance is the proliferation of fast-growing macroalgae that may replace slow-growing macrophytes and significantly decrease the areal extent of seagrass meadows, which decreases the ecological value of the entire estuary (Short and Wyllie - Echeverria 1996; Howarth 1988; Bricker et al. 1999; Valiela 2006; Patrício et al. 2009).

In addition to the impact of organic loading, estuarine ecosystems are also influenced by natural perturbations, such as extreme weather events (floods, droughts and heat waves), which can have a strong negative impact on key components of the estuarine system (e.g. Cardoso et al. 2008; Chainho et al. 2007). Apart from the hydrological conditions (e.g. flood and drought periods), variability due to small spatial scale habitat heterogeneity (e.g. plant presence/absence, sediment grain size and organic matter content) may have a large impact on communities, and thus on ecosystem functioning (Pearson and Rosenberg 1978; Gray 1997; Raffaelli et al. 1998; Cloern 2001; Patrício et al. 2009). For instance, seagrass meadows, in comparison to bare sediment, contribute greatly to estuarine functioning by sustaining longer and more complex food webs, which can affect ecosystem productivity and stability in periods of environmental change. Seagrasses increase sedimentation of fine sediment particles and thus enhance the quantity and quality of food for many macrofauna invertebrates, which are the fundamental trophic link between basal resources and predators such as fish and seabirds. Seagrass meadows are also a nursery for economically valuable fish (Pinto et al. in press) and a refuge from predators (Duarte 2002). Furthermore, seagrasses, particularly *Z. noltii*, can contribute to carbon and nutrient sequestration and storage for longer periods than macroalgae. Therefore, the water over the meadow is of higher quality than the water over bare sediment, and thus eutrophication effects are mitigated (Cebrian 1999).

For all the abovementioned reasons, estuaries are particularly challenging to model. Previous studies (e.g. Patrício and Marques 2006) have shown that an ecosystem

consists of so many interacting components that it is impossible to understand how it functions by examining the component relationships in isolation (Likens 1985; Allen 1988). To avoid this problem, simplified models can be developed that contain enough of the characteristics of the original system to resemble reality, but at the same time are simple enough to be understood (Brown 2004). Mass-balance models represent a “snapshot” of the trophic flows in the ecosystem, and can be used to describe at least part of the reality (Christensen 1994). Information gained from studying trophic webs can be used to make predictions of the impacts of natural or human-induced events on ecosystems, which can be used for management purposes.

Previous studies of Mondego estuary (Patrício et al 2004; Patrício and Marques 2006) described two areas in 1993-94: one characterized by a *Zostera* bed considered to be in a healthy condition as it had high biodiversity, and a bare sediment site described as highly stressed due to strong eutrophication that had led to the loss of the *Zostera* meadow and the associated species. In the present study, we return to the models established previously for these two sites and add diet information from natural stable isotope ratios to determine how the properties have evolved since the models were originally developed.

Therefore, six mass-balanced models were developed (with EcoNetwrk) using the “Ecopath with Ecosim” software package (v 5.1) to assess differences in benthic food web properties in the Mondego estuarine ecosystem (Portugal). The main study objective was to assess the effects of:

- 1) a period of anthropogenic enrichment of the system which led to overproduction of organic matter in the form of algal blooms (1993/94);
- 2) mitigation measures (e.g. reducing nutrient loading, protecting seagrass beds and enhancing hydrodynamic circulation) that were implemented with the aim of promoting the recovery of the seagrass beds and the entire surrounding environment following a long period of eutrophication (1999/2000);
- 3) a centenary flood (winter 2000/2001).

More specifically, the analysis of the properties of the 6 models allowed us to answer the following questions: 1) Did the stress events modify the structure of the primary producers?; 2) What was the impact of the stress on grazers and other consumers?; 3) Did different types of stress (eutrophication vs flood) have the same impact on the

communities?; 4) Did the habitat structure (*Zostera noltii* meadows vs bare sediment) influence the community response to stress?; and 5) Did the mitigation measures lead to the “recovery” of the food webs?

2. Materials and methods

2.1. Study site

The Mondego estuary is a relatively small (860 ha), warm-temperate, polyhaline, intertidal system located on the western Atlantic coast of Portugal. It consists of two arms: the northern and southern arms (Fig. 1). The southern arm is characterised by intertidal mudflats (almost 75% of the area) that are exposed at low tide. The tidal range varies between 0.35 and 3.3 m depending on the site and tide coefficient, while the water residence time varies between 1-2 (northern arm) and 3 days (southern arm).

From 1991 to 1997, the communication between the two arms of the estuary became totally interrupted in the upstream area, which caused the river discharge to flow essentially through the northern arm. Consequently, water circulation in the southern arm became mainly dependent on tides and on the small freshwater input from a tributary, the Pranto River, artificially controlled by a sluice (Marques et al. 2009). This led to clear eutrophication symptoms in the southern arm (e.g. green macroalgal blooms) (Leston et al. 2008; Patrício et al. 2009). In 1997, to decrease these eutrophication symptoms and test ways of improving the system's condition, the freshwater discharge from the Pranto River sluice into the southern arm was reduced to a minimum in order to decrease nutrient inputs, and was diverted to the northern arm by another sluice located further upstream. Moreover, the communication between the northern and southern arms was re-established to a very limited extent (periods of only 1.5 to 2 hours before and after each high tide peak through a section of only 1 m²) to improve water circulation (Neto 2004; Lillebø et al. 2007; Marques et al. 2009).

A long-term study of the Mondego estuary ecosystem carried out since the mid 1980s made it possible to determine the system's responses to these modifications in the physical conditions, such as the changes in the *Z. noltii* beds and green macroalgae distribution (e.g. Patrício et al. 2009). Following the interruption of the upstream communication between the two arms, the ecological conditions in the southern arm rapidly deteriorated. The combined effect of an increased water residence time and higher nutrient concentrations was a major driving force behind the seasonal *Ulva* spp. blooms and the consequent severe reduction in

the area occupied by *Z. noltii* beds due to competition with macroalgae (Marques et al. 2003; Patrício et al. 2009). The shift in benthic primary producers changed the benthic macrofauna trophic structure (e.g. Marques et al. 1997, 2003; Cardoso et al. 2004a; Patrício and Marques 2006; Dolbeth et al. 2007). According to Patrício et al. (2009), after experimental mitigation measures were applied in 1998, this trend appeared to reverse to a certain extent, as the area occupied by *Z. noltii* was partially regained, the green *Ulva* spp. blooms stopped, and the macrofauna assemblages gave signs of recovering their former condition of the late 80s.

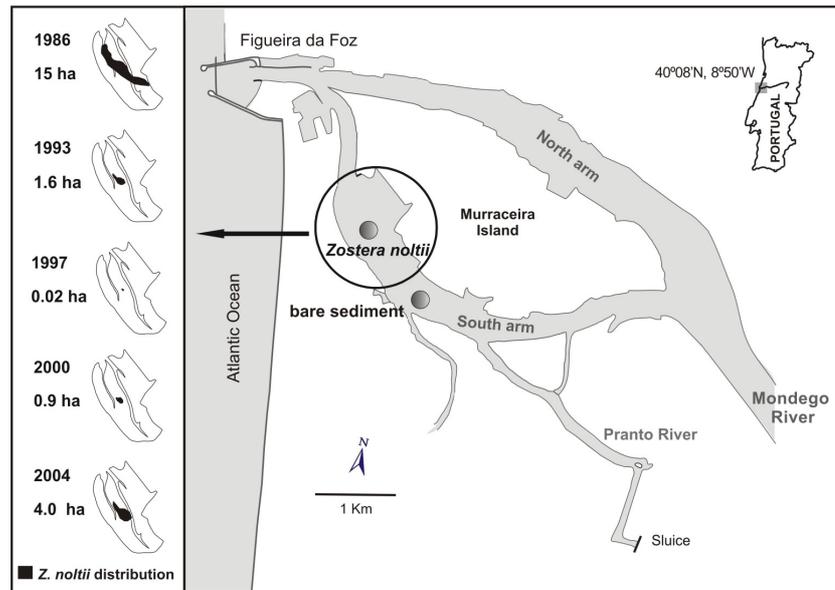


Fig. 1. Map of the Mondego estuary showing the sampling sites: *Zostera* and bare sediment sites (grey circles). Change in the area covered by *Zostera noltii* in the southern arm of the Mondego estuary. Mapping of benthic vegetation is based on field observations, aerial photographs and a GIS methodology (Arc View GIS version 8.2).

Winter 2000/01 was characterized by unprecedented high precipitation values compared to the average long-term precipitation (2000/01: 1802.1 mm; 1940 to 1997: 1030.6 mm), which caused one of the largest flood events of the century in the Mondego catchment area.

2.2. Sampling program and laboratory treatment

Intertidal communities were sampled fortnightly (February 1993-June 1994), and monthly (July 1994-December 1994; January 1999-December 2000; January 2001-December 2002). In all cases, sampling was conducted at two sites in the southern arm (Fig. 1), within the *Z. noltii* meadow, which persisted during the early 1990s, and in an area where the *Z. noltii* had disappeared, now characterized by bare sediment. Each time at each site, six replicate cores were taken to a depth of 20 cm (13 cm inner diameter) and then pooled together for biological material analyses. The sediment was washed through a 500 µm mesh sieve and the biological material preserved in 4% buffered formalin. Animals and plants were identified to species level and subsequently dried at 70°C for 72 h to estimate biomass as dry weight (DW), and ash free dry weight (AFDW) after combusting samples for 8 h at 450°C. For each time period, the weights of all taxa were summed to obtain an annual average standing stock. Although combining bacteria with detritus can be problematic using the Ecopath software package, the bacterial biomass was assigned to the detritus compartment, as recommended by Christensen and Pauly (1992).

To determine the Chl *a* concentration, sampled water (500-1000 ml) was filtered through GF/C Whatman glass fibre filters (4.7 cm diameter) followed by acetone extraction according to Parsons et al. (1985), and expressed as g Chl *a* m⁻³. In the absence of a calculated factor for the chlorophyll to carbon conversion, the generally accepted value of 50 (Eppley et al. 1977) was applied and considered constant.

To obtain an approximate value for the microphytobenthos biomass in the system, in June, July and September 2008, the microphytobenthos biomass was estimated at each sampling location by sampling the top 1 cm of the six 27 cm² replicates. The samples were carefully mixed, freeze-dried and kept in the dark at -20°C until further processing. The Chl *a* content of the dried sediment was extracted in 90% acetone over 20 h in the dark; Chl *a* was then measured using a fluorometer, and expressed as g Chl *a* m⁻². The C:Chl *a* ratio was considered constant and equal to 40 mg C mg Chl *a*⁻¹ (de Jonge 1980).

2.3. Modelling approach

2.3.1. Compartments

Different numbers of compartments were identified in each situation: 36, 31 and 24 in the *Zostera* site, and 25, 20 and 20 in the bare sediment site, for the three periods, 1993/94,

1999/2000 and 2001/02 respectively. Species that were not naturally present in one of the three years or sites or whose roles in the trophic network were unimportant (biomass < 0.001 g AFDW m⁻²) were not taken into account. The Detritus compartment reflects the standing stock of non-living organic matter in the sediments (including different types of plant tissue, dead microbes, faeces), the suspended organic matter, inputs from the river basin and from the ocean, and flows from living organisms.

2.3.2. Ecopath model equations

Ecopath trophic models are mass-balanced models that account for energy flows (to and from each compartment) in a food web. The parameterization of the Ecopath model is based on satisfying two ‘master’ equations. The first equation describes the production term for each compartment included in the system:

$$B_i \times (P/B)_i \times EE_i - \sum_j (B_j \times (Q/B)_j \times DC_{ji}) - Y_i - BA_i - E_i = 0 \quad (1)$$

where B_i and B_j are the biomasses of prey (i) and predators (j) respectively; P/B_i the production/biomass ratio; EE_i the ecotrophic efficiency, defined as the fraction of the production of each group that is used in the food web; Y_i the fisheries catch per unit area and time; Q/B_j the food consumption per unit biomass of j; DC_{ji} the fraction of prey i in the average diet of predator j; BA_i the biomass accumulation rate for i (the default value of zero was used to indicate no biomass accumulation); and E_i is the net migration of i, calculated as immigration (migration into the area covered by the model) minus emigration (migration out of the area) (the default value of zero was used).

Eq. (1) only includes the production. When a compartment in an ecosystem is balanced, other flows must be considered. Eq. (2) expresses the principle of conservation of matter within a compartment:

$$B_i \times (Q/B)_i = B_i \times (P/B)_i + R_i + U_i \quad (2)$$

where R_i is the respiration rate, and U_i the unassimilated food rate. The Ecopath software solves the set of Eq. (1) (one for each group i) for one of the following parameters: B , P/B , Q/B or EE . Therefore, at least three of these four parameters are required as input. As EE is

both difficult to measure and calculate it was estimated by the model for most of the groups, and the model quality was evaluated by checking if the *EE* value obtained was realistic (between 0 and 1). The software approach, its methods, capabilities and limitations are explained thoroughly in Christensen and Walters (2004).

2.3.3. The estimation procedure

Once the software had estimated the parameters, the system balanced the input and output of each group, using respiration (R_i) for adjustments. The relationship used is represented by Eq. (2):

where *consumption* is the intake of food by a compartment over a given interval of time, and was entered as the ratio of consumption over biomass (Q/B). For the heterotrophic compartments, the P/Q ratios were entered into the program in order to estimate the Q/B ratio indirectly (Hostens and Hamerlynck, 1994);

Production excludes primary production and refers to the elaboration of tissue (whether it survives or not) by a compartment over a given period. Thus, the production/biomass ratio (P/B) is the turnover rate of a species' biomass, meaning the amount of time it takes to replace the biomass of the population (McLusky, 1989; Cusson and Bourget, 2005). Long-lived species will have a lower P/B than short-lived species (McLusky, 1989). Previously calculated P/B ratios were used for *Hydrobia ulvae* (gastropoda), *Scrobicularia plana* (bivalvia), *Cyathura carinata* (isopoda), *Hediste diversicolor* (polichaeta), *Ampithoe valida* and *Melita palmata* (amphipoda) for each of the study sites and periods (Dolbeth et al., 2007). Brey's (2001) method, version 4-04 (Brey 2001, www.awi-bremerhaven.de/Benthic/Ecosystem/FoodWeb/Handbook/main.htm) was used to calculate the P/B for the other species (except for zooplankton), for the two study sites and the three periods. The weight-to-energy ratios needed in order to apply the empirical method were also provided by Brey (2001);

Respiration (R_i) is the part of the consumption that is not used for production or recycled as faeces or urine, and therefore is a nonusable currency. Following Christensen et al. (2000), our models assumed that autotrophs with a $Q/B = 0$ and detritus had zero respiration.

Respiration is only used in Ecopath for balancing the flows between groups; therefore, we were not able to enter respiration values.

Finally, the *Unassimilated food* (U_i) is an input fraction of food that is not assimilated (i.e. it is egested or excreted). Following Christensen et. al. (2000), our energy models used a U_i default value of 0.20 (i.e. 20% of the consumption for all groups), and the non-assimilated food was allocated to the detritus.

2.3.4. Diet composition

The diet matrices of the benthic species were built using data from a variety of sources (Appendices A and B). Quantitative information on diet composition from the study system was only available for the crab *Carcinus maenas* (Baeta et al. 2006), and qualitative information was available for *H. ulvae*, *S. plana*, *C. carinata*, *A. valida*, *M. palmata*. Baeta et al. (2009a) measured isotopic signatures (both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in the tissues of producers and consumers and obtained qualitative information on the trophic structure of the benthic and water column food webs in the Mondego estuary for both the *Zostera* and bare sediment sites. Moreover, possible food sources for the macrobenthos were investigated by using mixing models, and the mean dietary proportion of consumers was determined (Baeta et al. submitted). Complementary information on diet composition was gathered using dietary data from the literature (see Appendix B).

2.3.5. Catches (Y_i)

A complete network needs estimates of the export rates from the system, including the harvesting of economically important species. In the present work, the harvesting of *S. plana*, *Cerastoderma edule* and *C. maenas* for human consumption and the polychaete *H. diversicolor* and decapod *Crangon crangon* for bait for fisheries was considered small enough to be negligible.

2.3.6. Balancing the models

For each of the six models (one model per station and sampling period), the software calculated the missing parameters. As expected, initially none of the models were balanced (e.g. negative flows to detritus, EE higher than 1 – which indicates that the demand is too

high to be sustainable). According to Christensen et al. (2000) the normal procedure for building Ecopath models is to start with a low quality first model and then adjust the parameters entered within the range of possible values, trying to avoid changing the more reliable data. Therefore, changes were made one at the time, and each time the basic estimates routine was rerun and re-examined. Data were re-edited whenever necessary. In our study, the most reliable data were the macrofaunal biomass and production, determined *in situ*, and consequently these values were left unchanged. One exception was the decapod biomass (*C. crangon* and *C. maenas*); for the decapod species the biomass parameter was estimated by the software because the original values were underestimated owing to the sampling strategy used (sampling was carried out using a TASM rather than nets suitable for catching pelagic organisms). For subsequent balancing it was necessary to re-evaluate the diet composition of some compartments (e.g. *C. maenas*, *C. crangon*, *C. carinata*, *H. diversicolor*, *Littorina littorea*), since the feeding habits of these species are highly labile and mainly depend on the food sources that are available in the ecosystem. Adjustments were made within the range of the minimum and maximum contributions of each source in the consumers' diet, given by the mixing models (Baeta et al. submitted), as well as taking into account the trophic group of each species. A diet fraction is impossible if it pushes another diet fraction outside its feasible range. Therefore, we calculated the upper limit of the amount of a given prey item that a predator can consume (i.e. available prey biomass) using the highest possible production rate of the prey and the lowest total consumption of the prey by other predators. The highest fraction for prey *i* in the diet of predator *j* was then given by the highest available biomass of prey *i* divided by the lowest possible consumption rate of predator *j*. The lower limit for the diet fraction was obtained in a similar way (for more details see Baeta et al. submitted).

2.3.7. Summary of ecological statistics and indices

A number of statistics that describe an ecosystem as a whole were calculated for assessing the status of the ecosystem (Christensen et al. 2000) at the two study sites in the three time periods, that is, the sum of all consumptions, exports, respiratory flows, flows to detritus, production (all in g AFDW m⁻² y⁻¹), and total biomass (excluding detritus) (g AFDW m⁻²). Other measures were also estimated:

The *total system throughput*, TST ($\text{g AFDW m}^{-2} \text{y}^{-1}$), is the sum of all flows in a system (consumption + export + flows to detritus + respiration).

The *net primary production* ($\text{g AFDW m}^{-2} \text{y}^{-1}$) is calculated as the total primary production from all producers.

The *Net system production* ($\text{g AFDW m}^{-2} \text{y}^{-1}$) is the difference between total primary production and total respiration. According to Odum (1969), it will be large in immature systems and close to zero in mature ones. Moreover, systems with large imports may have negative system production.

The *total primary production/total biomass* (y^{-1}) is expected to be a function of the system's maturity. In immature systems, production exceeds respiration for most groups, and therefore the biomass can be expected to accumulate over time, which in turn will lead to a decline in the ratio.

The *total biomass/TST* (y) is expected to increase and reach a maximum in the most mature stages of the system (Odum 1971).

The *system omnivory index*, SOI , is the average omnivory index of all consumers weighted by the logarithm of each consumer's food intake. It is a measure of how the feeding interactions are distributed among trophic levels, and is useful for characterizing the extent to which a system displays web-like features (Christensen et al. 2000). When the value of the omnivory index is zero, the consumer in question is specialized (i.e. feeds on a single trophic level).

3. Results and discussion

3.1. Summary statistics: comparing three time periods at two intertidal sites

The basic input data and model estimations of the dietary composition matrices at each site and in each period are given in Table 1 and Appendix A respectively. The ecological system statistics and indices for the *Zostera* and bare sediment sites for the three periods are given in Table 2.

Table 1 Input data and calculated estimates (in parentheses) of all compartments in the food web network for the two areas (Z, *Zostera noltii*; bs, bare sediment area) for the three time periods (1994/94; 1999/00; 2001/02). Biomass given in g AFDW m⁻². (M, macrophytes; Am, amphipoda; B, bivalvia; D, decapoda; G, gastropoda; I, isopoda; P, polychaeta).

Compartment	Name	1993/1994								1999/2000								2001/2002							
		Biomass		P/B*		Q/B*		EE*		Biomass		P/B*		Q/B*		EE*		Biomass		P/B*		Q/B*		EE*	
		Z	bs	Z	bs	Z	bs	Z	bs	Z	bs	Z	bs	Z	bs	Z	bs	Z	bs	Z	bs	Z	bs	Z	bs
1	Phytoplankton	0.336	0.330	135.00	135.00			(0.55)	(0.79)	0.326	0.350	135.00	135.00			(0.54)	(0.97)	0.316	0.330	135.00	135.00			(0.57)	(0.94)
2	Microphytobenthos	4.200	6.000	40.00	40.00			(0.96)	(0.62)	4.200	6.000	40.00	40.00			(0.97)	(0.36)	4.200	6.000	40.00	40.00			(0.98)	(0.24)
3	Green macroalgae	2.201	64.371	3.20	3.20			(0.97)	(0.03)	1.679	2.178	3.20	3.20			(0.41)	(0.08)	2.462	0.515	3.20	3.20			(0.20)	(0.16)
5	<i>Zostera noltii</i> (M)	194.141		2.50				(0.01)		62.266		2.50				(0.00)		108.222		2.50				(0.00)	
6	Zooplankton	(0.069)	(0.030)	22.00	18.00	(88.00)	(90.00)	0.95	0.95	(0.025)	(0.056)	22.00	18.00	(88.00)	(90.00)	0.95	0.95	(0.042)	(0.027)	22.00	18.00	(88.00)	(90.00)	0.95	0.95
7	<i>Ampithoe valida</i> (Am)	(0.169)	0.080	6.40	7.05	(32.00)	(35.25)	0.95	0.75	0.039		6.47		(32.35)		0.98		0.018		5.38		(26.88)		(0.95)	
8	<i>Corophium multisetosum</i> (Am)																	0.006		6.87		(34.37)		(0.39)	
9	<i>Melita palmata</i> (Am)	0.116	0.059	7.10	11.33	(35.50)	(56.65)	(0.98)	(0.62)	0.007	(0.015)	7.79	3.42	(38.97)	(17.10)	(0.62)	0.95	0.021	0.007	5.95	2.11	(29.76)	(10.55)	(0.74)	(0.20)
10	<i>Cerastoderma edule</i> (B)	4.484	0.131	0.91	7.91	(4.60)	(39.53)	(0.17)	(0.05)	0.879	0.047	1.07	2.74	(5.36)	(13.70)	(0.07)	(0.14)	0.409	0.092	0.91	2.35	(4.57)	(11.77)	(0.62)	(0.16)
11	<i>Mytilus</i> sp.	0.015		4.10		(20.50)		(0.63)																	
12	<i>Scrobicularia plana</i> (B)	2.668	8.337	1.00	1.60	(5.00)	(8.00)	(0.40)	(0.06)	16.762	35.000	1.00	0.90	(5.00)	(4.50)	(0.14)	(0.07)	15.756	23.174	1.00	1.06	(5.00)	(5.30)	(0.33)	(0.09)
13	<i>Carcinus maenas</i> (D)	0.780	0.252	2.01	2.94	(10.10)	(14.72)	(0.56)	(0.86)	0.342	0.327	2.23	2.93	(11.13)	(14.65)	(0.86)	(0.63)	0.680	(0.138)	1.92	4.29	(9.62)	(21.43)	(0.94)	(0.51)
14	<i>Crangon crangon</i> (D)	(0.308)	(0.092)	3.97	6.25	(19.90)	(31.25)	0.95	0.95	(0.149)	(0.152)	3.97	7.57	(19.87)	(37.81)	0.95	0.95	(0.209)	(0.074)	4.87	6.25	(24.36)	(31.25)	0.95	0.95
15	<i>Gibulla umbilicallis</i> (G)	0.045		1.81		(9.00)		(0.45)																	
16	<i>Haminoea hydatis</i> (G)	0.187	0.066	2.12	2.51	(10.60)	(12.54)	(0.06)	(0.03)	0.101		3.84		(19.21)		(0.03)									
17	<i>Hydrobia ulvae</i> (G)	59.809	6.751	2.00	4.78	(8.00)	(23.90)	(0.02)	(0.01)	21.300	1.692	3.00	3.67	(15.00)	(18.35)	(0.03)	(0.03)	45.273	0.537	2.00	3.30	(10.00)	(16.65)	(0.08)	(0.20)
18	<i>Littorina</i> spp. (G)	2.117		1.10		(5.50)		(0.12)		0.578		1.03		(5.16)		(0.09)		0.204		1.27		(6.37)		(0.07)	
19	<i>Cyathura carinata</i> (I)	0.407	8.077	3.10	3.16	(15.50)	(15.80)	(0.39)	(0.01)	1.095	9.397	2.00	1.53	(10.00)	(7.65)	(0.12)	(0.03)	1.116	8.656	2.00	2.36	(10.00)	(11.80)	(0.12)	(0.02)
20	<i>Idotea chelipes</i> (I)	0.036	0.013	4.76	4.23	(23.79)	(21.15)	(0.93)	(0.63)	0.005		5.10		(25.48)		(0.94)		0.010		2.98		(14.89)		(0.77)	
21	<i>Lekanesphaera levii</i> (I)	0.002		5.87		(29.37)		(0.55)																	
22	<i>Alkmaria romijni</i> (P)	0.022	0.113	9.45	13.15	(47.23)	(65.73)	(0.81)	(0.39)	0.009	0.031	10.65	8.79	(53.25)	(43.94)	(0.31)	(0.63)	0.028	0.057	5.93	9.99	(29.67)	(49.95)	(0.52)	(0.46)
23	<i>Aonides oxycephala</i> (P)	0.013		4.90		(24.50)		(0.95)																	
24	<i>Capitella capitata</i> (P)	0.009	0.023	7.66	9.72	(38.28)	(48.61)	(0.78)	(0.44)	0.006	0.008	6.47	7.37	(32.35)	(36.83)	(0.37)	(0.39)	0.271	0.006	2.62	9.52	(13.11)	(47.60)	(0.70)	(0.59)
25	<i>Chaetozone setosa</i> (P)	0.118	0.008	7.73	13.82	(38.65)	(69.11)	(0.83)	(0.11)	0.032		6.46		(32.28)		(0.40)									
26	<i>Diopatra neapolitana</i> (P)	0.012		2.75		(13.76)		(0.59)																	
27	<i>Eteone flava</i> (P)	0.002		6.48		(32.40)		(0.66)		0.035		2.39		(11.94)		(0.61)									
28	<i>Glicera tridactyla</i> (P)	0.214	0.004	2.18	5.12	(10.89)	(25.62)	(0.99)	(0.62)	0.061		2.02		(10.09)		(0.94)		0.005		5.04		(25.18)		(0.78)	
29	<i>Hediste diversicolor</i> (P)	(0.795)	0.423	2.40	1.85	(12.00)	(9.25)	0.98	(0.99)	2.417	0.891	1.60	1.72	(8.00)	(8.60)	(0.97)	(0.92)	6.952	2.906	2.00	1.98	(10.00)	(9.90)	(0.37)	(0.96)
30	<i>Heteromastus filiformis</i> (P)	0.455	0.142	3.39	4.56	(16.97)	(22.81)	(0.94)	(0.79)	0.473	0.108	3.24	4.44	(16.19)	(22.20)	(0.96)	(0.84)	0.141	0.038	3.60	5.34	(18.01)	(26.72)	(0.90)	(0.74)
31	<i>Lagis koreni</i> (P)	0.017		3.40		(17.00)		(0.76)		0.008		3.77		(18.84)		(0.35)		0.005		4.73		(23.67)		(0.49)	
32	<i>Lumbrineris impatiens</i> (P)	0.081		2.72		(13.61)		(0.89)																	
33	<i>Mediomastus fragilis</i> (P)									0.004	0.005	5.75	6.60	(28.75)	(33.02)	(0.87)	(0.23)								
34	<i>Mista picta</i> (P)									0.023		4.21		(21.07)		(0.45)		0.049		2.46		(12.28)		(0.69)	
35	<i>Nephtys hombergii</i> (P)	0.034		2.55		(12.76)		(0.85)		0.040		2.27		(11.35)		(0.86)									
36	<i>Pygospio elegans</i> (P)	0.002	0.010	8.37	10.12	(41.85)	(50.60)	(0.53)	(0.12)		0.011		6.51		(32.53)		(0.21)								
37	<i>Streblospio shrubsolei</i> (P)	0.011	0.009	9.28	13.95	(46.39)	(69.76)	(0.96)	(0.09)	0.003	0.006	9.75	10.04	(48.73)	(50.21)	(0.89)	(0.13)	0.059	0.014	4.42	8.39	(2.08)	(41.94)	(0.59)	(0.44)
38	Nemertini	0.004	0.002	5.06	9.65	(25.31)	(48.24)	(0.89)	(0.45)	0.028		4.11		(20.59)		(0.91)									
39	Oligochaets	0.132	0.004	6.74	12.75	(33.70)	(63.75)	(0.92)	(0.52)	0.148	0.004	5.49	10.01	(27.45)	(50.03)	(0.82)	(0.19)	0.071	0.022	3.66	10.10	(18.28)	(50.27)	(0.80)	(0.61)
40	Detritus	511.28	305.80					(0.49)	(0.43)	511.28	305.8					(0.69)	(0.55)	527.31	305					(0.77)	(0.57)

* P, production; Q, consumption; EE, ecotrophic efficiency

Table 2 Summary of ecological statistics/indices for the two areas (*Zostera* site and bare sediment site) for the three time periods (1994/94; 1999/00; 2001/02).

Statistic/indices	<i>Zostera</i> site			Bare sediment site		
	1993/94	1999/00	2001/02	1993/94	1999/00	2001/02
Sum of all consumptions (g AFDW m ⁻² y ⁻¹)	592.255	470.232	642.164	395.542	289.694	277.682
Sum of all exports (g AFDW m ⁻² y ⁻¹)	350.632	91.879	105.462	253.583	121.821	120.367
Sum of all respiratory flows (g AFDW m ⁻² y ⁻¹)	356.904	282.031	385.115	237.927	173.816	16.6609
Sum of all flows into detritus (gvAFDW m ⁻² y ⁻¹)	733.167	358.96	533.388	450.049	269.277	286.576
Sum of all production (gvAFDW m ⁻² y ⁻¹)	823	468	618	571	350	342
Total system throughput (g AFDW m ⁻² y ⁻¹)	2036	1203	1 666	1337	855	851
Net primary production (g AFDW m ⁻² y ⁻¹)	705.756	373.048	489.093	490.537	294.22	286.198
Total primary production/total respiration	1.977	1.323	1.270	2.062	1.693	1.718
Net system production (g AFDW m ⁻² y ⁻¹)	348.852	91.016	103.979	252.611	120.403	119.589
Total primary production/total biomass (y ⁻¹)	2.576	3.300	2.622	5.146	5.228	6.718
Total biomass/total system throughput (y ⁻¹)	0.135	0.094	0.112	0.071	0.066	0.050
Total biomass (excluding detritus) (g AFDW m ⁻²)	274.01	113.042	186.514	95.327	56.279	42.604
Omnivory index	0.097	0.101	0.097	0.056	0.080	0.066

The total consumption, exports, respiration, flow to detritus and production were higher in the *Zostera* site for the three time periods compared to the bare sediment site, except for the total exports, which was lower at the *Zostera* site in 1999/2000 and 2001/2002. The *Zostera* site in the period 99/00 showed the lowest values for the total exports. For the bare sediment system, the lowest values for the sum of each type of flow were found in the 1999/2000 and 2001/2002 periods, which had very similar values. Similarly, the total system throughput was always higher at the *Zostera* site (Table 2), which shows that there is a higher level of system activity in this community. Figure 2 illustrates the six food webs (one for each site and time period).

Different numbers of compartments were identified in each situation: 36, 31 and 24 at the *Zostera* site, and 25, 20 and 20 at the bare sediment site, for the three periods, 1993/1994, 1999/2000 and 2001/2002 respectively. There was no aggregation of compartments; species that were not naturally present in one of the three areas or whose roles in the trophic network were unimportant (biomass < 0.001 g AFDW m⁻²) were not taken into account at a specific time and space.

This overall structure of flows is partially influenced by the number of compartments. Respiration, flow to detritus and exports are not sensitive to the number of compartments, but consumption and the TST are. Accordingly, the *Zostera* site, due to its complex community, had a larger number of compartments than the bare sediment site, and

showed a higher level of system activity (e.g. TST). Probably for the same reason, both areas showed their highest TST values during the period of nutrient enrichment (1993/1994).

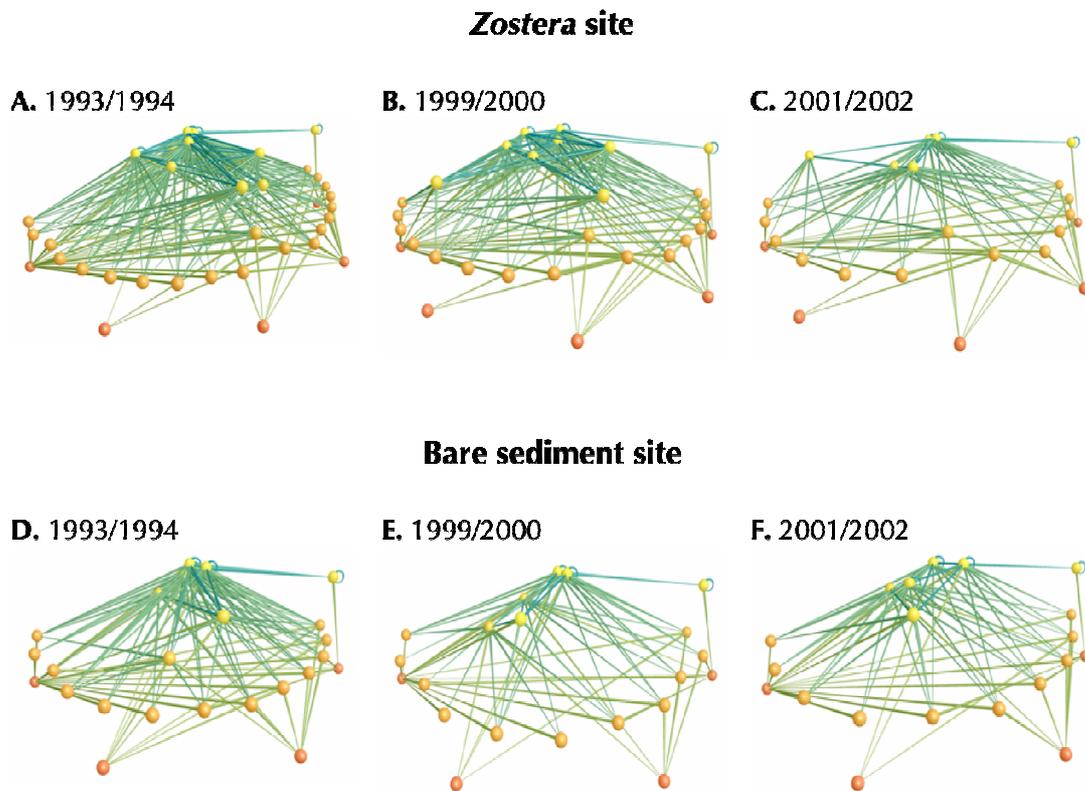


Figure 2. 3D representation of the six food webs from Mondego estuary. A to C: *Zostera* site in 1993/1994, 1999/2000 and 2001/2002 respectively. D to F: Bare sediment site in 1993/1994, 1999/2000 and 2001/2002 respectively. Images were produced with FoodWeb3D written by R.J. Williams, Pacific Ecoinformatics and Computational Ecology Laboratory. The different coloured dots represent functional groups from different trophic levels: red=primary producers, orange=primary consumers, and yellow=secondary consumers. The light and dark grey links represent feeding links.

To measure how the feeding interactions are distributed between trophic levels, Ecopath calculates the System Omnivory Index (SOI). When the SOI value is zero, the consumer in question is specialized (i.e. it feeds on a single trophic level). A large value indicates that the consumer feeds on many trophic levels. According to Heymans (2003), this index is dependent on the number of compartments in the model: more compartments

have more connections, and there is less omnivory when compartments are combined and diets consolidated. In this study, despite the disturbance events (nutrient enrichment or flood) and the different numbers of compartments, the *Zostera* site showed a similar SOI for all the time periods and always had higher values than the bare sediment site. Interestingly, the index decreased, although only slightly, during the disturbance periods (1993/1994 and 2001/2002).

In comparison with the previous preliminary attempt to model the food web characteristics of the two sites during the period of nutrient enrichment (1993/1994) (Patrício and Marques 2006), we found several differences with regard to community structure and flows in the present study (e.g. present study: lower TST, total production, total respiration, total exports, flow to detritus, SOI). In our models the species were not aggregated, the *P/B* ratios were calculated for the two sites for each period, and new information and measurements of the diets of benthic species (Baeta et al. 2006, 2009a, b; submitted) were used for model calibration, which probably modified the previous models' results. This allowed the quantitative contribution of the consumers' food sources to be estimated more accurately using mixing models and index values as well as the quantification information on nutrition sources of the estuarine invertebrates inhabiting the two study areas.

3.2. Effects of different ecological conditions on primary producers

The *Zostera* area in 1999/2000 and 2001/2002 showed similar partitioning of the total throughput: between around 39% of the total flow was due to consumption, approximately 6-8% was exported, about 30-32% flowed to detritus, and around 23% was respired (Figure 3). At the *Zostera* site, the major difference concerned a proportionally higher value (18%) for exports during the nutrient enrichment period (1993/1994).

The differences in the breakdown of throughput, with higher exports and flows to detritus in the period 1993/1994, were mainly due to differences in the macrophyte biomass. It is well known that macrophytes support two types of food webs: a herbivorous web, in which herbivores feed directly on the plant, and a detritivorous web, in which some species feed on the plant detritus. According to Enriques et al. (1993), macrophytes are major producers of organic matter; however, little of this production enters the grazing food chain because there is a time lag between production and utilisation since few animals feed on these plants directly. This production is usually used after decomposition and a

large proportion of the production decays to detritus or is washed away from the production area and used in other systems. This is consistent with the results of the current study (Figure 4).

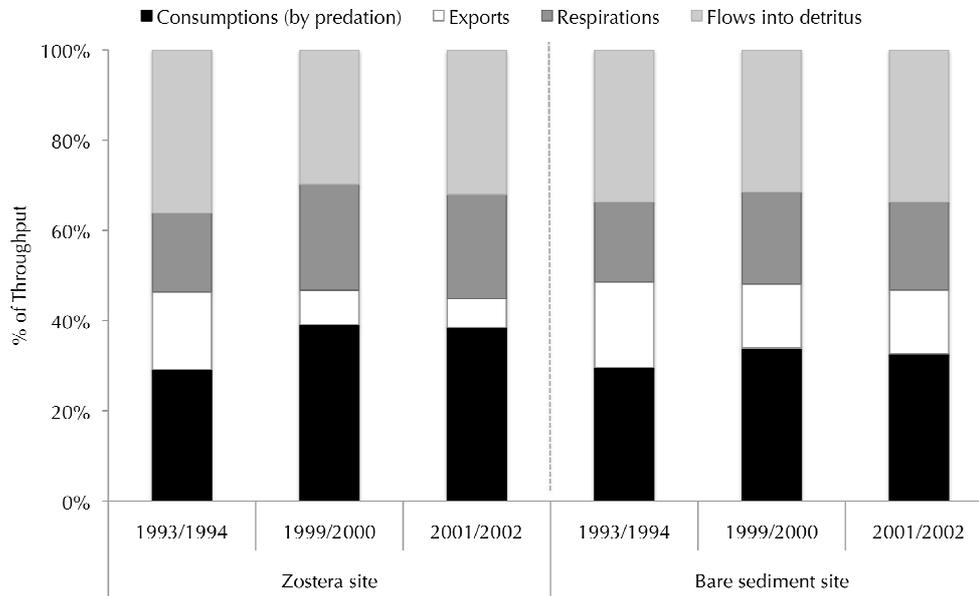


Figure 3. Partitioning of throughput among consumption by predators, exports, flow to detritus and respiration at the *Zostera* site and bare sediment site in the three time periods (1993/1994; 1999/2000 and 2001/2002).

Similar results were obtained over time in the bare sediment models, with a similar partitioning of the total throughput. Between 30 and 34% of the total throughput was due to consumption, about 32 to 34% flowed to detritus, and approximately 18 to 20% was respired (Figure 3). The major difference found for the three periods was a proportionally higher exports value in 1993/1994 compared to the other two periods (19% vs 14% respectively). At this site, the microphytobenthos played a crucial role in the system production. In 1993/1994, although the macroalgae biomass was extremely high (64.4 g AFDW m⁻²) (Figure 4), the consumption of this primary producer was very low. Consequently, at the bare sediment site, during the period of nutrient enrichment, a large percentage of the primary production (that led to excessive production of organic matter in the form of green algal blooms) passed to the Detritus compartment (Figure 4).

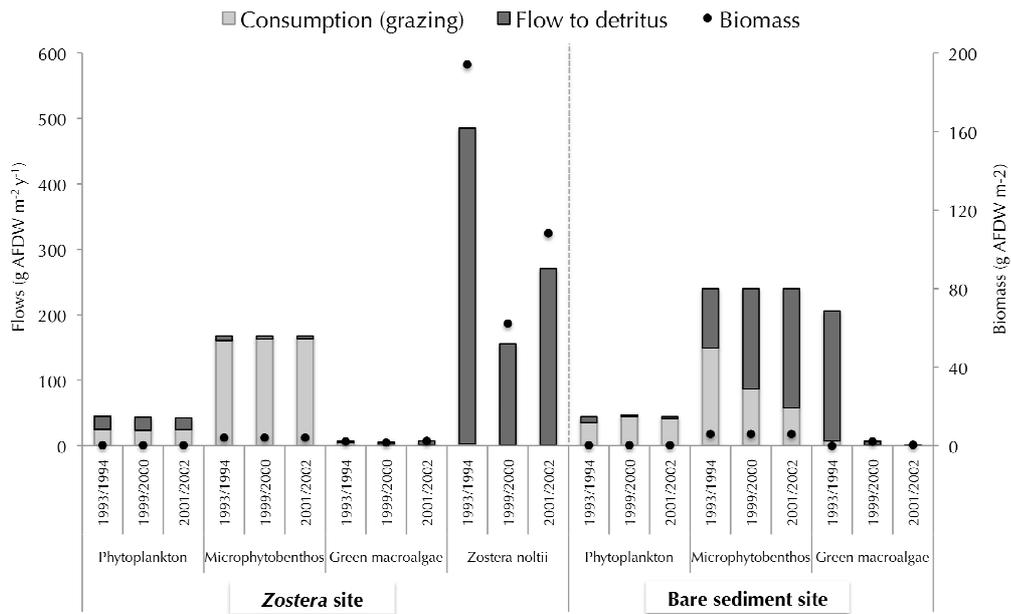


Figure 4. Flows (consumption, flow to detritus, exports and respiration) from each primary producer at the *Zostera* site and bare sediment site in the three time periods (1993/1994, 1999/2000 and 2001/2002).

The annual rate of net primary production showed higher values in 1993/1994 for both sites (Table 2), which clearly reflects the primary production dynamic in each time period (Figure 4). The *Zostera* site had higher production than the bare sediment site, which is consistent with the hypothesis that less impacted systems exhibit higher rates of net primary production (Odum 1969).

3.3. Effects of disturbances on grazers and other consumers

The grazers accounted for most of the consumer biomass (Figure 5). In particular at the *Zostera* site, independently of the time period, *H. ulvae*, *C. edule*, *S. plana*, *L. litorea*, *H. diversicolor* and *C. maenas* represented 99% of the total grazer biomass and approximately 95% of the total consumer biomass (Figure 5A). At the bare sediment site, *S. plana*, *H. diversicolor*, *H. ulvae* and *C. maenas* corresponded to 64-79% of the total consumer biomass (Figure 5B). It is also worth noting that in this habitat the other consumers play a relevant role in the system consumption flows, unlike what was observed in the *Zostera*

meadow community, in which the flows were totally dominated by grazers. In summary, *H. ulvae* is the key species in the macrophyte meadows and *S. plana* plays the dominant role in the bare sediment habitat. Not surprisingly, the consumption, respiration and flow to detritus are dominated by these two species, each in its preferred habitat (Figure 5C-H).

What was the effect of the different types of disturbances (i.e. nutrient enrichment, mitigation measures and centenary flood) on consumers?

At both sites, *H. ulvae* showed the highest biomass, consumption, respiration and flow to detritus during the period of organic enrichment. After preliminary mitigation measures were implemented, the structure and activity indices decreased, and then rose again after another disturbance event, i.e. the centenary flood during the winter of 2001. Patrício et al. (2009) used the Mondego estuary to test the performance and robustness of a set of ecological indicators to highlight the changes in the ecological state of intertidal areas over a period of 17 years (1985–2002). They tested differences over periods characterized by different anthropogenic disturbances. Indices were compared with biological and abiotic descriptors (macroalgae, macrophytes, benthic macrofauna, nutrient concentrations, sediment grain size and total organic carbon). Their results showed that during the period of works and closure of the channel between the 2 arms of the estuary (1993/1994), the abundance of some opportunistic polychaete species of Tubificidae and *H. ulvae* (*Alkmaria romijni*, *Heteromastus filiformis*, *Capitela capitata* and *Chaetozone setose*) was much higher than in the period before the disturbance (1986) and the period following mitigation measures (1998–2002). Our model results are in line with the abovementioned study.

Moreover, at both sites after mitigation measures were implemented there was an increase in *S. plana* and *H. diversicolor* biomass, consumption, respiration and flow to detritus. At the *Zostera* site, these species were apparently not affected by the flood event; however, at the bare sediment site, *S. plana* decreased in biomass and flows after the winter 2001 extreme weather event.

Finally, during the nutrient enrichment period, at the bare sediment site it was clearly visible that the other consumers played a substantial role in the system flows. Their contribution decreased after the mitigation measures then regained importance after a new disturbance event (i.e. the flood).

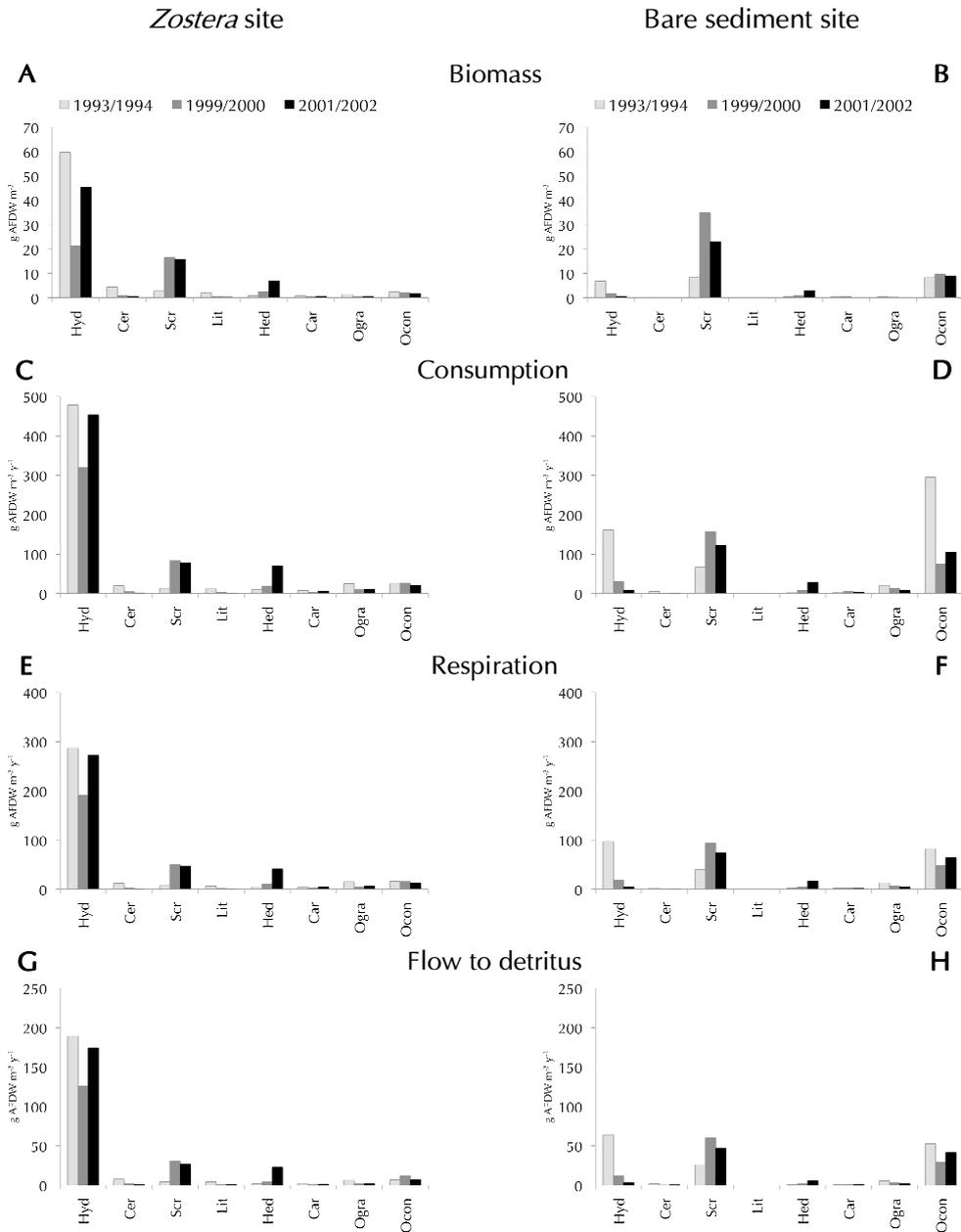


Figure 5. Biomass (A and B), Consumption (C and D), Respiration (E and F) and Flow to detritus (G and H) for *Hydrobia ulvae* (Hyd), *Cerastoderma edule* (Cer), *Scrobicularia plana* (Scr), *Littorina* spp. (Lit), *Hediste diversicolor* (Hed), *Carcinus maenas* (Car), other grazers (Ogra) and other consumers (Ocon) at the *Zostera* site and bare sediment site respectively in the three time periods (1993/1994, 1999/2000 and 2001/2002).

3.4. Can food web analysis have management implications?

In the early and mid 1990s, nutrient enrichment of the system led to macroalgal blooms which depressed the previously dominant macrophyte communities (Marques et al. 2003, Patrício et al. 2009). In 1998, mitigation measures were implemented in order to restore the *Z. noltii* beds and the overall quality of the system. A few species responded positively (e.g. higher biomass of *S. plana* and *H. diversicolor*; lower biomass of *H. ulvae*), which led to more structured and stable populations, closer to the less disturbed conditions observed in the 80s (Patrício et al. 2009). The results of the mass-balanced models show that the trophic structure of the benthic communities of Mondego estuary was affected differently by distinct disturbance events. Interestingly, in this study, a high system throughput seems to be associated with higher stress levels, which contradicts the idea that higher system activity is always a sign of healthier conditions (the period of mitigation measures presented lower TST). This observation should serve as a warning and require special precautions in terms of ecological quality assessment and management. Moreover, knowing that the direct and indirect responses to changes in hydrology, nutrient enrichment, and extreme weather events can be different in different habitats (Cloern 2001), realistic management actions need to be based on a new paradigm that takes the entire system into account. Our results show that some important properties are only revealed at the system level (Jørgensen 2002). Therefore, to take full advantage of the results, further research is needed to link the overall system indices (e.g. biomass/total system throughput, net system production and system omnivory index) with recognized theories of maturity and system development (e.g. Odum 1971).

Acknowledgments

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Appendix B. Model data sources.

Compartments	Parameter	Source
Phytoplankton	Biomass	Pardal (1998), Lillebø et al. (2005)
	C:Chl a ratio	Anderson and Williams (1998)
	<i>P/B</i>	Almeida et al. (2000), this study
Microphytobenthos	Biomass	this study
	C:Chl a ratio	de Jonge (1980)
	<i>P/B</i>	Brotas and Catarino (1995), Serôdio and Catarino (2000)
Green macroalgae	Biomass	Pardal (1998), this study
	<i>P/B</i>	Anibal (1998)
<i>Zostera noltii</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	Sand-Jensen (1975); Pérez-Lloréns and Niell (1993)
Zooplankton	Biomass	Azeiteiro (1999)
	<i>P/B</i>	Rosado-Salórzano and Próo (1998)
	Diet	Azeiteiro (1999), Baeta et al. (2009a)
<i>Ampithoe valida</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	Pardal (1998), Grilo et al. (2009)
<i>Melita palmata</i>	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Pardal et al. (2000), Baeta et al. (2009a)
<i>Corophium multisetosum</i>	Biomass	this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Cunha et al. (2000), Baird et al (2004), Baeta et al. (2009a)
<i>Cerastoderma edule</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	Dolbeth et al. (2003, 2007), this study
<i>Mytilus</i> sp.	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Eklöf et al. (2005), Garcia-Arberas and Rallo (2002), Verdelhos et al. (2005), Baeta et al. (2009a; submitted to ECSS)
<i>Carcinus maenas</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	Dolbeth et al. (2003), this study
<i>Crangon crangon</i>	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Oh et al. (2001), Baeta et al. (2006, 2009a)

<i>Gibulla umbilicalis</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Bode et al. (2006), Baeta et al. (2009a)
<i>Haminoea hydatis</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Malaquias et al. (2004)
<i>Hydrobia ulvae</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	Dolbeth et al. (2007)
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Baeta et al. (2009a; submitted to ECSS)
<i>Littorina littorea</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	Dolbeth et al. (2003), this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Bode et al. (2006), Baeta et al. (2009a)
<i>Cyathura carinata</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	Dolbeth et al. (2007)
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Ferreira et al. (2004), Baeta et al. (2009a)
<i>Idotea chelipes</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Bamber (2004), Baeta et al. (2009a)
<i>Lekanesphaera levii</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Mancinelli et al. (2005), Baeta et al. (2009a)
<i>Alkmaria romijni</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Bamber (2004), Baeta et al. (2009a)

<i>Aonides oxycephala</i>	Biomass	Pardal (1998)
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Dauer et al. (1981)
<i>Capitella capitata</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Gaston and Nasci (1988), Baeta et al. (2009a)
<i>Chaetozone setosa</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Oug et al. (1998)
<i>Diopatra neapolitana</i>	Biomass	Pardal (1998)
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Mangum et al. (1968)
<i>Eteone flava</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Oug et al. (1998), Volkenborn and Reise (2007)
<i>Glycera tridactyla</i>	Biomassa	Pardal (1998), this study
	<i>P/B</i>	Dolbeth et al. (2007), this study
<i>Hediste diversicolor</i>	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Garcia-Arberas and Rallo (2002), Baeta et al. (2009a)
<i>Heteromastus filiformis</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Volkenborn and Reise (2007), Baeta et al. (2009a)
<i>Lagis koreni</i>	Biomass	Pardal (1998)
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Mistri et al. (2001)
<i>Lumbrineris impatiens</i>	Biomass	Pardal (1998)
	<i>P/B</i>	this study

	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Hily et al. (2008)
<i>Mediomastus fragilis</i>	Biomass, <i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Rakocinski et al. 1997
<i>Mista picta</i>	Biomass, <i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Solis-Weiss et al. (2004)
<i>Nephtys hombergii</i>	Biomass	Pardal, 1998, this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Beukema (1991), Volkenborn and Reise (2007), Baeta et al. (2009a)
<i>Streblospio shrubsolii</i>	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
<i>Pygospio elegans</i>	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Mazik and Elliott (2000), Volkenborn and Reise (2007), Baeta et al. (2009a)
Nemertini	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	McDermott and Roe (1985), Thiel and Reise (1993)
Oligochaeta	Biomass	Pardal (1998), this study
	<i>P/B</i>	this study
	<i>P/Q</i>	Hostens and Hamerlynck (1994)
	Diet	Giere (1975)
Detritus	Biomass	Pardal (1998, 2004)
	(O.M in the sediment)	

General Discussion and Conclusions

The overall aim of this dissertation was to investigate the estuarine food web related to the eelgrass *Z. noltii* and quantify the ecological trophic role of benthic macrofauna consumers, in intertidal areas of the Mondego estuary (from 1993 to 2008). This study specifically focused (i) on the incorporation of the nitrogen derived from human activities, considering N uptake as an eutrophication index, and (ii) on the role of the eelgrass on the benthic food web. Six mass-balanced models, integrating diet information from natural stable isotope ratios, were developed to assess the effects of (i) a period of nutrient enrichment, (ii) the implementation of mitigation measures, and (iii) a centenary flood, on the benthic food web properties.

Nitrogen incorporation

Z. noltii may utilize several nitrogen sources, including the N derived from agriculture fertilizers, generally ^{15}N enriched, and store this nitrogen in its tissue; thereby transferring human-derived nitrogen in the food web (Cebrian 1999; Duarte 2002). Consequently, differences in the level of eutrophication between areas characterized by the presence/absence of the eelgrass were expected. This study showed that *Z. noltii* may rely on the nitrogen derived from human activities, since the concentration of nitrogen in the overlying water and the sources of nitrogen used by primary producers were very similar between bare sediment and sediment occupied by *Z. noltii* (2005/2006). In addition, the high nitrogen isotopic signatures of primary producers could indicate that the sources of nitrogen were from human activities (e.g. sewage discharges, agriculture). Indeed, $\delta^{15}\text{N}$ values ranging from +10 to +20‰ in primary producers strongly indicate anthropogenic sources (Kendall 1998). Furthermore, high values of nutrient concentration in the water column were observed in the Mondego estuary, compared to the values from other estuaries (Tomasky et al. submitted for publication). Despite mitigation procedures implemented in the Mondego estuary in 1998, and 2002 (Lillebø et al. 2007), high nitrogen loads are thus still entering the system and the eelgrass habitat is not able to buffer these inputs of nutrients. The measures currently employed seem thus insufficient to ensure high

environment quality. Larger eelgrass meadows could probably sequester part of these nutrients and more environmental protection might be needed to ensure a full recovery of the eelgrass in the system.

Trophic structure as determined by *Zostera noltii*

Z. noltii did not change considerably the trophic structure of the planktonic food web which was in part sustained by particulate organic matter and supported most predator fish. Overall, the benthic food web did not use food derived from the eelgrass. Suspension feeders used particulate and resuspended sediment organic matter, whereas the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the deposit feeders indicated that sometimes benthic microalgae played a key role as food sources. Other consumers also showed a large variability in their isotopic signature suggesting they could shift diet following some environmental changes (example: the detritivore *S. plana*, and five omnivores, *N. cirrosa*, *H. diversicolor*, *C. carinata*, *C. crangon* and *C. maenas*). We therefore hypothesised that these diet shifts could be related to the presence of the eelgrass, not because it is a direct source of food, but because *Z. noltii* meadows create a complex habitat structure due to the presence of rooted macrophytes, and can offer higher protection from predators and high levels of organic matter in the sediment (Duarte 2002). This work indeed showed that there were differences in the diet of some consumers among habitats, following the proximity of the habitat to the *Z. noltii*. Differences occurred especially between well-separated habitats, whereas differences were less clear between the eelgrass meadow and the habitat adjacent to it. We also observed that sometimes these habitat-related differences could occur at different stages of the animal development (*S. plana*), thereby affecting juvenile food uptake and, probably, recruitment.

Additionally, very little seasonal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of producers and consumers was found in the Mondego estuary, despite a marked seasonality in weather and water column related parameters (including nutrient supply and chlorophyll *a* concentration). Macrophytes showed high nitrogen isotopic signatures in July 2006, during a period of elevated temperatures and drought conditions, what may have resulted from seasonal changes in biogeochemical processes, such as denitrification. Also, the increased nitrogen isotope ratios in July for two grazers (*I. chelipes* and *L. levii*) might be the result of the fast turnover rate of isopod populations, since, excepting isopods, the other groups might not feed directly on fresh macroalgae (Bamber, 2004). If indeed global atmospheric

warming increases water temperatures in estuaries such as the Mondego, we can expect a gradually increase in $\delta^{15}\text{N}$ in the producers. $\delta^{15}\text{N}$ values then could therefore be thought of as indirect indicators of warming. This suggests that stable isotope values from macrophytes and selected grazers are useful as tracers of seasonal changes in nitrogen inputs into estuaries, and that those of consumers reflect other factors beyond seasonal variations in N and C sources.

Human-mediated and natural disturbances effects on the benthic food web properties

Human-mediated and natural disturbances such as nutrient enrichment, habitat modification, and flood events resulted in shifts in species composition and abundance that were translated into changes in the food web structure. Compared to the bare sediment area, the *Z. noltii* site, due to its complex community, presented higher number of compartments and higher level of system activity. Probably, the higher number of compartments for both sites during the period of nutrient enrichment can explain the highest Total System Throughput values during this period. Despite disturbance events (nutrient enrichment or flood) and the different number of compartments, *Z. noltii* showed similar System Omnivory Index for all the time periods and always had higher values than the bare sediment site.

During the period of nutrient enrichment, both areas showed higher exports and flows to detritus. At the *Z. noltii* site these differences in the breakdown of throughput were mainly due to the high biomass of the eelgrass, since a big proportion decays to detritus or is washed away from the production area, being used in other systems. At the bare-sediment a large percentage of the primary production (that lead to excessive production of organic matter in the form of green algal blooms) passed to the Detritus compartment. Higher values on the annual rate of net primary production in 1993/94 for both sites clearly reflect the primary production dynamic in each time period. The *Z. noltii* site had higher production than the bare sediment site.

For both sites, during the period of nutrient enrichment, the abundance of some species dramatically increased (e.g. *H. ulvae*). After the implementation of recovery measures a few species responded positively (e.g. higher biomass of *S. plana* and *H. diversicolor*), which led to more structured and stable populations, closer to the less

disturbed conditions observed in the 80s (Patrício et al. 2009). In the absence of macroalgal blooms and anoxic conditions, the organisms have opportunity to grow to larger sizes. Slower-growing species can be accommodated as can those with more complex morphology (Valiela 1995). In other words, K-selected species dominate to the detriment of r-selected species. This probably explains a faster recovery of the biomass of *S. plana* and *H. diversicolor*. Apparently, at the *Z. noltii* site these species were not affected by the flood event, but at the bare sediment site *S. plana* decreased its biomass and flows after the winter 2001 extreme climatic event.

Future Perspectives

This work shaped new challenges and suggested two main interesting avenues that could help to improve the analysis made, namely:

1. The rich benthic community of estuarine sediments thrives in an environment where organic carbon inputs of different origins are diluted with inedible sediment particles. Our knowledge on how the different carbon inputs are partitioned within the benthic community is limited, because of the intractability of the benthic environment, in particular due to difficulties with accessibility and sampling, and high heterogeneity. By combining stable isotope techniques with quantitative modelling approaches it will be possible to gain additional insight in the structure of marine benthic food webs.
2. Regarding the Ecopath models developed in this study, further research is needed to link the overall system indices with recognized theories of maturity and system development. In addition, developing new models that represent the present status of whole system would be essential to clarify some of the trends that we were just able to roughly see considering the study time periods.

Conclusions

The present study showed that the eelgrass *Z. noltii* plays a vital role in the Mondego estuary, by creating a complex habitat structure that offers higher protection from predators and high levels of organic matter in the sediment. Overall, organisms do not use food derived from the eelgrass, but its habitat affects animals by, for example, providing availability of recruits juveniles of many species for macrofauna invertebrates, which led to

more structured and stable populations. This study also showed that the trophic structure of the benthic communities of Mondego estuary was affected differentially by distinct disturbance events. The presence of *Z. noltii* affected the ecosystem productivity and stability under environmental change, by sustaining longer and more complex food webs.

Ongoing change in the environment is inevitable, and it is critically important to have tools to effectively quantify responses in the community. Characterizing predator-prey interactions is a very important component of ecosystem-level studies, because some species will modify their diet in response to environmental change or perturbation. A quantitative understanding of predator-prey dynamics and potential sources of food will better define trophic interactions and food web structure, as well as will help us to better understand ecosystem ecology at a fundamental level.

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Résumé

Cette thèse de Doctorat examine le réseau trophique estuarien lié aux herbiers à zostères, *Zostera noltii* et quantifie le rôle trophique des consommateurs de la macrofaune benthique, dans deux zones intertidales de l'estuaire du Mondego, à différentes périodes de 1993 à 2008. Elle s'intéresse spécifiquement (i) à l'incorporation de l'azote issu des activités humaines, en considérant l'assimilation d'azote comme un indicateur de l'eutrophication et (ii) au rôle des zostères dans le réseau trophique benthique. Six modèles à l'état stable ont été développés afin d'analyser les effets, (i) d'un enrichissement en nutriments, (ii) des mesures de mitigation, et (iii) d'une inondation centennale, sur les propriétés du réseau trophique benthique estuarien.

Il est possible que la zostère, *Z. noltii*, utilise l'azote issue des activités humaines, car la concentration en azote de l'eau dans l'estuaire et des sources utilisées par les producteurs primaires sont similaires entre la zone de sédiment nu et celle occupée par *Z. noltii*. Aucune différence dans le niveau d'eutrophication n'est observée entre les sites (2005/2006). Les fortes valeurs en signature isotopique de l'azote des producteurs primaires pourraient indiquer que les sources d'azote viennent des activités humaines.

La présence de *Z. noltii* ne change pas beaucoup la structure du réseau trophique planctonique, soutenu en partie par la matière organique particulaire et supporte principalement des poissons comme prédateurs. Globalement, le réseau trophique n'utilise pas de nourriture issue de la zostère. Les filtreurs utilisent la matière organique particulaire issue de la remise en suspension du sédiment, tandis que les valeurs de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ des dépositores montrent que les microalgues benthiques jouent parfois un rôle clé comme source de nourriture.

Les autres consommateurs montrent une grande variabilité de signature isotopique, ce qui suggère qu'ils peuvent changer de régime alimentaire en fonction des changements de l'environnement. Ces changements de régime alimentaire sont liés à la présence des herbiers, puisque les différences entre habitats observées chez certains consommateurs sont liées à la proximité de l'habitat, herbier à zostères. Ces différences de régime se rencontrent surtout entre habitats bien séparés, et sont moins marqués entre l'herbier à zostère et la zone adjacente. Parfois, ces différences entre habitats existent pour différents stades de développement (en particulier chez *Scrobicularia plana*), affectant par conséquent la prise de nourriture des juvéniles et probablement le recrutement.

Les $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ des producteurs et consommateurs de l'estuaire du Mondego montrent très peu de variation saisonnière, malgré une saisonnalité météorologique marquée, ainsi qu'une forte variation saisonnière des paramètres de la colonne d'eau (apports en sels nutritifs et concentration en chlorophylle a). Seules les macrophytes et deux brouteurs (*Idotea chelipes* et *Lekanesphaera levii*) montrent des signatures isotopiques de l'azote très hautes en juillet 2006, pendant une période de forte température et de sécheresse. Les forts ratios isotopiques de l'azote rencontrés chez les macrophytes peuvent être liés à des changements saisonniers des processus biogéochimiques, tels que la dénitrification. Pour les deux isotopes présentant de fortes valeurs de $\delta^{15}\text{N}$, il pourrait s'agir d'un taux de renouvellement plus rapide des populations d'isopodes.

Les perturbations liées à l'activité humaine ou naturelles, telles que celles liées aux enrichissements en sels nutritifs, aux modifications d'habitats, ou encore aux inondations, se traduisent par des changements dans la composition spécifique et dans les abondances des espèces présentes. Elles se traduisent ainsi par des changements dans la structure du réseau trophique. Trois modèles à l'état stable ont été développés pour chacun des deux sites d'étude : l'herbier à zostères et la zone de sédiments nus, afin de décrire les changements de propriétés du réseau trophique benthique dans la zone intertidale de l'estuaire du Mondego. Du fait de la forte complexité de sa communauté, l'herbier à zostères présente toujours un plus grand nombre de compartiments et de niveaux trophiques et une plus forte activité totale.

Durant la période d'enrichissement en sels nutritifs, les deux zones modélisées présentent des fortes valeurs d'exportation et de flux alimentant le compartiment des détritiques. Les différences entre les périodes modélisées, se retrouvant pour les deux sites, sont principalement des baisses d'activité liées à une chute de biomasse des producteurs primaires. Les flux de consommation, respiration et de formation de détritiques sont principalement dominés par les brouteurs *Hydrobia ulvae* et *Scrobicularia plana*, dans les sites d'herbiers à zostères et de sédiments nus, respectivement. Dans ces deux sites, les mesures mises en place afin d'améliorer la qualité du milieu, se sont traduites par une augmentation de la biomasse, de la consommation, de la respiration et de la formation de détritiques des espèces *S. plana* et *Hediste diversicolor* et une chute de ces variables chez *H. ulvae*. La population de cette dernière remonte cependant suite à l'épisode de forte crue.

Les modèles à l'état stable montrent ainsi que la structure trophique de la communauté benthique de l'estuaire du Mondego est affectée différemment par chacun des événements particuliers étudiés. Il est intéressant de noter pour finir que, dans notre système d'étude, une forte activité du réseau trophique semble être associée à des conditions correspondant à un système en bonne santé.