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## Insights into seascape ecology: Landscape patterns as drivers in coastal marine ecosystems

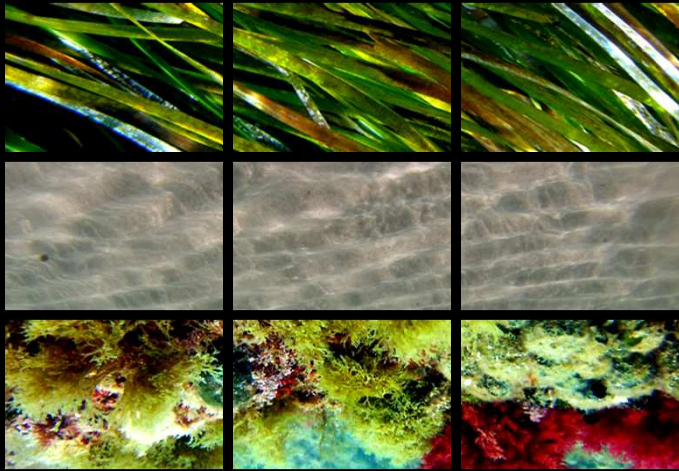
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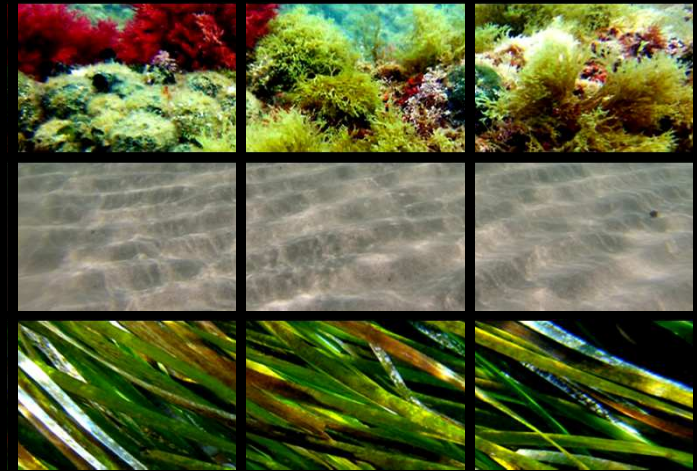
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AM Ricart

Insights into seascape ecology: Landscape patterns as drivers in coastal marine ecosystems

# Insights into seascape ecology: Landscape patterns as drivers in coastal marine ecosystems



Aurora Martínez Ricart

# **Insights into seascape ecology: Landscape patterns as drivers in coastal marine ecosystems**

*Perspectives en l'ecologia del paisatge marí: els patrons del paisatge com a factors de control en els ecosistemes marins costaners*

**Aurora Martínez Ricart**

Ricart AM, 2016. *Insights into seascape ecology: Landscape patterns as drivers in coastal marine ecosystems*. Universitat de Barcelona.

Cover design: Joan Pau Jordà i Aurora Martínez Ricart. Photos: Rocky bottoms with macroalgal cover (from Pol Capdevila), *Posidonia oceanica* seagrass leaves (from Jordi Pagès), Sandy bottoms (from the author).



TESI DOCTORAL



UNIVERSITAT DE  
BARCELONA

DEPARTAMENT D'ECOLOGIA

Programa de Doctorat en Ecologia Fonamental i Aplicada

# **Insights into seascape ecology: Landscape patterns as drivers in coastal marine ecosystems**

Memòria presentada per Aurora Martínez Ricart per optar  
al grau de Doctora per la Universitat de Barcelona

**Aurora Martínez Ricart**

Dept. d'Ecologia, Universitat de Barcelona  
Barcelona, Febrer del 2016

Vist-i-plau dels directors de la tesi:

**Javier Romero Martinengo**

Professor titular  
Dept. d'Ecologia  
Universitat de Barcelona

**Marta Pérez Vallmitjana**

Professora titular  
Dept. d'Ecologia  
Universitat de Barcelona



*A la meva família*



# AGRAÏMENTS

Després de tantíssimes hores d'aigua i d'altres tantes de laboratoris, de despatx, d'aprenentatge, de discussions agosarades, de lectura, d'escriptura i d'anar de bòlid quasi constantment, em trobe davant d'un full en blanc que és, probablement, el que més em costarà d'escriure de tots els que teniu a les mans.

Sou molts els que m'heu anat acompanyant en aquest camí cap a Ítaca. Molts els que m'heu fet evolucionar i créixer. I molts els que m'heu ensenyat i donat exemple de moltes coses que em pense emportar a la butxaca d'aquí en endavant. Us nombri o no, moltes gràcies a tots els que m'heu ajudat a arribar fins aquí.

Aquesta tesi no hagués estat possible sense els meus directors, Javier Romero i Marta Pérez. En primer lloc gràcies per confiar en mi i donar-me la oportunitat de portar-la a terme i, en segon lloc, per tot el suport i seguiment que m'heu donat de forma constant durant tot aquest temps. Formeu un equip fantàstic. Javier, has sigut una font de saviesa i, a part de donar-me un munt d'idees cada cop que entrava tremolant al teu despatx, m'has ensenyat a tenir una visió més crítica i constructiva, a deixar d'escriure telegrams i a trobar el punt mig (la virtut!) entre el generalitzar i l'anar massa al detall (sempre tan difícil de trobar). Marta, la teva practicitat et fa única, gràcies per ajudar-me a ser més estructurada en tot (la salvació dels teus esquemes i dibuixos!), per fer-me pensar i argumentar millor, per ser tan resolutiva i per ser sempre tan propera. Als dos, gràcies per tota la llibertat i empena que m'heu donat per desenvolupar la tesi, per posar-hi seny i per fer-me reduir marxos quan m'accelerava més del que podia assumir.

Una altra part ben important d'aquests agraïments és per la Neus, que quasi hauria de signar a la portada a sota meu per tota la seva ajuda en cadascun dels passos que he donat des de que vaig començar aquesta aventura. Companya de batalla i inestimable amiga. Et pense posar a la butxaca, i te m'emporte sense que te n'adonis allà on vagi. Gràcies per tot i més.

I una altra part ben gran és per tota la gent que hi era i hi és al GEMM. A la UB, a l'Oriol (encara tinc totes les teves fotos a la taula!), la Sílvia, la Izaskun i la Yaiza, recentment aterrada i que ens ha omplert el despatx de riures i globus de colors (no te me vuelvas a romper chiquilla!). Al CEAB, a la Teresa, per tot l'entusiasme que contagia fins i tot als que estem lluny i als *Posidonios*, els sempre somrients Jordi P i

Jordi B, als divertidíssims italians, l'Ale i el Simone i molt en especial al Guillem. I no em vull deixar a tots els estudiants que heu anat passant, sobretot a l'Andreu, la Marine, la Laura i la Júlia, perquè m'heu ensenyat més vosaltres a mi que jo a vosaltres. A tots mil gràcies.

Un dels més culpables de què em decidís a fer un doctorat en ecologia marina i que a dia d'avui escrigui aquestes línies és Fabián A. Rodríguez Zaragoza, professor a la Universitat de Guadalajara, Mèxic. Las campañas salvajes (¡MUY SALVAJES!) en islas remotas, en océanos que solo conocía por los libros, y que me llevaras de la oreja a presentar en mi primer congreso, provocó que quisiera dedicarme a esto de mayor. Gracias por el empujón. Un altre gran culpable és el Jesús Tomás Aguirre, professor de la Universitat de València qui també, entre tortugues i cetacis, sortides en vaixell i boges necròpsies en quiròfans gegants, em va animar a venir a Barcelona a buscar el que realment volia fer. Gràcies també al Bernat Hereu i a la Cristina Linares per acollir-me aquell primer any de màster, descobrir-me tots els secrets de les Illes Medes i fer-me conèixer a tanta i tantíssima gent que ara té i tindrà un pes importantíssim.

I am also very grateful to all the Australian people I have met on the way and introduce me in the world of the *carbon colours*. Thanks to Peter Macreadie for the warmest welcome in my first trip to Australia, for giving me the chance to develop one of the chapters of this thesis and for leading me to ride the east coast by plane, by boat and helicopter! Also to Paul York to watch over the crocodiles and to prepare chocolate during the cool night samplings. I am also very grateful to Paul Lavery for welcoming me in Western Australia and leading me to develop a crazy idea playing with seagrasses and kelps. And thank you very much to all the ECU people who joined me underwater, especially to Fede, Pierre, Flavia, Caitlin, Simone and Marta (¡nena! ¡no más sustos con tiburones!). Moltes gràcies també a l'Òscar i la Karina, el Pere, la Glòria i als peques per acollir-me tan bé a Perth i fer-me sentir com a casa.

A la Clara, Stefano, Maria (i Marcos!), Julia, Gema, Manu, Yolanda, Marta, Marina, Rut i Óscar, *invalorables* i grandíssims companys del màster, amb els que vam començar aquesta etapa (menuda panda!). Tots ben grillats (*cada cual peor*) i fascinats pel mar. En especial a l'Eneko, per anys d'esmorzars impuntuals i berenars a l'hora de sopar, discutint sense avorriment de pràcticament tot (definitivament, ets més cabut que jo!).

No em puc oblidar de tots els companys del doctorat al Departament d'Ecologia (en sou tants que si us nombre no acabem mai!!), per fer que cada dia a la Universitat fos una mica menys pesat, i els dinars ben divertits. Per haver compartit molts cursos,

seminaris, consells i neures i també infinites cerveses i balls de crancs fins a altes hores i tantes activitats multi-aventura els caps de setmana que podríem muntar una agència de viatges (quan ens toqui la loteria clar!). Gràcies per tota l'ajuda que m'esteu donant tots en aquest moment pre-dipòsit. I moltes gràcies també a totes aquelles girafes que em vaig trobant pel passadís i m'ofereixen te i que, de tant en tant, em convencen per deixar l'ordinador i anar a navegar o esquiar.

Gràcies a tots els amics per estar sempre disposats a tot. Els que he anat fent a Barcelona, *fresletes, gossos elegants i regals de reis fins a la sopa*. A la gallega més catalana que conec. Al suport incondicional de la meua companya de pis, que ha aguantat i patit aquesta tesi (i a mi!) més que ningú (ja ho tenim això Txell!!). I a les que heu passat pel pis, Ada i Sílvia, i també l'heu fet casa vostra. Als valencians (sobretot a l'Empar i a la Joana!) que també han deixat *la terreta* per venir a la gran ciutat i em fan sentir a casa. A tots els que he deixat a València i trobe tan a faltar (ni temps ni distància mestres!) i que celebren cadascuna de les meves alegries sigui on sigui. Als que ja no esteu. I a tots els que esteu repartits pel món però sempre ben a prop (menys mal que hi ha la tecnologia!).

A la mare i al pare, per que no hagués arribat ací sense vosaltres! Per la vostra infinita paciència, amor i suport, per fer-me estimar la natura i perquè sé que heu fet un gran esforç per entendre el que estudeie i deixar de mirar el mar amb por. A la meua germana Mar, el *charquito* que ja s'ha fet gran com l'oceà i sempre està per espavilar-me i fer-me riure quan no puc més.

Moltes gràcies a la Montse i el José per les correccions idiomàtiques. Al Juan i el Joan Pau per ajudar-me amb la portada. I al J. per fer-me tan dolç aquest final de tesi i perquè tu també acabaràs a l'aigua!

I gràcies a tu, per bussejar entre aquestes pàgines i a tots aquells inquiets qui, amb una gran mescla de curiositat, ganes de comprendre i motivació, han anat posant les bases per a què els que venim per darrere puguem continuar avançant.

Barcelona 19 de Febrer de 2016

He tingut el suport econòmic d'una Beca FPI del Ministerio de Economía y Competitividad (BES-2011-046849). Part de la meua recerca ha estat finançada pels projectes del Plan Nacional de I+D del Ministerio de Economía y Competitividad (CTM2010-22273- C02-01 i CTM2013-48027-C3-1-R). A més, he disposat de dues beques de mobilitat del Departament d'Ecologia, dues beques de mobilitat de la Unió Europea (Programa COST) i una beca de mobilitat de la World Seagrass Association per assistir a congressos i cursos, i de dues ajudes per estades breus, associades a la beca FPI.



# Directors' report

Dr Javier Romero Martinengo and Dr Marta Pérez Vallmitjana, professors in the Department of Ecology at the University of Barcelona, as supervisors of the Doctoral Thesis presented by Aurora Martínez Ricart entitled “Insights into seascape ecology: Landscape patterns as drivers in coastal marine ecosystems”,

INFORM,

That the research studies developed by Aurora Martínez Ricart for her Doctoral Thesis have been organized in four chapters and one appendix, which correspond to five scientific papers: two already published, one manuscript in revision, and two manuscripts to be sent in the next months.

The list of the published chapters or in revision is shown, indicating the Journal Impact Factor according to SCI of ISI Web of Science:

**Ricart AM**, Dalmau A, Pérez M, Romero J (2015) Effects of landscape configuration on the exchange of materials in seagrass ecosystems. *Mar Ecol Prog Ser* 532:89–100

The impact factor of the Journal *Marine Ecology Progress Series* in 2014 was 2.619. This journal was reported in the First Quartile of Marine and Freshwater Biology, being in the 17<sup>th</sup> position of the 103 journals included.

**Ricart AM**, Pérez M, Romero J (in revision) Landscape configuration modulates carbon storage in seagrass sediments. *Estuar Coast Shelf Sci*

The impact factor of the Journal *Estuarine and Coastal Shelf Science* in 2014 was 2.057. This journal was reported in the Second Quartile of Marine and Freshwater Biology, being in the 27<sup>th</sup> position of the 103 journals included.

**Ricart AM**, York PH, Rasheed MA, Pérez M, Romero J, Bryant C V., Macreadie PI (2015) Variability of sedimentary organic carbon in patchy seagrass landscapes. *Mar Pollut Bull* 100:476–482

The impact factor of the Journal *Marine Pollution Bulletin* in 2014 was 2.991  
This journal was reported in the First Quartile of Marine and Freshwater Biology, being in the 9<sup>th</sup> position of the 103 journals included.

The list of the manuscripts in preparation:

**Ricart AM**, Sanmartí N, Pérez M, Romero J (in preparation) Influence of landscape patterns at multiple scales on coastal fish assemblages

**Ricart AM**, Pitito F, Pagès J, Boada J, Pérez M, Alcoverro T, Sanmartí N, Roca G, Romero J (in preparation) Benthic habitat mapping in the Catalan coast using Side Scan Sonar

And CERTIFY,

That Aurora Martínez Ricart has participated actively on the development of the research and the elaboration associated to each of the papers listed. In particular, her contribution included the following tasks:

- Participation in setting objectives and in the experimental design of each one of the chapters developed.
- Sampling design and field work
- Sample processing and analyses in laboratory.
- Statistical data analyses and interpretation of results.
- Writing, reviewing and editing processes of the manuscripts.

Part of the work presented has been developed in a research stay in the University of Technology Sydney, Australia.

Finally, we certify that the co-authors of the papers that conforms this Doctoral Thesis, will not use any of the manuscripts in other Doctoral Thesis.

Barcelona, 19<sup>th</sup> February 2016

Javier Romero Martinengo

Marta Pérez Vallmitjana



# ABSTRACT

Habitats commonly occur as more or less interconnected patches constituting spatial heterogeneous mosaics. Connectivity and interactions among habitats can determine their functioning. Therefore, ecological patterns and processes within these habitats, and among them, are modulated by the spatial structure of the entire mosaic. Landscape ecology is aimed at understanding how the composition (patch types and sizes) and configuration (how those patches are arranged spatially) of habitats in a mosaic influence the functioning of the overall system and its individual components (the patches and the species moving across), at a panoply of spatial scales. Interactions at landscape level are usually associated either to the movement of organisms (active mobile entities) or to the transfer of materials (passive mobile entities) from one habitat to another. Applying landscape ecology, the interactions among and within ecosystems can be identified and evaluated to optimize conservation efforts across broad spatial scales. This approach, although has been successfully used in terrestrial ecosystems, remains poorly explored in the marine realm. This thesis is an attempt to assess the influence of landscape patterns in the ecological function of marine habitats. The thesis focus on the study of coastal landscapes (or seascapes) due to its resemblance to terrestrial ones, and because they are among the most vulnerable to human action and climate change. With this objective we focus on the study of three main processes of special importance in the coastal systems. First, we focus on the study of landscape patterns influence on fluxes of material among habitats of the coastal seascape and resource-driven processes, such as trophic webs and recycling of nutrients. Secondly, we study how landscape patterns can influence on sediment carbon storage in coastal habitats, particularly in seagrass ecosystems. And in third place we study the influence of landscape patterns in shaping communities. In particular we study the role of landscape patterns in shaping littoral fish assemblages, as due to its mobility they can be strongly affected by the landscape structure and their biology could also play an important role.). Doing this, we attempt not only to improve general knowledge on seascape ecology, but also to provide new insights useful for management. Therefore, this thesis provide new evidence emphasizing the importance of landscape-level interactions and processes in driving coastal ecosystems, and underlining how the relative abundance of habitats and their spatial arrangement influence ecological functions across the coastal ecosystem mosaic.



# RESUM

Al medi natural els hàbitats es troben normalment en forma de taques més o menys connectades, formant mosaics heterogenis. La connectivitat entre hàbitats i les interaccions que tenen lloc entre ells poden ser determinants en el seu funcionament. Per tant, els patrons de distribució d'espècies i els processos ecològics que hi tenen lloc vénen influïts tant per la composició d'aquest mosaic (és a dir, la mida i el tipus de les taques d'hàbitats que s'hi troben), com per la seva configuració (com les taques es disposen espacialment). L'ecologia del paisatge busca esbrinar com s'exerceix aquesta influència. Les interaccions entre els hàbitats s'associen normalment al moviment d'organismes (elements de moviment actiu) o a la transferència de materials (elements de moviment passiu). Entendre aquestes interaccions pot ajudar a millorar el coneixement de la dinàmica ecològica dels ecosistemes implicats, i a la vegada optimitzar els esforços de conservació que s'hi esmercen. Les aproximacions basades en aquests conceptes i, en general, en l'ecologia del paisatge, tot i que s'ha desenvolupat molt als sistemes terrestres, encara resten per explorar al medi marí. Aquesta tesi pretén contribuir a atenuar aquest biaix, tot avaluant la influència dels patrons del paisatge en la funció ecològica dels hàbitats marins. La tesi es centra en l'estudi dels paisatges marins costaners, per la seva semblança amb els paisatges terrestres i per la seva vulnerabilitat a l'acció humana i al canvi climàtic. Amb aquest objectiu, ens centrem en l'estudi de tres grans processos d'especial importància en els sistemes costaners. En primer lloc, estudiem com els patrons del paisatge marí costaner poden influir sobre els fluxos de materials entre hàbitats i les conseqüències que se'n deriven per processos ecològics clau, com el funcionament de les xarxes tròfiques i el reciclatge de nutrients. En segon lloc estudiem com els patrons del paisatge poden influir en l'emmagatzemament de carboni orgànic en sediments marins, concretament el carboni que queda retingut al sediment de praderes d'angiospermes marines. En tercer lloc analitzem com, o fins a quin punt, els patrons del paisatge expliquen l'estructuració de les comunitats d'organismes, tot aplicant una aproximació multiescala. Concretament, estudiem la comunitat de peixos litorals, espècies mòbils que es poden veure afectades per aquests patrons de maneres diverses i on la biologia de cada espècie pot jugar un paper molt important. Aquesta tesi pretén no només aprofundir en el coneixement que es té de l'ecologia del paisatge marí, sinó també proveir de noves aproximacions i eines que millorin la gestió i conservació dels hàbitats marins costaners. Així doncs, la tesi aporta noves evidències que emfatitzen la importància de processos i interaccions a escala de paisatge (quan més d'un hàbitat entra en joc), destacant que, l'abundància dels

diferents hàbitats i la seva disposició espacial tenen un fort paper en el funcionament i l'ecologia dels hàbitats marins.



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# CHAPTER 1

## General Introduction



Photo: Patch of *Posidonia oceanica* in a rocky bottom with macroalgal cover, from Jordi Boada.

## A conceptual approach to landscape ecology

Heterogeneity and scale are core tenets of the ecological sciences. Heterogeneity, understood as the non-uniformity in a system property in space and/or time, is intrinsic to most ecological systems and to nature in general. Although initially disregarded by some ecologists in order to simplify theories, spatial heterogeneity is now recognized as a central driver to many ecological processes (Pickett & Cadenasso 1995). How spatial heterogeneity is perceived strongly depends on the particular scale of study at which it is assessed (Levin 1992, Schneider 2001). Yet, spatial heterogeneity acts in ecological systems at various scales, often influencing important functions, ranging from population structure through community composition to ecosystem processes (Pickett & Cadenasso 1995, Thornton et al. 2011). Landscape ecology, which regards spatial patterns as main drivers in ecological systems, tries to understand the influence of both spatial heterogeneity and scale in ecological functions (Levin 1992, Turner 2005, Wu 2007, Thornton et al. 2011).

While the perception of phenomena or patterns at the human eye scale can be based on direct observation, the approach to both smaller and larger scales needs the help of technology. This is maybe the cause of a limited attention to landscape-scale processes until a relatively recent date (Odum 1953). Although the term *landscape ecology* was first coined in the late 30's (Troll 1939), this discipline emerged strongly in the 80's, arising from geography and the study of vegetation, when spatial data and analytical methods became more widely available (Turner 2005, Hinchey et al. 2008). The appearance of aerial photographs, taken vertically from great altitudes, enabled the inventory and distribution assessment of landscape elements (e.g. ecosystems, habitats, species assemblages), and provided the basis for the development of landscape ecology (Troll 1970).

Many definitions of the term landscape have been proposed, depending on the research or management context. For example, Forman and Godron (1986) defined landscape as a heterogeneous area composed of a cluster of interacting ecosystems that is repeated in similar form throughout. Turner et al. (2005) defined the landscape as a spatially heterogeneous area in at least one factor of interest, while for Dunning et al.

(1992) landscape was an area containing a mosaic of habitat patches, often within which a particular "focal" or "target" habitat is embedded. As it can be seen, all of these definitions are, to a greater or lesser extent, based on the concept of spatial heterogeneity at scales higher than the habitat (or community).

The relevance of the concept of scale clearly appears when realizing the existence of the two distinct approaches that have evolved within landscape ecology (Pickett & Cadenasso 1995). One is based on the anthropogenic perspective, used commonly in land planning, which considers a landscape as a specific area perceived at human scales (i.e. kilometres or tens of kilometres), where the smallest unit of the landscape is relatively homogeneous in its biological-ecological content (Troll 1970). The other is the organism-centred perspective, where there is no absolute size for a landscape, and where the landscape is defined relative to a particular organism's perception and scaling of the environment, according to its needs or its movement capacity (Wiens 1976). The size of a landscape varies thus depending on what constitutes a mosaic of habitats or resource patches meaningful to a particular organism (McGarigal & Cushman 2002). In fact, the former perspective is only a particular case (for a given species) of the later.

Landscape patterns result from complex interactions among natural biotic and abiotic factors, as well as with anthropogenic pressures. The activity of ecosystem engineers such as trees or corals, or processes such as herbivory, among biotic factors, and climate conditions or hydrodynamics, among abiotic ones, should be mentioned as key drivers of landscape patterns. To these, the ways in which humans use the landscape, or have used it historically, should be added. For instance, historical land use (e.g. agriculture, urbanization) has modified landscape patterns, and current characteristics of most ecosystems are a legacy of that use (Riitters et al. 2002) (Fig. 1.1). Landscape patterns could be defined by three main aspects or properties: composition (what habitats are present and how much surface they occupy), configuration (how those habitats are arranged spatially) and connectivity (Turner 2005, Grober-Dunsmore et al. 2009). Connectivity, used here in both structural and functional senses, is an emergent property of the landscape, and refers, respectively, to the proximity of the physical structures in the landscape, and to the degree to which



the landscape allows the movement of materials, energy and organisms between its different elements (Crooks & Sanjayan 2006, Grober-Dunsmore et al. 2009, Sheaves 2009).



Fig. 1.1 Agricultural landscape of Kansas, US. From Image USDA Farm Service Agency.

Disturbances, either natural- or human-induced, play also a major role in shaping landscape patterns and properties. Their importance depends on their spatial extent, their persistence and their return time. Disturbances can create patchy landscapes, by inducing fragmentation, which is considered a landscape-level process in which a continuous habitat is progressively sub-divided into smaller, geometrically more complex and more isolated habitat fragments or patches (McGarigal & McComb 1995). Fragmentation reduces functional connectivity among patches of the same nature, and it can also stimulate the interactions among different habitat types by increasing edge abundances. Fragmentation processes have been usually associated to negative effects on species abundance and diversity, and on ecosystem resilience, as they imply habitat loss (Bender et al. 1998). However, some authors have reported positive effects, such as a biodiversity increase (see review of Fahrig 2003). In any case, the ecological mechanisms and effects of habitat fragmentation, especially at large scales and in the long term, remain still poorly understood (McGarigal &

Cushman 2002). On the other hand, patchy landscapes can also be the result of a natural processes of habitat expansion or coalescence (Cebrián et al. 2000). A common characteristic of both fragmented and patchy landscapes is to present high amount of edges per unit of habitat area. Yet, habitat edges constitute important ecological transitional gradients (ecotones) or boundaries that can influence biotic or abiotic processes (Puth & Wilson 2001), as widely investigated in both terrestrial (see review of Murcia 1995) and, to a lesser extent, marine environments (Smith et al. 2008, Macreadie et al. 2010, Pagès et al. 2014).

Landscape attributes related to composition, configuration and connectivity, as well as fragmentation processes, patchiness and edge effects have been extensively investigated (e.g. Hovel 2003, Solé et al. 2004, Jha et al. 2005, Deza & Anderson 2010). However, in spite of these research efforts explaining their structural and functional consequences on ecosystems and predicting future scenarios of landscape patterns in changing environments remain surprisingly difficult (Turner 2005).

### The study of landscape patterns

Landscape ecologists tend to view the landscape as a hierarchy of elements (Fig. 1.2) nested at different scales, from broader to smaller: (i) landscape level, (ii) habitat or ecosystem level and (iii) patch level (Pittman et al. 2004). Different models are used for heuristic purposes in landscape ecology, which help to identify and explore these levels (Fig. 1.3). The patch matrix model, derived from the theory of island biogeography (MacArthur & Wilson 1967), uses a binary classification where the landscape is represented as an homogeneous matrix with patches of a focal habitat viewed as 'islands' embedded in (Boström et al. 2011). In contrast, the patch mosaic model understands the landscape as a collection of different habitat patch types, where the interactions of the parts influence the ecological function of the whole (Wiens et al. 1993). In the patch mosaic model, a homogeneous matrix does not exist and the composition and spatial configuration of the mosaic are of central importance (Turner 2005). More recently, a third model approaching landscape as a continuous gradient, lacking discrete boundaries among landscape elements, has been proposed (Frazier & Wang 2013).

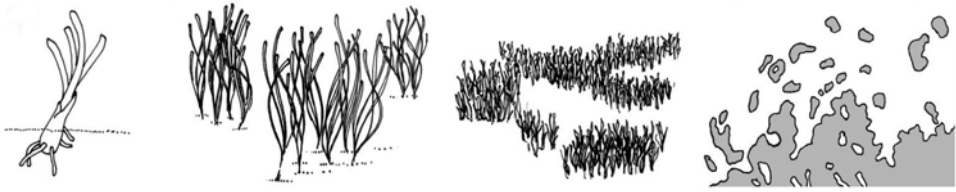


Fig. 1.2 Representation of the hierarchical structure of a seagrass habitat in the marine coastal seascape, modified from Boström et al. (2011). From left to right seagrasses viewed at increasing scales from centimetres to kilometres.

These models allow quantitative descriptions of landscape patterns, which are mostly based on the wide availability of classified habitat maps (or on the availability of tools to obtain such maps, for instance aerial photographs, satellite imagery and hydroacoustic methods, among others). This information, combined with advances in Geographic Information Systems (GIS) and spatial statistics, which are routinely used tools in this field (Grober-Dunsmore et al. 2009), allow computing indexes (metrics) aimed at expressing different spatial properties of the landscape (Fig. 1.3). A high amount of metrics have been developed to this end, and incorporated to several software GIS-based packages. However, the use of landscape metrics has several shortcomings. First, no single metric can adequately capture all the complexity of a given landscape, and the statistical properties and behaviour of many metrics remain poorly understood (Turner 2005). Moreover, many of them are sensitive to changes in the spatial resolution of the data extracted from maps, and can be correlated (Riitters et al. 1995). Despite these limitations, landscape metrics are widely applied, being advisable the use of a group of uncorrelated metrics to optimally embrace the complexity of a landscape (Cushman et al. 2008). As ecological properties may not respond to a unique scale, and landscape extent is often defined depending on biological traits (e.g. movement capacity) of the species of interest, it is relatively common to evaluate these metrics at different spatial scales. Multi-scale approaches allow a comprehensive analysis of the interaction of factors across scales and facilitates more comprehensive ecological interpretations (Cushman & McGarigal 2002). Landscape ecology provides a well developed conceptual and operational framework

to address complex multi-scale questions regarding the influence of spatial heterogeneity on ecological patterns and processes (Grober-Dunsmore et al. 2009).

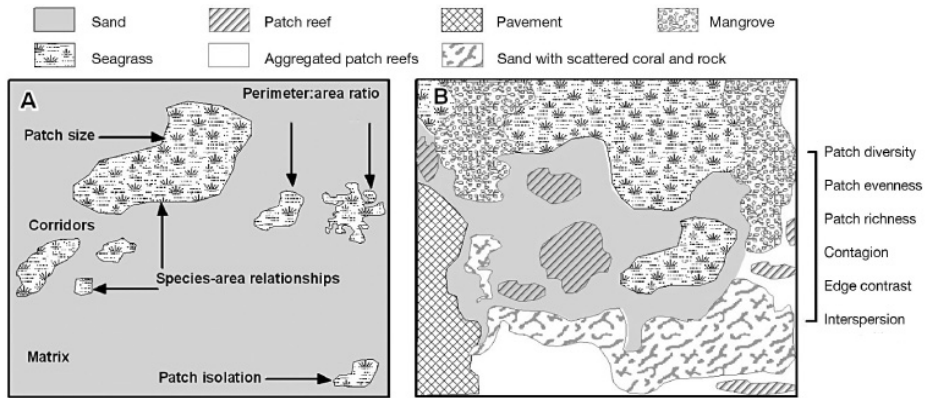


Fig. 1.3 Examples of the models and metrics used to represent and characterize the landscape, modified from Wedding et al. 2011. (a) Patch matrix model, with focal seagrass patches embedded in a homogeneous matrix of sand; (b) Patch mosaic model, with several habitats in a complex spatially and compositionally landscape.

## Landscape ecology in marine systems: the new-comer

Landscape ecology is traditionally associated to terrestrial systems (Hinchey et al. 2008, Jelinski 2014). This explains why most definitions of the term landscape are framed into a terrestrial context, and in fact, the term itself (at least in English) suggests etymologically (“land”) such terrestrial context (Jelinski 2014).

The physical limitations on our ability of observation and measurement underwater represents a handicap for ecological research on the marine realm, relative to that on terrestrial systems (Pen et al. 2005). Thus, while terrestrial ecologists can study nature in real time or space, the logistic challenges that marine media presents make its study extremely difficult, and often mediated by observation technologies. This handicap is even more evident when referring to large spatial scales such as the landscape. Despite these difficulties, in the last years studies adapting concepts and tools of landscape ecology to answer ecological questions within marine systems have increasingly appeared (e.g. Hovel et al. 2002, Grober-Dunsmore et al. 2008, Pagès et al. 2014).

Nevertheless, there is still a huge gap in relation to landscape ecology on land and landscape ecology underwater (probably better called *seascape ecology*), to the extent that we still do not know if the patterns and principles observed in the former apply in the same way to the latter (Wedding et al. 2011).

Most of these studies have been developed in coastal areas, with few examples in the pelagic domain, probably because coastal ecosystems have structural characteristics similar to the ones in land and are more accessible than the open ocean (Jelinski 2014). Coastal ecosystems embrace wetland, estuarine and marine habitats in the interface between terrestrial systems and the open sea. Their characteristic time and spatial scales, aside with many other abiotic and biotic features, largely differ from those found on land (Steele 1989). Thus, and unlike many terrestrial landscapes, coastal seascapes consist on a mosaic of habitat patches settled in relatively small, shallow and highly dynamic areas, often with complex spatial patterns and a high degree of connectivity among them (Grober-Dunsmore et al. 2009, Sheaves 2009, Boström et al. 2011, Wedding et al. 2011).

Coastal areas are hotspots of productivity and concentrate in a small proportion of the ocean a large part of marine biodiversity, playing a key role not only in local ecological and biogeochemical processes, but also in global cycles. For this reason, it is a matter of big concern the fact that coastal ecosystems are strongly threatened worldwide due to the direct and indirect effects of human actions, including the consequences of climate change. Among the plethora of human-induced changes, it is worth noting that human impacts have altered landscape patterns (Short & Wyllie-Echeverria 1996, Waycott et al. 2009). Therefore, it seems urgent the incorporation of a landscape perspective to develop not only a proper assessment of human impacts, but also to implement adequate and effective management and conservation strategies (Turner et al. 1999).

## **Ecological processes in the coastal seascape**

A wide array of ecological processes in coastal waters are influenced by the spatial patterning of the coastal seascape, and are based on phenomena operating at multiple

and nested spatial and temporal scales (Sheaves 2009). Among them, fluxes or linkages through landscape elements, carbon sequestration capacities and structure of coastal communities appear among the most relevant ones.

### **Fluxes among elements of the coastal seascape: the importance of landscape patterns on nutrient cycling and trophic webs**

Coastal areas contain highly productive ecosystems, such as mangroves, kelp forests, seagrasses and coral reefs. Their production not only fuels local food webs, but a large part of it is exported to a range of marine (and even terrestrial) areas (Mumby et al. 2004, Wernberg et al. 2006, Heck et al. 2008, Hyndes et al. 2014). These fluxes from donor to recipient habitats allow an asymmetrical energy flow that is important for the long term sustainability of ecosystems (Polis et al. 1997, Reiners & Driese 2003, Turner 2005) and for the overall coastal seascape (Marczak et al. 2007, Mellbrand et al. 2011, Hyndes et al. 2012). The spatial arrangement of donor and recipient habitats, their relative abundances in terms of surfaces and the permeability of edges constrain these fluxes, with important effects to all levels of ecological organization (Puth & Wilson 2001). It has been shown that a spatially explicit and quantitative examination of these fluxes is key to improve our understanding of how connectivity among habitats influences food web structure (Crooks & Sanjayan 2006), and, consequently, how a potential modification of landscape patterns can have effects across the whole coastal system. However, fluxes among habitats of the coastal seascape and how they are controlled at large spatial scales have been scarcely studied (Wernberg et al. 2006, Hyndes et al. 2012).

### **Coastal ecosystems as part of the global carbon budgets: carbon sequestration within the coastal seascape modulated by landscape patterns**

Some of the most productive coastal habitats, such as mangroves, saltmarshes and seagrass meadows, have recently been acknowledged for their large and long-term carbon storage potential. The basis for this is the organic carbon buried in the sediments (Mcleod et al. 2011, Pendleton et al. 2012, Duarte et al. 2013). Their high carbon sequestration rates highlight the important role that coastal ecosystems play as

natural carbon sinks (Macreadie et al. 2014), to the point that these habitats have been included in the IPCC 2013 Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Moreover, the Fifth Assessment Report of IPCC (AR5) (2014) includes for the first time mangrove conservation and replanting as an ecosystem-based measure to mitigate climate change. This should foster the research on carbon budgets in those habitats (see Macreadie et al. 2014, for seagrass meadows). Issues that need clarification, at this respect, include a proper quantification of carbon inputs and remineralization in sediments, the harmonisation of methodologies based on firm standards and the understanding of the sources of variability in carbon storage, as this can vary within and across species, over time and environmental conditions (see, again for seagrasses, Lavery et al. 2013).

Organic carbon produced in coastal vegetated ecosystems can be exchanged throughout the food chain, transported to other marine or terrestrial habitats, deposited into the ocean's deeper layers or stored in the sediments (Hyndes et al. 2014). The origin, transfer and fate of this carbon may be influenced by large scale processes and the spatial heterogeneity of the habitats concerned. The extent to which carbon related processes are influenced by landscape patterns is probably a major contribution from the field of seascape ecology to the study of biosphere resilience to global change.

### **Species-environment relationships within the coastal seascape: the role of landscape patterns in shaping communities at multiple scales**

Mobile organisms can be strongly influenced by landscape patterns. Landscape structure favours/impedes movement, enhances/deters resource search and modifies habitat use, and, therefore, plays a key role in determining the abundance and distribution of mobile species (Meyer et al. 2010). Movement capacity (e.g. maximum distance, range of achievable speeds) is highly variable among species, resulting in a variability of home-ranges from few cm to thousands of kilometres (Chittaro 2004). Consequently, landscape patterns influence on mobile species will be largely scale-dependent, and the empirical results about those influences will

depend on the scale used in the experimental approach (Cushman & McGarigal 2004, Turner 2005).

Fishes, as mobile and conspicuous components of coastal ecosystems, have been typically used as model taxa to assess the influence of the environment on species assemblages. While fish-environment relationships have been widely studied in marine ecology, particularly at the habitat-level, the use of a landscape spatially explicit approach remains less explored. Existing studies linking fish assemblages and landscape patterns (Pittman et al. 2007, Grober-Dunsmore et al. 2007, 2008, Gullström et al. 2011, Yeager et al. 2011 among others) have been done, for the most, in coral reefs, while temperate areas have received less attention (Jackson et al. 2006). Most of these studies have used multi-scale approaches, as organisms may not respond to processes occurring at a unique scale, and the same environmental predictor or landscape attribute can present different behaviour depending on the scale of observation. The recent appearance of multilevel models in landscape studies, sometimes referred to as hierarchical modelling (Finch et al. 2014), seems a promising direction for research on this topic.

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Overall, landscape ecology holds great promise on the development of hypothesis and theories at broad scales, with also potential benefits for management, planning and conservation of natural resources (Grober-Dunsmore et al. 2009). For these reasons, it seems urgent to increase efforts to build a solid basis on which to develop the conceptual framework of seascape ecology.



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The background of the slide is an underwater photograph. The top portion shows clear, greenish-blue water. The bottom portion shows a sandy seabed with distinct, wavy sand ripples. A black rectangular box is overlaid on the upper part of the image, containing the chapter title and subtitle in white text.

# CHAPTER 2

Objectives and study systems

Photo: Sandy bottoms, from the author.



## Objectives

This thesis is an attempt to assess the influence of landscape patterns in the ecological function of coastal seascapes. Doing this, we attempt not only to improve general knowledge on seascape ecology, but also to provide new insights useful for management. Under this general objective, the research presented here focus on several important ecological patterns and processes in coastal waters: transfer of resources and trophic interactions (Chapter 3), carbon storage (Chapter 4 and 5), and structure of communities (Chapter 6).

The work presented in this thesis is mainly developed in field experiments. Conceptually, throughout the thesis distinct model approaches are used, which are the patch matrix model (Chapter 3, 4 and 5) and the patch mosaic model (Chapter 6). Different landscape attributes are evaluated, such as patch size and matrix type (Chapter 3 and 4), edge distance (Chapter 5) and landscape composition and configuration (Chapter 6), and their effects on selected processes and patterns assessed. The specific objectives are the following:

I. Assessing the effect of landscape patterns in mediating fluxes and resource-driven processes

The main objective is to assess the effect of landscape patterns on the exchange of materials among habitats in the coastal seascape and to evaluate to which extent this exchange could affect the associated food webs. Given the importance of detritus on matter and energy transfer in benthic vegetated habitats, we quantify detritus stocks in different seagrass landscape configurations, differing mostly in patch size and matrix type, as well as its potential impact in detritivore food chains (Chapter 3).

II. Assessing the effect of landscape patterns in mediating carbon storage in seagrass sediments

The main objective is to assess how landscape patterns affect carbon storage variability in seagrass sediments. First, we study the variability in sediment carbon content and its sources among seagrass meadows, as a function of the landscape configuration, differing mostly in seagrass patch size and matrix type where seagrasses are embedded

in (Chapter 4). Second, we assess the variability in sediment carbon content and its sources within the meadow, as a function of the distance to the boundary (edge) between the seagrass habitat and bare sediments (Chapter 5).

### III. Assessing the effect of landscape patterns in species assemblages

The main goal is to evaluate to which extent communities of fishes in coastal areas depend on landscape patterns and how this relation varies as a function of the spatial scale of assessment. For that purpose, we quantify several landscape metrics using a multi-scale approach, and assess their influence in different features of the fish assemblages structure (Chapter 6).

## Study systems

The work presented in this thesis is developed in temperate (Chapters 3, 4 and 6) and sub-tropical areas (Chapter 5), where seagrasses represent important elements of the coastal seascape.

The temperate area is situated in the NW Mediterranean, where coastal seascapes are mainly characterized by the presence of seagrasses, macroalgal communities on rocky reefs and sandy bottoms. Among the four seagrass species known for this Mediterranean area (Procaccini et al. 2003) we work with the endemic and long-lived species *Posidonia oceanica*, which is commonly found in different landscape configurations (i.e. continuous and patchy meadows interspersed with other habitats) (Gera et al. 2013, Pagès et al. 2014). Details are provided in the specific section of each chapter.

The sub-tropical area is situated in the NE Pacific coast of Australia, where in general, coastal seascapes found are highly diverse (e.g. coral reefs, mangroves, seagrasses and rocky reefs) (Coles et al. 2003). We work in a macro-tidal estuary with presence of the seagrass *Zostera muelleri* in most of its intertidal areas, forming a mosaic of large *Z. muelleri* seagrass patches interspersed with naturally occurring unvegetated (bare) sediments. More details are provided in the specific section of the chapter.

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An underwater photograph showing a sandy seabed with several clumps of dead or decaying seagrass. The seagrass blades are long, thin, and translucent, some showing a brownish or darkened color, indicating they are dead or decomposing. The sand is light-colored and fine-grained. The lighting is natural, coming from above, creating soft shadows.

# CHAPTER 3

Effects of landscape configuration on the exchange  
of materials in seagrass ecosystems

Photo: Holothurians covered by detrital seagrass leaves in sandy bottoms, from the author.

## Abstract<sup>1</sup>

Landscape (or seascape) attributes play an important role in modulating the flow rates of materials between habitats in the coastal marine environment. Seagrass meadows are known to both export and import organic matter, thus, establishing links with other habitats. Most of those links remain unexplored, and little is known about the relevance of landscape configuration on these flows. We studied the relationships between landscape configuration (continuous meadows, patchy meadows in a sand matrix and patchy meadows in a rock matrix) and the exchange and accumulation of detrital material. Moreover, we evaluated the impact of landscape configuration on (i) plant nutrient content and (ii) the diet of a model deposit feeder (holothurians). We determined detritus stocks in seagrass meadows as well as the carbon and nitrogen elemental and isotopic composition of plants, detritus and other food sources (e.g. suspended organic matter). Based on this, we identified, by applying mixing models, the different contributions of these sources to the diets of deposit feeders. Our results showed that landscape configuration influences the exchange of materials across the coastal seascape. Less accumulation of detrital seagrass leaves was found in patchy meadows, although no effects were found for allochthonous materials. In addition, patchy seagrass meadows showed significantly lower nitrogen concentrations in leaves compared to continuous meadows. Landscape configuration had no effect on the diet of the deposit feeder studied. These findings highlight the importance of landscape-level processes in coastal waters and specifically warn of the possible effects of changes in meadow size on seagrass performance.

<sup>1</sup>See original publication in Appendix 3

**Ricart** AM, Dalmau A, Pérez M, Romero J (2015) *Effects of landscape configuration on the exchange of materials in seagrass ecosystems*. Marine Ecology Progress Series 532:89–100





## Introduction

Landscape ecology has made important contributions to our understanding of ecosystem dynamics (Pittman et al. 2011). The view that spatial properties are critical for functional and evolutionary aspects of the ecosystems is a central tenet of terrestrial ecology, and concepts from landscape ecology are increasingly being applied to the marine environment. Among them, the ecological consequences of broad-scale spatial heterogeneity are receiving increasing attention (Grober-Dunsmore et al. 2007, Hinchey et al. 2008, Boström et al. 2011).

Underwater marine landscapes, or seascapes, and specifically those in the coastal zone, usually consist of an intermingled set of habitat patches that are settled in relatively small areas and often exhibit complex spatial patterns. Overall ecosystem functions in coastal seascapes and the delivery of associated ecosystem services are dependent not only on the intrinsic properties of individual habitat patches but also on the spatial configuration and functional links between the patches and their properties (Grober-Dunsmore et al. 2008, Pittman et al. 2011, Hyndes et al. 2014).

Habitat patches are linked by a range of mechanisms that function on broad spatial and temporal scales (Sheaves 2009). Habitat boundaries are usually crossed by organisms and materials that can carry energy, matter or information. The degree to which a given landscape facilitates such flows is called connectivity (Taylor 1993). Cross-habitat linkages allow asymmetrical energy flows (Valiela et al. 2001) that can have important ecological consequences, especially by increasing productivity in the recipient area (Marczak et al. 2007, Heck et al. 2008, Mellbrand et al. 2011, Hyndes et al. 2012), which has been called a spatial subsidy (Polis et al. 1997). Habitat linkages depend on the nature and spatial arrangement of the habitats and the general setting of the physical environment. Landscape attributes such as the perimeter of focal habitats, the distance between habitats or the matrix within which they are embedded modulate such fluxes (Polis et al. 1997, Bellido et al. 2008, Pagès et al. 2014). Thus, landscape composition and configuration will influence ecological connectivity (Mumby 2006, Grober-Dunsmore et al. 2008).

It is generally accepted that coastal ecosystems are experiencing an unprecedented set of alterations due to human pressures (Halpern et al. 2008). Most of the effects of these changes have been studied in depth, at levels ranging from the sub-individual (biochemical, physiological) through the individual and population to the community and ecosystem levels (Worm et al. 2006, Pérez et al. 2007, Vergés et al. 2014, Hyndes et al. 2014). However, much less attention has been paid to the effects of such changes on the spatial structure at the large scale; that is, to the modification of the composition and configuration of coastal seascapes resulting from human activities.

Valuable habitats in the coastal ecosystem mosaic are losing cover and becoming increasingly fragmented (Macreadie et al. 2009). Fragmentation is a modification of the landscape configuration that reduces continuous ranges of habitat to small, isolated patches and can drastically change habitat structural complexity (Montefalcone et al. 2010). Habitat fragmentation increases the patchiness, which in turn causes a dramatic reduction in connectivity within habitats (Bender et al. 1998, Hovel et al. 2002) and can seriously modify the way organisms use the seascape (Boström et al. 2011, Gera et al. 2013). Changes in landscape configuration caused by human action can disrupt the natural direction or magnitude of matter and energy flows between habitats, with consequences for trophic food webs and ecosystem productivity (Howe & Simenstad 2011).

Common habitats in the coastal seascape include rocky reefs dominated by different species of macroalgae, seagrass beds, and unvegetated sedimentary bottoms dominated by infauna as well as mangroves and coral reefs, among others. Different linkages have been explored between such habitats and their importance for the functioning of the overall system assessed (Wernberg et al. 2006, Howe & Simenstad 2011, Hyndes et al. 2014, Pagès et al. 2014). However, despite these recent efforts, the effects of landscape configuration and its ecological significance for the linkages between habitats within the coastal seascape are still poorly understood.

While coral reefs and mangroves in tropical areas seem to play a central role in ecosystem functioning, seagrass meadows constitute one of the most important structural and productive habitats in coastal waters worldwide (Hemminga & Duarte

2000). Seagrass meadows are linked to other coastal habitats through multiple processes, both as subsidizer and as subsidized habitat. Seagrasses play an important nursery role (Boström et al. 2011) and can play an important role in the ontogenetic migrations of many organisms (Mumby et al. 2004). Together, primary and derived secondary seagrass production represents an important trophic subsidy for several coastal habitats (Heck et al. 2008) and even for deep sea (Vetter & Dayton 1998) and terrestrial systems (Mellbrand et al. 2011). Seagrass meadows are in turn subsidized by other habitats, such as rocky reefs with macroalgae (Hyndes et al. 2012), which are frequently detached during periods of high wave action and transported passively to seagrass habitats (Wernberg et al. 2006). Moreover, they also induce deposition of particles suspended in the water column (Gacia et al. 1999, Kennedy et al. 2010), thus stretching the benthopelagic links. All of these imported allochthonous materials have the potential to enhance primary seagrass productivity by supplying nutrients but also to feed trophic webs by increasing food availability to consumers. Among consumers, deposit feeders can benefit greatly, as allochthonous sources are generally more palatable and easily assimilated and therefore preferred over seagrass as a food resource (Olsen et al. 2011, Poore & Gallagher 2013).

Seagrasses can grow either as large, continuous meadows or in the form of patches of different shapes and sizes interspersed with unvegetated sand or rocky habitats with macroalgal cover (Robbins & Bell 1994, Jackson et al. 2006), with the latter especially common in shallow waters. These patterns are driven by natural biotic (e.g. growth rate and the expansion of rhizomes) and abiotic (e.g. hydrodynamics) factors (Fonseca & Bell 1998, Hovel 2003, Mills & Berkenbusch 2009), to which anthropogenic forcing (e.g. eutrophication or physical removal) is added (Short & Wyllie-Echeverria 1996, Duarte 2002, Boström et al. 2006). Therefore, these ecosystems (and the seascapes they are embedded in) are excellent models to explore and test hypotheses about the influence of landscape attributes on ecosystem processes.

The fate of the high production of seagrass meadows has been widely studied (Mateo & Romero 1997, Cebrián et al. 2000, Pérez et al. 2001). Aboveground production (mostly leaves) can either enter the food web directly through leaf grazing or temporarily accumulate as leaf litter and then decompose or be exported (Romero et al.

1992, Cebrián et al. 1997). Meanwhile, the bulk of belowground production remains buried as dead organic matter (OM) in the sediment (Mateo et al. 1997). Despite previous work, little is known about how landscape configuration modulates energy flow via the export of seagrass detritus or the import of allochthonous OM, such as particles suspended in the water column or macroalgae from surrounding habitats within the coastal seascape (Valiela et al. 2001, Heck et al. 2008).

To help fill this gap, in this study we explored the relationships between landscape configuration and the exchange of materials across a temperate coastal seascape dominated by *Posidonia oceanica* (L.) Delile seagrass meadows as well as the impact of landscape configuration on both plant nutrient content and the diets of consumers. *P. oceanica* is a habitat-forming seagrass species that can grow as patchy meadows, especially in shallow areas, and can be found growing either between rocky substrates or in sandy areas (Pagès et al. 2014). In this study, we used a patch matrix model approach (Boström et al. 2011) with the seagrass *P. oceanica* as the focal habitat to compare the three most frequent landscape configurations: large continuous meadows, small patches intermingled in rocky habitats (patches embedded within a rock matrix) and small patches in sedimentary bottoms (patches embedded within a sand matrix).

We evaluated detritus stocks accumulated in seagrass meadows to understand how landscape configuration modulates the flux of materials between seagrasses and the surrounding habitats. We hypothesized that (i) detrital seagrass leaves will accumulate in greater quantities in continuous meadows than in seagrass patches and (ii) landscape matrix composition will influence the type of material entering the focal habitat. At the community level, we assessed whether landscape configuration influenced the nutrient content of seagrass leaves and the proportions of food sources in the diet of a model deposit feeder (holothurians). We hypothesized that (i) nutrient content will be lower in seagrass patches due to low material accumulation rates and (ii) the proportions of food sources in the diets of deposit feeders will change by differences in the flux of materials between habitats.

## Materials and Methods

### Study site

The study was performed at 3 sites along the NE coast of Spain (NW Mediterranean): Aiguablava (41°56'N, 3°12'E), Giverola (41°44'N, 2°57'E) and Rustella (42°14'N, 3°13'E) (Fig. 3.1). These sites were selected for their similar geomorphological conditions (e.g. area, bathymetry and degree of exposure) and also for having a similar underwater ecosystem mosaic including rock, sand and seagrass habitats. The 3 landscape configurations under study were present in all 3 sites, thus minimising sources of variability among configurations other than the configuration itself. We considered continuous seagrass meadows where seagrasses covered an area of more than 100 x 100 m, while seagrass patches, in either a rock or a sand matrix covered approximately 2 x 2 m. All landscape configurations were situated at similar depths in all sites (5 to 8 m) and, for each site, at a maximum distance of 50 m from each other. At these depths, fragmentation of seagrass habitats has been associated with, apart from anthropic impacts, big storms that occur sporadically and in general have a long return time (Montefalcone et al. 2010; Alcoverro et al. 2012).

To discard confounding factors (other than landscape configurations), shoot density was measured in 3 replicate 40 x 40 cm quadrats in each landscape configuration within each site. Results were analysed using a 2-way mixed-effects ANOVA, with site and landscape configuration as factors, and there were no significant differences among sites and configurations ( $p > 0.05$ , see Table SM 3.1 in the Appendix 2), with an overall mean of  $548 \pm 29$  shoots  $m^{-2}$  ( $\pm$  SE).

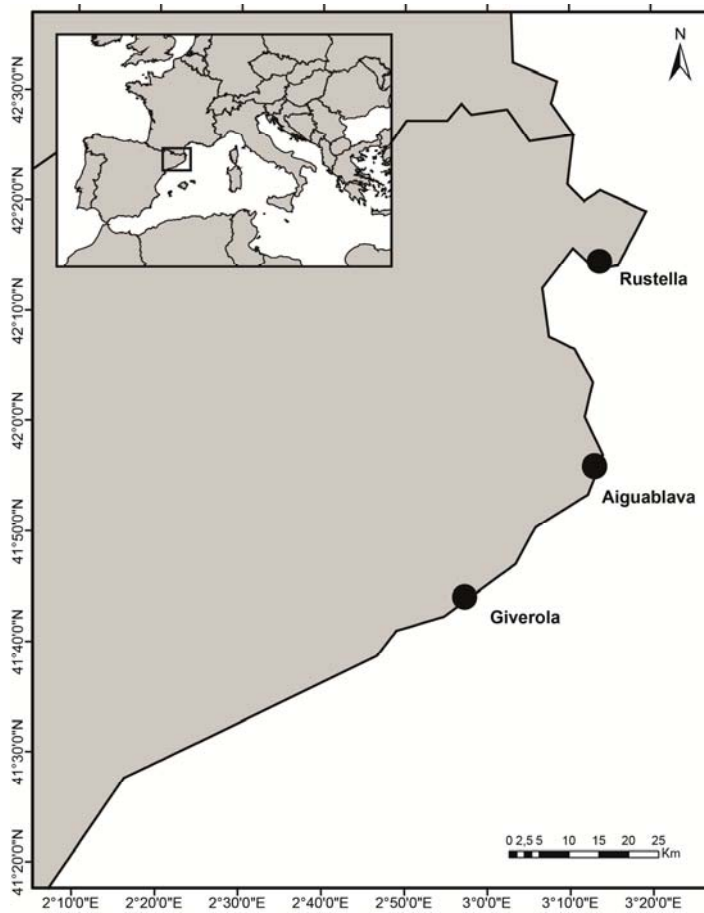


Fig. 3.1. Sampling locations along the Catalan coast, Spain, NW Mediterranean. Each of the sites presented similar seagrass landscapes, with continuous meadows and small patches in sand and rock habitats at the same depth range.

## Sampling

Samples were collected in October 2012 (fall season in the northern hemisphere), at the end of the period of leaf fall and, consequently, the period for which leaf litter accumulation is at its maximum (Romero et al. 1992). No storm or high hydrodynamic event, potentially distorting results, occurred before or during the sampling. At each site, 5 replicate sampling points (each consisting of 1 to 1.5 m<sup>2</sup>) were randomly selected inside the continuous meadows, and 1 sampling point was selected

in each of 5 randomly selected seagrass patches in both a rock and a sand matrix, for a total of 15 sampling points per site. Scuba divers haphazardly placed a 40 x 40 cm square at each sampling point and used hand-held corers (40 mm diameter) to collect undisturbed sediment (upper 2 cm) for OM determination. Then, all of the detrital material inside the square was collected using a suction device for one minute, and sieved through a 1 mm mesh attached to the collector end of the suction device. Each sample was then placed inside a plastic bag, sealed and later transported chilled to the laboratory. Five seagrass shoots were then collected from within each square for elemental and isotopic composition analysis of living plants. Finally, one individual sea cucumber, either *Holothuria poli* or *Holothuria tubulosa-mamatta* complex (Borrero-Pérez et al. 2009), was captured as close as possible to the sampling square for elemental and isotopic composition analysis. Holothurians were used as a model organism as they are known to feed on a variety of detrital sources (bulk sediment and leaf litter of different sizes), and they constitute the largest and most conspicuous detritivore in seagrass habitats (Massin & Jangoux 1976).

The entire sampling programme resulted in a total of 45 samples of each class, corresponding to 3 landscape configurations in 3 replicated sites, with  $n = 5$  replicates per experimental condition. Additionally, 2 litres of seawater (from 1 m depth) was collected in triplicate from each site and filtered into prewashed and precombusted (450°C, 4 h) Whatman GF/F filters within 2 h of collection for later elemental and isotopic composition analysis of suspended particulate organic matter (SPOM). All samples were kept frozen at -20°C until analysis in the laboratory.

### **Laboratory processing**

In the laboratory, the detritus samples were sieved again through a 1 cm sieve and sorted into 1 fine (particles between 1 mm and 0.9 cm) fraction and 4 coarse (1 cm or more) detrital fractions: *P. oceanica* leaves, *P. oceanica* roots and rhizomes, macroalgae, and material of terrestrial origin. Subsamples from the fine fraction were inspected under a dissecting microscope to estimate its origin. Detrital *P. oceanica* leaves have very low epiphytes loads, but when necessary epiphytes were removed manually as much as possible. These fractions were dried at 60°C and weighed. The leaves from the

5 living shoots collected at each sampling point were scraped with a razor blade to remove epiphytes (Alcoverro et al. 1997a), which were kept for subsequent analysis. Once cleaned, we separated the second youngest leaf from each of the 5 shoots and pooled them. Both the epiphytes and these leaves were dried as above (Martínez-Crego et al. 2008).

Isotopic and elemental analysis was performed on samples of the coarse detrital fractions, in epiphytes and in living leaves. We did not analyse the fine detritus, as it was a mixture of the coarse detrital fractions (see Results). After drying, the samples were ground to a fine powder, placed in a tin capsule and analysed for carbon (C) and nitrogen (N) elemental and isotopic composition. Prior to analysis, the detrital macroalgae and epiphytes were acidified drop by drop with HCl 2 N to remove carbonates, re-dried without rinsing and ground (Jacob et al. 2005, Carabel et al. 2006). As this chemical procedure has been reported to alter  $\delta^{15}\text{N}$  values (Bunn et al. 1995), each sample was split into 2 subsamples: half of the sample was washed with acid, and the other half remained untreated. For the isotopic and elemental analysis of seston, the SPOM sample filters were dried to constant weight, split into 2 subsamples and weighed, and half of the filter was fumed under concentrated HCl fumes (12 N) overnight at room temperature (Lorrain et al. 2003). The subsamples treated with acid were used to analyse  $\delta^{13}\text{C}$ , and the untreated subsamples were used to analyse  $\delta^{15}\text{N}$ .

The holothurians were dissected, and the retractor muscles were carefully removed and used for isotopic analysis after being rinsed in distilled water, oven dried to constant weight (for 72 h at 45°C) and ground to a fine powder. As lipids are depleted in  $\delta^{13}\text{C}$  and may influence carbon isotope ratios in animal tissues (DeNiro & Epstein 1978, Post 2002), 5 samples were reanalysed after lipid removal by chloroform-methanol (2:1 ratio) extraction (Folch et al. 1957). No significant differences were found in the  $\delta^{13}\text{C}$  results (data not shown) between untreated tissue and that with lipids removed, probably due to the low lipid content, and therefore untreated samples were used.

Stable isotope ratios and elemental C and N composition were measured using a MAT253 continuous-flow isotope ratio mass spectrometer (Thermo Finnigan) coupled to an EA1108 elemental analyser (Carlo Erba Instruments) through a ConFlo III



interface (Thermo Finnigan). C and N isotope ratios are expressed as  $\delta$  values in parts per thousand (‰) relative to VPDV (Vienna Pee Dee Belemnite) and the atmospheric air standard respectively, according to standard notation ( $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where R is the ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ). International Atomic Energy Agency standards were inserted every 12 samples for calibration. Sediment OM content was measured in triplicate as loss on ignition from sediment dry weight after combustion at 450°C in a muffle furnace for 4 h.

### Data analysis

The dry weights of each detritus fraction, sediment OM content and C and N isotopic and elemental composition of living leaves and epiphytes were analysed using a 2-way mixed-effects ANOVA with site (Aiguablava, Giverola and Rustella) and landscape configuration (continuous, patches in a rock matrix and patches in a sand matrix) as factors. Site was considered random, and landscape configuration was considered fixed. Where a significant ( $p < 0.05$ ) difference occurred, a post hoc Tukey's HSD test was used to distinguish between groups. When necessary, the data were fourth root transformed to meet the requirements of homogeneity of variance and normality. Non-transformed values (means  $\pm$  SE) are shown in the figures and tables. These analyses were performed using Statistica 8 software (StatSoft).

The Bayesian mixing model SIAR 4.2 (Parnell & Jackson 2013) running with R software (R Core Team 2014) was used to estimate the contribution of potential food sources to the diets of deposit feeders. The greatest advantage of this procedure is the incorporation of uncertainty linked to sources, consumers and trophic enrichment factors within the model (Parnell et al. 2010). This leads to the inclusion of an overall residual error term and to the generation of potential dietary solutions as true probability distributions. The model was run with 3 sources: detrital macroalgae, SPOM and a combined source of epiphytes and detrital *P. oceanica* leaves. The isotope signatures of this combined source were obtained using a weight ratio of 36:64 (epiphytes to leaves), as derived for old leaves from Alcoverro et al. (2004) and Pérez & Romero (unpubl. data). This procedure allowed us to avoid bias by reducing the

number of food sources and to include epiphytes as part of the detrital material, as it was difficult to sort epiphytes from leaves in the detritus compartment. Separate mixing models were computed for each site and landscape configuration based on each corresponding set of isotope values. Within each mixing model simulation, holothurians were treated as individual consumers. We refer throughout the paper to *Holothuria* spp. (*H. poli* and *H. tubulosa-mamatta* complex), as no differences in isotope signatures were found between species (data not shown). The isotope ratios of the holothurians and food sources were analysed considering a trophic enrichment of  $1.3 \pm 0.3\text{‰}$  for  $\delta^{13}\text{C}$  and  $2.9 \pm 1.8\text{‰}$  for  $\delta^{15}\text{N}$  (adapted from McCutchan et al. 2003). Concentration dependence was incorporated into the model, as element concentrations differed between sources (Phillips & Koch 2002).

## Results

Concerning coarse material, detrital *P. oceanica* leaves accumulated in quantities 3-fold higher in continuous meadows than in patchy ones in either a rock or a sand matrix. No landscape configuration effects were found on the accumulation of other detrital fractions (Fig. 3.2, Table 3.1, Table SM 3.1 in the Appendix 2). Autochthonous material (detrital seagrass leaves and root and rhizome debris) accounted for most of the total dry weight accumulated in all of the landscape configurations studied, from 74 to 79%, while allochthonous material, including detrital macroalgae and material of terrestrial origin, represented the rest. The fine fraction (not represented) accounted for, on average, ca. 50 g dry wt m<sup>-2</sup>, irrespective of the site or the configuration (Table 3.1, Table SM 3.1 in the Appendix 2). As indicated by the observation using the dissecting microscope, the fine fraction was made mostly of leaf fragments (especially in samples from continuous meadows) and also included algal fragments and small debris from belowground organs.

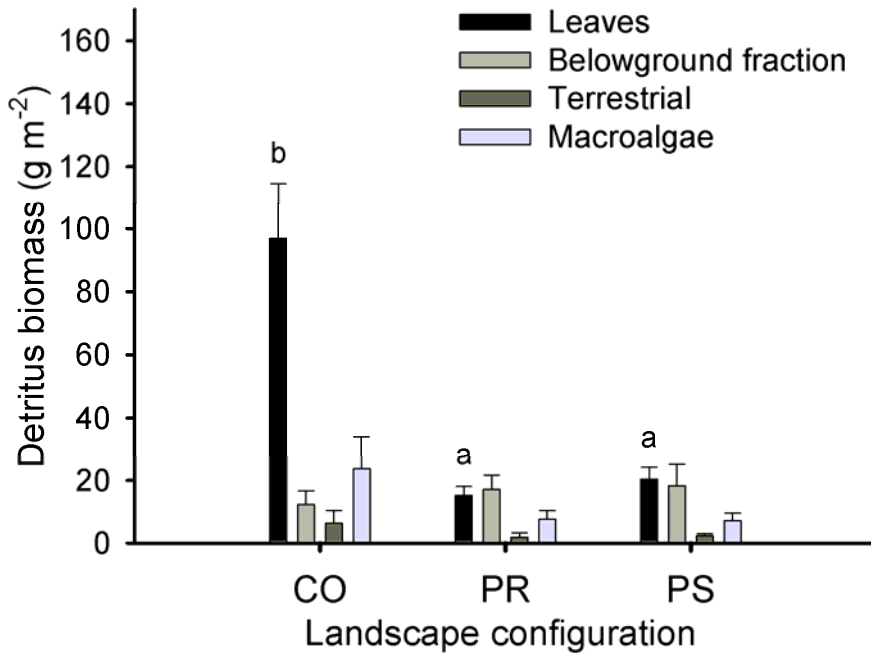


Fig. 3.2. Mean dry weight ( $\pm$ SE) of detritus stocks fractions in *Posidonia oceanica* meadows from the different landscape configurations (n=15). Bars labelled with the same letter and unlabelled bars do not differ significantly according to the Tukey's HSD post hoc test. CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix.

The N content of living leaves showed clear differences between landscape configurations (Fig. 3.3, Table 3.1, Table SM 3.1 in the Appendix 2), with plants from continuous meadows having a higher N content than those from patches in either a rock or a sand matrix. There was no evidence of an effect of landscape configuration on the N content of epiphytes (Fig. 3.3, Table 3.1, Table SM 3.1 in the Appendix 2). In the same way, there was no evidence of an effect of landscape configuration in the C content of living seagrass leaves (overall mean of  $39.99 \pm 0.09$  %, relative to dry weight) and epiphytes (overall mean of  $5.39 \pm 0.18$  %, relative to decalcified dry weight) (Table 3.1, Table SM 3.1 in the Appendix 2). Similarly, there was no evidence of an effect of landscape configuration on sediment OM content, with an overall mean of  $1.39 \pm 0.09$  %, relative to dry weight (Table 3.1, Table SM 3.1 in the Appendix 2).

Table 3.1. Results of 2-way mixed-effects ANOVAs, with landscape configuration as the fixed factor and site as the random factor. Comparisons for (a) dry weight of detrital fractions and sediment data and (b) elemental composition of living *Posidonia oceanica* leaves and epiphytes. Significant p-values ( $p < 0.05$ ) in **bold**. Df values between and within groups separated with a comma. Data were fourth root transformed to meet ANOVA assumptions

<b>(a) Detrital fractions biomass and sediment data</b>				
	<b>Df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
<i>P. oceanica</i> detrital leaves	2, 36	10.39	64.62	<b>&lt;0.001</b>
<i>P. oceanica</i> belowground fraction	2, 36	0.10	0.15	0.869
Terrestrial fraction	2, 36	1.64	1.20	0.390
Macroalgae	2, 36	1.39	0.48	0.640
Fine fraction	2, 36	32183	1.15	0.404
Sediment organic matter (%)	2, 36	0.02	0.01	0.986
<b>(b) <i>P. oceanica</i> leaves and epiphytes variables</b>				
<b>Nitrogen content (%)</b>	<b>Df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
<i>P. oceanica</i> live leaves	2, 36	0.02	10.37	<b>0.026</b>
Epiphytes	2, 36	0.25	0.69	0.554
<b>Carbon content (%)</b>	<b>Df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
<i>P. oceanica</i> live leaves	2, 36	2.01	0.15	0.869
Epiphytes	2, 36	1.43	0.13	0.885

Potential food sources for deposit feeders (detrital *P. oceanica* leaves and epiphytes, detrital macroalgae and SPOM) were well distinguishable using both C and N stable isotope values (Fig. 3.4; Table SM 3.2 in the Appendix 2). The  $\delta^{13}\text{C}$  values of the sources ranged between -12.26 and -25.50‰. SPOM was the most  $^{13}\text{C}$ -depleted source (-22.30 to -25.50‰) and showed low C:N ratios (9.60 to 10.74). The  $\delta^{13}\text{C}$  values of detrital macroalgae showed intermediate values, although they presented a high variability (from -14.87 to -24.56‰), probably due to the presence of a range of species in different amounts. Furthermore, detrital macroalgae had low C:N ratios (9.13 to 11.97). The combined source of detrital *P. oceanica* leaves plus epiphytes showed the most  $\delta^{13}\text{C}$  enriched values (-12.26 to -14.98‰) and high C:N ratios (27.12 to 30.78). The  $\delta^{15}\text{N}$  values of the potential food sources were more homogeneous, between 2.10 and 5.24‰. SPOM values ranged from 2.60 to 4.60‰; detrital macroalgae ranged from 2.10 to 4.96‰; and detrital *P. oceanica* leaves plus

epiphytes presented slightly higher  $\delta^{15}\text{N}$  values, from 3.30 to 5.24‰, probably due to the presence of small sessile animals among the epiphytes, such as hydrozoans (*Aglaophenia harpago*, *Sertularia perpusilla*) or bryozoans (e.g. *Electra posidoniae*), among others (Prado et al 2007).

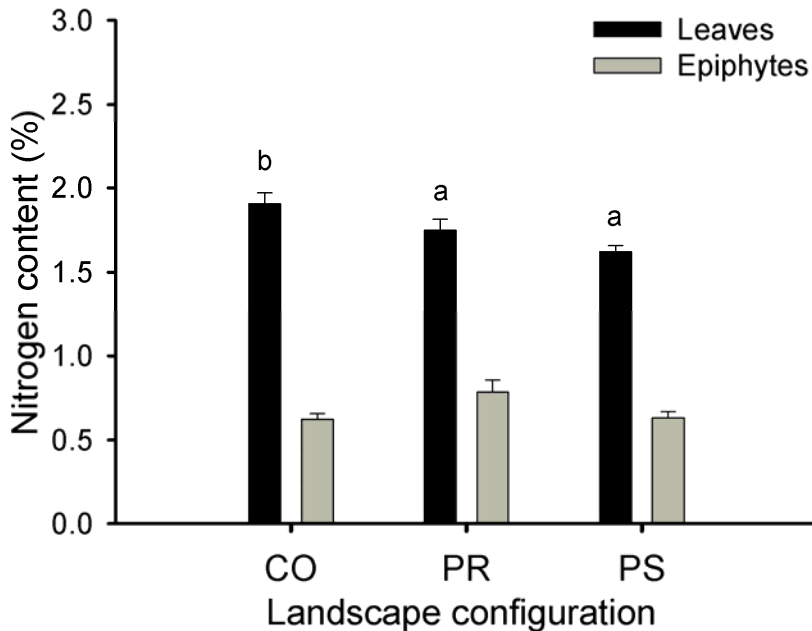


Fig. 3.3. Mean nitrogen content ( $\pm$ SE) of *Posidonia oceanica* living leaves and epiphytes from the different landscape configurations (n=15). Bars labelled with the same letter and unlabelled bars do not differ significantly according to Tukey's HSD post hoc test. CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix.

The isotopic composition of *Holothuria* spp. was similar in the different landscape configurations studied (Fig. 3.4; Table SM 3.2 in the Appendix 2). The  $\delta^{13}\text{C}$  signatures of *Holothuria* spp. ranged from -13.43 to -17.72‰ and the  $\delta^{15}\text{N}$  values ranged from 6.94 to 9.53‰. The ranges of feasible contributions from each food source to *Holothuria* spp. diets varied slightly between landscape configurations (Fig. 3.5; Table SM 3.3 in the Appendix 2). The models suggested that the combined source

of *P. oceanica* leaves and epiphytes constitutes the majority of the diet at all sites and landscape configurations, with mean values of the proportions ranging from 41 to 63%. Detrital macroalgae appeared as the second source in all of the models applied, with mean proportions in the narrow range, 29 to 36%. SPOM was also a potentially significant contributor to *Holothuria* spp. diets, with mean contributions ranging from 5 to 28%.

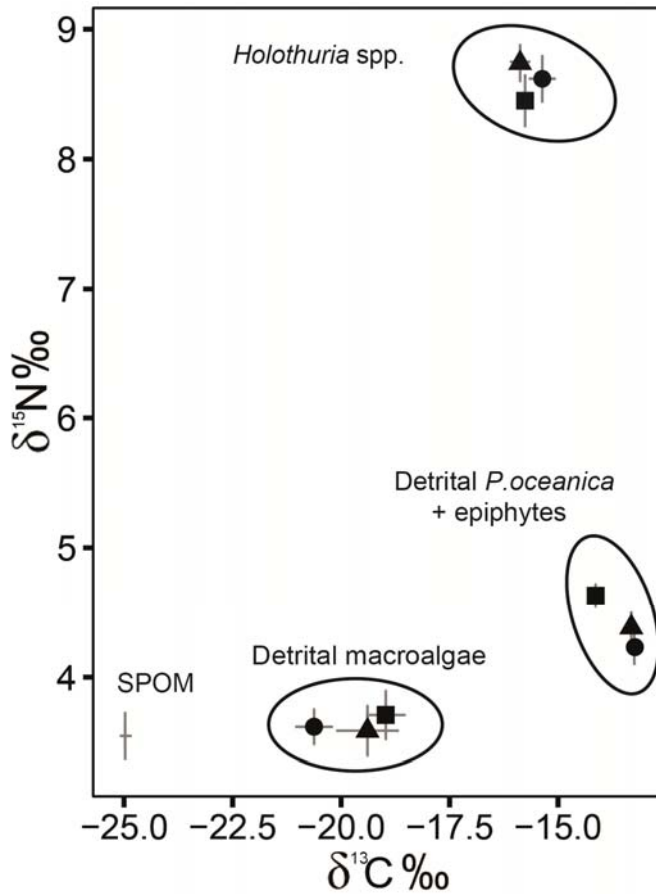


Fig. 3.4. Isotope plot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in parts per thousand (‰) for consumers, *Holothuria* spp. and their potential food sources (mean and standard deviation) in each landscape configuration. Circles, continuous meadows; triangles, patchy meadows in a rock matrix; squares, patchy meadows in a sand matrix. SPOM, suspended particulate organic matter.

## Discussion

As hypothesized, landscape configuration plays a role in modulating the flows of material between habitats in coastal marine ecosystems. Specifically, in continuous meadows, the accumulation of detrital seagrass leaves was enhanced (up to 3-fold) relative to the accumulation in seagrass patches in either a rock or sand matrix. Under such high leaf litter accumulation, *in situ* mineralization could increase nutrient availability, as suggested by the nutrient content found in leaves from plants collected in continuous meadows, which was significantly higher than that from plants in patchy configurations. Detrital seagrass leaves, including their epiphyte loads, were the main food source for deposit feeders. However, the accumulation of such materials in continuous meadows was not paralleled by a shift in the isotopic composition of the model deposit feeder (*Holothuria* spp.). This did not support the hypothesis proposed that the proportion of food sources in the diets of the deposit feeders could be modulated by differences in the flux of materials between habitats.

Accumulated detritus within *P. oceanica* meadows (only coarse fraction) accounted for high organic stocks, up to 220 g dry wt m<sup>-2</sup> in continuous meadows and approximately 100 g dry wt m<sup>-2</sup> in seagrass patches. These values are of the same order as those for standing litter stocks or even higher at that time of the year (e.g. Romero et al. 1992). These values are 10-fold higher when compared with other seagrass species (e.g. *Cymodocea nodosa*) (Pérez et al. 2001) and 5-fold higher when compared with different coastal habitats such as mangroves (Woodroffe 1985). The bulk of the detritus is autochthonous, including leaf litter and rhizome and root debris. Allochthonous materials such as terrestrial detritus and macroalgae detached from rocky reefs were equally present in all of the landscape configurations in lower proportions (2 and 13%, respectively).

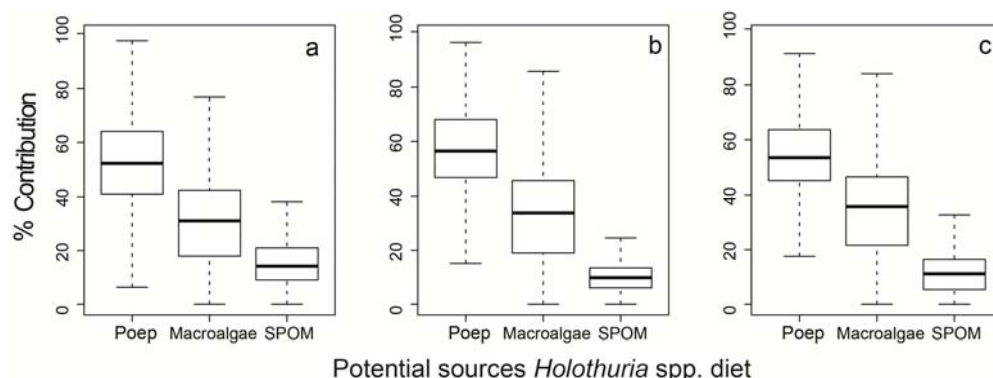


Fig. 3.5. Percentage dietary contributions of the 3 potential food sources for *Holothuria* spp. diets in the landscape configuration studied: (a) Continuous meadows; (b) patches in a rock matrix; (c) patches in a sand matrix. Plots show the distribution of feasible contributions from each food source to the species diet resulting from the application of the SIAR isotope model. Boxplot from top to bottom: largest observation, upper interquartile, median, lower interquartile and lowest observation. Poep, compounded source of detrital *P. oceanica* leaves and epiphytes. SPOM, suspended particulate organic matter.

Detritus accumulation in seagrass meadows is a complex process in which biological and physical forcing interact (Romero et al. 1992). Standing litter is the result of the balance between local detritus production (e.g. leaf fall), import (of allochthonous materials but also probably of seagrass leaves from neighbouring meadows), export and decay (both mechanical, leading to the fine fraction, and biological, i.e. decomposition). All of these factors seem relevant, except maybe import. The effects of landscape configuration (specifically meadow size) seem to be restricted to the accumulation of detrital leaves, while the other fractions (macroalgae and terrestrial detritus) seem to vary randomly across configurations. The leaf canopy of seagrass meadows attenuates water flow and reduces turbulence (Pujol & Nepf 2012). The below-canopy habitat, where detached leaves accumulate, presents low shear stress and reduced turbulence compared to the canopy–water interface region (Hendriks et al. 2008). In this study, under the same environmental conditions and with no differences in shoot density, the higher detrital leaf stocks found in continuous seagrass meadows suggest that the continuous meadows attenuate turbulence to a greater extent than patches, thus reducing the export of materials. In contrast, this does not seem to affect the import rates of allochthonous detritus.



The relatively low accumulation of allochthonous detritus suggests that seagrass meadows could act as barriers, making the arrival of external materials less likely. In agreement with this, it has been shown that in relatively dense seagrass meadows, the bulk of water flow is directed to the roof of the canopy (Granata et al. 2001), and detached algae do not percolate into the canopy but pass above it (Wernberg et al. 2006). Input rates of drifting materials are considered a function of landscape variables such as patch perimeter and the distance between habitats (Polis et al. 1997), but this is not reflected in our results. This may be because the most important issue is that the probability of a moving entity entering a given habitat once intercepted is determined by the boundary permeability of the habitat (Polis et al. 1997).

Interestingly, the nutrient content of seagrass leaves was higher in continuous meadows than in patchy ones, suggesting an association between nutrients and leaf detritus accumulation. Seagrasses meet their N requirement mainly via two mechanisms (Romero et al. 2006): uptake from either the water column or pore water (through leaves and roots, respectively), and internal recycling (i.e. resorption and remobilization of nutrients from old leaves or rhizome pools). For the species *P. oceanica*, internal recycling has been estimated to be high enough to meet 40% of annual needs (Alcoverro et al. 2000, Lepoint et al. 2002). As demonstrated by Hyndes et al. (2012), seagrass uptake of leached nutrients from detrital sources can account for part of the remaining 60%, thus linking detritus accumulation and nutrient availability, as is known for terrestrial systems (Swift et al. 1979, Vogt et al. 1986).

The differences found in this study in the N content of plant leaves, albeit small, could have profound consequences for plant performance. As in other seagrasses, growth rates of shallow *P. oceanica* meadows are usually limited by nutrients, usually N (Alcoverro et al. 1997b, Lepoint et al. 2002, Invers et al. 2004). The N content threshold suggested to indicate N limitation, either for this species or for seagrasses in general (Duarte 1990, Alcoverro et al. 1997b, Invers et al. 2002, Lepoint et al. 2002), is within the range of values reported here for small patches. This suggests that even small increases in N availability, if these take place close to the values involving N limitation, can stimulate plant performances (e.g. leaf growth, Alcoverro et al. 1997b), thus linking patch size and nutrient deficiency, as proposed by Gera et al. (2013) and Pagès

et al (2014) in previous works. Those authors attributed their results to increased fish herbivore activity in small patches. However, we found low densities of herbivorous fish in our study areas (authors' pers. obs.), suggesting that although the herbivorous hypothesis cannot be ruled out, detritus accumulation seems to be a better explanation in our case. Regardless, the explanations are not mutually exclusive, and further studies are needed to evaluate their (probably) site-specific relative importance. The conclusion emerging from the available evidence is that meadow fragmentation, in addition to other functional and structural effects (Montefalcone et al. 2010), could result in reduced plant performance due to nutrient shortage.

The feeding behaviour of holothurians is still poorly understood, and traditionally they have been considered non-selective feeders (Massin & Jangoux 1976). In this study, the main food source for *Holothuria* spp. was detrital *P. oceanica* leaves, including epiphytes, followed by macroalgae and SPOM in all landscape configurations. Interestingly, the contribution of detrital leaves and macroalgae to the diets did not parallel the standing biomass found in the landscape configurations studied. Some studies stress the capacity of holothurians to discriminate between nutrient-rich and nutrient-poor particles and also their particle size selection capacity, at least in some species (Massin & Jangoux 1976, Mercier et al. 1999, Mezali & Soualili 2013). Our results suggest that the diets of holothurians, irrespective of landscape configuration, are mainly supported by materials detached from the seagrass meadow, particularly leaves and epiphytes. These materials can be ingested not only as very small decaying fragments mixed with the sediments but also as large (up to 1 cm<sup>2</sup>) pieces, as confirmed by our observation of holothurian stomach contents. Both seagrass leaves and epiphytes seem to contribute to holothurian nutrition. However, the assimilation of epiphytes seems to be prevalent, as suggested by the isotope signatures found in holothurians, maybe due to their higher nutritional value (Tomas et al. 2006). This confirms previous findings, in which the contribution of seagrass epiphytes to seagrass trophic webs is not in strict relation to their abundance (Tomas et al. 2006, Park et al. 2013). Although it has been pointed out that alterations at the landscape scale could potentially disrupt or divert the natural direction of energy flows between adjacent ecosystems and hence influence food web pathways (Polis et al. 1997, Puth & Wilson

2001, Howe & Simenstad 2011), this does not seem to be occurring in the seagrass deposit feeders studied here, at least not at the spatial scale studied.

The findings reported here confirm the importance of landscape configuration in modulating flows of material within the coastal ecosystem mosaic. These effects mainly concern seagrass leaf litter accumulation and the associated effects on nutrient availability for plants but not on the food sources for deposit feeders. Importantly, seagrass patches accumulating less foliar detritus are poorer in N content than continuous meadows, which could lead to nutrient limitation. This is of particular concern in future scenarios where synergistic effects between water quality and climate change could further modify coastal seascapes, especially under the assumed worldwide seagrass decline (Waycott et al. 2009), where habitat loss will promote habitat fragmentation or increasing seagrass patchiness and potentially reduce plant performance, with consequences for the entire ecosystem.

## Acknowledgements

We thank Neus Sanmartí, Jordi Pagès, Max Galindo, Oriol Mascaró, Alessandro Gera, Owen S Wangensteen and Marine Baud for their help in the field and laboratory. Carbon–nitrogen analyses were performed at the Unidade de Técnicas Instrumentais de Análise (Universidade de Coruña), and we thank María Lema for her assistance. This study was performed with the financial support of the Spanish government (projects CTM2010-22273-C02-01 and CTM2013-48027-C3-1-R). The Spanish government supported AMR (scholarship BES-2011-046849).

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An underwater photograph of a seagrass meadow. The water is a clear, vibrant turquoise. In the foreground and middle ground, there are dense, dark green seagrass plants with long, thin leaves. The seagrass is growing on a sandy and rocky seabed. A large, solid black rectangular box is overlaid on the upper portion of the image, containing white text. The text is arranged in three lines: 'CHAPTER 4' in a large, bold, sans-serif font; 'Landscape configuration modulates carbon' in a smaller, regular, sans-serif font; and 'storage in seagrass sediments' in the same smaller, regular, sans-serif font.

# CHAPTER 4

Landscape configuration modulates carbon  
storage in seagrass sediments

Photo: Habitat edge between a *Posidonia oceanica* seagrass meadow and sandy bottoms, from the author.

## Abstract

Due to the imminence of climate change there is growing interest in seagrass systems as natural carbon sinks. Recent studies have provided reasonable estimates of the carbon stocks of seagrass ecosystems. However, the factors that affect its variability remain poorly understood. In this paper, we assess how landscape-level attributes (patch size and matrix composition) influence the carbon storage in seagrass sediments. We quantified organic carbon ( $C_{\text{org}}$ ) content and other geochemical properties ( $\delta^{13}\text{C}$ , particle size) in the surface sediments of continuous meadows, patchy meadows interspersed with rocky-algal reefs and patchy meadows in sedimentary bottoms. We also sampled the potential sources of carbon and applied Bayesian mixing models to determine their relative contribution. The results obtained indicate that continuous meadows accumulate larger amounts of  $C_{\text{org}}$  than patchy meadows, whether embedded in a rock or sand matrix. The  $C_{\text{org}}$  from continuous meadows was also more  $^{13}\text{C}$  enriched, which suggests that a high proportion of carbon comes from plant material (autochthonous sources), whereas patchy meadows (especially in a sand matrix) showed a higher contribution from allochthonous sources (mainly SPOM). These findings indicate that continuous meadows store more  $C_{\text{org}}$  in the sediment than patchy meadows, and this is probably due to the higher contribution from seagrass leaves, which are much more refractory than SPOM. In general, landscape configuration, and especially patchiness, would appear to reduce the carbon storage capacity of seagrasses. Since the current decline of seagrass is leading to habitat fragmentation, our results constitute an additional argument for the promotion of effective measures to preserve the integrity of these natural carbon sinks.





## Introduction

The urgency to reduce atmospheric CO<sub>2</sub> levels in order to mitigate climate change has led to considerable interest in quantifying the capacity of natural systems to trap and store carbon. Vegetated coastal habitats have been reported to present a relatively high carbon storage potential (Mcleod et al. 2011, Pendleton et al. 2012, Duarte et al. 2013), despite the minor proportion (<2%) of the surface they occupy (Duarte et al. 2005). These ecosystems, particularly mangroves, saltmarshes and seagrass beds, have a disproportionately high contribution to carbon sequestration, since, unlike terrestrial ecosystems, they store large amounts of organic carbon (C<sub>org</sub>) in their sediments, accounting for more than 50-70% of all carbon stored in ocean sediment (Nellemann et al. 2009).

Among these ecosystems, seagrasses are the highest contributors in relation to their global area (Duarte et al. 2005), and recent estimates indicate as much as 19.9 Pg C stored in the first meter of sediment (Fourqurean et al. 2012). Similarly, their carbon accumulation rates range from 48 to 112 Tg C yr<sup>-1</sup> (Duarte et al. 2005, Kennedy et al. 2010). This high capacity is partially the result of their high primary production rates, combined with the refractory nature of seagrass tissues (Mateo & Romero 1997). Seagrass canopies are also highly efficient at trapping particles and associated carbon from outside the ecosystem, and this key mechanism contributes to the importance of seagrasses as carbon sinks (Gacia & Duarte 2001, Hendriks et al. 2008, Kennedy et al. 2010). Moreover, since seagrasses promote regular sediment accretion, their carbon storage potential is maintained over time, unlike carbon-saturated terrestrial soils (Mcleod et al. 2011).

Many recent studies, mindful of the significant contribution of seagrasses to the carbon budget of the oceans, have attempted to quantify their carbon stocks and carbon storage potential (Kennedy et al. 2010, Duarte et al. 2010, Fourqurean et al. 2012, Greiner et al. 2013, Lavery et al. 2013, Serrano et al. 2014). These studies highlight the high variability of carbon accumulation on seagrass sediments. Species identity and abiotic habitat characteristics, such as depth and sediment properties, have been suggested as factors behind this variability (Greiner et al. 2013, Lavery et

al. 2013, Serrano et al. 2014). However, our understanding of the factors that regulate this variation is still greatly limited (Nellemann et al. 2009, Duarte et al. 2010, Serrano et al. 2014) and the influence of other factors (e.g. the surrounding habitats and the spatial complexity of habitats) have rarely been considered.

In general terms, carbon stored in seagrass sediments depends on the amount of carbon deposited and the proportion of this bulk carbon that is not remineralised. The  $C_{org}$  deposited in seagrass sediments comes from different sources, and its origin could play a critical role in both processes. Part of the carbon deposited is produced in the meadow itself (Hemminga & Mateo 1996, Papadimitriou et al. 2005, Kennedy et al. 2010), including the below-ground organs (roots, rhizomes and, in some genera, leaf sheaths) and the part of the above-ground tissues (leaves and, eventually, their epiphytes) not exported outside the meadow (Romero et al. 1992, Hyndes et al. 2014). Other sources are allochthonous materials, such as macroalgae, land-derived detritus, suspended particulate organic matter (SPOM) and, to a lesser extent, the macrofauna inhabiting the meadows (Kennedy et al. 2004).

Seagrass sediments are generally reduced and less than 10% of the overall organic materials entering the sediment is decomposed (Gacia et al. 2002). The different sources of  $C_{org}$  pools typically have different decomposition rates. Long-term carbon sinks have been associated with refractory materials with C/N ratios that no longer exhibit changes over time (Mateo et al. 2006). In most seagrass species, this applies mainly to the carbon derived from below-ground tissues, especially in the Mediterranean species *Posidonia oceanica* (Mateo & Romero 1997), but also to seagrass leaves with intermediate decomposition rates (Mateo et al. 2006), while epiphytes, allochthonous materials such as SPOM and macroalgal detritus are more labile (Mateo & Romero 1997, Cebrián 2002, Trevathan-Tackett et al. 2015). Shifts in the relative proportions of  $C_{org}$  sources with different decomposition rates could determine the amount of carbon stored on seagrass sediments.

The relative contribution of the  $C_{org}$  sources accumulated in seagrass sediments vary widely (Kennedy et al. 2004, 2010, Papadimitriou et al. 2005). In most cases, SPOM appears to be the main contributor, followed by autochthonous sources (Gacia et al.

2002, Kennedy et al. 2004, Papadimitriou et al. 2005). However, little is known about the factors that modulate their relative importance. Specifically, recent findings suggest that the configuration of the coastal seascape influences the magnitude of carbon fluxes among its habitats (Hyndes et al. 2014) likely affecting sediment carbon storage.

Seagrass beds exist naturally forming either homogeneous landscapes (large, continuous meadows) or heterogeneous landscapes, in the form of patches of varying shapes and sizes interspersed with unvegetated sand areas or rocky-algal reefs (Robbins & Bell 1994, Jackson et al. 2006). These patterns are driven by natural processes, which are either physical (e.g. hydrodynamics), geological (e.g. sediment transport) or biological (e.g. growth rate and expansion of rhizomes; (Fonseca & Bell 1998, Hovel 2003, Mills & Berkenbusch 2009). Anthropogenic disturbances (e.g. eutrophication and physical removal) constitute additional factors leading to fragmentation of seagrass habitats (Macreadie et al. 2009, Montefalcone et al. 2010). Landscape ecology has made a major contribution to the knowledge of seagrass ecosystem dynamics (Pittman et al. 2011). Yet the interaction between landscape attributes and carbon storage remains largely unexplored, despite the fact that understanding these processes is crucial for improving global carbon sink estimates and future projections (Nellemann et al. 2009, Ricart et al. 2015b).

In order to explore the variability of the carbon storage in seagrasses, we assessed the influence of landscape configuration on the carbon content and carbon sources of seagrass sediments. To achieve this, we used a patch matrix model approach (Boström et al. 2011), with *Posidonia oceanica* seagrass meadows as the focal habitat, to compare continuous meadows with patchy meadows interspersed among rocky reefs with macroalgal cover (patches in a rock matrix) and patchy meadows in sedimentary bottoms (patches in a sand matrix). We hypothesised that landscape configuration will influence the relative importance of the sediment  $C_{org}$  sources, with autochthonous sources contributing most in continuous meadows and allochthonous sources most in seagrass patches, with differences associated to the matrix type. This variability would have implications for the total amount of carbon stored in the different landscape configurations.

## Materials and Methods

### Study site

The present study was performed at six locations on the Catalan coast and Balearic Islands, in the NW Mediterranean (Fig. 4.1). These locations were selected because of their similar geomorphological conditions (e.g. depth range, shape and exposure) and the presence of the different seagrass landscape configurations. The continuous meadows were larger than 100 x 100 m, while the seagrass patches measured about 2 x 2 m. All landscape configurations were situated at similar depths in all sites (5-8 m), and, for each site, at a maximum distance of 50 m one from each other.

### Sampling

Sediments were collected manually by inserting open-barrel PVC pipes (20 cm length, 4 cm internal diameter). At each location 5 replicate cores were randomly taken from the continuous meadows and 1 core was taken from the centre of each of 5 randomly selected patches in a rock matrix and 5 patches in a sand matrix. Detached detrital leaves of *P. oceanica* and detrital macroalgae were collected for elemental and isotopic analysis using a suction device in a 40x40 cm quadrat near each core replicate sample. In addition, 5 living shoots of *P. oceanica* were collected in order to obtain epiphytic material (see below). In each location, 2 litres of seawater were collected 1 m below the surface in triplicate and filtered into prewashed and precombusted (450°C, 4 h) Whatman GF/F filters within 2 h of collection for the isotopic analysis of SPOM. All samples were kept frozen at -20°C until analysis in the laboratory.

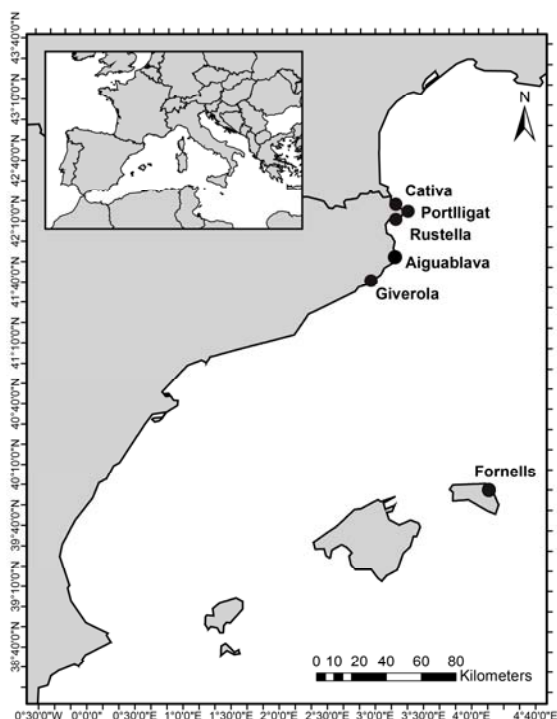


Fig. 4.1. Sampling locations along in the NW Mediterranean. Each of the sites presented similar seagrass landscapes, with continuous meadows and small patches in rock and sand habitats at the same depth range

### Laboratory processing

In the laboratory, the upper 2 cm of each sediment core was sectioned and weighed before and after drying at 60°C for 48 h. Each sample was then split into 2 subsamples; one subsample was retained for grain size analysis and the other was dry-sieved through a 1-mm mesh to remove the coarse inorganic particles and living plant material (Lavery et al. 2013, Miyajima et al. 2015). The sieved subsample was used for  $C_{org}$  isotopic and elemental analysis.

Grain size analysis was conducted for a 10 ml subsample of homogenised sediment of each core. A 0.5% of sodium hexametaphosphate solution was used as a dispersant to homogenise the sample (Poppe et al. 2003). Fine organic matter (OM) was previously

removed from the subsample by the addition of several attacks (until eliminate all organic matter) of 50ml hydrogen peroxide 10% during 24h at ambient temperature (Poope et al. 2003; Frigola et al. 2008), while large organic material was removed by hand. The grain size analysis was done on an LS 230 Laser Diffraction Particle Size Analyser (PSA; Beckman Coulter). The PSA reports grain size distribution as % volume for particle diameters of 0  $\mu\text{m}$  to 1000  $\mu\text{m}$ . The  $d_{50}$  (i.e. the diameter corresponding to the median of the particle volumes, assuming that all particles are spherical) was used as an estimator of the particle size distribution.

Samples of detrital macroalgae (mainly *Halopteris scoparia*, *Dictyota spp.* *Corallina elongata*, *Padina pavonica* and *Cystoseira spp.*) and detrital seagrass leaves were carefully sorted and cleaned. Leaves from the living *P. oceanica* shoots were separated and epiphytes were carefully removed with a razor blade (Alcoverro et al. 1997). Epiphytes from the 5 shoots collected from each replicate sampling point were then pooled for isotopic analysis (Martínez-Crego et al. 2008). All samples (sediment, detrital macroalgae, detrital seagrass leaves and epiphytes) were dried at 60°C and ground to a fine powder before being acidified with HCl 2N to remove carbonates (with exception of detrital seagrass leaves) (Carabel et al. 2006) and then re-ground, involved in two tin capsules (Mateo et al. 2008) and analysed for carbon elemental and isotopic composition. The filters in which SPOM had been collected were dried to constant weight and fumed under concentrated HCl fumes (12 N) overnight at room temperature (Lorrain et al. 2003), then placed in a tin capsule and analysed for carbon elemental and isotopic composition.

Carbon elemental composition and stable isotope ratio measurements were performed with the sieved and acidified sub-sample using a continuous-flow isotope-ratio mass spectrometer MAT253 (Thermo Finnigan) coupled to an EA1108 elemental analyser (Carlo Erba Instruments) through a ConFlo III interface (Thermo Finnigan). The  $C_{\text{org}}$  content was then referred to the bulk (pre-sieved and pre-acidified) sediment weight, expressed as a percentage (%) (Lavery et al. 2013). This percentage represents, thus, the amount of  $C_{\text{org}}$  in the fraction < 1mm per unit of dry bulk weight of sediment. Carbon isotope ratios were expressed as  $\delta$  values in parts per thousand (‰) relative to VPDB (Vienna Pee Dee Belemnite). Standing carbon stocks per volume unit (carbon

density) were calculated using dry bulk density (mass of dried soil (g) / original volume sampled (cm<sup>3</sup>) data and C<sub>org</sub> content and expressed as mgC<sub>org</sub>cm<sup>-3</sup> (Howard et al. 2015)

## Data analysis

The differences among landscape configurations (continuous, patches in a rock matrix, patches in a sand matrix) in particle size (d<sub>50</sub>), dry bulk density, C<sub>org</sub> content (%), carbon density (mg C<sub>org</sub> cm<sup>-3</sup>) and δ<sup>13</sup>C (‰) were assessed using a 2-way mixed-effects ANOVA with site as random factor and landscape configuration as fixed factor. Post-hoc Tukey's HSD tests were conducted to distinguish between the groups. The relationships between the elemental and isotopic carbon of the sedimentary organic matter and fine-sized particle abundance (<63µm) variables were analysed using the Pearson correlation coefficient (r). When necessary, the data were log transformed to improve linearity. Non-transformed values (means ± SE) are shown in the figures and tables. All statistical analyses were performed using R standard core package (R Core Team, 2014).

The Bayesian mixing model SIAR 4.2 (Parnell & Jackson 2013) in R software was used to estimate the relative contribution of the potential sources to sedimentary C<sub>org</sub>. The model was run with three sources: detrital macroalgae, SPOM and a combined source of epiphytes and detrital *P. oceanica* leaves. These sources were chosen as they represent the most important biomass carbon stocks in *P. oceanica* seagrass systems (Ricart et al. 2015a). We used detrital seagrass leaves instead of living leaves because there is no direct carbon transfer from living leaves to sediment. Instead, after leaf fall, dead leaves spend some time as leaf litter, as documented and reflected in carbon flux models in *P. oceanica* (e.g. Pergent et al. 1994, Mateo & Romero 1997) and, thus, dead leaves represent better the carbon that could potentially be buried into sediments. However, epiphyte load on detrital leaves is very low, indicating that epiphyte loss takes place when (or soon after) leaf detaches. Thus, we used data from fresh epiphytes to obtain a combined source (detrital leaves + epiphytes). To do this, we averaged the values of detrital leaves and epiphytes, weighed using the ratio 36/64 (epiphytes:leaves; Alcoverro et al. 2004). This procedure allowed us to avoid bias by

reducing the number of carbon sources and to include epiphytes as part of the detrital material. As many isotope mixing problems are underdetermined, with too many sources and not enough tracers (Fry 2013) it is recommended that the number of sources are aggregated and reduced in order to reduce this uncertainty (Phillips & Gregg 2003). Thus, the model was run again excluding macroalgae, and retaining SPOM and the combined source of epiphytes and detrital *P. oceanica* leaves. Macroalgae were excluded because of their lower abundance in the detritus relative to seagrass leaf litter (Ricart et al. 2015a). A mixing model was computed separately for each landscape configuration based on its own isotopic values. We did not consider any trophic enrichment, because the published data suggest that during decomposition of recently fallen leaves there are only small diagenetic shifts for  $\delta^{13}\text{C}$  (up to  $\sim 1\text{‰}$ ) (Zieman et al. 1984, Freudenthal et al. 2001). Concentration dependence was incorporated into the model, since the carbon content differed between sources (Phillips & Koch 2002, Parnell et al. 2010). The mean and the lowest and highest 95% credible bounds of the high-density regions (HDR) generated were detailed (Phillips et al. 2014).

## Results

### Sediment properties

All sediments sampled were well sorted and composed by medium to coarse sands, as indicated by the median ( $d_{50}$ ) grain size diameter, which ranged from 220  $\mu\text{m}$  to 1800  $\mu\text{m}$  (Table 4.1). Sediments from patchy meadows in a sand matrix were significantly finer ( $d_{50}=455 \mu\text{m}$ ;) than those in the other configurations ( $d_{50}$  around 740  $\mu\text{m}$ ) (Table 4.1 and Table 4.2). The silt and clay fractions ( $<63 \mu\text{m}$ ) were more abundant in continuous meadows than in patchy ones (Table. 4.1 and Table 4.2). While continuous meadows presented lower bulk density values than patchy meadows, in both the sand and rock matrix (Table 4.1 and Table 4.2).



Table 4.1. Sediment properties of *Posidonia oceanica* meadows in each landscape configuration (CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix). Mean and standard error (SE) are given.

	CO		PR		PS	
	Mean	SE	Mean	SE	Mean	SE
Median grain size ( $d_{50}$ )	717.11	101.19	770.31	140.65	455.04	49.15
<63 $\mu\text{m}$ (%)	3.27	0.82	0.55	0.14	0.17	0.06
Bulk density ( $\text{g cm}^{-3}$ )	0.94	0.05	1.12	0.05	1.22	0.04
Carbon density ( $\text{mgC}_{\text{org}}\text{cm}^{-3}$ )	16.75	6.96	8.18	2.19	3.39	1.14

The  $\text{C}_{\text{org}}$  content of the surface sediments (Table 4.1, Fig. 4.2) was higher in continuous meadows (Table 4.2) than in patchy ones. Carbon density also differed among the landscape configurations (Table 4.1; Table 4.2), with higher values in surface sediments of continuous meadows (up to 3 times) than patches in both the rock and sand matrix.

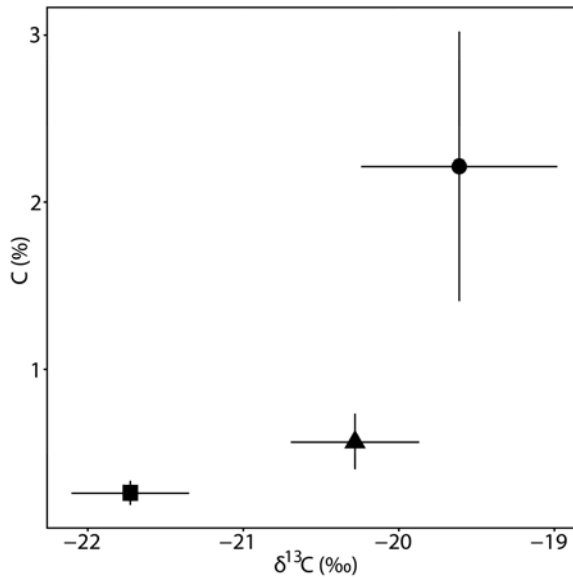


Fig. 4.2. Sedimentary organic carbon content (%) and  $\delta^{13}\text{C}$  (‰) of *Posidonia oceanica* meadows. Mean and standard deviation are given for each landscape configuration: patches in a sand matrix (squares), patches in a rock matrix (triangles), continuous meadows (circles).

The carbon isotopic ratios of the surface sediments from patches in a sand matrix were significantly more depleted in  $^{13}\text{C}$  (from -18.0 to -24.5‰) than sediments from

continuous meadows (from -16.1 to -22.8‰), while patches in a rock matrix (from -16.8 to -23.6‰) did not differ from the other two configurations (Table 4.1, Table 4.2, Fig. 4.2).

The  $C_{org}$  content (log transformed) was positively correlated with  $\delta^{13}C$  values ( $r=0.71$ ;  $P\leq 0.001$ ) and the amount of fine particles ( $<63\mu m$ ) ( $r=0.81$ ;  $P\leq 0.001$ ). The  $\delta^{13}C$  values were also positively correlated with the amount of fine particles ( $<63\mu m$ ) ( $r=0.56$ ;  $P\leq 0.001$ ).

### Source contributions to sedimentary $C_{org}$

Potential carbon sources (detrital *P. oceanica* leaves and epiphytes, and SPOM) were easily distinguishable using carbon signatures (Fig. 4.3). The combined source of detrital *P. oceanica* leaves and epiphytes showed the most  $^{13}C$ -enriched values (-12.3 to -14.9‰), while SPOM had the most  $^{13}C$ -depleted values (-21.2 to -25.5‰). Macroalgae showed great variability and presented values between the other two sources (-14.97 to -23.9‰).

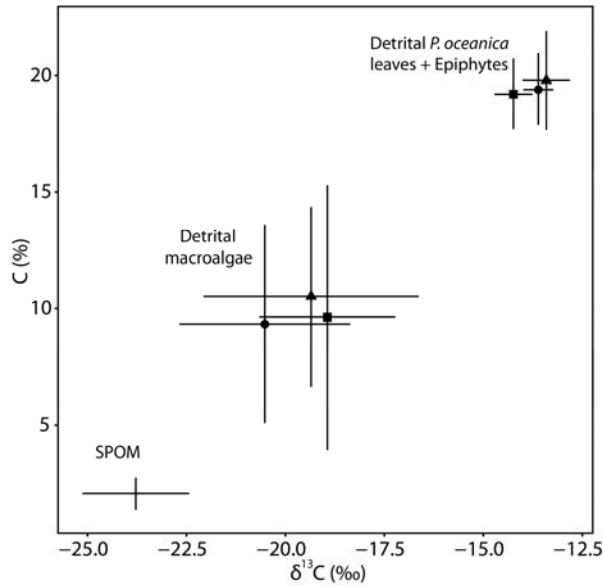


Fig. 4.3. Organic carbon content (%) and  $\delta^{13}C$  (‰) for potential sources of sedimentary organic carbon: Patches in a sand matrix (squares), patches in a rock matrix (triangles), continuous meadows (circles).

The relative contribution of sources to the  $C_{org}$  in the sediment varied across the landscape configurations (Fig. 4.4). SPOM constituted an important source of sedimentary carbon in all of them, although its contribution in patches in a sand matrix was higher than in the other two configurations (Table SM 4.1 in the Appendix 2). The contribution of the combined source of detrital *P. oceanica* leaves and epiphytes also accounted for a large fraction in continuous meadows followed by patches in a rock matrix. However, the proportion decreased in patches in a sand matrix (Table SM 4.1 in the Appendix 2). The contribution of macroalgae did not vary among the landscape configurations (Table SM 4.1 in the Appendix 2). Similar results were obtained using only two sources (not including detrital macroalgae, see Methods section) (Fig. 4.4).

Table 4.2. Results of the 2-way mixed-effects ANOVA for sediment properties, with landscape configuration as fixed factor and site as random. Df values within groups and between groups separated with a comma. Results of post hoc TukeyHSD test are given according to the following notation: CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix (Tukey's  $p < 0.05$ ).

	df	SS	F	P	Tukey
Median grain size $d_{50}$	2,5	1364886	7.09	<b>&lt;0.01</b>	CO=PR;CO≠PS;PR≠PS
<63 $\mu$ m	2,5	89.11	19.86	<b>&lt;0.01</b>	CO≠PR;CO≠PS;PR=PS
Bulk density	2,5	0.73	14.29	<b>&lt;0.01</b>	CO≠PR;CO≠PS;PR=PS
$C_{org}$ content	2,5	79.66	21.81	<b>&lt;0.01</b>	CO≠PR;CO≠PS;PR=PS
$\delta^{13}C$	2,5	33.55	5.53	<b>&lt;0.01</b>	CO=PR;CO≠PS;PR=PS
Carbon density	2,5	3429	17.93	<b>&lt;0.01</b>	CO≠PR;CO≠PS;PR=PS

## Discussion

Landscape configuration influences the carbon storage of seagrass meadows. In this study, we have shown how patchy meadows, independently of the matrix type, store less  $C_{org}$  in their surface sediments than continuous ones. This fact has obvious implications for future projections of the carbon sinks associated with these ecosystems. The precise mechanisms underlying these differences appear to be associated primarily with a greater capacity for the retention, in continuous meadows, of autochthonous, more refractory materials.

The amount of  $C_{\text{org}}$  stored in seagrass sediments depends on two key factors: the availability of raw materials for burial (including autochthonous production and imported materials) and their susceptibility to decomposition. Landscape attributes can interact with both, and are thus an important driver of carbon storage.

The availability of raw materials is controlled by the magnitude of seagrass production, the fraction of this production that remains within the meadow and the capacity of the meadow to trap allochthonous materials (Kennedy et al. 2010). Although some studies have found a significant correlation between leaf biomass and the  $\delta^{13}\text{C}$  of the sediment  $C_{\text{org}}$  (Kennedy et al. 2004), which would suggest a link between seagrass productivity and carbon storage, it is important to remember that a significant and highly variable proportion of the above-ground production may be exported outside the system in the form of leaf litter (Romero et al. 1992, Hyndes et al. 2014). The remaining portion, together with the below-ground organs, constitutes the bulk of seagrass materials entering the sediment. Therefore, the export rates of seagrass production may have more influence than production rates *per se* on the amount of  $C_{\text{org}}$  input in seagrass sediment. Material exchange rates are modulated by landscape variables (Polis et al. 1997). In previous work (Ricart et al. 2015a), we quantified biomass stocks at the same sites, revealing a much higher value of seagrass leaf detritus in continuous meadows relative to the other two configurations, which was mainly attributed to hydrodynamic properties related to the integrity/fragmentary state of the meadows. This is consistent with the data reported here, which show less negative  $\delta^{13}\text{C}$  values in the sediment under continuous meadows than that of the patchy meadows, especially on those in a sand matrix. In line with the results of our mixing models, this implies that the detrital *P. oceanica* leaves have a high contribution to the stored carbon in continuous meadows.

Matrix composition has a small effect on the carbon storage in seagrass meadows as there is no variation in any variable assessed between patches in a rocky or sandy substrate, with exception on  $\delta^{13}\text{C}$  in sedimentary organic carbon, which present less negative values indicating higher inputs of autochthonous materials, maybe caused by the high topographic complexity of rocky bottoms.

SPOM presented a greater contribution in all landscape configurations, which is consistent with previous data reported for *P. oceanica* (Gacia et al. 2002, Papadimitriou et al. 2005) and other seagrass species (see review of Kennedy et al. 2010). Gacia et al. (2002) conducted a study on a single *P. oceanica* meadow using two carbon sources and found values of 57% for SPOM and 43% for *P. oceanica* tissues. This was confirmed by Papadimitrou et al. (2005) in a study of 22 *P. oceanica* seagrass beds along the Spanish Mediterranean coast that reported a similarly high SPOM contribution, with values between 51% and 86%. Again, we found that the contribution of SPOM was modulated by landscape-level attributes, with higher values in patchy meadows. This is likely to be a direct consequence of the variability in the contribution of the seagrass fraction.

Our results concerning the contribution of macroalgae to sediment carbon are inconclusive, and this is due in part to the uncertainty introduced by the number of sources relative to the isotopes used as tracers in the mixing models. Macroalgal sources have seldom been taken into account in previous studies, despite the fact that their presence in seagrass meadow litter is relatively common (Pérez et al. 2001, Como et al. 2008). This is most probably because their  $\delta^{13}\text{C}$  signatures could sometimes be confused with those of epiphytes (Moncreiff & Sullivan 2001). Our results suggest contributions from macroalgae to sediment organic carbon in the range 10-50%, without apparent differences among configurations. Given the small amounts of macroalgae found in the detritus (ca. 20% of the weight of leaf detritus: Ricart et al. 2015a) and their relatively high decay rates (see below), it seems reasonable that the real values would be close to the lower part of the range. In any case, further work should be developed to elucidate the role of detrital macroalgae as carbon sources for seagrass sediments.

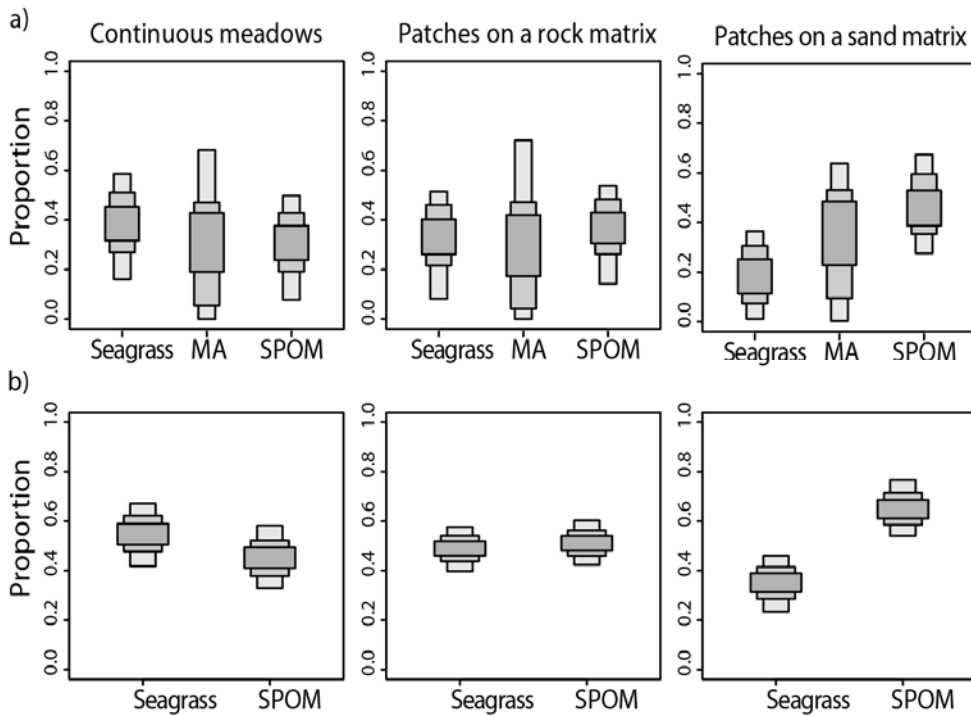


Fig. 4.4. Relative contribution (%) of each source of carbon in seagrass sediment for the different landscape configurations studied; (a) Results of the mixing model for each landscape configuration using three sources; (b) Results of the mixing model for each landscape configuration using two sources. Grey scale (light to dark) indicates 95,75,25% credible intervals of the high-density regions generated. MA, macroalgae.

There is a high variability in the decay rates of the different materials that potentially reach seagrass sediments. The decay rates are usually ranked as follows: SPOM > macroalgae > seagrass (Gacia et al. 2002). In fact, seagrass tissues contain relatively high amounts of refractory organic compounds (Klap et al. 2000, Torbatinejad et al. 2007), which delays their mineralisation, while macroalgal detritus, and particularly SPOM particles, are richer in labile  $C_{org}$  compounds (Mateo & Romero 1997, Cebrian 2002), and this leads to early decomposition during diagenesis. Consequently, it seems reasonable to assume that the higher the seagrass contribution to the carbon input, the higher the amount of carbon stored. This assumption is also supported by our finding of a positive correlation between carbon storage and  $\delta^{13}C$ . Overall, the variability in the contribution of seagrass sources (and other

autochthonous materials) to carbon accumulated may be the link between landscape-level processes and carbon storage.

In addition to aspects related to leaf detritus retention, the highest storage of continuous meadows also seems to be associated with the presence of fine sediment ( $<63\mu\text{m}$ ). Fine sediment, which was more abundant in continuous meadows, retains more organic matter than coarser fractions and ensures its preservation by impeding oxygen diffusion and, hence, mineralisation (Burdige 2007). The significant and positive correlation found between the abundance of fine particles ( $<63\mu\text{m}$ ) and carbon content in the sediment supports this theory.

The present work confirms that, in addition to the  $C_{\text{org}}$  stored in the form of massive underground organs (mainly rhizomes), *P. oceanica* meadows have a great capacity for  $C_{\text{org}}$  storage in sediment. Areal estimates of carbon stocks in the upper 2 cm (the depth of the cores we used in this study) give mean values as high as  $1.67 \text{ Mg C ha}^{-1}$ ,  $0.81 \text{ Mg C ha}^{-1}$ ,  $0.33 \text{ Mg C ha}^{-1}$  in continuous meadows, patches in a rock matrix and patches in a sand matrix, respectively. The values of the continuous meadows are similar to those reported elsewhere for this same species (Serrano et al. 2012, Lavery et al. 2013), when normalised to a soil thickness of 2 cm, and are among the highest standing stocks of total carbon, on a unit-area basis, reported in seagrasses. The carbon storage of *P. oceanica* sediments occurs in two main forms: in the form of underground organs (rhizomes, leaf sheaths) and in the form of a fine, undifferentiated fraction of diverse origins (Mateo & Romero 1997). In this work, we have ignored the former and concentrated on the latter, based on the assumption that the accumulation of massive deposits based on rhizome growth would be a much longer process (in the order of millennia) (Mateo et al. 1997) than those related to habitat fragmentation. Moreover, the fine, undifferentiated fraction has been found to represent an important fraction (ca. 40%) of total carbon stored in *P. oceanica* sediments (Serrano et al. 2012).

The findings reported here confirm the importance of landscape configuration in controlling carbon storage in seagrass sediments. This control seems to be exerted primarily through the capacity for leaf detritus retention, which is higher in

continuous meadows than in patchy ones (Ricart et al. 2015a), but also by sediment characteristics (higher fine fraction in continuous meadows) that potentially delay  $C_{org}$  remineralisation. Overall, patchy meadows, especially those in a sand matrix, clearly presented a lower carbon density per unit of vegetated area (about one third) than continuous meadows. This has obvious implications for the refinement of estimates of the global carbon stocks of seagrasses, since patchy seagrass meadows are common in all regions where seagrasses are present (Robbins & Bell 1994). Moreover, despite the patchiness of our study sites is likely due to a combination of environmental and anthropogenic factors (Gera et al. 2014, Marbà et al. 2014), this point becomes even more relevant in future scenarios, where the synergistic effects of water quality and climate change may further modify coastal seascapes, especially in the context of the assumed worldwide seagrass decline (Waycott et al. 2009), thus promoting habitat fragmentation and increasing seagrass patchiness. Apart of other effects of fragmentation (Bell et al. 2001, Macreadie et al. 2009, Gera et al. 2013), in terms of blue carbon, this creates significant concern, as it could lead to not only a significant loss of the carbon stored as a result of the reduction in vegetated surface (Duarte et al. 2010), but also a considerable loss in this storage potential due to the shift from continuous to patchy meadows. The implementation of effective protection measures is therefore imperative for preserving these natural carbon sinks.

## Acknowledgements

We thank Neus Sanmartí, Andreu Dalmau, Jordi Pagès, Max Galindo and Laura Ros for their help in the field and laboratory. Carbon-nitrogen analyses were performed at the Unidade de Técnicas Instrumentais de Análise (Universidade de Coruña); we thank María Lema for her assistance. This study was performed with the financial support of the Spanish government (projects CTM2010-22273-C02-01 and CTM2013-48027-C3-1-R). The Spanish government supported AMR (scholarship BES-2011-046849).



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An underwater photograph showing a dense patch of seagrass. The seagrass blades are long, narrow, and green, with some showing signs of wear or damage. The background is a soft, out-of-focus blue-green, suggesting the water column. A black rectangular box is overlaid on the upper right portion of the image, containing the chapter title and subtitle in white text.

# CHAPTER 5

Variability of sedimentary organic carbon in  
patchy seagrass landscapes

Photo: *Zostera muelleri* seagrass meadow, from Peter Macreadie



## Abstract<sup>1</sup>

Seagrass ecosystems, considered among the most efficient carbon sinks worldwide, encompass a wide variety of spatial configurations in the coastal landscape. Here we evaluated the influence of the spatial configuration of seagrass meadows at small scales (metres) on carbon storage in seagrass sediments. We intensively sampled carbon stocks and other geochemical properties ( $\delta^{13}\text{C}$ , particle size, depositional fluxes) across seagrass-sand edges in a *Zostera muelleri* patchy seagrass landscape. Carbon stocks were significantly higher (ca. 20%) inside seagrass patches than at seagrass-sand edges and bare sediments. Deposition was similar among all positions and most of the carbon was from allochthonous sources. Patch level attributes (e.g. edge distance) represent important determinants of the spatial heterogeneity of carbon stocks within seagrass ecosystems. Our findings indicate that carbon stocks of seagrass areas have likely been overestimated by not considering the influence of meadow landscapes, and have important relevance for the design of seagrass carbon stock assessments.

<sup>1</sup>See original publication in Appendix 3

**Ricart** AM, York PH, Rasheed MA, Pérez M, Romero J, Bryant CV, Macreadie PI (2015) *Variability of sedimentary organic carbon in patchy seagrass landscapes*. Marine Pollution Bulletin 100:476–482



## Introduction

The necessity to reduce CO<sub>2</sub> fluxes to mitigate climate change has generated considerable interest in quantifying the capacity of natural ecosystems to trap and sequester carbon (Mcleod et al. 2011). Most efforts have focussed on quantifying carbon sinks in terrestrial ecosystems, but recently vegetated coastal habitats have been highlighted for their carbon storage potential (Pendleton et al. 2012, Duarte et al. 2013a). The carbon sequestered by vegetated coastal habitats, referred commonly as "blue carbon", provides long-term organic carbon (C<sub>org</sub>) storage once it has become bound within sediments. The large amount of C<sub>org</sub> stored in coastal sediments, combined with their high carbon sequestration rates, highlights the important role that coastal ecosystems play as natural carbon sinks (Macreadie et al. 2014a,b). These ecosystems, particularly mangroves, saltmarshes and seagrasses, have a disproportionately large C<sub>org</sub> storage potential relative to their global area (Duarte et al. 2005, Mcleod et al. 2011, Duarte et al. 2013a).

Seagrasses develop organic-rich soils derived from both autochthonous (produced internally - e.g. seagrass detritus) and allochthonous (of external origin - e.g. sestonic particles) sources (Gacia & Duarte, 2001; Kennedy et al. 2010). Seagrass carbon storage capacity is a result of a high primary productivity, the refractory nature of seagrass tissues and its capacity to trap particles from the water column and incorporate them into the sediment (Hendriks et al. 2008; Kennedy et al. 2010). These facts, in combination with high sedimentation rates and the anaerobic nature of these soils, that promotes slow microbial decomposition rates, provide environments where carbon can be buried and preserved over long time frames, such as the millennial carbon deposits found in temperate seagrass meadows formed by persistent, long-lived species (Mateo et al. 2006, 1997).

The relevance of seagrasses as a potential mitigating agent of CO<sub>2</sub> emissions was already suggested in the last decades (Smith 1981), although, the paucity of data on the carbon budgets from seagrass ecosystems hindered the inclusion of seagrasses in models of global carbon transfer and global green-house gas abatement schemes (Macreadie et al. 2014a). Over the past five years, however, there has been a major

increase in the accuracy of regional and global estimates of carbon stocks and accumulation rates in seagrass ecosystems (Kennedy et al. 2010, Duarte et al. 2010, Fourqurean et al. 2012, Serrano et al. 2012, 2014, Greiner et al. 2013, Lavery et al. 2013).

Seagrass ecosystems encompass a wide variety of species across a range of depositional environments and depths (Carruthers et al. 2007). Species identity as well as abiotic habitat characteristics have been highlighted as factors driving variability of sedimentary carbon stocks of seagrass meadows (Lavery et al. 2013; Serrano et al. 2014). However, our understanding of the factors regulating this variability is still limited (Duarte et al. 2010; Nellemann et al. 2009; Serrano et al. 2014).

Moreover, seagrass meadows present high spatial heterogeneity (Jackson et al. 2006). They can occur either as large, continuous meadows or in the form of patches of various shapes and sizes with unvegetated or macroalgal regions interspersed among more homogenous seagrass areas (Robbins & Bell 1994). Patchiness is an intrinsic feature of most seagrass meadows, especially in shallow and estuarine areas where natural and anthropogenic forcing is severe (Montefalcone et al. 2010). Patchiness increases the amount of edge associated to the habitat (Smith et al. 2008), which constitutes an important transitional gradient from one structural type to another. Habitat edges influence patterns in biological and physical activity by promoting interactions with the surrounding landscape (Puth & Wilson 2001, Macreadie et al. 2010a,b). In particular for seagrasses, the edge has been defined as a boundary that stops the water flow, increasing turbulence on the edge of the canopy (Granata et al. 2001) and enhancing deposition of particles inside the seagrass meadow (Macreadie et al. 2010a, Zong & Nepf 2011). Over time, these processes could potentially generate spatial heterogeneity in  $C_{org}$  accumulation, both in the seagrass patches and in adjacent bare sediments.

Carbon burial rates on seagrasses can be as much as threefold higher when compared to bare sediments (Duarte et al. 2005), as seagrass canopies promote sedimentation and reduce particle resuspension (Gacia & Duarte, 2001; Gacia et al. 1999). But this is unlikely to be uniform inside such seagrass meadows, where significant differences

in burial rates due to the meadow architecture and spatial configuration occurs (Granata et al. 2001; Gruber & Kemp, 2010). Small-scale variability (e.g. one seagrass patch might be denser than another patch) could influence carbon sequestration at the patch level thus affecting carbon stocks estimations at the habitat and landscape scale.

Despite previous works quantifying seagrass carbon sinks, little is known about the within-meadow variability on sediment carbon storage. In this study we selected a patchy seagrass distribution to evaluate the small-scale spatial variability on carbon storage within seagrass ecosystems. We evaluated horizontal spatial variability by quantifying carbon stocks and sources both in seagrass patches and adjacent bare sediments at increasing distances from the seagrass-sand edge. We also evaluated vertical variability by sampling at different depth sections in the sediment. We hypothesized that (1) sediment carbon stocks will be highly variable across seagrass patches, with carbon stocks increasing across the transition from bare sediments to seagrass patch edges and to seagrass patch interiors; and (2) there will be greater contribution of autochthonous (plant-produced) carbon to the carbon stocks within seagrass patches than in bare sediments.

## Materials and Methods

The study was conducted in the middle of the growing season (September 2013), well before the seasonal dieback, on Pelican Banks inside the entrance to Port Curtis Harbour (23°46'S; 151°18'E). Port Curtis is a macro-tidal estuary in central Queensland (Australia) characterized by the presence of *Zostera muelleri* Irmisch ex Asch. beds in most of its intertidal areas. The selected area for this study represented a patchy landscape characterized by a mosaic of large seagrass patches (diameter about hundreds of meters) interspersed with naturally occurring unvegetated (bare) sediments.

To test for variability in carbon storage, 4 different locations on the boundary among seagrass patches and bare sediments were selected haphazardly at the same water depth. The seagrass patches selected for the study were determined to be persistent for

at least the last 10 years by using satellite imagery (Google Earth 2013). At each location, cores were taken at 6 different positions along a transect perpendicular to a seagrass-bare sediment edge: at 0.5 m, 10 m and 20 m into seagrass (S0.5, S10 and S20) and at 0.5 m, 10 m and 20 m into bare sediment (B0.5, B10 and B20). We sampled 4 replicated transects, resulting in a total of 24 (6 positions x 4 transects) sampling points. Seagrass cover, measured in three replicate 50 x 50 cm quadrates at each seagrass position, was similar among all positions ( $p > 0.05$ ) with an overall mean of  $22.5 \pm 3.06\%$  ( $\pm$  SE).

Sediments were collected manually by inserting open-barrel PVC pipes (20 cm length, 5 cm internal diameter) into sediments to a depth of 10 cm, and using a piston to provide suction as cores were withdrawn. Compaction during coring was low ( $<10\%$ ). Once extracted, cores were capped at both ends and transported to the laboratory. In the laboratory, the sediments were extruded, sliced into 10 sections at 0-0.5, 0.5-1, 1-1.5, 1.5-2, 2-3, 3-4, 4-5, 5-6, 6-8, 8-10 cm intervals, dried at  $60^\circ\text{C}$  and weighed in order to calculate dry bulk density (Howard et al. 2015).

Each sediment section was split into 2 sub-samples, with grain size particle distribution analysed from the first subsample using a Malvern Mastersizer 2000 laser microgranulometer. Organic matter (OM) was removed from the subsample by addition of hydrogen peroxide 10%, while large organic material was removed by hand. Particle size distribution was expressed as % volume for particle diameters from 0 to 2000  $\mu\text{m}$ . The  $d_{50}$  (i.e. the diameter corresponding to the median of particle volumes assuming that all particles are spherical) and skewness (Folk & Ward 1957) were used as an indicator of the particle size distribution.

The second sub-sample was used for  $C_{\text{org}}$  elemental and isotopic analysis after being sieved through a 1 mm mesh to remove living plant material and coarse inorganic particles (i.e. carbonate material). Samples were then ground and acidified with HCl 1 M to remove any carbonates that were too small to be sieved. After drying, samples were re-ground and then analyzed for carbon elemental and isotopic composition.

The apparent depositional flux (considered as the result of both deposition and resuspension processes) was measured in each position sampled along the transects

using sediment traps consisting of cylindrical centrifuge tubes (20.5 ml) with a height versus diameter ratio of 5, with the aperture of the tube positioned at 5 cm above the sediment surface. Sediment traps were removed after 7 days of deployment. These short-term measurements were used only to capture relative spatial differences, and not to elaborate annual budgets. In the laboratory contents of the sediment traps were filtered through pre-combusted (450°C for 4h) 25 mm GF/F filters and dried (60°C for 48h) to obtain dry weight. Samples were analyzed for  $C_{org}$  elemental and isotopic composition, after acidification, using the method described above.

Measurements of carbon elemental composition and stable isotope ratios (as a tracer of carbon sources) were performed using a continuous-flow isotope-ratio mass spectrometer MAT253 (Thermo Finnigan) coupled to an elemental analyser EA1108 (Carlo Erba Instruments) through a Conflo III interface (ThermoFinnigan). Carbon isotope ratios are expressed as  $\delta$  values in parts per thousand (‰) relative to VPDV (Vienna Pee Dee Belemnite) according to standard notation ( $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$ , where R is the ratio  $^{13}C/^{12}C$ ). Standing carbon stocks per volume unit were calculated using dry bulk density data and  $C_{org}$  content and expressed as  $mgC_{org}cm^{-3}$ .

Differences in dry bulk density, particle size median diameter ( $d_{50}$ ), sedimentary  $C_{org}$  elemental and isotopic composition, and carbon stocks were analyzed using a 2-way analysis of variance (ANOVA) with position along the transects and depth section as fixed factors. The apparent depositional flux and the carbon elemental and isotopic composition of the material collected in the sediment traps were compared using a 1-way ANOVA with position along the transects as a fixed factor. Where a significant ( $p < 0.05$ ) difference occurred, post hoc Tukey's HSD tests were used to distinguish differences among groups. When necessary, data were fourth root transformed to meet ANOVA assumptions. Non-transformed values (means  $\pm$  SE) are shown in figures and tables.

## Results

Dry bulk density of sediments was similar across all the positions along the transects and depth sections (Table 5.1) with an overall mean of  $1.57 \pm 0.08 \text{ g cm}^{-3}$ .

Table 5.1. Results of the two-way ANOVAs, with position (P) and depth (D) as fixed factors (a) Comparisons for sediment cores variables (b) Comparisons for sediment traps variables. (\*) Data were fourth root transformed to meet ANOVA assumptions

<b>(a) Sediment cores variables</b>		<b>df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
Dry bulk density ( $\text{g cm}^{-3}$ )	P	5	0.24	1.09	0.37
	D	9	0.76	1.90	0.06
	P*D	45	2.01	0.01	0.48
	Residuals	180	8.05		
Particle size median diameter ( $d_{50}$ )	P	5	14.10	0.30	0.91
	D	9	133.10	1.56	0.13
	P*D	45	327.50	0.77	0.84
	Residuals	1	1116.10		
Carbon content ( $\%C_{\text{org}}$ )	P	5	4.31	13.45	<b>&lt;0.01</b>
	D	9	0.45	0.78	0.63
	P*D	45	1.71	0.59	0.98
	Residuals	180	11.53		
Carbon stocks ( $\text{mgCorgcm}^{-3}$ )	P	5	1059.00	6.93	<b>&lt;0.01</b>
	D	9	113.00	0.41	0.93
	P*D	45	9.77	0.71	0.91
	Residuals	180	5503.00		
$\delta^{13}\text{C}$ (‰)	P	5	48.85	19.38	<b>&lt;0.01</b>
	D	9	4.76	1.05	0.40
	P*D	45	7.73	0.34	0.01
	Residuals	180	90.74		
<b>(b) Sediment traps variables</b>		<b>df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
Deposition rates ( $\text{mg m}^{-2} \text{ day}^{-1}$ ) (*)	P	5	0.06	1.90	0.15
	Residuals	18	0.11		
Carbon content ( $\%C_{\text{org}}$ ) (*)	P	5	0.61	11.82	<b>&lt;0.01</b>
	Residuals	14	0.15		
$\delta^{13}\text{C}$ (‰)	P	5	0.64	0.10	0.99
	Residuals	14	18.76		



The median diameter of sediment particles ( $d_{50}$ ) was similar among all positions along the transects and depth sections (Table 5.1), ranging from 103 to 120  $\mu\text{m}$ , while skewness was positive (overall mean  $0.83 \pm 0.01$ ) indicating that sediments can be characterized as fine sands.

The  $C_{\text{org}}$  content, carbon stocks and  $\delta^{13}\text{C}$  showed significant differences among positions but not among depth sections, and a significant interaction between both factors was found for  $\delta^{13}\text{C}$  (Table 5.1). The profile values of  $C_{\text{org}}$  content and  $\delta^{13}\text{C}$  through the top 10 cm of the sediment cores remain fairly constant through depth in the innermost seagrass positions, and showed more variability in all other positions, although this variability neither followed any trend nor provided any indication of past seagrass presence (Fig. 5.1a and Fig. 5.1b). The  $C_{\text{org}}$  content in the sediment was significantly higher in the innermost seagrass positions than in seagrass edges. The lowest  $C_{\text{org}}$  content was found in bare sediment positions (Fig. 5.2a). Carbon stocks showed a similar pattern as  $C_{\text{org}}$  content, with higher values in seagrass positions than in bare sediments positions (Fig. 5.2b). The  $\delta^{13}\text{C}$  values were significantly more negative in bare sediments than in the rest of positions. Inside seagrass positions,  $\delta^{13}\text{C}$  values were more negative in the seagrass edge than in the other two innermost seagrass positions (Fig. 5.2c).

The apparent depositional flux showed high variability in bare sediment positions compared to seagrass positions resulting in the lack of significant differences among positions (Table 5.1, Fig. 5.3a). The  $C_{\text{org}}$  content in the material deposited was significantly lower in bare sediments than in seagrass patches. Within seagrass positions, the seagrass edge showed values ca. 3-fold higher relative to bare sediments and the innermost seagrass positions showed values ca. 5-fold higher relative to bare sediments (Table 5.1, Fig. 5.3b). The  $\delta^{13}\text{C}$  values of the material deposited did not show differences among positions (Table 5.1, Fig. 5.3c).

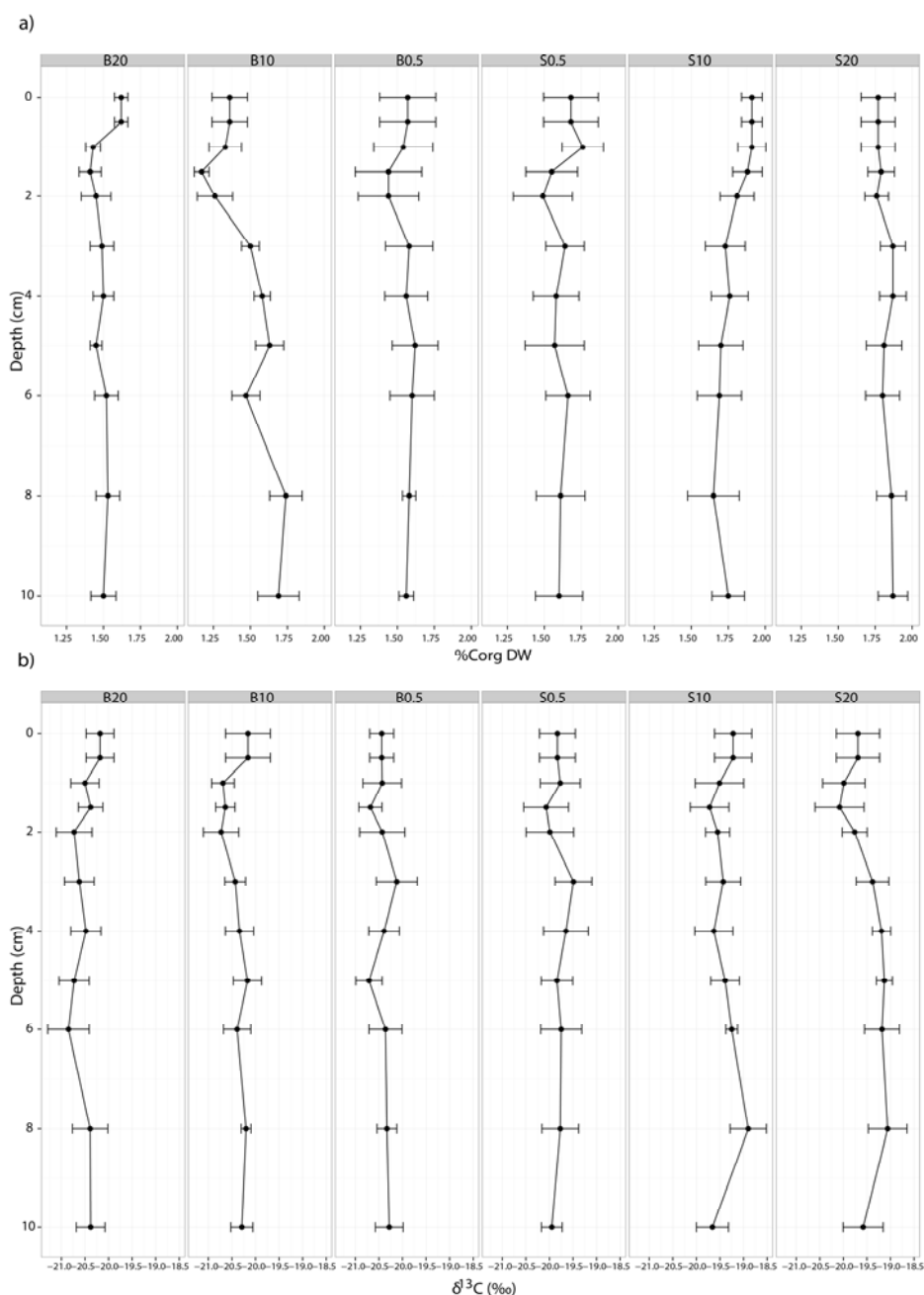


Fig. 5.1. Depth profiles of the top 10cm of sediment cores from the different positions along the transects (Mean and SE). (a) Carbon organic content ( $C_{org}$ ) expressed as percentage of the total sample dry weight (%); (b) Carbon isotopic ratios. Labels: S0.5, S10 and S20 positions at 0.5 m, 10 m and 20 m into seagrass patches and B0.5, B10 and B20 positions at 0.5 m, 10 m and 20 m into bare sediment

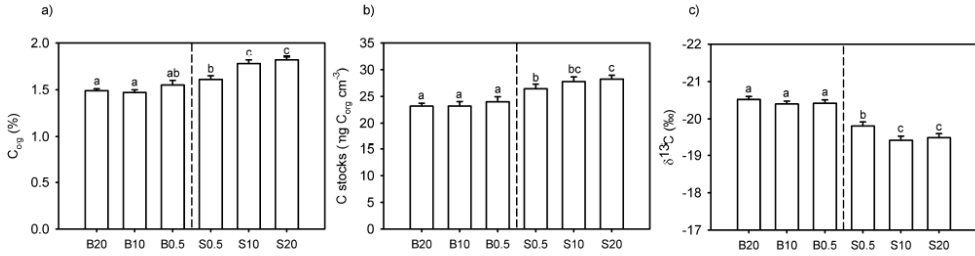


Fig. 5.2. Results from sediment variables (Mean and SE) in the top 10 cm of sediment cores from the different positions along the transects. (a) Carbon organic content ( $C_{org}$ ) expressed as percentage of the total sample dry weight (%); (b) Carbon stocks; (c) Carbon isotopic ratios. Labels: S0.5, S10 and S20 positions at 0.5 m, 10 m and 20 m into seagrass patches and B0.5, B10 and B20 positions at 0.5 m, 10 m and 20 m into bare sediment. Dotted line represents the seagrass/sand boundary. Bars labelled with the same letter do not differ significantly according to a Tukey's HSD post hoc test.

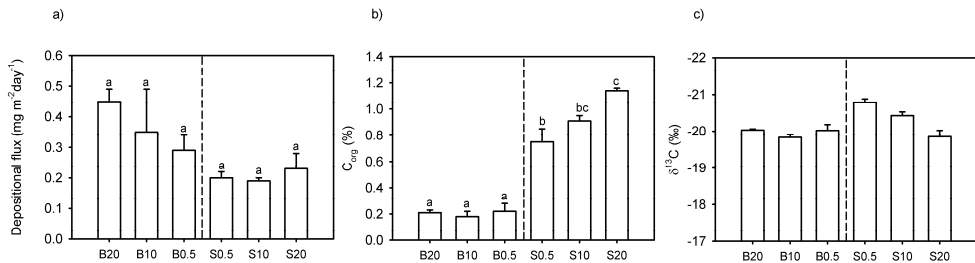


Fig. 5.3. Results from sediment traps on the different positions (Mean and SE). (a) Apparent depositional flux; (b) Carbon organic content ( $C_{org}$ ) in deposited materials expressed as percentage of the total sample dry weight (%); (c) Carbon isotopic ratios of deposited material. Labels: S0.5, S10 and S20 positions at 0.5 m, 10 m and 20 m into seagrass patches and B0.5, B10 and B20 positions at 0.5 m, 10 m and 20 m into bare sediment. Dotted line represents the seagrass/sand boundary. Bars labelled with the same letter do not differ significantly according to a Tukey's HSD post hoc test.

## Discussion

Our results show that seagrass carbon storage varied at small scales (metres). Seagrass patches showed a clear pattern of variation with lower carbon stocks at seagrass edges than in the innermost parts. Carbon stocks in bare sediments were lower than in seagrass vegetated areas, but higher than expected because of the influence of the seagrasses. Patch level attributes (distance to the edge) represent an important determinant of the spatial heterogeneity of carbon storage within seagrass ecosystems.

Variability on spatial distribution of carbon stocks needs to be taken into account for the accurate estimations on blue carbon assessments for seagrasses.

The present work confirms that *Z. muelleri* meadows have a high capacity for  $C_{org}$  storage in the sediments. Areal estimates of carbon stocks in the upper 10 cm (the depth of the cores we used in this study) give values as high as  $600 \text{ g C m}^{-2}$  in the innermost parts of seagrass patches. The values of seagrass patches are higher to those reported elsewhere for this same species (Lavery et al. 2013), and are among the highest standing stocks of total carbon, on a unit-area basis, reported in tropical seagrasses (Lavery et al. 2013).

The lower carbon stocks found in the edges of seagrass patches, relative to the innermost parts, could be caused by several processes related with habitat boundaries. Habitat boundaries are recognized to have patterns differing from the main part of the habitat (Puth & Wilson, 2001; Smith et al. 2011) and these patterns will be influenced by the nature of the adjacent habitats (Sheaves 2009). The boundary from sand to dense seagrass cover enhances flow acceleration over the canopy (Folkard 2005), while producing a secondary circulation cell at the seagrass meadow edge (Granata et al. 2001). Thus, turbulence increases at the interface between sand and seagrass, likely promoting higher resuspension of sediments and export of seagrass derived materials in the seagrass edge, and also less deposition of suspended particles, reducing carbon storage capacity when compared with the innermost parts of the seagrass patches.

On the other hand, the lower carbon stocks found in the seagrass edge could be related with the development stage of the seagrass patches. As far as we know from satellite imagery, the seagrass patches studied in this work have been stable for the past decade, however, the history of each seagrass patch sampled (i.e. the permanency of the boundary) is not known prior to this study. Different stages of patch development could affect carbon storage (Duarte et al. 2013b, Greiner et al. 2013, Marbà et al. 2015). If the patch is growing, the edge is younger than the central part of the patch and is formed by fast-growing rhizomes and new leaf clusters (Duarte & Sand-Jensen 1990). During the first years after patch establishment, most of the

carbon is stored as aboveground biomass, however, during the middle-aged and old stages of development, the importance of below-ground biomass and below-ground detrital mass as carbon stores increase (Cebrián et al. 2000). Thus, the seagrass-derived carbon that is available for burial in the edge is likely to be lower if the plant is still colonizing the sediments. It is also possible that the meadow could be in regression and the lower carbon stocks at seagrass edges were related with a prior loss of seagrass habitat cover in the adjacent bare sediments. Carbon stocks in the sediments could be lost after the regression of the seagrass habitat by physical removal, promoting a higher resuspension of sediments or even remineralisation (Fourqurean et al. 2012). However, evidence of a reduction in stored  $C_{org}$  from sediment after seagrass loss is inconclusive (Macreadie et al. 2014b).

In this study, bare sediments on the patchy seagrass landscape showed less carbon storage than seagrass patches, however, their carbon stocks were higher than those previously reported for continuous *Z. muelleri* meadows approximately 1000 km to the north of our study in Queensland, Australia (Lavery et al 2013). Seagrass beds are known to modify the  $C_{org}$  content of nearby sediments (Miyajima et al. 1998) influencing its  $C_{org}$  content in two different ways; by direct supply of materials detached from the plants in a mechanism of seagrass-derived carbon spill over (Miyajima et al. 1998); and also by reducing the velocity of the water flow, because of the structural influence of the seagrass canopy, thus depositing suspended particulate materials not only within the seagrass but also in adjacent bare sediments areas (Fonseca & Koehl 2006, Pujol & Nepf 2012).

Resuspension, transport and resettlement of seagrass-derived organic matter have been suggested as factors controlling the horizontal distribution of  $C_{org}$  in the sediment (Miyajima et al. 1998). Evidence for export of the organic matter produced within the seagrass meadow to adjacent bare sediments has been reported in several studies (Kennedy et al. 2010; Miyajima et al. 1998). However, in our case, values of  $\delta^{13}C$  presented in both habitats were extremely  $^{13}C$  depleted, suggesting that most of the  $C_{org}$  was from an allochthonous origin. Potential allochthonous sources of  $C_{org}$  like seston (ca.  $\delta^{13}C$  -20‰), epiphytes, microphytobenthos and macroalgae (all with typical ca.  $\delta^{13}C$  -17‰), and terrestrial organic matter (ca.  $\delta^{13}C$  -27‰) are all

isotopically light relative to  $\delta^{13}\text{C}$  seagrass (ca.  $\delta^{13}\text{C} -12\text{‰}$  for *Z. muelleri*) (Moncreiff and Sullivan 2001, Leduc et al. 2009, Kennedy et al. 2010, Dubois et al. 2012, Oakes & Eyre 2014, Ricart et al. 2015). It is likely that the high  $C_{\text{org}}$  content found on the bare sediments on the seagrass patchy landscape may be due to the seagrass canopy promoting deposition of allochthonous sources. A fact that is also suggested by the similarity in  $\delta^{13}\text{C}$  values on the trapped material in all positions along the transects. While the less negative  $\delta^{13}\text{C}$  values in sediments of seagrass patches indicated a slightly greater contribution of seagrass-produced carbon. The higher carbon content found in particles trapped inside seagrass sediments, were probably due to the higher deposition of mineral fraction in bare sediments areas. This is in accordance with previous published data which show that particles trapped and deposited in seagrass sediments are often richer in organic matter (Kennedy et al. 2010; Miyajima et al. 1998) when compared with those deposited in bare sediments.

This study provided insights on the spatial variability of sediment carbon storage of seagrasses. Patchy seagrass landscapes have a great carbon storage capacity as plants not only promote carbon storage inside the seagrass patches but also in the adjacent bare sediments. The distribution of carbon stocks within a patchy seagrass landscape could be linked to the spatial configuration of the habitats within the landscape (sand and seagrass in this study), which in time determines the carbon sources to the sediments and processes to which  $C_{\text{org}}$  accumulated in seagrass sediments could be subjected (e.g. less resuspension). Tropical areas, as the one in this study, could be subjected to temporal and spatial fluctuations on seagrass abundance both seasonally and between years (Rasheed et al. 2013, York et al. 2015), although this study did not address the issue of temporal variability of carbon storage, due the short term of the measurements.

Total carbon storage of seagrass ecosystems will depend in part on the amount of seagrass vegetated coverage and the amount of edges found. This fact has to be taken into account when attempting global blue carbon estimates, especially in naturally patchy meadows or in human induced fragmented ones. The major issue in carbon stocks assessments at large scales come down to how researchers scale up their data. Usually sediment samples are taken in core areas of the seagrass meadows and then

extrapolated to the landscape scale based on the measured seagrass vegetated areas. This could lead to overestimation of carbon stocks, because, as shown in this study, seagrass edges stored less carbon than the innermost parts of seagrass meadows. To avoid overestimation in continuous seagrass meadows researchers should not be taking into account seagrass edges (e.g. first 10 m to the interior of seagrass areas) when scaling up the data. While for patchy seagrass landscapes a correct estimation of the surface of vegetated areas is required. We recommend the use of high resolution cartography to localize core areas of seagrass meadows, seagrass edges and bare areas interspersed, and the sampling of sediment cores in these three parts to scale up the data for each part separately. Landscape configuration and patch dynamics are factors that are often overlooked by managers when assessing the level of ecosystem function provided by seagrasses, as happens with meadow form, habitat and life history of each species (Kilminster et al. 2015). Detailed seagrass mapping needs to be used, and seagrass ecology and functional aspects of these ecosystems need to be taken into account in environmental decision making, management and policy.

Findings reported in this study demonstrated that spatial heterogeneity of the seagrass meadows affect its sediment carbon storage. These results also highlight concerns for future changes to seagrass ecosystems in the face of increasing anthropogenic impacts and seagrass decline (Waycott et al. 2009). The increasing rate of degradation of seagrass ecosystems will promote habitat fragmentation and increase the patchiness, which in turn will influence seagrass carbon storage. Gaining insights on the variability of carbon storage of seagrasses is important to properly manage and protect carbon stocks of these valuable coastal ecosystems.

## Acknowledgements

We thank Jessie Jarvis for her assistance in the field. PM was supported by an Australian Research Council DECRA Fellowship (DE130101084) and the CSIRO Coastal Climate Cluster. AMR was supported by the Spanish Government (fellowships BES-2011-046849 and EEBB-I-13-06916 and project CTM2013-48027-C3-1-R).

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The background of the entire page is an underwater photograph. It shows a sandy seabed with patches of green and brown algae. Several fish are visible: in the foreground, a large silver fish with a black spot on its tail and a black stripe on its face; behind it, another similar fish; to the right, a fish with a prominent green stripe; and in the upper left, a fish with a dark body and a white stripe. The water is clear and blue.

# CHAPTER 6

Influence of landscape patterns at multiple scales  
on coastal fish assemblages

Photo: *Diplodus sargus*, *Diplodus vulgaris* and *Thalassoma pavo* in rocky bottoms with macroalgal cover, from Pol Capdevila

## Abstract:

Coastal habitats commonly occur as heterogeneous landscapes of interconnected habitat patches, in which species-environment interactions occur at different spatial scales. In this study we assessed the influence of landscape patterns in fish assemblages in temperate (Mediterranean) coastal seascapes composed by *Posidonia oceanica* seagrass meadows, macroalgal communities on rocky reefs and sandy bottoms. Using a multi-level approach, we evaluated several landscape metrics measured at three spatial scales (from meters to kilometres) in a several sites and analysed their statistical association with different features of fish assemblages (abundance, species richness and abundance of prevalent families) obtained from visual censuses. Cover of vegetated habitats, especially cover of macroalgal habitats on rocky reefs, and landscape spatial heterogeneity stand out as overall important variables structuring fish assemblages. Species richness was influenced by small scale landscape patterns (cover of rocky reefs), while total abundance was associated to patterns measured at small and intermediate scales, encompassing metrics associated to vegetated habitat cover (rocky reefs: positive association; seagrass: negative association) and those indicating landscape heterogeneity (negative association). Interestingly, the spatial scale at which metrics had the higher influence on the abundance of specific families was positively correlated to the home ranges of those families. Results from this study support the need to consider small to large-scale landscape patterns for a better understanding and management of coastal fish assemblages.





## Introduction

Habitats commonly occur as more or less interconnected patches constituting spatial heterogeneous mosaics. While community ecology usually focus on structural and functional characterization of homogeneous patches, landscape ecology is aimed at understanding how the landscape composition (patch types and sizes) and configuration (how those patches are arranged spatially) influence not only the overall system, but also its individual components (the patches and the species moving across the mosaic), at a panoply of spatial scales (Turner 2005, Thornton et al. 2011, Pittman et al. 2011, Fuller 2014).

The concept of scale is a core tenet of the ecological sciences (Levin 1992, Yeager et al. 2011). Ecological systems are structured hierarchically, each level having a specific suite of patterns and interactions, which appear more or less important depending on the scale of observation. Therefore, our perception of species-environment relationships is strongly dependent on the scale at which forcing functions and response variables are measured (Cushman & McGarigal 2004a). Under the landscape approach, there is no preferred or characteristic scale at which ecological patterns and processes are studied (Levin 1992). A number of authors contend that studies focussing on a single-scale would probably miss important interactions occurring at others, especially in the studies of multi-species assemblages (Chittaro 2004, Pittman et al. 2007). Thereby, to combine observations at different spatial scales is common in landscape ecology studies (Cushman & McGarigal 2004b, Canavelli et al. 2014). This approach, if achieved successfully, has obvious advantages for a better understanding of ecological and evolutionary issues in natural communities. In addition, it can provide quantitative and spatially explicit information on the scales influencing the most the community structure, with obvious implications for management (Wiens 1999, Grober-Dunsmore et al. 2009). Classically, patterns of species distribution and their environmental drivers have been explored at the habitat level in terrestrial and marine environments (e.g. Risk 1972, Downes et al. 1998). More recently, approaches at multiple spatial scales have been successful in predicting the distribution and abundance of mobile species over habitat

mosaics in terrestrial systems (Jaberg & Guisan 2001, Cushman & McGarigal 2004b, Clough et al. 2005, Thornton et al. 2011, Su et al. 2015). In contrast, similar approaches are less explored in the marine environment (see reviews of Hinchey et al. 2008 and Jelinski 2014).

Fishes, and specifically littoral fishes, are one of the most conspicuous and mobile groups among marine organisms. Apart from being an important food resource, they are a good indicator of coastal biodiversity and are considered emblematic species (Allen 2007). For these reasons, they are main targets of conservation initiatives, such as marine protected areas (MPA). Fishes are essential drivers of matter and energy flow in coastal seascapes, playing a central role in maintaining ecosystems resilience (Ledlie et al. 2007) and exerting a key control in trophic cascades (Shears & Babcock 2002, Arias-González et al. 2006). Fish species display a wide range of functional and biological traits, habitat specializations and ontogenic habitat shifts (Mumby et al. 2004, Arias-González et al. 2006), thereby exhibiting a high diversity of distribution patterns and responses to environmental drivers. Littoral fish species body sizes encompass two orders of magnitude, and their home ranges are from the very small (ca.  $\text{cm}^2$ ) to the very large (ca. several  $\text{km}^2$ ), depending on the family (Chittaro 2004). The combination of the great variety of biological traits and home ranges with their ecological importance makes fishes excellent targets for studies attempting to establish how organisms interact with the landscape. Such an approach has been less used in fishes than in mobile terrestrial organisms (e.g. Pittman et al. 2007, Grober-Dunsmore et al. 2008).

Previous research has demonstrated the importance of habitat and landscape-level patterns in shaping fish assemblages (i.e. distribution, composition, abundance and species richness) (Pittman et al. 2004, 2007, Grober-Dunsmore et al. 2007, 2008, Gullström et al. 2008, Moore et al. 2011, Yeager et al. 2011). On the one hand, abundance and composition of fish assemblages have been usually associated to the characteristics of the benthic habitats (Luckhurst & Luckhurst 1978, Arias-González et al. 2011, Risk 1972). Rocky and coral reefs provide high availability of food resources and shelter for fishes due to its high topographic complexity (Risk 1972, Roberts & Ormond 1987), and a similar role has been invoked for seagrass canopies

(Yeager et al. 2011). In addition, these habitats can act as nurseries (García-Rubies & Macpherson 1995, Heck et al. 2003, Mumby et al. 2004). On the other hand, landscape patterns have also been shown to contribute to explain variability in fish assemblages (Grober-Dunsmore et al. 2007, 2008). Generally a high number of habitat types promote a high number of fish species (Leopold 1933; Jackson et al. 2006), while homogeneous landscapes seem to provide a more stable environment (less predation, less disturbance) where more species can survive (Jackson et al. 2006a). In addition, edges or boundaries among habitat types have been shown to have ecological effects, either positive and negative (Smith et al. 2008, 2011). At this respect, it has been proposed that negative effects over fish assemblages are associated to patchiness and fragmentation processes (e.g. Macreadie et al. 2009) suggesting that low extension and high dispersion of habitats (either seagrasses or rocky reefs) with high abundance of edges are not beneficial for the overall fish assemblage.

Most of the studies on fish assemblages at the landscape-level have been conducted in tropical systems, with coral reefs, mangroves and seagrasses as the main habitats of the coastal seascape. In contrast, fewer research has been executed in temperate areas (Jackson et al. 2006b, Jackson et al. 2006a, Moore et al. 2011). There, the main habitats are seagrass meadows and macroalgal communities on rocky reefs. These coastal vegetated habitats commonly occur as parts of a landscape that can adopt different configurations, from large, continuous habitat patches to complex mosaics of both habitats interspersed in sandy bottoms (Robbins & Bell 1994, Pagès et al. 2014, Ricart et al. 2015). While several studies on spatial variation of coastal fish assemblages have been done at the habitat level (García-Charton & Pérez-Ruzafa 2001, La Mesa et al. 2011), studies using a quantitative landscape approach at several scales are still scarce. Yet a landscape-framed approach to littoral fishes is needed, not only to deepen our basic knowledge on such an important community, but also to better manage human impacts. In effect, coastal ecosystems are among the most threatened worldwide (Halpern et al. 2008, Waycott et al. 2009). While studies focussing on direct effects of pressures (and, specifically, overfishing) are abundant (Jackson et al. 2001), the assessment of indirect effects through habitat loss and, specially, through habitat fragmentation and other landscape-level effects are less common (Jelinski 2014). In addition, the scale at which fish assemblages respond to

the landscape and, therefore, at processes altering landscape, have important implications for their conservation(Chittaro 2004).

To help to fill these gaps, here we explore how the variability in fish assemblage features (abundance, species richness and abundance of prevalent families) is determined by landscape patterns measured at three hierarchically nested spatial scales spanning about 4 orders of magnitude, i.e. small (metric scale), intermediate (i.e. scale of hundreds of meters) and large (i.e. scale of kilometres) in a temperate coastal seascape. We use a correlative approach, combining visual censuses of fishes and estimates of landscape composition and configuration metrics. Our objectives fall into two categories: (i) assessing which landscape metrics have the greatest association with fish assemblages features and (ii) identifying the most relevant spatial scales at which these associations occur.

## Materials and Methods

### Study area

Our study was conducted in the Catalan coast (NW Mediterranean). We selected 8 sites (inlets) along the coast (from 42°19'14"N-3°19'18"E to 41°44'09"N-2°57'17"E), where the main habitats were *Posidonia oceanica* seagrass meadows, rocky reefs with macroalgal cover and unvegetated sandy bottoms (seagrass, rocky reefs and sand thereafter). *P. oceanica* is a habitat-forming seagrass species that can grow either as continuous or patchy meadows, intermingled with sand or rocks (Pagès et al. 2014, Ricart et al. 2015). Macroalgal communities were dominated by species such as *Cystoseira* spp., *Jania* spp., *Corallina elongata*, *Padina pavonica*, *Acetabularia acetabulum*, *Codium* spp., *Lithophyllum* spp., *Halopteris* spp., *Dictyota* spp. and *Peyssonnelia* spp. among others (Vigo et al. 2008). The sites were chosen for their apparent similarity in habitat types and spatial configuration.

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### **Benthic habitat maps**

At each site, a map of the benthic habitats was elaborated using a high resolution Side Scan Sonar (Tritech StarFish 990F) system, working at a frequency of 1000kHz. The device had a full coverage of 20m of lateral range, obtaining a basic resolution of 2.5 cm. The sonar data were georeferenced using the GPS position provided by a Lowrance Elite 3x, taking into account the offset (distance between the towed fish and the GPS receptor). The bathymetry of each site was derived from the Lowrance Elite 3x echosounder data, obtained in parallel to the position and sonar data. Inlets were fully covered (overlap of ca. 30% among adjacent lines), until 15m isobaths. Mapped areas encompassed 3 to 8 ha in surface, depending on the inlet size and bathymetry. We performed ground-truthing recording video-transects, by following the side scan sonar transects throughout the entire study area. Once validated, bathymetry and habitat maps were integrated into a vector based Geographic Information System (GIS) (ArcGis 10.1). Substrate boundaries were manually digitized around areas of uniform sonar signature by visual interpretation of the images. Digitized lines were converted to polygons and assigned to a habitat class. To better define the location of the coastline and upper limits of seagrass meadows, we integrated aerial photographs (ICC 2012) into the GIS (see Appendix 1 for more information). Finally, we obtained a two-dimensional map of benthic habitats where the classes considered were seagrasses (clearly identifiable *P.oceanica* homogeneous seagrass patches independently of their size), rocky reefs and sandy bottoms (Appendix 1). To this, two additional categories were added when the intermingling of habitats did not allow the identification of individual habitats. These were mosaics dominated by seagrasses and rocky reefs (habitat classes <sup>(1)</sup> in Appendix 1) and mosaics dominated by seagrasses and sandy bottoms (habitat classes <sup>(2)</sup> in Appendix 1).

### **Underwater sampling**

With the help of benthic maps and according to habitat classes, we identified five landscape configurations (thereinafter called structures) in order to perform censuses of fish assemblages. Three of them were continuous habitats at least 1000 m<sup>2</sup> in

surface (*P. oceanica* seagrass meadows, rocky reefs and sandy bottoms), and the other two were mosaics (seagrass patches interspersed with rocky reefs and seagrass patches interspersed with sandy bottoms).

Sampling of fishes was conducted in June-July 2012 (early summer season) using standard methodology for underwater visual transects fish census with SCUBA diving equipment (e.g. Harmelin-Vivien 1989). Five (replicated) parallel 25 m long transects, were randomly placed inside each one of the five structures identified at each site (Fig. 6.1). For each transect, a visual census was performed, by the same observer in all cases, recording the number of fish species and individuals present at 1 m at each side of the transect line. All transects were restricted to depths between 5 and 8 m, to avoid bathymetric variability. To characterise the transect, a second diver recorded the transect with a digital camera held perpendicular to the bottom and 3 m above it at a constant speed, and using a metric tape for spatial reference (McDonald et al. 2006). A total of 166 transects were considered for subsequent analysis, discarding those with no fishes observed.

From the census, a series of variables were obtained to characterise the fish assemblages. These included: (a) total abundance of fishes (total number of individuals per transect), (b) species richness (number of species per transect) and (c) abundance of prevalent families (total number of individual belonging to a given family per transect). These families were Pomacentridae, Mullidae, Serranidae, Labridae and Sparidae, which encompass more than 95% of total fish abundance recorded.

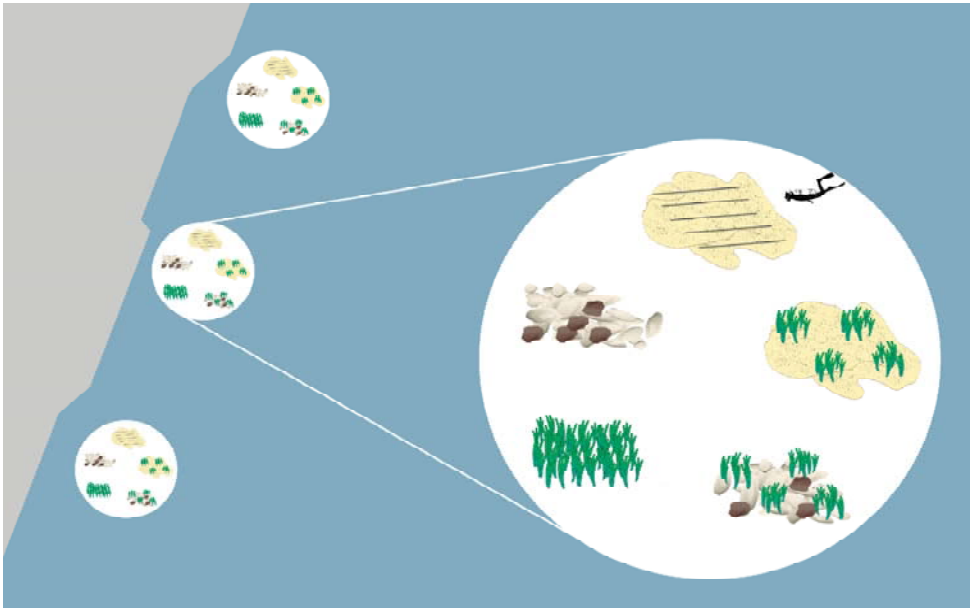


Fig. 6.1. Conceptual scheme of the sampling design used in this study at three spatial scales: Fish censuses were performed in five replicated transects within each one of the five structures (see text) for each of the eight sites. Figure represents one site with five structures and the five transects developed in each structure. Small scale metrics were measured for each transect, and reflect variability within each structure. Intermediate scale metrics were measured for areas embracing the five transects of the same structure, reflecting the variability among structures. Finally, large-scale metrics were measured for all the area mapped at each site, and thus reflect overall variability among sites.

### Characterization of landscape patterns

We evaluated landscape metrics at three different spatial scales from small (meters) to intermediate (hundreds of meters) and large (kilometres), with the aid of the video recording for the small scale and the benthic habitat maps for the other two.

**Small scale (meters):** The video-transects were carefully visualized and the three main habitats (seagrasses, rocky reefs and sand) were identified. At this scale, we also distinguished other two secondary habitats (dead matte and leaf litter accumulations) that we took into account to describe the small-scale landscape patterns. We located the position of the transitions between the different habitats, for any habitat occupying more than 0.1 m over the transect line. From these data, and for each transect, we quantified habitat richness (number of habitat types present in the

transect), and the number of transitions along the transect. In addition, we estimated the seagrass percent cover and rocky reefs percent cover for the whole transect. To do this, 12 video-frames (the minimum number of frames needed to absorb all transect variability) (Bros & Cowell 1987), encompassing ca. 1.8 m x 1.2 m of seafloor each, were selected for each video-transect at constant time intervals, and rocky reefs and seagrass cover were estimated for each frame using an image processing software (Adobe Photoshop). Values for the 12 frames were averaged to obtain a cover value for each transect.

**Intermediate scale (hundreds of meters):** We used rasters (cell size 0.1m) of the benthic habitat maps. For each structure, previously identified for fish censuses as explained above, we defined a polygon 0.3 ha in surface which included the five transects performed. The rationale for selecting such a surface was based on Claudet et al. (2011), who based their choice on home-range considerations. For each of these polygons, and using the spatial statistical software FRAGSTATS version 4.2 (McGarigal et al. 2012), we quantified seagrass percent cover and rocky reefs percent cover (percentage of surface of the polygon occupied by the habitat map classes “seagrasses” or “rocky reefs”, respectively). We also estimated the patch richness density (number of patches of the different habitat classes on a per area basis) and the contagion index (indicating the aggregation of patches of the different habitat classes; see Table 6.1).

**Large scale (kilometres):** We used, as above, rasters of the benthic habitat maps (cell size 0.1m), and defined a single polygon per site encompassing all the area mapped. From these polygons, and using the spatial statistical software FRAGSTATS version 4.2 (McGarigal et al. 2012), we computed seagrass percent cover and rocky reefs percent cover (percentage of surface of the polygon occupied by the habitat map classes “seagrasses” or “rocky reefs”, respectively). We also estimated the seagrass patch density (number of seagrass patches on a per area basis) and the landscape shape index (perimeter to area ratio integrated over the polygon; see Table 6.1). For more details on specific calculations of the metrics, see McGarigal et al. 2002.



It has to be noted that the small scale metrics were measured for each transect, and reflect variability among transects within each of the five structures defined, continuous habitats at least 1000 m<sup>2</sup> in surface (*P.oceanica* seagrass meadows, rocky reefs and sandy bottoms,), and mosaics (seagrass patches interspersed with rocky reefs and seagrass patches interspersed with sandy bottoms). In contrast, intermediate scale metrics were measured for each structure, reflecting thus the variability among structures. Finally, large-scale metrics were measured for each site, and thus reflect variability among sites in their overall landscape patterns. Therefore, some metrics measured at different scales (e.g. seagrass cover, rocky reefs cover), although having the same name, represented different magnitudes, as they were measured either at the small scale (for each transect), at the intermediate scale (for polygons encompassing five different transects within the same structure) or at the large scale (for the overall site, including all structures and transects). Metrics were chosen on the basis that they could be quantified in all sampling units at each spatial scale, and that they were, by construction, size independent to allow comparisons between different sized landscapes.

### **Data analysis**

Differences among structures and sites in total abundance, species richness and abundance of the most prevalent families were analysed using Generalized Linear Models (GLM). Then we modelled the association between dependent variables (i.e. those related to fish assemblages) and independent ones (landscape metrics) using two approaches. We first examined the statistical association between variables describing fish assemblages and landscape metrics separately for each spatial scale, to identify important drivers at each scale. Then we constructed multi-level models combining as independent variables all the landscape metrics, irrespectively from the scale, to compare the relative importance of the different scales.

Therefore, for each dependent variable, we fitted a set of four generalised linear mixed models (GLMM). Each one of the first three models included only the metrics relevant to each one of the three spatial scales considered, while the last one included all the metrics. We used structures and sites as random effects (i.e. grouping

variables), which allowed us to account for potential spatial autocorrelation within transects and structures (Dormann et al. 2007, Canavelli et al. 2014, Finch et al. 2014). All statistical analyses were performed in R software (R Core Team 2015). We used package "glmulti" (Calcagno 2013) to screen the landscape metrics and select those to be included in each model and we used the package "lme4" to fit GLMMs (Bates et al. 2014). The best performing model was selected using Bayesian Information Criterion (BIC) (Raftery 1995). Similar to Akaike's Information Criterion (AIC), models with lower BIC values are better supported by the data. We preferred BIC over AIC corrected by small sample size (AICc) to avoid over-parameterized models (Villaseñor et al. 2015). We interpreted differences in BIC between models of 0-2 as weak, 2-6 as positive, 6-10 as strong and >10 as very strong (Raftery 1995). Models were considered when differences on BIC were higher than 2. We also ran a null model for each set of models to examine the degree to which variability of dependent variables was explained by random effects. As response variables followed a typical counting data distribution, GLM and GLMM were used with a Poisson distribution and log link function. All predictor variables were standardized prior to analysis, and interactions among variables were not considered. We tested for normality of GLMM by visual inspection of model residuals. As regression based models can be sensitive to predictors (independent variables) that are correlated, in the multi-level models only variables with no collinearity (Pearson correlations of  $R^2 < 0.7$ ) were included in the analysis. As seagrass and rocky reefs cover presented co-linearity among scales, all multi-levels models were run three times, where each time, seagrass and rocky reefs cover were assessed in only one scale and only kept in the scale where the model presented the lowest BIC.

## Results

### **Fish assemblages description**

We identified a total of 46 fish species belonging to 14 families (Table SM 6.1 in the Appendix 2). Their distribution among the different structures was uneven ( $p < 0.05$ ), being more abundant in rocky reefs and in mosaics of seagrass patches

interspersed with rocky reefs, and less abundant in sandy bottoms (Fig. 6.2a). Species richness was also influenced by the structure ( $p < 0.05$ ), with the highest values in rocky reefs and the lowest in sandy bottoms (Fig. 6.2a). Fish abundance showed significant variability at large scale (i.e. among sites,  $p < 0.05$ ), while species richness did not (Fig. 6.2b).

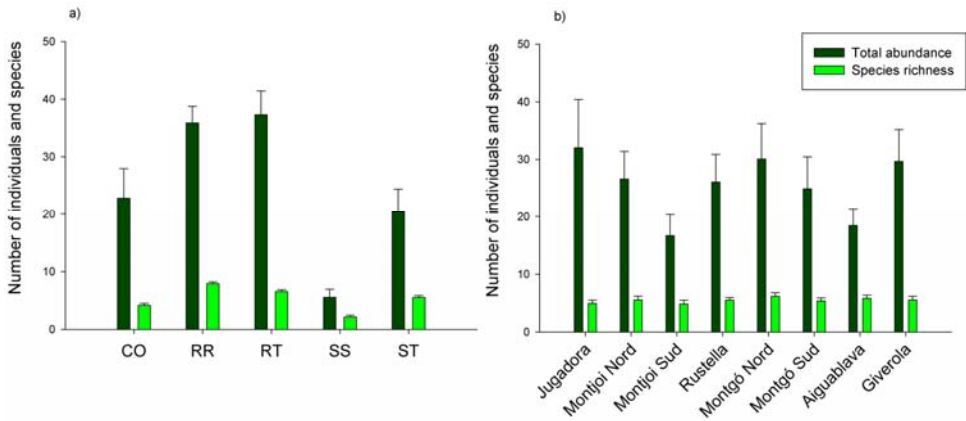


Fig. 6.2. Total abundance and species richness of fish assemblages (a) by structures (b) by sites. CO, continuous *P. oceanica* seagrass meadows; RR, continuous rocky reefs habitats, RT; seagrass patches interspersed with rocky reefs; SS, continuous sandy bottoms; ST, seagrass patches interspersed with sandy bottoms.

The abundance of one (out of five) of the families considered, Mullidae (with only one species, *Mullus surmuletus*) did not show significant differences among structures ( $p > 0.05$ , Fig. 6.3a). Pomacentridae (with only one species, *Chromis chromis*) was abundant in all structures with the exception of sandy bottoms; Serranidae (with the species *Serranus scriba* and *Serranus cabrilla*) and Labridae (which included *Coris julis*, *Thalassoma pavo*, and *Labrus spp* and *Symphodus spp*) seemed associated to rocky reefs (either as continuous habitats or in mosaics interspersed with seagrass patches); while the abundance of Sparidae (with the species *Sarpa salpa* and *Diplodus spp.*, among others) was higher in continuous seagrass meadows and in mosaics of seagrass patches interspersed with rocky reefs. The abundance of Mullidae, Pomacentridae, Labridae and Sparidae differed significantly among sites ( $p < 0.05$ ), while Serranidae did not (Fig. 6.3b).

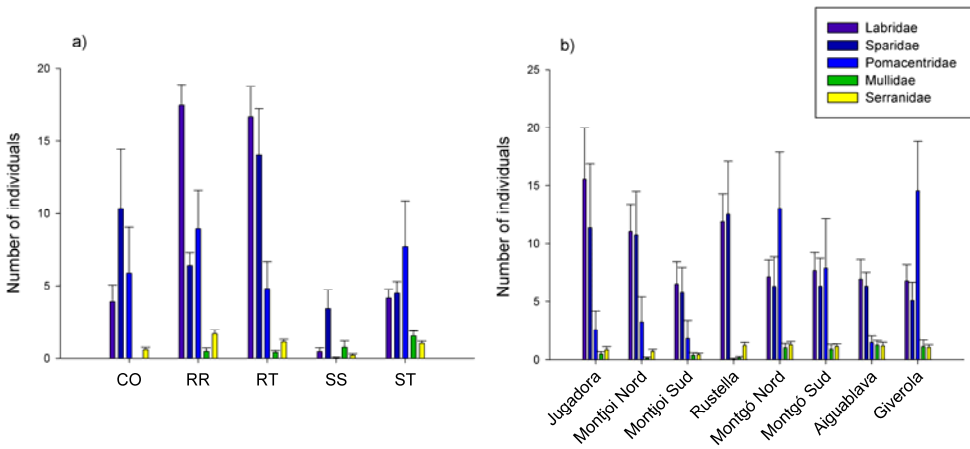


Fig. 6.3. Abundance of the most prevalent families of fish assemblages (a) by structures (b) by sites. CO, continuous *P. oceanica* seagrass meadows; RR, continuous rocky reefs habitats; RT, seagrass patches interspersed with rocky reefs; SS, continuous sandy bottoms; ST, seagrass patches interspersed with sandy bottoms.

### Influence of landscape metrics in total fish abundance and species richness

At small spatial scale (i.e. among transects), rocky reefs cover was the metric explaining the largest part of the variability in total fish abundance and species richness (as inferred from regression coefficients, Table 6.2), showing positive effects. Fish abundance was also weakly and negatively associated to the number of transitions between habitats. At intermediate spatial scale (i.e. among structures), seagrass cover was the metric explaining the largest part of fish abundance variability (Table 6.2), with a negative association over it. Rocky reef cover (positive association) and patch richness density (negative association) also explained part of fish abundance variability. At this level, species richness was not related to any landscape metric measured. At large spatial scale (i.e. among sites) landscape metrics did not explain neither fish abundance nor species richness variability, as derived from the fact that the null model had the lowest BIC among all the models fitted (Table 6.2).

The multi-level model was the best performing model to predict total fish abundance, combining metrics at small and intermediate scales, being the intermediate scale metrics the most influential (seagrass cover and patch richness density, with negative association, and rock cover, with positive association) (Table 6.2). In addition to this,

the number of transitions (measured within transects, i.e. at small spatial scale) also showed a negative influence. The multi-level model confirmed the positive association already mentioned between rock cover (measured at small spatial scale) and species richness.

Table 1. Description of the landscape metrics used in this study, including mean, standard deviation (SD) and observed range

Small scale metrics		Description	Units	Mean	SD	Observed range
Seagrass cover	Cover of <i>P.oceanica</i> seagrass		%	38.62	2.72	0-100
Rocky reefs cover	Cover of rocky reefs with macroalgal vegetation		%	29.65	2.94	0-100
Number of transitions	Number of transitions among habitat types along the transect		num per 25m	5.42	0.38	0-17
Habitat richness	Total number of habitats in the transect		num per 25m	2.36	0.08	0-5
<b>Intermediate scale metrics</b>						
Seagrass cover	Cover of <i>P.oceanica</i> seagrass		%	19.26	0.80	1-36
Rocky reefs cover	Cover of rocky reefs with macroalgal vegetation		%	12.94	0.72	1-30
Contagion index	Index of aggregation of patches. Measures patch dispersion and interspersion. Approaches 0 when the patch types are highly disaggregated and interspersed		%	76.76	17.78	48.6-99.7
Patch richness density	Number of the different patch types to per area basis		num per 100 ha	19.23	0.83	1-33
<b>Large scale metrics</b>						
Seagrass cover	Cover of <i>P.oceanica</i> seagrass		%	35.56	1.64	5-68
Rocky reefs cover	Cover of rocky reefs with macroalgal vegetation		%	15.74	0.72	3-26
Seagrass patch density	Density of seagrass patches		num per 100 ha	1447.97	77.37	403-3173
Landscape Shape Index	Perimeter to area ratio of all patches in the landscape as a whole. It increases without limit as landscape shape becomes more irregular and/or the length of edges increases		none	6.10	0.06	4.9-7.4

**Influence of landscape metrics on the abundance of the main families**

At small spatial scale (i.e. among transects, Table 6.3a) the abundance of Pomacentridae, Serranidae, Labridae and Sparidae increased with rocky reefs cover. The number of transitions among habitats was associated positively to the abundance of Pomacentridae, and negatively to that of Labridae and Sparidae. Habitat richness appeared to increase the abundance of Serranidae and Labridae, and decrease the abundance of Sparidae. At this scale, seagrass cover appeared to decrease the abundance of Mullidae.

At intermediate spatial scale (i.e. among structures, Table 6.3a) the abundance of all families was negatively associated with seagrass cover, while rock cover was positively related to the abundance of Pomacentridae and Sparidae. Moreover, the high contagion index (indicating a low degree of dispersion and interspersed habitat patches) was associated to an increase in Pomacentridae and a decrease in Serranidae and Sparidae abundances. Finally, Labridae and Sparidae abundances were negatively related with the patch richness density.

At large spatial scale (i.e. among sites, Table 6.3b), we detected only weak associations between the abundance of several families and landscape metrics, such as that found between seagrass patch density (negative) and landscape shape index (positive) with Sparidae abundance.

For all the families, except for the Serranidae, the best results were obtained when using the multi-level model (Table 6.3b). Thus, the abundance of Pomacentridae was related positively to the number of transitions among habitats at small scale; and also to a decrease in the degree of the dispersion of habitat patches (increased contagion index), an increase in the cover of rocky reefs, and a decrease in the seagrass cover at intermediate scale. The abundance of Mullidae was related negatively with seagrass cover at intermediate scale, and positively with seagrass patch density at large scale. The abundance of Labridae was related negatively to the number of transitions and, positively to habitat richness at small scale, and negatively to patch richness density and seagrass cover at intermediate scale. The abundance of Sparidae was related

negatively to habitat richness, and the number of transitions at small scale; also was negatively associated to contagion index, patch richness density and seagrass cover at intermediate scale; and positively with an increase in the irregularity of the landscape configuration (an increase in the Landscape Shape Index) and negatively to seagrass patch density at larger scale.



Table 6.2. Results of the GLMM's used to describe the association among total abundance and species richness of fish assemblages with landscape metrics at small, intermediate and large scales separately and together (multi-level). Coefficients and associated standard error for each independent variable were derived from multi-model inferences and Bayesian Information Criteria (BIC). Coef, regression coefficient; SE, Standard error of regression coefficient; - parameter discarded from the model using model parameter selection BIC criteria in package glmulti; NULL, when the null model has the best performance; In bold the lowest BIC between the model performed and its null model; \*best performing model among the set of four models performed for each dependent variable

		Small scale				Intermediate scale				Large scale				Multi-level			
Dependent variables →		Abundance		Richness		Abundance		Richness		Abundance		Richness		Abundance		Richness	
Independent variables ↓		Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE
Small scale	Seagrass cover	-	-	-	-									-	-	-	-
	Rocky reefs cover	0.35	0.05	0.26	0.08									-	-	0.26	0.08
	Number of transicions	-0.14	0.03	-	-									-0.19	0.03	-	-
	Habitat richness	-	-	-	-									-	-	-	-
Intermediate	Contagion Index					-	-							-	-	-	-
	Patch Richness Density					-0.28	0.03							-0.29	0.03	-	-
	Rocky reefs cover					0.22	0.04							0.20	0.04	-	-
	Seagrass cover					-0.60	0.05							-0.60	0.05	-	-
Large scale	Landscape Shape Index													-	-	-	-
	Seagrass Patch density									NULL		NULL		-	-	-	-
	Rocky reefs cover													-	-	-	-
	Seagrass cover													-	-	-	-
BIC final model		3128.00		722.68*		3043.80		729.50		3223.50		729.14		2995*		722.68	
BIC null model		3218.60		725.43		3218.60		725.43		3218.60		725.43		3218.60		725.43	

Table 6.3a. Results of the GLMM's used to describe the association among the most abundant families of fish assemblages with landscape metrics at small and intermediate scales separately. Coefficients and associated standard error for each independent variable were derived from multi-model inferences and Bayesian Information Criteria (BIC). Coef, regression coefficient; SE, Standard error of regression coefficient; - parameter discarded from the model using model parameter selection BIC criteria in package glmulti; NULL, when the null model has the best performance; In bold the lowest BIC between the model performed and its null model; \*best performing model among the set of four models performed for each dependent variable

Dependent variables →		Small scale										Intermediate scale									
		Pomacentridae		Serranidae		Mullidae		Labridae		Sparidae		Pomacentridae		Serranidae		Mullidae		Labridae		Sparidae	
Independent variables ↓		Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE
Small scale	Seagrass cover	-	-	-	-	-0.54	0.20	-	-	-	-										
	Rocky reefs cover	0.95	0.14	0.43	0.08	-	-	0.28	0.07	0.26	0.08										
	Number of transicions	0.12	0.05	-	-	-	-	-0.15	0.05	-0.25	0.06										
	Habitat richness	-	-	0.32	0.08	-	-	0.19	0.04	-0.55	0.06										
Intermediate	Contagion Index											0.26	0.05	-0.27	0.11	-	-	-	-	-0.13	0.06
	Patch Richness Density											-	-	-	-	-	-	-0.43	0.05	-0.24	0.05
	Rocky reefs cover											0.23	0.08	-	-	-	-	-	-	0.72	0.11
	Seagrass cover											-2.35	0.17	-0.39	0.16	-0.88	0.25	-0.45	0.08	-0.28	0.09
Large scale	Landscape Shape Index																				
	Seagrass Patch density																				
	Rocky reefs cover																				
	Seagrass cover																				
BIC final model		<b>2519.30</b>		<b>428.36*</b>		<b>406.94</b>		<b>1198.20</b>		<b>2219.20</b>		<b>2271.20</b>		<b>434.80</b>		<b>401.80</b>		<b>1156.30</b>		<b>2439.00</b>	
BIC null model		2558.90		435.74		408.37		1212.90		2489.50		2558.90		<b>435.74</b>		408.37		1212.90		2489.50	

Table 6.3b. Results of the GLMM's used to describe the association among the most abundant families of fish assemblages with landscape metrics at large scale separately and all the scales together (multi-level). Coefficients and associated standard error for each independent variable were derived from multi-model inferences and Bayesian Information Criteria (BIC). Coef, regression coefficient; SE, Standard error of regression coefficient; - parameter discarded from the model using model parameter selection BIC criteria in package glmulti; NULL, when the null model has the best performance; In bold the lowest BIC between the model performed and its null model; \*best performing model among the set of four models performed for each dependent variable

		Large scale										Multi-level									
Dependent variables →		Pomacentridae		Serranidae		Mullidae		Labridae		Sparidae		Pomacentridae		Serranidae		Mullidae		Labridae		Sparidae	
Independent variables ↓		Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE	Coef	SE
Small scale	Seagrass cover											-	-	-	-	-	-	-	-	-	-
	Rocky reefs cover											-	-	0.43	0.08	-	-	-	-	-	-
	Number of transicions											0.22	0.05	-	-	-	-	-0.21	0.05	-0.27	0.06
	Habitat richness											-	-	0.32	0.08	-	-	0.18	0.04	-0.58	0.06
Intermediate	Contagion Index											0.24	0.05	-	-	-	-	-	-	-0.19	0.06
	Patch Richness Density											-	-	-	-	-	-	-0.46	0.06	-0.29	0.05
	Rocky reefs cover											0.26	0.08	-	-	-	-	-	-	-	-
	Seagrass cover											-2.43	0.17	-	-	-0.88	0.23	-0.43	0.08	-0.42	0.09
Large scale	Landscape Shape Index	-1.62	0.40	NULL		-	-	-	-	0.29	0.06	-	-	-	-	-	-	-	-	0.34	0.10
	Seagrass Patch density	1.68	0.40			0.49	0.18	-0.24	0.09	-0.35	0.06	-	-	-	-	0.44	0.14	-	-	-0.42	0.10
	Rocky reefs cover	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Seagrass cover	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BIC final model		<b>2559.90</b>		438.20		<b>408.00</b>		<b>1213.30</b>		<b>2487.20</b>		<b>2256.7*</b>		<b>428.36</b>		<b>400.1*</b>		<b>1147.2*</b>		<b>2194.6*</b>	
BIC null model		<b>2558.90</b>		<b>435.74</b>		<b>408.37</b>		<b>1212.90</b>		2489.50		2558.90		435.74		408.37		1212.90		2489.50	

## Discussion

Fish assemblages are influenced by landscape patterns at a range of spatial scales, from meters to kilometers, including landscape features related with its composition (e.g. cover of vegetated habitats), and its configuration (e.g. number of transitions between habitats, patch richness density). The spatial scale at which landscape patterns best explain fish assemblages depends on the assemblage descriptor being analyzed. Thus, total abundance and species richness are mostly associated to landscape patterns reflected by metrics measured at small and intermediate spatial scales, with rocky reefs cover having a strong positive effect. While the abundance of the different families is explained by different landscape metrics at different spatial scales, depending on the biology and ecology of each group. Thereby, the abundance of Serranidae is mostly associated to small-scale metrics, that of Pomacentridae and Labridae to small and intermediate-scale metrics, that of Mullidae to intermediate and large-scale metrics and that of Sparidae to all three spatial scales. The effect of each metric varies depending on the family.

Metrics related to landscape composition, such as cover of vegetated habitats, clearly influenced fish assemblages. On the one hand, rocky reefs cover appeared as an important predictor at the small and intermediate spatial scales, with a strong positive effect over fish abundances and species richness. This is in accordance with many studies about spatial variation of fish populations (García-Charton & Pérez-Ruzafa 2001, Tunesi et al. 2006, La Mesa et al. 2011), and is likely caused by the high complexity (e.g. crevices and holes) of this substrate, further increased by macroalgal canopies, overall providing multiple ecological niches and refuges (García-Rubies & Macpherson 1995, Vega Fernández et al. 2009). On the other hand, seagrass cover, when significant, appeared to be negatively related to fish abundances at small and intermediate scales. This is in contrast with the supposed role of seagrass meadows as fish habitat, due to their food and shelter provision (Yeager et al. 2011). The relative scarcity of fishes observed in our transects in continuous seagrass meadows was probably caused by the eventual use that fishes make of this habitat, only in specific hours of the day, season of the year or phase of the life cycle (e.g. diel foraging

movements to surrounding habitats, seasonal behaviour, etc.) (March et al. 2010, Aguzzi et al. 2013). Moreover, fishes are known to be more abundant near seagrass meadows boundaries than in their internal parts (Macreadie et al. 2010). Finally, it has to be noted that neither rock cover nor seagrass cover presented any effect at the large spatial scale.

Landscape configuration metrics influenced negatively total fish abundance at small and intermediate scales (i.e. number of transitions and patch richness density, both indicating spatial heterogeneity). This seems in agreement with the decreased fish abundance associated to landscapes with high degree of patchiness or fragmentation (Jackson et al. 2006a). However, these metrics did not present any effect on species richness, while the importance of the biology of different species in their interactions with landscape, appears when assessing the statistical association between these landscape metrics and the abundance of different fish groups (i.e. families). In these cases, the specific traits (e.g. foraging behaviour, trophic role, home range) determine a differential response to landscape patterns.

On the one hand, Pomacentridae (*C. chromis*) and Serranidae (*S. cabrilla* and *S. scriba*) were mostly associated to small-scale metrics. Pomacentridae were more abundant in landscapes dominated by rocky reefs, patchy (high number of transitions between habitats) but with a clumped spatial structure (high contagion index). This is in accordance with the preference of *C. chromis* for rocky habitats and landscapes with low spatial heterogeneity (Coll et al. 1998, Vega Fernández et al. 2005). This species is very common in rocky reefs, a habitat they use for recruitment and shelter (García-Rubies & Macpherson 1995, Guidetti 2000), being less abundant in *P. oceanica* seagrass meadows (Guidetti 2000, La Mesa et al. 2011). Moreover, it is known that Pomacentridae species display territorial behaviour, with home ranges in the order of meters or hundreds of meters (Jones 2005), thus explaining the lack of influence on their abundances of landscape patterns at large scales. Similarly, the abundance of Serranidae increased with rocky reefs cover, and seemed also favoured by the presence of other habitats (positive association with habitat richness at small scale), with no influence of seagrass cover, despite the two species, *S. cabrilla* and, specially, *S. scriba*, are commonly found in seagrass meadows and use it for recruitment (García-Rubies

& Macpherson 1995, Coll et al. 1998, Guidetti 2000). This could be due in part by their nycthemeral behaviour, with a nocturnal resting phase in the meadow and a diurnal foraging phase in nearby rocky reefs habitats (March et al. 2010). Species belonging to this family also have small home ranges, from meters to hundreds of meters (March et al. 2010), which explain the limited influence of intermediate scale metrics and the null influence of large scale ones on their abundance.

On the other hand, Mullidae (with the species *M. surmuletus*) have home ranges larger than those of the previous two families (from hundreds to thousands of meters: Meyer et al. 2000, Strübin et al. 2011), which is consistent with the influence of large scale metrics on their abundance. *M. surmuletus* was more abundant in landscapes with high seagrass patch density, while it was really scarce in landscapes with continuous seagrass cover, as previously shown by Vega-Fernández et al. (2005). This is coherent with the recruitment patterns, which take place mainly in *P. oceanica* seagrass meadows (García-Rubies & Macpherson 1995), but mostly with the foraging behaviour, associated to sandy bottoms (Palmer et al. 2011, Soares 2016) and to sandy-seagrass boundaries (De Pirro et al. 1999), where this species is commonly observed (García-Charton & Pérez-Ruzafa 2001, Vega Fernández et al. 2005).

Labridae were mostly associated to small- and intermediate-scale metrics, in accordance with their home-ranges of hundreds of meters or less (Palmer et al. 2011). However, their abundance, higher in homogeneous landscapes (low number of transitions and low patch richness) with low cover of seagrass is in disagreement with what is known about this family. Labridae are inhabitants of rocky habitats and seagrass meadows (Guidetti 2000, Tunesi et al. 2006, La Mesa et al. 2011), and their abundance has been reported to be high in heterogeneous and patchy landscapes (García-Charton et al. 2004, Vega Fernández et al. 2005). This discrepancy can be attributed to the relatively large number of species within the group (14 species, see Table SM 6.1 in the Appendix 2), with different biological traits and habitat uses, that weakens statistical correlations when assessed as a whole.

Finally, Sparidae, including mostly carnivorous-omnivorous species (e.g. *Diplodus* spp., *Sparus aurata*, *Spondyliosoma cantharus*, among others, see Table SM 6.1 in the

Appendix 2) but also herbivorous (*Sarpa salpa*) and planktophagous-omnivorous (*Boops boops*) have as a common feature their generally large home ranges (in the order of kilometres, D'Anna et al. 2011, Pagès et al. 2013). In agreement with that, they are influenced by a wide array of landscape patterns from all three scales, being the group with the most clearly association to metrics measured at large spatial scales. Their abundance was higher in transects with low spatial heterogeneity at the small scale (low number of transitions and low habitat richness in transects), but with higher complexity (in terms of a high Landscape Shape Index) at the large scale. This result is probably linked to the fact that predation is usually enhanced at the edge of seagrass habitats (Macreadie et al. 2012, Farina et al. 2014) due to the high density of resources in there (Macreadie et al. 2010). Moreover, their preference for heterogeneous landscapes with presence of rocky reefs and seagrass habitats in which they move around for foraging purposes has been already described (García-Charton et al. 2004, D'Anna et al. 2011).

Overall, these results suggest a clear correspondence between the home range of the different families and the scale(s) at which landscape patterns influence their abundance. Groups more strictly linked to the habitat (e.g. Serranidae) respond mostly to small-scale patterns of the landscape, while those having great movement capacities and larger home ranges (e.g. Sparidae) show the influence of large-scale ones.

This study confirms the importance of the scale in the assessment of landscape effects on the abundance and distribution of mobile organism such as fishes. Studies combining measures at different spatial scales are especially important in landscape ecology research and in management actions and conservation policies. Multi-scale approaches allow the comparison of the strength of the species-environment association across scales, helping to the identification of the scale where the strongest influences are generated (Pittman et al. 2007). Most of the models applied improved their goodness-of-fit when landscape metrics of different scales were assessed together, as previously shown for other organisms (Canavelli et al. 2014, Villaseñor et al. 2015). In the present study, the multi-scale approach combined with the multi-level models, has led to the conclusion that, concerning overall features of the fish

assemblages (i.e. total abundance and species richness), the main landscape drivers seem to be found at small or intermediate scales. However, when working with subgroups (families) with specific biological and ecological traits, the large-scale metrics become increasingly important for groups with large home-ranges. This has serious practical implications, as the objectives of management, to be effective, should determine the scale at which the efforts should be realized (Pittman et al. 2007). For instance, when planning a MPA for increasing fish populations, the scale at which the landscape is considered will have direct consequences on the success of protection applied (Grober-Dunsmore et al. 2007). Following the results of this study, if the main purpose of a MPA is a general increase in fish richness and abundance, selection criteria should focus on small-scale landscape patterns. However, if the MPA is more targeted to species of commercial interest (such as Sparidae), then the focus should be widened to include also large-scale landscape patterns.

This study has two main limitations. One is the different statistical power associated to the spatial scales assessed, and, specifically, the loss of statistical power (due to the reduction in the number of cases) as the spatial scale increased (166 transects, 36 polygons representing structures, 8 sites). This is a shortcoming that should be taken into consideration and improved in future works. The other is the correlative nature of our approach, that may not provide sufficient background to build appropriate predictive models (Macreadie et al. 2010, Yeager et al. 2011). However, and acknowledging that further studies are required to understand the specific mechanisms linking coastal fishes and the seascape, this study highlights the need for taking into account the landscape structure in the understanding, modelling and management of the coastal zone.



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An underwater photograph showing a dense patch of green seagrass on a sandy and rocky seabed. The water is clear and blue, with sunlight filtering down from the surface. A large black rectangular box is overlaid on the upper half of the image, containing the chapter title and subtitle in white text.

# CHAPTER 7

General discussion

Photo: Habitat edge between a *Posidonia oceanica* seagrass meadow and rocky bottoms with macroalgal cover, from the author.

## **Landscape patterns influence the functioning of the coastal system by modulating the interactions among its parts**

Although ecosystems are often studied separately for practical reasons, none of them exists in complete isolation from its pairs (Vanni et al. 2004). Thereby, the spatial arrangement of the different ecosystems (or habitats) within a landscape influences its overall function (Gillis et al. 2014). Interactions at landscape level, taking place between habitats spatially segregated, are usually associated either to the movement of organisms (active mobile entities) or to the transfer of materials (passive mobile entities) from one habitat to another (e.g. Howe and Simenstad, 2015; Hyndes et al., 2014; Milner et al., 2007). In this thesis we provide new experimental (observational) evidence emphasizing the importance of landscape-level interactions in driving coastal ecosystems, and underlining how the relative abundance of habitats and their spatial arrangement can influence ecological functions across the coastal ecosystem mosaic. We enlarge thus the existing body of knowledge at this respect (see reviews of Bostrom et al. 2006 and Grober-Dunsmore et al. 2009).

A first aspect refers to marine ecosystems with high primary productivity, from which an important part of the biomass is exported as detrital materials to other submersed habitats (Romero et al. 1992, Mateo & Romero 1997, Wernberg et al. 2006, Krumhansl & Scheibling 2011) or to the shoreline (Kirkman and Kendrick 1997) (Mateo et al. 2003). The rate at which these transport processes take place depends, among other factors, on landscape attributes such as habitat patch size, as demonstrated in Chapter 3. The transfer of detrital materials acts as a subsidy for the recipient habitats, enhancing their productivity (Heck et al. 2008, Mellbrand et al. 2011), but it also weakens the nutrient recycling capacity in the donor habitat. This mechanism links some landscape patterns with primary production, consumers and sediment organic carbon capacity of seagrass habitats, as pointed in Chapter 3 and Chapter 4.

Specifically, we have shown that seagrasses living in small patches were nutrient-depleted, in comparison with those from continuous meadows, and that this depletion could be associated to the low leaf litter stock found in small patches

(Chapter 3). The low leaf litter retention capacity in small patches is likely due to the combination of abiotic factors, such as waves and currents, and a high permeability of habitat boundaries (Polis et al. 1997, Puth & Wilson 2001), which entails that detached seagrass leaves are washed away, and decay takes place out of the seagrass patches (Cebrian & Duarte 2001). This low nitrogen content of seagrass tissues can induce a different habitat use by key organisms such as herbivores (Hovel et al. 2002, Haynes & Cronin 2004, Pagès et al. 2014), which in turn can limit seagrass long-term primary production, decrease reproductive success (Gera 2013, Planes 2011) and act as allogenic engineers triggering trait-mediated indirect interactions (Pagès et al. 2012). Moreover, a patchy seagrass landscape, by facilitating litter export, can promote higher accumulation of detrital materials in the shoreline or in other marine ecosystems serving there as subsidy (Heck et al. 2008). Therefore, a specific feature of the landscape can have cascading effects and propagate across different elements of the coastal system.

Trophic subsidies have been previously related to landscape patterns (Polis et al. 1997, Howe & Simenstad 2011). The work of Polis et al. (1997) was probably the first to suggest the need for integration of landscape ecology and food web ecology, noting that flows of energy, materials, or organisms from one habitat to another could strongly influence the structure and dynamics of local food webs. Since then, spatially subsidized food webs have been documented across a wide variety of ecosystems, including terrestrial (Edwards & Sugg 1993, Wallace et al. 2015), marine (Mumby et al. 2004, Wernberg et al. 2006), and those at the land-sea interface (Polis & Hurd 1995, Sanchez-Pinero & Polis 2000), demonstrating how widespread and important these mechanisms are. However, we failed in demonstrating such kind of landscape-level process in our study (Chapter 3). Although we found differences in the availability of food sources for detritivores associated to landscape configuration, these differences were not reflected in the effective food sources (assimilated carbon and nitrogen) identified for our organisms model (sea cucumber), putatively a non selective feeder (Massin & Jangoux 1976). This can be explained by several hypotheses. First, holothurians could be more selective than usually thought (Mercier et al. 1999 ). Second, the results of the method we used, stable isotopes analyses and mixing models with a high number of food sources and two elements as tracers, are

affected by a component of uncertainty (Parnell et al. 2010, Phillips et al. 2014). Moreover, it has to be considered that the proportions at which different elements are assimilated by a consumer can vary across food sources (Tomas et al. 2006), which adds further uncertainty to the mixing models used. In any case, as there are many evidences reporting diet shifts linked to resource availability and to landscape patterns (Wernberg et al. 2006, Deudero et al. 2011, Howe & Simenstad 2015), the question applied to our study system remains open.

The influence of landscape patterns on material flow among habitats (Chapter 3) generates differences in the organic carbon pool in seagrass sediments (Chapter 4), linking thus a landscape-level process with local mechanisms of carbon storage. Although connectivity through multiple vectors across coastal seascape and its importance on carbon transfer have been studied at multiple-scales (Hyndes et al. 2014), in this thesis we present what is probably a first attempt to merge landscape ecology and blue carbon research. We have shown how landscape patterns influence sediment carbon storage within coastal seascapes (Chapters 4 and 5). Overall, we conclude that patchy seagrass landscapes store less carbon and of less quality (regarding blue carbon, i.e. less refractory) due to the meadow size effect (as shown in Chapter 4) and to the higher presence of edges (as shown in Chapter 5). This finding underlines the great variability in seagrass carbon storage, usually neglected in estimates of global carbon budgets (Lavery et al. 2013), and emphasizes the need to incorporate landscape considerations in future estimates of seagrass carbon sinks.

A second aspect concerns the influence of landscape patterns in the distribution and abundance of organisms considered as active mobile entities, as we show in Chapter 6 for fish assemblages. Our results indicate that a high spatial heterogeneity (at different spatial scales, from metric to kilometric) decreases total fish abundance, although not species richness, which is coherent with previous works on patchiness and fragmentation effects (Jackson et al. 2006a, Jackson et al. 2006b, Macreadie et al. 2009). Moreover, we also showed that abundance of rocky habitats increased fish abundance, while that of seagrass meadows decreased it. Results from Chapter 6 suggest that the spatial arrangement of habitats in the landscape and the nature of

these habitats are equally important in shaping fish assemblages, while their action depend on the scale.

Although the role of landscape in shaping communities has been widely studied in terrestrial systems (Jaberg & Guisan 2001, Forbes & Chase 2002, Thornton et al. 2011), the potential of this approach in marine ecology has still room for further development. The assessment of landscape effects has usually been attempted based on community level descriptors (e.g. species richness, abundance, diversity), which can preclude the detection of species-specific interactions at landscape level (Gullström et al. 2008). In Chapter 6 we indeed work with community-level descriptors, evidencing the importance of the spatial scale in assessing the effects of the landscape. Further, we break down the fish assemblage, although only to the family level due to statistical power limitations. Even if families can encompass species differing in their ecology, we found a panoply of different responses to the landscape patterns depending on the biological traits of the family considered, as has been shown in other studies on fish assemblages (Jackson et al. 2006a, Pittman et al. 2007, Drew & Eggleston 2008). Interestingly, the spatial scale at which landscape patterns were more influential in the abundance of a given family was positively correlated with the family home range. This suggests that studying the community, considering each species individually at landscape-level, although requiring a considerable effort, will have promising results. Moreover, this also confirms how much scale matters, and how important is choosing the right scale (or, alternatively, using a multi-scale approach) in experimental landscape research.

## **Importance of the landscape approach for the management and conservation of marine systems**

Applying landscape ecology, the interactions among and within ecosystems can be identified and evaluated to optimize conservation efforts across broad spatial scales. This approach has been successfully used for management purposes in terrestrial ecosystems, such as the assessment of environmental impacts, the design of protected areas or corridors, or in the restoration of wildlife populations (e.g. Gaston et al. 2002, Lunt & Spooner 2005, Dauber et al. 2006). In contrast, the tools provided by

landscape ecology are rarely used in the management of coastal waters (Boström et al. 2006). Conservation policies in the marine environment tend to protect individual habitats rather than mosaics of habitats (Grober-Dunsmore et al. 2009), or, at best, entire areas, although disregarding functional linkages among habitats, despite the growing evidence on the relevance of landscape level processes in marine ecosystem dynamics (Mumby et al. 2004, Boström et al. 2006). Landscape ecology offers great potential to managers facing environmental problems, such as to maintain connectivity patterns among marine ecosystems, identify suitable locations for restoration of threatened populations of mobile species of special interest, or to predict the effects of habitats loss, or the spread of marine invasive species, among others (Mumby 2006, Grober-Dunsmore et al. 2009). However, this potential remains, to the best of our knowledge, unexplored.

Beyond local or regional management scales, landscape level processes are also relevant at global management scales. The recently emerged interest shown by policy makers and scientific community on blue carbon as a part of global carbon sinks, has promoted studies to evaluate those sinks, and, specifically, addressing the variability in carbon stocks in coastal vegetated ecosystems among which seagrasses stand (Lavery et al. 2013, Serrano et al. 2014, Miyajima et al. 2015). However, this growing interest, and the huge investments that some governments are doing in research efforts on this matter, could represent a double-edged sword. On the one hand, it gives an added value to coastal habitats, as providers of a valuable service (carbon storage: Sutton-Grier et al. 2014), which should help to apply more conservation efforts over the threatened coastal ecosystems (Orth et al. 2006, Waycott et al. 2009). However, on the other hand we see how natural ecosystems are being commodified, and the recent interest of governments about natural carbon sinks could lead to the rising of an undesirable business. While it is necessary to apply mitigation measures to reduce the high rates of CO<sub>2</sub> emissions and global warming, researchers should be prudent in the carbon estimates they provide and critical with the mitigation measures they propose, such as restoration (Greiner et al. 2013, Marbà et al. 2015), or nutrient enrichment (Armitage & Fourqurean 2016), and warn society against decision making based only in simple cost-benefit arguments. A robust knowledge of factors causing variability in

seagrass carbon storage, incorporating landscape considerations, can be of great help at this respect.

## **Open questions and future directions**

In this thesis we aimed to examine a series of questions or hypothesis. While along the work progress, new questions and new hypothesis have emerged. Some of them derive specifically from results we have found or problems we have encountered, while others correspond to gaps detected in the knowledge published so far in the field.

### **Nitrogen spatial heterogeneity in the seagrass landscape**

The low nitrogen content in seagrass leaves found in small patches relative to continuous ones (Chapter 3) inside the same area, would deserve a more detailed exploration. Beyond the most parsimonious explanation proposed here (low accumulation of detached seagrass leaves in small patches), there is a number of interacting processes and feedbacks that would merit a closer look out. Among them, decreased leaf production or high herbivory rates over small seagrass patches (Gera et al. 2013), plant response to differential herbivory (Vergés et al. 2008, Sanmartí et al. 2014) or plant quality influencing herbivores action (Haynes & Cronin 2004) could also play a relevant role. All this offers a stimulating field for an experimental approach to landscape patterns effects on spatial resource distribution.

### **Trophic relationships modulated by landscape attributes depend on the biology of each species**

The lack of association between feeding ecology of Holothurians and seagrass landscape-level features (Chapter 3), remains an open question, especially at the light of similar studies (e.g. Howe & Simenstad 2011) where landscape patterns modulate trophic interactions. A number of possible explanations have been proposed in previous sections, but their experimental assessment for eventual refutation or validation is needed. The potential trophic shifts due to landscape processes (e.g. fragmentation) should further be investigated, among other reasons to better manage



food webs in a future scenario of changes in landscape patterns driven by climate change or other human-made impacts.

### **Processes at landscape level driving carbon storage**

The seagrass species *Posidonia oceanica* (Chapter 4) and *Zostera muelleri* (Chapter 5) have a completely different morphology, biology and ecology (Carruthers et al. 2007), and different patterns of carbon storage in their sediments (Lavery et al. 2013). An important part of the carbon stored by *P. oceanica* is in form of dead organs, with high refractory tissues, therefore having the longest carbon stocks worldwide among seagrasses (Mateo et al. 1997, Fourqurean et al. 2012, Serrano et al. 2012). While *Z. muelleri*, and also *P. oceanica*, also store a huge amount of organic carbon derived from allocthonous sources deposited and bounded in sediments (as seen in Chapters 4 and 5). Yet these carbon pools bound to the sediment represents a huge carbon sink, and seem, following our results, not only species-specific but also clearly associated to landscape patterns, specially to patchiness and to edge effects modulating inputs of carbon through deposition processes. While further research should be done to deepen our knowledge in how landscape patterns affect other local processes of sediment carbon storage, such as remineralization. Coupling these evidences with patch dynamics models or fragmentation simulations could help to predict the time-changes in the associated carbon stocks in the coming years, when a correct assessment of carbon sinks will become a critical necessity for management and mitigation strategies related to global change.

### **Mechanisms shaping fish assemblages at landscape level**

Habitat richness is not positively related to species richness in fish communities, at least as shown by different works in seascape ecology (Jackson et al. 2006a, Worm et al. 2006, Wedding et al. 2011) and also by our results in Chapter 6. This seems counter-intuitive, and is in disagreement with the ‘habitat heterogeneity hypothesis’ (MacArthur & MacArthur 1961) proposed in terrestrial systems. This intriguing difference between marine and terrestrial studies has not yet been sufficiently explored. Addressing the issue under a common conceptual framework and through a

comparative approach in both terrestrial and marine landscapes would seem a promising matter.

Another possible way forward in the understanding of the controls exerted by the environment on multi-specific assemblages, as coastal fish assemblages, could be merging community and landscape ecology approaches. Both statistical and mechanistic models describing species-specific relationships with the habitat and landscape (and with other features of the environment) would be of great help at this respect. Of course, this would require a huge experimental, sampling and analytical effort, but it will be likely rewarded by a more in-depth knowledge of processes shaping the abundance and richness of species guilds of special interest, either due to their ecological functions or to their socio-economic relevance, or to both.

### **Fragmentation: pattern and process**

We have referred to the concept of fragmentation repeatedly throughout the thesis. While this concept evokes a dynamic process, through which a continuous habitat suffer surface losses and shifts to a mosaic of habitat patches, it is often used to describe a static state (i.e. patchiness), not necessarily linked to habitat loss (Macreadie et al 2009). Studies on the effects of fragmentation, including those presented in this thesis, are usually conducted in steady-state arrangements of patches and at the patch scale (Boström et al. 2006). The basic assumption behind many fragmentation studies is that differences in attributes between habitat patches of different sizes are a proxy for the assessment of fragmentation effects, irrespectively of the implicit dynamics (steady state or not, patches growing or regressing). Therefore, results reflect mostly a pattern, ignoring (for acceptable practical reasons much often) the process behind. Despite few studies have attempted to study fragmentation from manipulative experiments (e.g. Macreadie et al. 2009), more research should be done to further understand fragmentation of marine landscape as a process of critical importance.

### **Functional landscape ecology: from patterns to mechanisms**

Throughout this thesis and in most similar studies, the mechanistic understanding of landscape level processes are based predominantly on correlative studies (Macreadie et al. 2010). A step needed is thus probably the demonstration of causality through experimental and manipulative studies (Yeager et al. 2011). Because of the large spatial scale necessary for landscape studies, and the inherent problems of this approach (Grober-Dunsmore et al. 2009), this next step will be a great challenge for the coming years.

Patch size, matrix type, edge effects, landscape composition and configuration are perhaps the most basic aspects of seascape patterns. In addition to the effects documented in this thesis, they may also affect a number of important ecological processes such as colonization, reproduction, mortality, predator-prey interactions and transport of organisms, materials and energy across seascapes (Grober-Dunsmore et al. 2009). Despite there are already several studies that have assessed how these landscape attributes affect ecological functions in marine landscapes (e.g. Smith et al. 2010, 2011, Pagès et al. 2014), more steps towards integration of landscape consideration in functional ecology are needed to improve our understanding and our modelling and predicting capacity over coastal marine ecosystems.

### **Seascape ecology as an emerging discipline**

In accordance with Hinchey et al. (2008) and Jelinsky (2014), our overall impression of the state of the application of landscape ecology to marine systems is that there is still a lot to do. Seascape ecology is an emerging field that holds great promise. At present, a key issue is the need to adapt theories, concepts, tools and methods developed in terrestrial landscapes (Wedding et al. 2011, Jelinski 2014) to physical features of marine environment and to the biological traits of marine organisms. The new perspectives thus obtained should be integrated not only into the study of marine ecological dynamics but also into general ecological theory (Hinchey et al. 2008). This will, hopefully, help to fill the gap, sometimes too large, between terrestrial and marine ecologists (Steele 1989, Underwood 2005). Maybe the consolidation of

seascape ecology will stimulate terrestrial ecologists to also look at the ocean to answer their questions in the near future (Pen et al. 2005, Ruttenberg & Granek 2011).

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Given that seascape ecology is still in its early beginning, the list of ideas for further research is endless - or it appears to be so. Once a question is answered, many others (too many) arise in a kind of cascading bloom; or, to say it in scientific terms, one is submerged by an exponential outbreak of new ones. The quest for knowledge is probably infinite, only limited by our minds and perception, or even by the short duration of our lives. It has been an exciting pleasure finishing this thesis and having contributed with this tiny fraction of knowledge to the huge heritage of the ecological science.

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# CHAPTER 8

Main conclusions

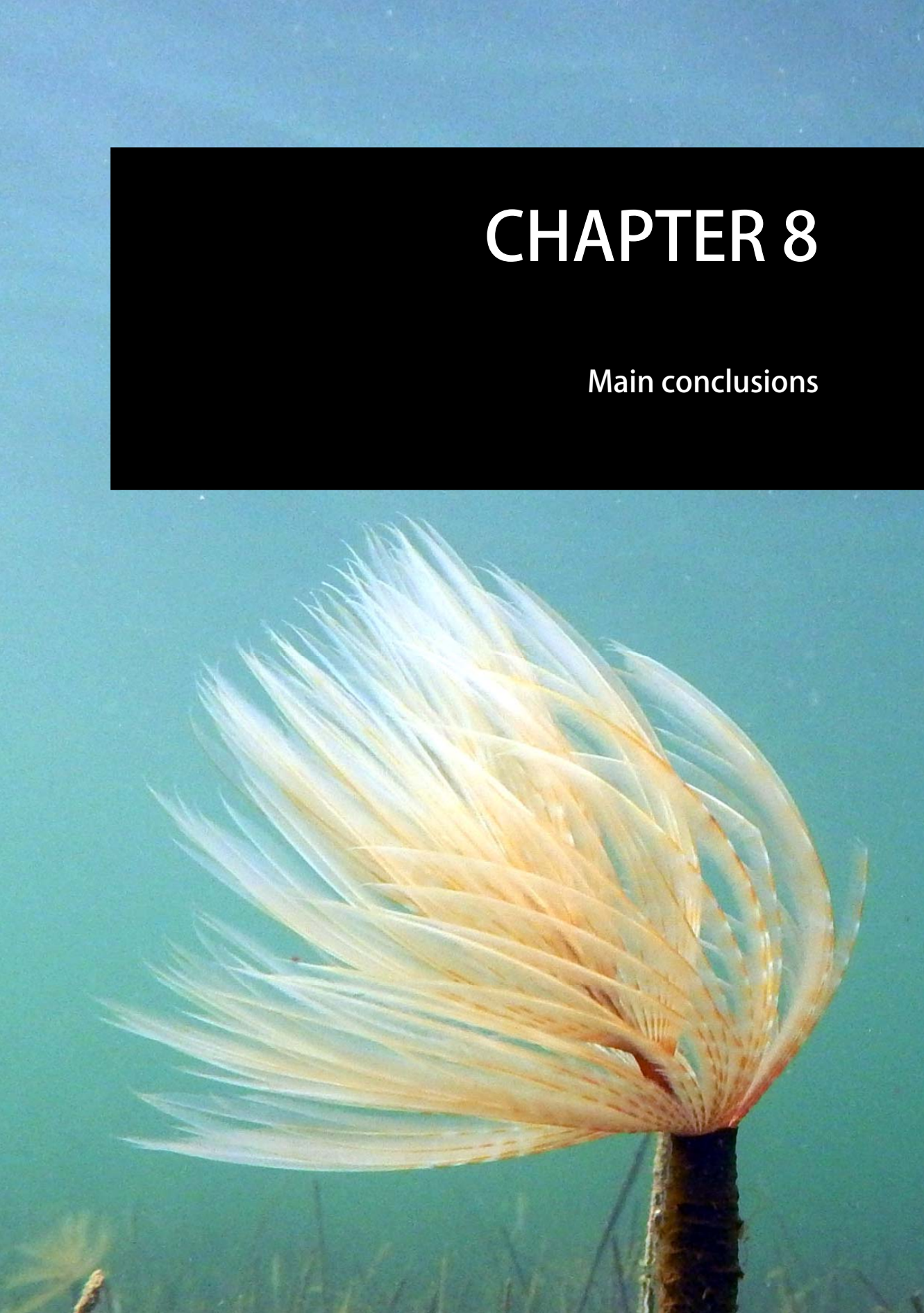


Photo: Polychaete *Sabella spallanzanii* in a *Cymodocea nodosa* seagrass meadow, from Neus Sanmartí.

## Chapter 3

Landscape patterns modulate flow rates of materials across the coastal seascape, as shown by the effects of landscape attributes (i.e. patch size and matrix type) on the accumulation of detritus. Specifically, seagrass leaf litter accumulation is higher in continuous meadows than in small patches, independently of the surrounding habitats (matrix type). This could have potential implications on both, the nutrient availability for the system itself, and communities associated (i.e. plants and secondary consumers). We demonstrate that, indeed, leaves from shoots in small patches are poorer in N content than leaves from shoots in continuous meadows. However, we failed to detect any effect concerning the diets of deposit feeders (holothurians).

## Chapter 4

Landscape patterns influence carbon stocks in coastal vegetated ecosystems, as shown by the effects of landscape attributes (i.e. patch size and matrix type) in sedimentary organic carbon of *Posidonia oceanica* seagrass meadows. Specifically, organic carbon density per unit of vegetated area was higher in continuous meadows than in small patches, independently of the surrounding habitat (matrix type). Landscape influence seems to be exerted primarily through the capacity for retention of autochthonous detritic materials, and also sediment characteristics, such as a fine fraction, potentially delaying organic carbon remineralisation in continuous meadows. Overall, patchy meadows, especially those in a sand matrix, presented lower carbon density (about one third) than continuous meadows.

## Chapter 5

Landscape patterns influence carbon stocks in coastal vegetated ecosystems, as revealed by the importance of edge effects as sources of within habitat variability on sedimentary organic carbon in a *Zostera muelleri* patchy seagrass landscape. Organic carbon density per unit area was higher inside seagrass patches than at seagrass–sand

edges and in adjacent bare sediments, suggesting that, for a same vegetated surface, carbon storage capacity is higher in continuous than in patchy meadows.

## Chapter 6

Landscape patterns are important drivers of coastal communities at multiple spatial scales, from meters to kilometers, as shown by the effects of landscape composition and landscape configuration on shaping coastal fish assemblages. Overall, the total abundance and species richness of fish assemblages are mostly influenced by small to medium scale landscape metrics, while the influence over the abundance of specific groups (i.e. families) is heterogeneous and depends on the biological traits of the species concerned. In general terms, rock cover has a positive effect on fish abundance, while the heterogeneity of the landscape has a negative one.



An underwater photograph of a coral reef. The scene is dominated by a large, dense patch of yellowish-green seaweed or soft coral in the center. To the left, there are vibrant red coral structures. The bottom is covered with various types of coral and rocks. The water is clear and blue, with some light rays visible. A black rectangular box is overlaid on the upper right portion of the image, containing the word 'APPENDIX' in white capital letters.

# APPENDIX

Photo: Rocky bottom with macroalgal cover, from Pol Capdevila

# APPENDIX 1

Benthic habitat mapping in the Catalan coast  
using Side Scan Sonar



## Background

Maps, and specially habitat maps, are key tools in landscape ecology research, and are extremely valuable for research and management purposes. In marine systems obtaining underwater habitat maps is notably difficult and costly for a number of reasons (e.g. the low transparency of water, impossibility of transmission of electromagnetic signals, difficulties for direct observations). Side Scan Sonar (SSS) provides a cost efficient method for remotely sensing the sea floor and, specifically, the existing habitats. The SSS has been defined as an acoustic imaging device used to provide wide-area, high resolution sonograms of the seabed. The system emits ultrasonic pulses toward the seafloor, across a wide angle perpendicular to the path of a towed fish navigating underwater (which acts, simultaneously, as transmissor and transducer of signal) (Fig. A.1). The acoustic reflections are then recorded and georeferenced to obtain the full coverage of the area of interest. Here we used this technique to draw up the benthic habitat maps required for the research conducted in Chapter 6.

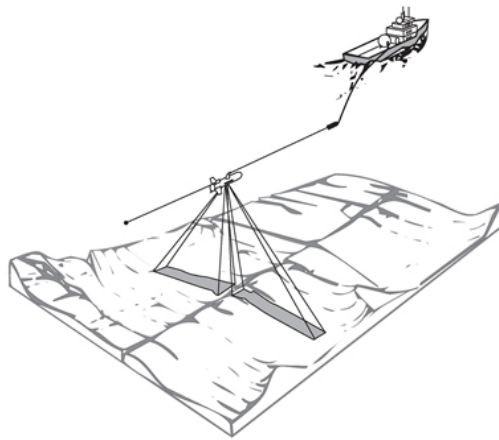


Fig. A1. Side Scan Sonar system in operation over the sea bed

## Mapped sites

We produced benthic habitat maps of six bays or inlets located along the northern part of the Catalan coast (also known as Costa Brava, NW Mediterranean), encompassing from 2 to 20 ha in surface. All the bays were characterized by the presence of seagrass meadows, rocky reefs covered by macroalgae and sandy bottoms. Benthic habitat maps were limited by the 0 and the -15m isobaths, or to the bay limits when the bay was shallower than 15m. Mapped areas covered surfaces from 2 to 16 ha (Table A.1).

Table A.1. Total mapped surface in each bay

<b>Bay</b>	<b>Total area mapped</b>	<b>Latitude</b>	<b>Longitude</b>
Jugadora	2.98	42°18'57"N	3°18'46"E
Montjoi	13.67	42°15'01"N	3°13'42"E
Rustella	2.07	42°14'36"N	3°13'36"E
Montgó	16.76	42°06'22"N	3°10'17"E
Aiguablava	2.61	41°56'10"N	3°12'57"E
Giverola	3.48	41°44'12"N	2°57'16"E

## Sonar survey

We used Tritech StarFish 990F system to scan the sea bottom at each bay. We selected a lateral range of 20 m and an overlap of almost 30% among adjacent lines. The sonar has a nominal frequency of 1000kHz and a basic resolution of 2.5 cm. The surveys were carried out in July 2013. During the operations, the fish was towed by a small rubber-boat with all the surface equipment installed on board. The tow-fish was connected to the surface unit by a communication cable where the sonar data was georeferenced by integrating the GPS position, provided each second, by a Lowrance Elite 3x using the Hypack navigation software. The navigation software allowed to manually insert the layback (the difference in the positioning between the boat and the tow-fish, which was up to 20 m behind the boat). Data acquisition was carried out at a constant speed of about 2-2.5 knots. The bathymetry of each site was

obtained using the Lowrance Elite 3x echosounder and elaborated using Hypack navigation software.

## Sonar data processing and ground-truthing

Raw sonar data were elaborated and exported into georeferenced images (geotiff) using SonarWiz 5 software. The images were then imported into the Geographic Information System (GIS) ESRI ArcGis 10.1 for further processing. In the areas in which navigation with SSS was impossible, satellite photos (ICC 2012) having a 10 cm pixel resolution were integrated into the GIS project (Fig. A.2a) to incorporate the data on the shallowest part of each bay. Then, for each bay, one mosaic composed by merging together all the surveyed sonar transects was produced (Fig. A.2b). The raster resolution scale was 5 cm and allowed to identify, in some specific cases, minimum mapping units (MMU) of about 0.01 m<sup>2</sup>. To verify the doubts on interpretation of sonar imagery and to complement the remote sensing survey, a second field campaign was conducted to video record the transects done with SSS. For that purpose we used a GoPro camera fixed under the boat, mounted on a pole. Navigation software allowed matching video data with the position of the boat (Fig. A.2c).

## Map production

Bathymetric data were exported to GIS, with isobaths spaced 1 m depth (Fig. A.2d). Sonar images were interpreted using texture, tone, shape, pattern and association to distinguish and classify the different habitat classes. The polygons representing different habitat classes were manually digitized in GIS (Fig. A.2e). The classification scheme included twelve predominant habitat classes based on combinations of sandy bottoms, rocky reefs with macroalgal vegetation (thereinafter rocky reefs) and seagrass habitats (Table A.2). Additionally, a point shapefile was included to account for poorly resolved areas or sonar shadows from the photomosaic. Sonar shadows are dark areas appearing behind solid objects which reflect acoustic signals back to the transducer, preventing those signals from 'imaging' areas beyond them. Bathymetry was also incorporated to benthic habitat maps in GIS as a line shapefile. All maps

were completed in March 2015 and comprised: a polygon layer representing habitat classes and a line layer representing bathymetry (Fig. A.2f), besides the point shapefile for poorly resolved areas commented above.

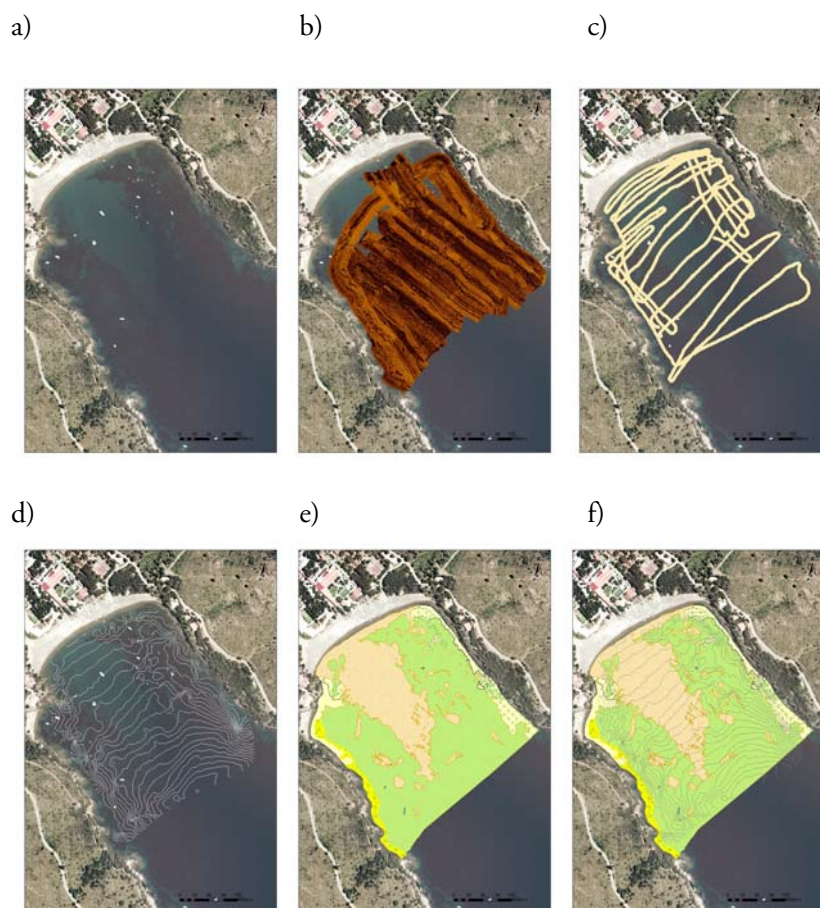


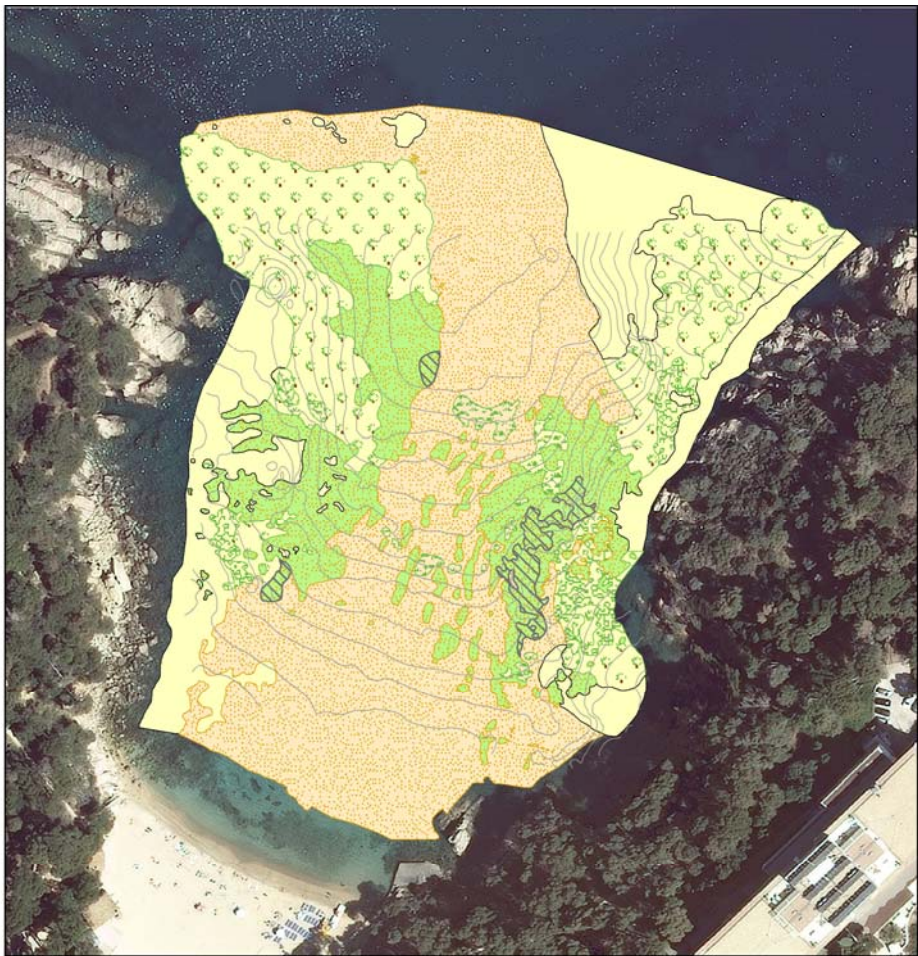
Fig. A.2. Elaboration process of benthic habitat maps (Montjoi Bay). (a) Satellite photo; (b) Sonar transects; (c) Transects for ground-truthing with video camera; (d) Bathymetry; (e) Benthic habitat map; (f) Benthic habitat map with bathymetry



Table A.2. Definitions of habitat classes used in benthic maps. 1, all these categories were merged in a new habitat class (mosaics dominated by seagrasses and rocky reefs) used in Chapter 6; 2, all these categories were merged in a new habitat class (mosaics dominated by seagrasses and sandy bottoms) used in Chapter 6

Habitat classes used in benthic maps
<i>Posidonia oceanica</i> homogeneous seagrass patches
Rocky reefs
Sandy bottoms
Mosaic of <i>P.oceanica</i> (50% cover) and rocky reefs (50% cover) <sup>1</sup>
Mosaic of <i>P.oceanica</i> (50% cover) and rocky reefs (50% cover) with presence of matte <sup>1</sup>
Mosaic of <i>P.oceanica</i> (75% cover) and rocky reefs (25% cover) <sup>1</sup>
Mosaic of rocky reefs (75% cover) and presence of <i>P. oceanica</i> seagrass patches (25% cover) <sup>1</sup>
Mosaic of <i>P.oceanica</i> (50% cover) and matte (50% cover) <sup>2</sup>
Mosaic of sandy bottoms (75% cover) and presence of <i>P. oceanica</i> seagrass patches (25% cover) <sup>2</sup>
Mosaic of sandy bottoms (75% cover) and presence of <i>P. oceanica</i> seagrass patches and rocky reefs (25% cover) <sup>2</sup>
Unknown objects
Emerged rock
<i>Zostera noltei</i> homogeneous seagrass patches

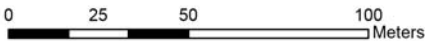
Benthic habitat maps

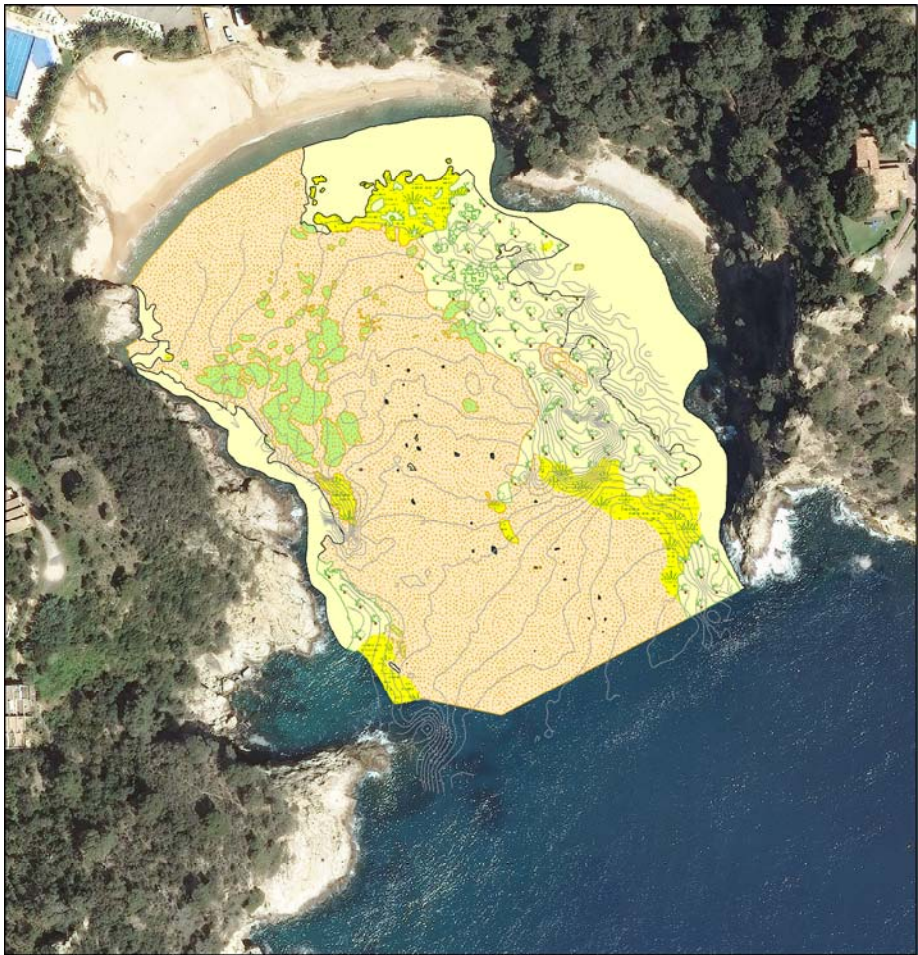


Aiguablava









- Rocky reefs
- Mosaic of rocky reefs (75%) and presence of *P. oceanica* seagrass patches (25%)
- Sandy bottoms
- Mosaic of sandy bottoms (75%) and presence of *P. oceanica* seagrass patches (25%)
- Mosaic of sandy bottoms (75%) and presence of *P. oceanica* seagrass patches and rocky reefs (25%)
- P. oceanica* homogeneous seagrass patches
- Mosaic of *P.oceanica* (50%) and rocky reefs (50%)
- Mosaic of *P.oceanica* (50%) and matte (50%)

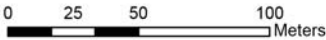




**Giverola**



-  Rocky reefs
-  Mosaic of rocky reefs (75%) and presence of *P. oceanica* seagrass patches (25%)
-  Sandy bottoms
-  *P. oceanica* homogeneous seagrass patches
-  Mosaic of *P. oceanica* (75%) and rocky reefs (25%)
-  Mosaic of *P. oceanica* (50%) and rocky reefs (50%)



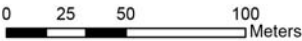




Jugadora



- Rocky reefs
- Mosaic of rocky reefs (75%) and presence of *P. oceanica* seagrass patches (25%)
- Sandy bottoms
- Mosaic of sandy bottoms (75%) and presence of *P. oceanica* seagrass patches (25%)
- P. oceanica* homogeneous seagrass patches
- Mosaic of *P. oceanica* (50%) and rocky reefs (50%)
- Emerged rock
- Zostera noltei* homogeneous seagrass patches





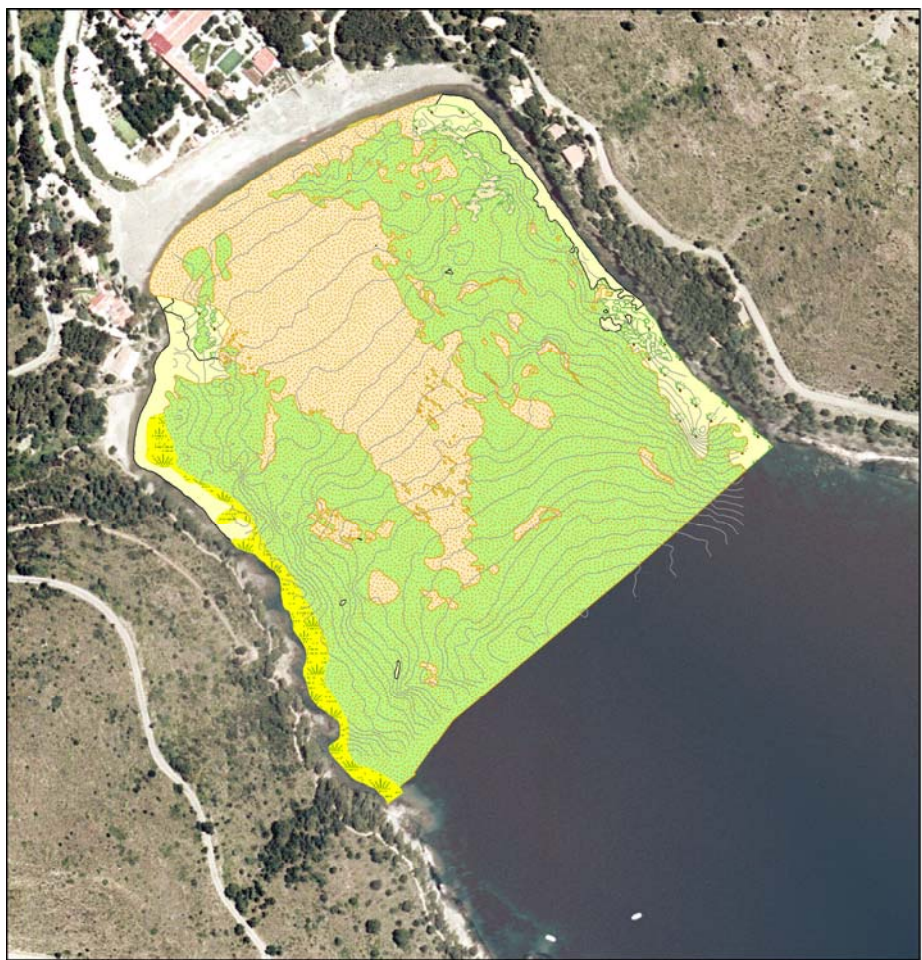
Montgó



- Rocky reefs
- Mosaic of rocky reefs (75%) and presence of *P. oceanica* seagrass patches (25%)
- Sandy bottoms
- Mosaic of sandy bottoms (75%) and presence of *P. oceanica* seagrass patches (25%)
- Mosaic of sandy bottoms (75%) and presence of *P. oceanica* seagrass patches and rocky reefs (25%)
- P. oceanica* homogeneous seagrass patches
- Mosaic of *P. oceanica* (50%) and rocky reefs (50%)

0 25 50 100  
Meters

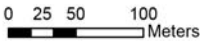




Montjoi











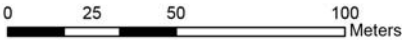
- Rocky reefs
- Mosaic of rocky reefs (75%) and presence of *P. oceanica* seagrass patches (25%)
- Sandy bottoms
- Mosaic of sandy bottoms (75%) and presence of *P. oceanica* seagrass patches (25%)
- P. oceanica* homogeneous seagrass patches
- Mosaic of *P.oceanica* (75%) and rocky reefs (25%)
- Mosaic of *P.oceanica* (50%) and rocky reefs (50%)





**Rustella**

-  Rocky reefs
-  Mosaic of rocky algal reefs (75%) and presence of *P. oceanica* seagrass patches (25%)
-  Sandy bottoms
-  Mosaic of sandy bottoms (75%) and presence of *P. oceanica* seagrass patches (25%)
-  *P. oceanica* homogenous seagrass patches
-  Mosaic of *P. oceanica* (50%) and rocky areefs (50%)
-  Mosaic of *P. oceanica* (50%) and rocky reefs (50%) with presence of matte
-  Mosaic of *P. oceanica* (50%) and matte (50%)







# APPENDIX 2

## Supplementary Materials

Table SM 3.1. Complete outcome of the 2-way mixed-effects ANOVA's, with landscape configuration (Conf) as fixed factor and site as random factor. (a) Comparisons for dry weight of detrital fractions and sediment data (b) Comparisons for elemental composition of living leaves and epiphytes (c) Comparisons for shoot density. (\*) Data were fourth root transformed to meet ANOVA assumptions. The random factor site significantly influenced the amount of detrital leaves and N content of seagrass leaves, but did not alter the significant effects of landscape configuration (non-significant configuration x site interactions).

<b>(a) Detrital fractions biomass and sediment data</b>					
		<b>df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
<i>P.oceanica</i> detrital leaves (*)	Conf	2	10.39	64.62	<b>&lt;0.001</b>
	Site	2	2.67	16.62	<b>&lt;0.001</b>
	Conf*Site	4	0.32	0.43	0.783
	Error	36	6.66		
<i>P.oceanica</i> belowground fraction (*)	Conf	2	0.10	0.15	0.869
	Site	2	1.45	2.04	0.245
	Conf*Site	4	1.42	0.72	0.583
	Error	36	17.74		
Terrestrial fraction (*)	Conf	2	1.64	1.20	0.390
	Site	2	3.29	2.41	0.206
	Conf*Site	4	2.73	1.87	0.138
	Error	36	13.18		
Macroalgae (*)	Conf	2	1.39	0.48	0.640
	Site	2	6.26	2.16	0.231
	Conf*Site	4	5.78	3.97	<b>0.009</b>
	Error	36	13.10		
Fine fraction	Conf	2	32182.60	1.15	0.404
	Site	2	57533.80	2.05	0.244
	Conf*Site	4	56143.60	2.20	0.080
	Error	36	230018.20		
Sediment organic matter (%)	Conf	2	0.02	0.01	0.986
	Site	2	3.13	2.53	0.194
	Conf*Site	4	2.47	2.52	0.060
	Error	36	8.84		

<b>(b) <i>P.oceanica</i> leaves and epiphytes variables</b>					
<b>Nitrogen content</b>		<b>df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
<i>P.oceanica</i> living leaves (*)	Conf	2	0.02	10.37	<b>0.026</b>
	Site	2	0.03	20.90	<b>0.008</b>
	Conf*Site	4	0.00	1.65	0.1833
	Error	36	0.02		
Epiphytes	Conf	2	0.25	0.69	0.554
	Site	2	0.02	0.06	0.947
	Conf*Site	4	0.72	7.52	<b>&lt;0.001</b>
	Error	36	0.85		
<b>Carbon content</b>		<b>df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
<i>P.oceanica</i> living leaves	Conf	2	2.01	0.15	0.869
	Site	2	17.59	1.27	0.373
	Conf*Site	4	27.63	3.65	<b>0.014</b>
	Error	36	68.16		
Epiphytes	Conf	2	1.43	0.13	0.885
	Site	2	5.48	0.48	0.649
	Conf*Site	4	22.69	5.54	<b>0.001</b>
	Error	36	36.87		
<b>(c) Shoot density</b>					
		<b>df</b>	<b>SS</b>	<b>F</b>	<b>P</b>
Shoot density	Conf	2	438541.00	6.18	0.060
	Site	2	84615.00	1.19	0.392
	Conf*Site	4	141990.00	1.96	0.123
	Error	35	635357.00		

Table SM 3.2. Carbon and nitrogen stable isotopic and elemental values and C:N ratios in sources and consumers collected in each site and landscape configuration (CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix). n: number of replicates. sd: standard deviation

Site	Landscape configuration		n	$\delta^{13}\text{C}$	sd	$\delta^{15}\text{N}$	sd	C	sd	N	sd	C:N	sd
Aiguablava	CO	Epiphytes	5	-16.29	0.52	6.34	0.28	5.87	1.43	0.64	0.03	9.12	2.22
		<i>P. oceanica</i> detrital leaves	5	-11.58	0.48	3.58	0.14	32.07	1.42	0.73	0.09	44.36	3.66
		Macroalgae	5	-19.45	2.17	4.21	0.14	6.34	3.92	0.81	0.32	7.38	1.78
		SPOM	3	-24.37	1.03	3.10	0.71	na	na	na	na	8.50	2.41
		<i>Holothuria</i> spp.	5	-15.99	0.43	9.11	0.09	44.85	6.29	13.24	1.67	3.38	0.09
	PR	Epiphytes	5	-16.84	0.53	6.99	0.55	4.64	0.95	0.70	0.14	6.68	1.03
		<i>P. oceanica</i> detrital leaves	5	-12.16	0.38	3.65	0.20	30.34	2.62	0.78	0.05	38.82	2.29
		Macroalgae	5	-17.33	1.81	4.45	0.28	11.39	4.35	0.87	0.18	13.25	5.47
		SPOM	3	-24.37	1.03	3.10	0.71	na	na	na	na	8.50	2.41
		<i>Holothuria</i> spp.	5	-16.41	0.32	9.17	0.43	45.38	2.04	13.26	0.69	3.42	0.03
	PS	Epiphytes	5	-16.91	0.79	6.73	0.32	5.91	1.57	0.77	0.18	7.92	2.45
		<i>P. oceanica</i> detrital leaves	5	-12.41	0.18	3.85	0.25	27.66	1.14	0.71	0.07	39.36	4.30
		Macroalgae	5	-17.53	1.93	4.48	0.37	16.09	5.62	0.93	0.28	17.19	2.51
		SPOM	3	-24.37	1.03	3.10	0.71	na	na	na	na	8.50	2.41
		<i>Holothuria</i> spp.	5	-15.96	0.46	9.14	0.36	46.44	3.02	13.62	0.84	3.41	0.05

Giverola	CO	Epiphytes	5	-17.17	0.48	5.03	0.79	5.16	0.58	0.75	0.09	7.02	1.32
		<i>P. oceanica</i> detrital leaves	4	-11.52	0.39	2.75	0.17	26.93	1.11	0.61	0.02	44.44	1.80
		Macroalgae	5	-21.14	0.31	3.08	0.27	6.86	2.70	0.69	0.12	9.70	2.71
		SPOM	3	-25.03	0.25	4.30	0.26	na	na	na	na	9.56	2.82
		<i>Holothuria</i> spp.	5	-16.71	0.21	8.77	0.32	48.02	8.02	13.86	2.36	3.47	0.02
	PR	Epiphytes	5	-16.69	0.23	5.97	0.31	5.03	0.73	0.63	0.11	7.96	0.44
		<i>P. oceanica</i> detrital leaves	5	-10.44	0.64	2.88	0.19	24.17	1.40	0.67	0.03	36.00	1.61
		Macroalgae	5	-21.29	2.78	3.01	0.46	8.15	3.41	1.06	0.34	7.68	2.79
		SPOM	3	-25.03	0.25	4.30	0.26	na	na	na	na	9.56	2.82
		<i>Holothuria</i> spp.	2	-16.27	2.04	8.24	0.16	38.46	4.34	10.73	1.64	3.60	0.15
	PS	Epiphytes	5	-17.67	0.46	6.71	0.93	4.60	0.38	0.58	0.07	8.00	0.54
		<i>P. oceanica</i> detrital leaves	5	-12.05	0.82	3.64	0.16	27.18	2.24	0.68	0.04	39.96	3.29
		Macroalgae	5	-20.05	1.08	3.61	0.28	5.40	2.15	0.65	0.17	8.33	3.11
		SPOM	3	-25.03	0.25	4.30	0.26	na	na	na	na	9.56	2.82
		<i>Holothuria</i> spp.	5	-16.18	0.83	8.03	0.71	49.88	2.72	13.53	1.11	3.69	0.16
Rustella	CO	Epiphytes	5	-18.08	0.58	6.72	0.91	4.49	0.89	0.48	0.07	9.36	1.06
		<i>P. oceanica</i> detrital leaves	5	-10.60	0.33	3.21	0.07	25.84	0.95	0.64	0.07	40.80	3.11
		Macroalgae	5	-21.05	1.54	3.69	0.33	12.01	1.63	1.19	0.28	10.30	1.46

	SPOM	3	-24.53	0.46	3.13	0.35	na	na	na	na	10.74	1.40
	<i>Holothuria</i> spp.	5	-14.21	0.68	8.11	1.00	41.22	5.59	12.11	1.77	3.41	0.13
PR	Epiphytes	5	-17.13	0.29	6.25	0.31	7.17	1.34	1.02	0.36	7.40	1.42
	<i>P. oceanica</i> detrital leaves	5	-11.85	0.26	2.94	0.19	28.17	1.44	0.71	0.06	39.93	3.14
	Macroalgae	4	-19.44	2.08	3.25	0.29	12.41	2.92	0.84	0.18	14.93	2.95
	SPOM	3	-24.53	0.46	3.13	0.35	na	na	na	na	10.74	1.40
	<i>Holothuria</i> spp.	5	-15.27	0.37	8.51	0.55	53.68	5.57	13.83	1.67	3.89	0.09
PS	Epiphytes	5	-19.29	0.70	6.51	0.50	5.66	0.43	0.55	0.06	10.39	1.30
	<i>P. oceanica</i> detrital leaves	5	-11.96	0.39	3.00	0.19	25.27	2.31	0.59	0.05	43.12	5.25
	Macroalgae	5	-19.31	0.87	3.03	0.59	8.36	1.70	0.80	0.12	10.39	0.92
	SPOM	3	-24.53	0.46	3.13	0.35	na	na	na	na	10.74	1.40
	<i>Holothuria</i> spp.	5	-15.55	0.43	8.19	0.80	48.08	4.60	13.37	1.54	3.60	0.18

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Table SM 3.3. Relative contributions of potential sources to *Holothuria* spp. diet requirements per site and landscape configuration (CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix) as modeled by SIAR. Mean and lower and upper 95% credible interval (CI95) for all the range of feasible solutions in each bayesian mixing model.

Source	<i>P.oceanica</i> +Epiphytes			Macroalgae			SPOM		
	mean	CI95		mean	CI95		mean	CI95	
Aiguablava									
CO	<b>0.50</b>	0.28	0.75	<b>0.36</b>	0.01	0.65	<b>0.15</b>	0.05	0.25
PR	<b>0.51</b>	0.28	0.77	<b>0.32</b>	0.00	0.61	<b>0.16</b>	0.08	0.25
PS	<b>0.54</b>	0.29	0.81	<b>0.35</b>	0.00	0.65	<b>0.12</b>	0.03	0.19
Giverola									
CO	<b>0.54</b>	0.34	0.76	<b>0.30</b>	0.00	0.59	<b>0.15</b>	0.06	0.25
PR	<b>0.41</b>	0.03	0.73	<b>0.32</b>	0.00	0.62	<b>0.27</b>	0.00	0.57
PS	<b>0.55</b>	0.31	0.82	<b>0.33</b>	0.00	0.63	<b>0.11</b>	0.02	0.21
Rustella									
CO	<b>0.60</b>	0.39	0.87	<b>0.36</b>	0.03	0.60	<b>0.04</b>	0.00	0.11
PR	<b>0.63</b>	0.42	0.85	<b>0.29</b>	0.00	0.54	<b>0.09</b>	0.02	0.15
PS	<b>0.60</b>	0.37	0.87	<b>0.34</b>	0.01	0.61	<b>0.06</b>	0.00	0.12

Table SM 4.1a. Relative contributions of potential sources to *Posidonia oceanica* sediments per landscape configuration (CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix) as modeled by SIAR. Mean and lower and upper 95% credible interval (CI95) for all the range of feasible solutions in each bayesian mixing model.

Source	<i>P. oceanica</i> +Epiphytes			SPOM			Macroalgae		
	mean	CI95		mean	CI95		Mean	CI95	
CO	<b>0.37</b>	0.16	0.59	<b>0.29</b>	0.08	0.5	<b>0.32</b>	0.00	0.68
PR	<b>0.31</b>	0.08	0.51	<b>0.36</b>	0.14	0.54	<b>0.32</b>	0.00	0.72
PS	<b>0.19</b>	0.01	0.36	<b>0.47</b>	0.28	0.67	<b>0.33</b>	0.01	0.64

Table SM 4.1b. Relative contributions of potential sources to *Posidonia oceanica* sediments per landscape configuration (CO, continuous meadow; PR, patches in a rock matrix; PS, patches in a sand matrix) as modeled by SIAR. Mean and lower and upper 95% credible interval (CI95) for all the range of feasible solutions in each bayesian mixing model.

Source	<i>P. oceanica</i> +Epiphytes			SPOM		
	mean	CI95		mean	CI95	
CO	<b>0.55</b>	0.42	0.67	<b>0.45</b>	0.32	0.58
PR	<b>0.49</b>	0.4	0.58	<b>0.51</b>	0.42	0.6
PS	<b>0.34</b>	0.23	0.46	<b>0.65</b>	0.54	0.77



Table SM 6.1. List of species and families found of coastal fish assemblages in the eight bays studied. Total number of individuals of each species, mean abundance among the 166 transects developed and standard error (SE).

Family	Species	Total number of individuals	Mean abundance	SE
Apogonidae	<i>Apogon imberbis</i>	13	0.79	0.06
Blenniidae	<i>Parablennius incognitus</i>	2	0.11	0.01
Blenniidae	<i>Parablennius pilicornis</i>	4	0.15	0.01
Blenniidae	<i>Parablennius rouxi</i>	2	0.11	0.01
Blenniidae	<i>Parablennius sanguinolentus</i>	2	0.11	0.01
Blenniidae	<i>Parablennius tentacularis</i>	2	0.11	0.01
Gobiidae	<i>Gobius bucchichi</i>	8	0.21	0.02
Gobiidae	<i>Gobius geniporus</i>	3	0.13	0.01
Gobiidae	<i>Gobius paganellus</i>	1	0.08	0.01
Gobiidae	<i>Gobius xanthocephalus</i>	6	0.22	0.02
Gobiidae	<i>Pomatoschistus bathi</i>	21	1.10	0.09
Labridae	<i>Coris julis</i>	1182	9.37	0.73
Labridae	<i>Ctenolabrus rupestris</i>	19	1.47	0.11
Labridae	<i>Labrus merula</i>	1	0.08	0.01
Labridae	<i>Labrus viridis</i>	7	0.34	0.03
Labridae	<i>Symphodus cinereus</i>	40	0.72	0.06
Labridae	<i>Symphodus doderleini</i>	79	0.48	0.14
Labridae	<i>Symphodus mediterraneus</i>	30	0.50	0.04
Labridae	<i>Symphodus melanocercus</i>	26	0.43	0.03
Labridae	<i>Symphodus melops</i>	4	0.19	0.01
Labridae	<i>Symphodus ocellatus</i>	14	0.40	0.03
Labridae	<i>Symphodus roissali</i>	72	1.01	0.08
Labridae	<i>Symphodus rostratus</i>	8	0.24	0.02
Labridae	<i>Symphodus tinca</i>	112	1.16	0.09
Labridae	<i>Thalassoma pavo</i>	5	0.17	0.01
Mullidae	<i>Mullus surmuletus</i>	110	1.57	0.12
Sciaenidae	<i>Sciaena umbra</i>	2	0.01	0.01
Scorpaenidae	<i>Scorpaena porcus</i>	7	0.23	0.02
Serranidae	<i>Serranus cabrilla</i>	132	1.01	0.08
Serranidae	<i>Serranus scriba</i>	30	0.44	0.03
Sparidae	<i>Boops boops</i>	168	8.25	0.64
Sparidae	<i>Diplodus annularis</i>	79	3.66	0.28
Sparidae	<i>Diplodus puntazzo</i>	9	0.23	0.02
Sparidae	<i>Diplodus sargus</i>	145	2.15	0.17
Sparidae	<i>Diplodus vulgaris</i>	245	2.15	0.17
Sparidae	<i>Lithognathus mormyrus</i>	6	0.33	0.03
Sparidae	<i>Oblada melanura</i>	152	4.94	0.38
Sparidae	<i>Sarpa salpa</i>	501	10.03	0.78
Sparidae	<i>Sparus aurata</i>	15	0.38	0.03
Sparidae	<i>Spondylisoma cantharus</i>	17	0.55	0.04
Centracanthidae	<i>Spicara maena</i>	56	1.55	0.12

Centracanthidae	<i>Spicara smaris</i>	5	0.39	0.03
Haemulidae	<i>Pomadasys incisus</i>	15	0.84	0.06
Mugilidae	<i>Chelon labrosus</i>	25	1.57	0.12
Pomacentridae	<i>Chromis chromis</i>	963	15.15	1.18
Sphyraenidae	<i>Sphyraena viridensis</i>	7	0.39	0.03

# APPENDIX 3

Pdf's of published articles

# Effects of landscape configuration on the exchange of materials in seagrass ecosystems

Aurora M. Ricart\*, Andreu Dalmau, Marta Pérez, Javier Romero

Facultat de Biologia, Departament d'Ecologia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

**ABSTRACT:** Landscape (or seascape) attributes play an important role in modulating the flow rates of materials between habitats in the coastal marine environment. Seagrass meadows are known to both export and import organic matter, thus establishing links with other habitats. Most of those links remain unexplored, and little is known about the relevance of landscape configuration on these flows. We studied the relationships between landscape configuration (continuous meadows, patchy meadows in a sand matrix and patchy meadows in a rock matrix) and the exchange and accumulation of detrital material. Moreover, we evaluated the impact of landscape configuration on (1) plant nutrient content and (2) the diet of a model deposit feeder (holothurians). We determined detritus stocks in seagrass meadows as well as the carbon and nitrogen elemental and isotopic composition of plants, detritus and other food sources (e.g. suspended organic matter). Based on this, we identified, by applying mixing models, the different contributions of these sources to the diets of deposit feeders. Our results showed that landscape configuration influences the exchange of materials across the coastal seascape. Less accumulation of detrital seagrass leaves was found in patchy meadows, although no effects were found for allochthonous materials. In addition, patchy seagrass meadows showed significantly lower nitrogen concentrations in leaves compared to continuous meadows. Landscape configuration had no effect on the diet of the deposit feeder studied. These findings highlight the importance of landscape-level processes in coastal waters and specifically warn of the possible effects of changes in meadow size on seagrass performance.

**KEY WORDS:** Coastal seascape · Habitat linkages · Spatial subsidy · Deposit feeder diets · Stable isotopes · Holothuria

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## INTRODUCTION

Landscape ecology has made important contributions to our understanding of ecosystem dynamics (Pittman et al. 2011). The view that spatial properties are critical for functional and evolutionary aspects of the ecosystems is a central tenet of terrestrial ecology, and concepts from landscape ecology are increasingly being applied to the marine environment. Among them, the ecological consequences of broad-scale spatial heterogeneity are receiving increasing attention (Grober-Dunsmore et al. 2007, Hinchey et al. 2008, Boström et al. 2011).

Underwater marine landscapes, or seascapes, and specifically those in the coastal zone, usually consist of an intermingled set of habitat patches that are settled in relatively small areas and often exhibit complex spatial patterns. Overall ecosystem functions in coastal seascapes and the delivery of associated ecosystem services are dependent not only on the intrinsic properties of individual habitat patches but also on the spatial configuration and functional links between the patches and their properties (Grober-Dunsmore et al. 2008, Pittman et al. 2011, Hyndes et al. 2014).

Habitat patches are linked by a range of mechanisms that function on broad spatial and temporal

\*Corresponding author: auroramartinez@ub.edu

scales (Sheaves 2009). Habitat boundaries are usually crossed by organisms and materials that can carry energy, matter or information. The degree to which a given landscape facilitates such flows is called connectivity (Taylor et al. 1993). Cross-habitat linkages allow asymmetrical energy flows (Valiela et al. 2001) that can have important ecological consequences, especially by increasing productivity in the recipient area (Marczak et al. 2007, Heck et al. 2008, Mellbrand et al. 2011, Hyndes et al. 2012), which has been called a spatial subsidy (Polis et al. 1997). Habitat linkages depend on the nature and spatial arrangement of the habitats and the general setting of the physical environment. Landscape attributes such as the perimeter of focal habitats, the distance between habitats or the matrix within which they are imbedded modulate such fluxes (Polis et al. 1997, Bellido et al. 2008, Pagès et al. 2014). Thus, landscape composition and configuration will influence ecological connectivity (Mumby 2006, Grober-Dunsmore et al. 2008).

It is generally accepted that coastal ecosystems are experiencing an unprecedented set of alterations due to human pressures (Halpern et al. 2008). Most of the effects of these changes have been studied in depth, at levels ranging from the sub-individual (biochemical, physiological) through the individual and population to the community and ecosystem levels (Worm et al. 2006, Pérez et al. 2007, Hyndes et al. 2014, Vergés et al. 2014). However, much less attention has been paid to the effects of such changes on the spatial structure at the large scale; that is, to the modification of the composition and configuration of coastal seascapes resulting from human activities. Valuable habitats in the coastal ecosystem mosaic are losing cover and becoming increasingly fragmented (Macreadie et al. 2009). Fragmentation is a modification of the landscape configuration that reduces continuous ranges of habitat to small, isolated patches and can drastically change habitat structural complexity (Montefalcone et al. 2010). Habitat fragmentation increases the patchiness, which in turn causes a dramatic reduction in connectivity within habitats (Bender et al. 1998, Hovel et al. 2002) and can seriously modify the way organisms use the seascape (Boström et al. 2011, Gera et al. 2013). Changes in landscape configuration caused by human action can disrupt the natural direction or magnitude of matter and energy flows between habitats, with consequences for trophic food webs and ecosystem productivity (Howe & Simenstad 2011).

Common habitats in the coastal seascape include rocky reefs dominated by different species of macro-

algae, seagrass beds and unvegetated sedimentary bottoms dominated by infauna as well as mangroves and coral reefs, among others. Different linkages have been explored between such habitats and their importance for the functioning of the overall system assessed (Wernberg et al. 2006, Howe & Simenstad 2011, Hyndes et al. 2014, Pagès et al. 2014). However, despite these recent efforts, the effects of landscape configuration and its ecological significance for the linkages between habitats within the coastal seascape are still poorly understood.

While coral reefs and mangroves in tropical areas seem to play a central role in ecosystem functioning, seagrass meadows constitute one of the most important structural and productive habitats in coastal waters worldwide (Hemminga & Duarte 2000). Seagrass meadows are linked to other coastal habitats through multiple processes, both as subsidizer and as subsidized habitat. Seagrasses play an important nursery role (Boström et al. 2011) and can play an important role in the ontogenetic migrations of many organisms (Mumby et al. 2004). Together, primary and derived secondary seagrass production represents an important trophic subsidy for several coastal habitats (Heck et al. 2008) and even for deep sea (Vetter & Dayton 1998) and terrestrial systems (Mellbrand et al. 2011). Seagrass meadows are in turn subsidized by other habitats, such as rocky reefs with macroalgae (Hyndes et al. 2012), which are frequently detached during periods of high wave action and transported passively to seagrass habitats (Wernberg et al. 2006). Moreover, they also induce deposition of particles suspended in the water column (Gacia et al. 1999, Kennedy et al. 2010), thus stretching the benthopelagic links. All of these imported allochthonous materials have the potential to enhance primary seagrass productivity by supplying nutrients but also to feed trophic webs by increasing food availability to consumers. Among consumers, deposit feeders can benefit greatly, as allochthonous sources are generally more palatable and easily assimilated and therefore preferred over seagrass as a food resource (Olsen et al. 2011, Poore & Gallagher 2013).

Seagrasses can grow either as large, continuous meadows or in the form of patches of different shapes and sizes interspersed with unvegetated sand or rocky habitats with macroalgal cover (Robbins & Bell 1994, Jackson et al. 2006), with the latter especially common in shallow waters. These patterns are driven by natural biotic (e.g. growth rate and the expansion of rhizomes) and abiotic (e.g. hydrodynamics) factors (Fonseca & Bell 1998, Hovel 2003, Mills & Berken-

busch 2009), to which anthropogenic forcing (e.g. eutrophication or physical removal) is added (Short & Wyllie-Echeverria 1996, Duarte 2002, Boström et al. 2006). Therefore, these ecosystems (and the seascapes they are embedded in) are excellent models to explore and test hypotheses about the influence of landscape attributes on ecosystem processes.

The fate of the high production of seagrass meadows has been widely studied (Mateo & Romero 1997, Cebrián et al. 2000, Pérez et al. 2001). Aboveground production (mostly leaves) can either enter the food web directly through leaf grazing or temporarily accumulate as leaf litter and then decompose or be exported (Romero et al. 1992, Cebrián et al. 1997). Meanwhile, the bulk of belowground production remains buried as dead organic matter (OM) in the sediment (Mateo et al. 1997). Despite previous work, little is known about how landscape configuration modulates energy flow via the export of seagrass detritus or the import of allochthonous OM, such as particles suspended in the water column or macroalgae from surrounding habitats within the coastal seascape (Valiela et al. 2001, Heck et al. 2008).

To help fill this gap, in this study we explored the relationships between landscape configuration and the exchange of materials across a temperate coastal seascape dominated by *Posidonia oceanica* (L.) Delile seagrass meadows as well as the impact of landscape configuration on both plant nutrient content and the diets of consumers. *P. oceanica* is a habitat-forming seagrass species that can grow as patchy meadows, especially in shallow areas, and can be found growing either between rocky substrates or in sandy areas (Pagès et al. 2014). In this study, we used a patch matrix model approach (Boström et al. 2011) with the seagrass *P. oceanica* as the focal habitat to compare the 3 most frequent landscape configurations: large continuous meadows, small patches intermingled in rocky habitats (patches embedded within a rock matrix) and small patches in sedimentary bottoms (patches embedded within a sand matrix).

We evaluated detritus stocks accumulated in seagrass meadows to understand how landscape configuration modulates the flux of materials between seagrass and the surrounding habitats. We hypothesized that (1) detrital seagrass leaves will accumulate in greater quantities in continuous meadows than in seagrass patches and (2) landscape matrix composition will influence the type of material entering the focal habitat. At the community level, we assessed whether landscape configuration influenced the nutrient content of seagrass leaves and the propor-

tions of food sources in the diet of a model deposit feeder (holothurians). We hypothesized that (1) nutrient content will be lower in seagrass patches due to low material accumulation rates and (2) the proportions of food sources in the diets of deposit feeders will change by differences in the flux of materials between habitats.

## MATERIALS AND METHODS

### Study site

The study was performed at 3 sites along the NE coast of Spain (NW Mediterranean): Aiguablava (41° 56' N, 3° 12' E), Giverola (41° 44' N, 2° 57' E) and Rustella (42° 14' N, 3° 13' E) (Fig. 1). These sites were selected for their similar geomorphological conditions (e.g. area, bathymetry and degree of exposure) and also for having a similar underwater ecosystem mosaic including rock, sand and seagrass habitats. The 3 landscape configurations under study were present in all 3 sites, thus minimising sources of variability among configurations other than the configuration itself. We considered continuous seagrass meadows where seagrasses covered an area of more than 100 × 100 m, while seagrass patches in either a rock or a sand matrix covered approximately 2 × 2 m. All landscape configurations were situated at similar

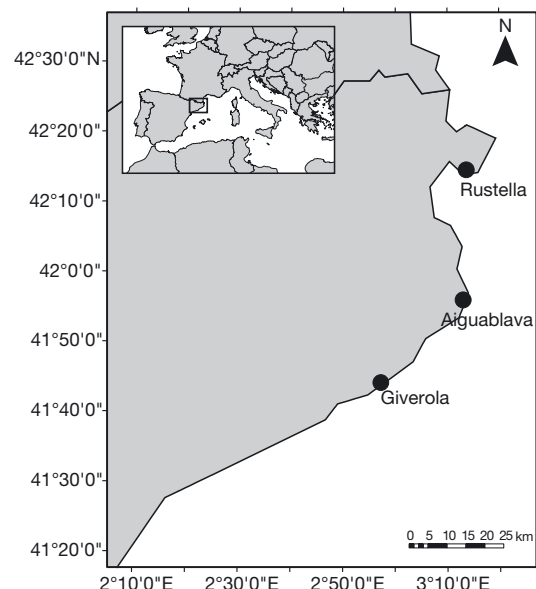


Fig. 1. Sampling locations along the Catalan coast, Spain, NW Mediterranean. Each of the sites presented similar seagrass landscapes, with continuous meadows and small patches in sand and rock habitats at the same depth range

depths in all sites (5 to 8 m) and, for each site, at a maximum distance of 50 m from each other. At these depths, fragmentation of seagrass habitats has been associated with, apart from anthropic impacts, big storms that occur sporadically and in general have a long return time (Montefalcone et al. 2010, Alcoverro et al. 2012). To discard confounding factors (other than landscape configurations), shoot density was measured in 3 replicate 40 × 40 cm quadrats in each landscape configuration within each site. Results were analysed using a 2-way mixed-effects ANOVA, with site and landscape configuration as factors, and there were no significant differences among sites and configurations ( $p > 0.05$ , see Table S1 in the Supplement at [www.int-res.com/articles/suppl/m532p089\\_supp.pdf](http://www.int-res.com/articles/suppl/m532p089_supp.pdf)), with an overall mean of  $548 \pm 29$  shoots  $m^{-2}$  ( $\pm$  SE).

### Sampling

Samples were collected in October 2012 (fall season in the northern hemisphere), at the end of the period of leaf fall and, consequently, the period for which leaf litter accumulation is at its maximum (Romero et al. 1992). No storm or high hydrodynamic event, potentially distorting results, occurred before or during the sampling. At each site, 5 replicate sampling points (each consisting of 1 to 1.5  $m^2$ ) were randomly selected inside the continuous meadows, and 1 sampling point was selected in each of 5 randomly selected seagrass patches in both a rock and a sand matrix, for a total of 15 sampling points per site. Scuba divers haphazardly placed a 40 × 40 cm square at each sampling point and used hand-held corers (40 mm diameter) to collect undisturbed sediment (upper 2 cm) for OM determination. Then, all of the detrital material inside the square was collected using a suction device for 1 min and sieved through a 1 mm mesh attached to the collector end of the suction device. Each sample was then placed inside a plastic bag, sealed and later transported chilled to the laboratory. Five seagrass shoots were then collected from within each square for elemental and isotopic composition analysis of living plants. Finally, 1 individual sea cucumber, either *Holothuria poli* or *H. tubulosa-mamatta* complex (Borrero-Pérez et al. 2009), was captured as close as possible to the sampling square for elemental and isotopic composition analysis. Holothurians were used as a model organism, as they are known to feed on a variety of detrital sources (bulk sediment and leaf litter of different sizes), and they constitute the largest and most

conspicuous detritivore in seagrass habitats (Massin & Jangoux 1976).

The entire sampling programme resulted in a total of 45 samples of each class, corresponding to 3 landscape configurations in 3 replicated sites, with  $n = 5$  replicates per experimental condition. Additionally, 2 l of seawater (from 1 m depth) was collected in triplicate from each site and filtered into prewashed and precombusted (450°C, 4 h) Whatman GF/F filters within 2 h of collection for later elemental and isotopic composition analysis of suspended particulate organic matter (SPOM). All samples were kept frozen at  $-20^\circ\text{C}$  until analysis in the laboratory.

### Laboratory processing

In the laboratory, the detritus samples were sieved again through a 1 cm sieve and sorted into 1 fine (particles between 1 mm and 0.9 cm) fraction and 4 different coarse (1 cm or more) detrital fractions: *Posidonia oceanica* leaves, *P. oceanica* roots and rhizomes, macroalgae and material of terrestrial origin. Subsamples from the fine fraction were inspected under a dissecting microscope to estimate its origin. Detrital *P. oceanica* leaves have very low epiphyte loads, but when necessary, epiphytes were removed manually as much as possible. These fractions were dried at  $60^\circ\text{C}$  and weighed. The leaves from the 5 living shoots collected at each sampling point were scraped with a razor blade to remove epiphytes (Alcoverro et al. 1997a), which were kept for subsequent analysis. Once cleaned, we separated the second youngest leaf from each of the 5 shoots and pooled them. Both the epiphytes and these leaves were dried as above (Martínez-Crego et al. 2008).

Isotopic and elemental analysis was performed on samples of the coarse detrital fractions, in epiphytes and in living leaves. We did not analyse the fine detritus, as it was a mixture of the coarse detrital fractions (see 'Results'). After drying, the samples were ground to a fine powder, placed in a tin capsule and analysed for carbon (C) and nitrogen (N) elemental and isotopic composition. Prior to analysis, the detrital macroalgae and epiphytes were acidified drop by drop with HCl 2 N to remove carbonates, re-dried without rinsing and ground (Jacob et al. 2005, Carabel et al. 2006). As this chemical procedure has been reported to alter  $\delta^{15}\text{N}$  values (Bunn et al. 1995), each sample was split into 2 subsamples: half of the sample was washed with acid, and the other half remained untreated. For the isotopic and elemental analysis of seston, the SPOM sample filters were

dried to constant weight, split into 2 subsamples and weighed, and half of the filter was fumed under concentrated HCl fumes (12 N) overnight at room temperature (Lorrain et al. 2003). The subsamples treated with acid were used to analyse  $\delta^{13}\text{C}$ , and the untreated subsamples were used to analyse  $\delta^{15}\text{N}$ .

The holothurians were dissected, and the retractor muscles were carefully removed and used for isotopic analysis after being rinsed in distilled water, oven dried to constant weight (for 72 h at 45°C) and ground to a fine powder. As lipids are depleted in  $\delta^{13}\text{C}$  and may influence carbon isotope ratios in animal tissues (DeNiro & Epstein 1978, Post 2002), 5 samples were reanalysed after lipid removal by chloroform–methanol (2:1 ratio) extraction (Folch et al. 1957). No significant differences were found in the  $\delta^{13}\text{C}$  results (data not shown) between untreated tissue and that with lipids removed, probably due to the low lipid content, and therefore untreated samples were used.

Stable isotope ratios and elemental C and N composition were measured using a MAT 253 continuous-flow isotope ratio mass spectrometer (Thermo Finnigan) coupled to an EA 1108 elemental analyser (Carlo Erba Instruments) through a ConFlo III interface (Thermo Finnigan). C and N isotope ratios are expressed as  $\delta$  values in parts per thousand (‰) relative to Vienna Pee Dee Belemnite and the atmospheric air standard, respectively, according to standard notation ( $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R$  is the ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ). International Atomic Energy Agency standards were inserted every 12 samples for calibration. Replicate assays of standards indicated measurement errors of  $\pm 0.1$  and  $\pm 0.2$ ‰ for C and N, respectively. Sediment OM content was measured in triplicate as loss on ignition from sediment dry weight after combustion at 450°C in a muffle furnace for 4 h.

### Data analysis

The dry weights of each detritus fraction, sediment OM content and C and N isotopic and elemental composition of living leaves and epiphytes were analysed using a 2-way mixed effects ANOVA with site (Aiguablava, Giverola and Rustella) and landscape configuration (continuous, patches in a rock matrix and patches in a sand matrix) as factors. Site was considered random, and landscape configuration was considered fixed. Where a significant ( $p < 0.05$ ) difference occurred, a post hoc Tukey's HSD test was used to distinguish between groups. When neces-

sary, the data were fourth root transformed to meet the requirements of homogeneity of variance and normality. Non-transformed values (means  $\pm$  SE) are shown in the figures and tables. These analyses were performed using Statistica 8 software (StatSoft).

The Bayesian mixing model SIAR 4.2 (Parnell & Jackson 2013) running with R software (R Development Core Team 2014) was used to estimate the contribution of potential food sources to the diets of deposit feeders. The greatest advantage of this procedure is the incorporation of uncertainty linked to sources, consumers and trophic enrichment factors within the model (Parnell et al. 2010). This leads to the inclusion of an overall residual error term and to the generation of potential dietary solutions as true probability distributions. The model was run with 3 sources: detrital macroalgae, SPOM and a combined source of epiphytes and detrital *P. oceanica* leaves. The isotope signatures of this combined source were obtained using a weight ratio of 36:64 (epiphytes to leaves), as derived for old leaves from Alcoverro et al. (2004) and M. Pérez & J. Romero (unpubl. data). This procedure allowed us to avoid bias by reducing the number of food sources and to include epiphytes as part of the detrital material, as it was difficult to sort epiphytes from leaves in the detritus compartment. Separate mixing models were computed for each site and landscape configuration based on each corresponding set of isotope values. Within each mixing model simulation, holothurians were treated as individual consumers. We refer throughout the paper to *Holothuria* spp. (*H. poli* and *H. tubulosa-mamatta* complex), as no differences in isotope signatures were found between species (data not shown). The isotope ratios of the holothurians and food sources were analysed considering a trophic enrichment of  $1.3 \pm 0.3$ ‰ for  $\delta^{13}\text{C}$  and  $2.9 \pm 1.8$ ‰ for  $\delta^{15}\text{N}$  (adapted from McCutchan et al. 2003). Concentration dependence was incorporated into the model, as element concentrations differed between sources (Phillips & Koch 2002).

## RESULTS

Concerning coarse material, detrital *Posidonia oceanica* leaves accumulated in quantities 3-fold higher in continuous meadows than in patchy ones in either a rock or a sand matrix. No landscape configuration effects were found on the accumulation of other detrital fractions (Fig. 2, Table 1, Table S1 in the Supplement). Autochthonous material (detrital seagrass leaves and root and rhizome debris)



accounted for most of the total dry weight accumulated in all of the landscape configurations studied, from 74 to 79 %, while allochthonous material, including detrital macroalgae and material of terrestrial origin, represented the rest. The fine fraction (not represented) accounted for, on average, ca. 50 g dry wt m<sup>-2</sup>, irrespective of the site or the configuration (Table 1, Table S1 in the Supplement). As indicated by the observation using the dissecting microscope, the fine fraction was made mostly of leaf fragments (especially in samples from continuous meadows) and also included algal fragments and small debris from belowground organs.

The N content of living leaves showed clear differences between landscape configurations (Fig. 3, Table 1, Table S1 in the Supplement), with plants from continuous meadows having a higher N content than those from patches in either a rock or sand matrix. There was no evidence of an effect of landscape configuration on the N content of epiphytes (Fig. 3, Table 1, Table S1 in the Supplement). In the same way, there was no evidence of an effect of landscape configuration in the C content of living seagrass leaves (overall mean of 39.99 ± 0.09 %, relative to dry weight) and epiphytes (overall mean of 5.39 ± 0.18 %, relative to decalcified dry weight) (Table 1, Table S1 in the Supplement). Simi-

Table 1. Results of 2-way mixed effects ANOVAs, with landscape configuration as the fixed factor and site as the random factor. Comparisons for (a) dry weight of detrital fractions and sediment data and (b) elemental composition of living *Posidonia oceanica* leaves and epiphytes. Significant p-values ( $p < 0.05$ ) in **bold**. Df values between and within groups separated with a comma. When necessary, data were fourth root transformed to meet ANOVA assumptions

	df	SS	F	p
<b>(a) Detrital fractions biomass and sediment data</b>				
<i>P. oceanica</i> detrital leaves	2, 36	10.39	64.62	<b>&lt;0.001</b>
<i>P. oceanica</i> belowground fraction	2, 36	0.10	0.15	0.869
Terrestrial fraction	2, 36	1.64	1.20	0.390
Macroalgae	2, 36	1.39	0.48	0.640
Fine fraction	2, 36	32 183	1.15	0.404
Sediment organic matter (%)	2, 36	0.02	0.01	0.986
<b>(b) <i>P. oceanica</i> leaves and epiphytes variables</b>				
<b>Nitrogen content (%)</b>				
<i>P. oceanica</i> living leaves	2, 36	0.02	10.37	<b>0.026</b>
Epiphytes	2, 36	0.25	0.69	0.554
<b>Carbon content (%)</b>				
<i>P. oceanica</i> living leaves	2, 36	2.01	0.15	0.869
Epiphytes	2, 36	1.43	0.13	0.885

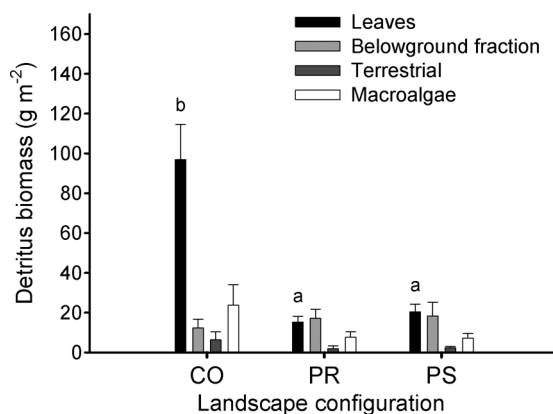


Fig. 2. Mean dry weight (±SE) of detritus stock fractions in *Posidonia oceanica* meadows from the different landscape configurations (n = 15). Bars labelled with the same letter and unlabelled bars do not differ significantly according to Tukey's HSD post hoc test. CO: continuous meadow; PR: patches embedded in a rock matrix; PS: patches embedded in a sand matrix

larly, there was no evidence of an effect of landscape configuration on sediment OM content, with an overall mean of 1.39 ± 0.09 %, relative to dry weight (Table 1, Table S1 in the Supplement).

Potential food sources for deposit feeders (detrital *P. oceanica* leaves and epiphytes, detrital macroalgae and SPOM) were well distinguishable using both C and N stable isotope values (Fig. 4, Table S2 in the Supplement). The δ<sup>13</sup>C values of the sources ranged between -12.26 and -25.50 ‰. SPOM was the most <sup>13</sup>C-depleted source (-22.30 to -25.50 ‰) and

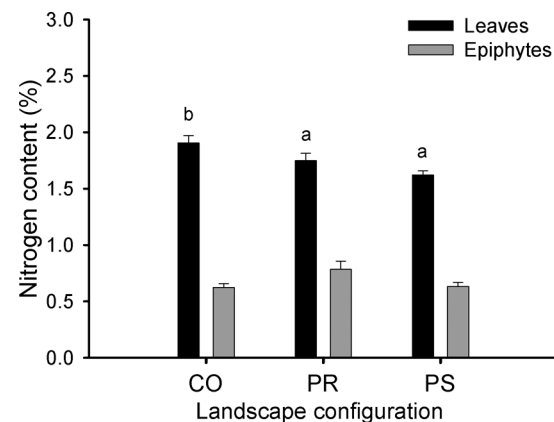


Fig. 3. Mean nitrogen content (±SE) of *Posidonia oceanica* living leaves and epiphytes from the different landscape configurations (n = 15). Bars labelled with the same letter and unlabelled bars do not differ significantly according to Tukey's HSD post hoc test. CO: continuous meadow; PR: patches in a rock matrix; PS: patches in a sand matrix

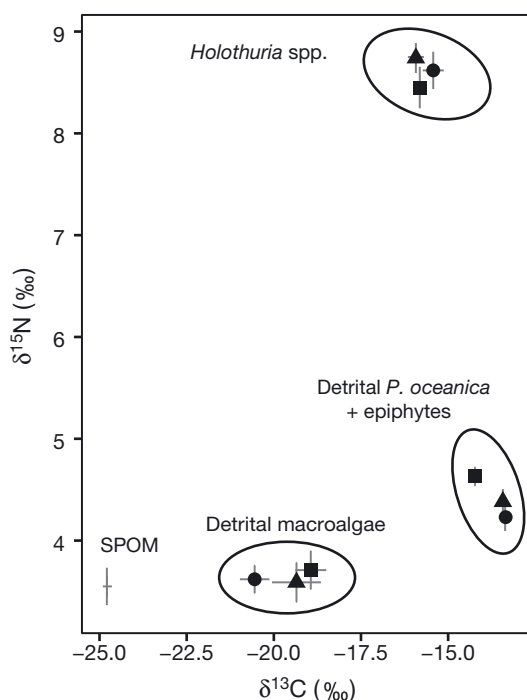


Fig. 4. Isotope plot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in parts per thousand (‰) for consumers, *Holothuria* spp. and their potential food sources (mean and standard deviation) in each landscape configuration. ●: continuous meadows; ▲: patchy meadows in a rock matrix; ■: patchy meadows in a sand matrix. SPOM: suspended particulate organic matter

showed low C:N ratios (9.60 to 10.74). The  $\delta^{13}\text{C}$  values of detrital macroalgae showed intermediate values, although they presented a high variability (from  $-14.87$  to  $-24.56$ ‰), probably due to the presence of a range of species in different amounts. Furthermore,

detrital macroalgae had low C:N ratios (9.13 to 11.97). The combined source of detrital *P. oceanica* leaves plus epiphytes showed the most  $\delta^{13}\text{C}$ -enriched values ( $-12.26$  to  $-14.98$ ‰) and high C:N ratios (27.12 to 30.78). The  $\delta^{15}\text{N}$  values of the potential food sources were more homogeneous, between 2.10 and 5.24‰. SPOM values ranged from 2.60 to 4.60‰; detrital macroalgae ranged from 2.10 to 4.96‰; and detrital *P. oceanica* leaves plus epiphytes presented slightly higher  $\delta^{15}\text{N}$  values, from 3.30 to 5.24‰, probably due to the presence of small sessile animals among the epiphytes, such as hydrozoans (*Aglaophenia harpago*, *Sertularia perpusilla*) or bryozoans (e.g. *Electra posidoniae*), among others (Prado et al. 2007).

The isotopic composition of *Holothuria* spp. was similar in the different landscape configurations studied (Fig. 4, Table S2 in the Supplement). The  $\delta^{13}\text{C}$  signatures of *Holothuria* spp. ranged from  $-13.43$  to  $-17.72$ ‰, and the  $\delta^{15}\text{N}$  values ranged from 6.94 to 9.53‰. The ranges of feasible contributions from each food source to *Holothuria* spp. diets varied slightly between landscape configurations (Fig. 5, Table S3 in the Supplement). The models suggested that the combined source of *P. oceanica* leaves and epiphytes constitutes the majority of the diet at all sites and landscape configurations, with mean values of the proportions ranging from 41 to 63%. Detrital macroalgae appeared as the second source in all of the models applied, with mean proportions in the narrow range, 29 to 36%. SPOM was also a potentially significant contributor to *Holothuria* spp. diets, with mean contributions ranging from 5 to 28%.

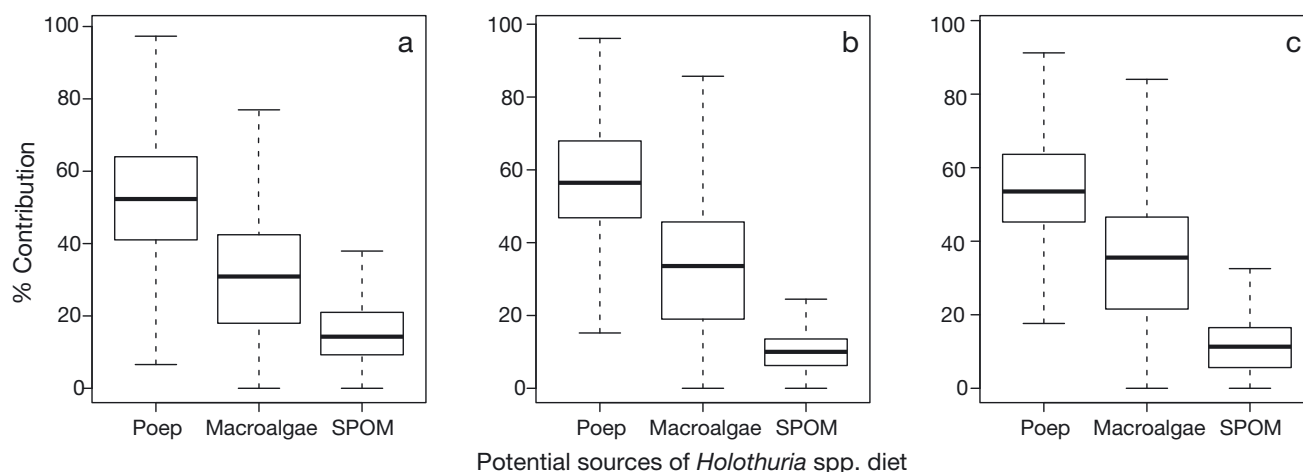


Fig. 5. Percentage dietary contributions of the 3 potential food sources for *Holothuria* spp. diets in the landscape configuration studied. (a) Continuous meadows; (b) patches in a rock matrix; (c) patches in a sand matrix. Plots show the distribution of feasible contributions from each food source to the species diet resulting from the application of the SIAR isotope model. Boxplot from top to bottom: largest observation, upper interquartile, median, lower interquartile and lowest observation. Poep: compounded source of detrital *Posidonia oceanica* leaves and epiphytes; SPOM: suspended particulate organic matter

## DISCUSSION

As hypothesized, landscape configuration plays a role in modulating the flows of material between habitats in coastal marine ecosystems. Specifically, in continuous meadows, the accumulation of detrital seagrass leaves was enhanced (up to 3-fold) relative to the accumulation in seagrass patches in either a rock or sand matrix. Under such high leaf litter accumulation, *in situ* mineralization could increase nutrient availability, as suggested by the nutrient content found in leaves from plants collected in continuous meadows, which was significantly higher than that from plants in patchy configurations. Detrital seagrass leaves, including their epiphyte loads, were the main food source for deposit feeders. However, the accumulation of such materials in continuous meadows was not paralleled by a shift in the isotopic composition of the model deposit feeder (*Holothuria* spp.). This did not support the hypothesis proposed that the proportion of food sources in the diets of the deposit feeders could be modulated by differences in the flux of materials between habitats.

Accumulated detritus within *Posidonia oceanica* meadows (only coarse fraction) accounted for high organic stocks, up to 220 g dry wt m<sup>-2</sup> in continuous meadows and approximately 100 g dry wt m<sup>-2</sup> in seagrass patches. These values are of the same order as those for standing litter stocks or even higher at that time of the year (e.g. Romero et al. 1992). These values are 10-fold higher when compared with other seagrass species (e.g. *Cymodocea nodosa*) (Pérez et al. 2001) and 5-fold higher when compared with different coastal habitats such as mangroves (Woodroffe 1985). The bulk of the detritus is autochthonous, including leaf litter and rhizome and root debris. Allochthonous materials such as terrestrial detritus and macroalgae detached from rocky algal reefs were equally present in all of the landscape configurations in lower proportions (2 and 13%, respectively).

Detritus accumulation in seagrass meadows is a complex process in which biological and physical forcing interact (Romero et al. 1992). Standing litter is the result of the balance between local detritus production (e.g. leaf fall), import (of allochthonous materials but also probably of seagrass leaves from neighbouring meadows), export and decay (both mechanical, leading to the fine fraction, and biological, i.e. decomposition). All of these factors seem relevant, except maybe import. The effects of landscape configuration (specifically meadow size) seem to be

restricted to the accumulation of detrital leaves, while the other fractions (macroalgae and terrestrial detritus) seem to vary randomly across configurations. The leaf canopy of seagrass meadows attenuates water flow and reduces turbulence (Pujol & Nepf 2012). The below-canopy habitat, where detached leaves accumulate, presents low shear stress and reduced turbulence compared to the canopy–water interface region (Hendriks et al. 2008). In this study, under the same environmental conditions and with no differences in shoot density, the higher detrital leaf stocks found in continuous seagrass meadows suggest that the continuous meadows attenuate turbulence to a greater extent than patches, thus reducing the export of materials. In contrast, this does not seem to affect the import rates of allochthonous detritus.

The relatively low accumulation of allochthonous detritus suggests that seagrass meadows could act as barriers, making the arrival of external materials less likely. In agreement with this, it has been shown that in relatively dense seagrass meadows, the bulk of water flow is directed to the roof of the canopy (Granata et al. 2001), and detached algae do not percolate into the canopy but pass above it (Wernberg et al. 2006). Input rates of drifting materials are considered a function of landscape variables such as patch perimeter and the distance between habitats (Polis et al. 1997), but this is not reflected in our results. This may be because the most important issue is that the probability of a moving entity entering a given habitat once intercepted is determined by the boundary permeability of the habitat (Polis et al. 1997).

Interestingly, the nutrient content of seagrass leaves was higher in continuous meadows than in patchy ones, suggesting an association between nutrients and leaf detritus accumulation. Seagrasses meet their N requirement mainly via 2 mechanisms (Romero et al. 2006): uptake from either the water column or pore water (through leaves and roots, respectively) and internal recycling (i.e. resorption and remobilization of nutrients from old leaves or rhizome pools). For the species *P. oceanica*, internal recycling has been estimated to be high enough to meet 40% of annual needs (Alcoverro et al. 2000, Lepoint et al. 2002). As demonstrated by Hyndes et al. (2012), seagrass uptake of leached nutrients from detrital sources can account for part of the remaining 60%, thus linking detritus accumulation and nutrient availability, as is known for terrestrial systems (Swift et al. 1979, Vogt et al. 1986).

The differences found in this study in the N content of plant leaves, albeit small, could have profound

consequences for plant performance. As in other seagrasses, growth rates of shallow *P. oceanica* meadows are usually limited by nutrients, usually N (Alcoverro et al. 1997b, Lepoint et al. 2002, Invers et al. 2004). The N content threshold suggested to indicate N limitation, either for this species or for seagrasses in general (Duarte 1990, Alcoverro et al. 1997b, Invers et al. 2002, Lepoint et al. 2002), is within the range of values reported here for small patches. This suggests that even small increases in N availability, if these take place close to the values involving N limitation, can stimulate plant performances (e.g. leaf growth, Alcoverro et al. 1997b), thus linking patch size and nutrient deficiency, as proposed by Gera et al. (2013) and Pagès et al. (2014) in previous works. Those authors attributed their results to increased fish herbivore activity in small patches. However, we found low densities of herbivorous fish in our study areas (authors' pers. obs.), suggesting that although the herbivorous hypothesis cannot be ruled out, detritus accumulation seems to be a better explanation in our case. Regardless, the explanations are not mutually exclusive, and further studies are needed to evaluate their (probably) site-specific relative importance. The conclusion emerging from the available evidence is that meadow fragmentation, in addition to other functional and structural effects (Montefalcone et al. 2010), could result in reduced plant performance due to nutrient shortage.

The feeding behaviour of holothurians is still poorly understood, and traditionally they have been considered non-selective feeders (Massin & Jangoux 1976). In this study, the main food source for *Holothuria* spp. was detrital *P. oceanica* leaves, including epiphytes, followed by macroalgae and SPOM in all landscape configurations. Interestingly, the contribution of detrital leaves and macroalgae to the diets did not parallel the standing biomass found in the landscape configurations studied. Some studies stress the capacity of holothurians to discriminate between nutrient-rich and nutrient-poor particles and also their particle size selection capacity, at least in some species (Massin & Jangoux 1976, Mercier et al. 1999, Mezali & Soualili 2013). Our results suggest that the diets of holothurians, irrespective of landscape configuration, are mainly supported by materials detached from the seagrass meadow, particularly leaves and epiphytes. These materials can be ingested not only as very small decaying fragments mixed with the sediments but also as large (up to 1 cm<sup>2</sup>) pieces, as confirmed by our observation of holothurian stomach contents. Both seagrass leaves

and epiphytes seem to contribute to holothurian nutrition. However, the assimilation of epiphytes seems to be prevalent, as suggested by the isotope signatures found in holothurians, maybe due to their higher nutritional value (Tomas et al. 2006). This confirms previous findings, in which the contribution of seagrass epiphytes to seagrass trophic webs is not in strict relation to their abundance (Tomas et al. 2006, Park et al. 2013). Although it has been pointed out that alterations at the landscape scale could potentially disrupt or divert the natural direction of energy flows between adjacent ecosystems and hence influence food web pathways (Polis et al. 1997, Puth & Wilson 2001, Howe & Simenstad 2011), this does not seem to be occurring in the seagrass deposit feeders studied here, at least not at the spatial scale studied.

The findings reported here confirm the importance of landscape configuration in modulating flows of material within the coastal ecosystem mosaic. These effects mainly concern seagrass leaf litter accumulation and the associated effects on nutrient availability for plants but not on the food sources for deposit feeders. Importantly, seagrass patches accumulating less foliar detritus are poorer in N content than continuous meadows, which could lead to nutrient limitation. This is of particular concern in future scenarios where synergistic effects between water quality and climate change could further modify coastal seascapes, especially under the assumed worldwide seagrass decline (Waycott et al. 2009), where habitat loss will promote habitat fragmentation or increasing seagrass patchiness and potentially reduce plant performance, with consequences for the entire ecosystem.

**Acknowledgements.** We thank Neus Sanmartí, Jordi Pagés, Max Galindo, Oriol Mascaró, Alessandro Gera, Owen S Wangensteen and Marine Baud for their help in the field and laboratory. Carbon–nitrogen analyses were performed at the Unidade de Técnicas Instrumentais de Análise (Universidade de Coruña), and we thank María Lema for her assistance. This study was performed with the financial support of the Spanish government (projects CTM2010-22273-C02-01 and CTM2013-48027-C3-1-R). The Spanish government supported A.M.R. (scholarship BES-2011-046849).

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*Editorial responsibility: Kenneth Heck Jr,  
Dauphin Island, Alabama, USA*

*Submitted: December 9, 2014; Accepted: June 6, 2015  
Proofs received from author(s): July 15, 2015*



Contents lists available at ScienceDirect

## Marine Pollution Bulletin

journal homepage: [www.elsevier.com/locate/marpolbul](http://www.elsevier.com/locate/marpolbul)

Baseline

## Variability of sedimentary organic carbon in patchy seagrass landscapes

Aurora M. Ricart<sup>a,\*</sup>, Paul H. York<sup>d</sup>, Michael A. Rasheed<sup>d</sup>, Marta Pérez<sup>a</sup>, Javier Romero<sup>a</sup>, Catherine V. Bryant<sup>d</sup>, Peter I. Macreadie<sup>b,c</sup><sup>a</sup> Departament d'Ecologia, Universitat de Barcelona, Av Diagonal 643 6a planta, 08028 Barcelona, Spain<sup>b</sup> Plant Functional Biology and Climate Change Cluster, University of Technology Sydney, PO Box 123, Sydney, NSW 2007, Australia<sup>c</sup> Centre for Integrative Ecology, School of Life and Environmental Sciences, Faculty of Science Engineering and Built Environment, Deakin University, Burwood, VIC 3125, Australia<sup>d</sup> Centre for Tropical Water and Aquatic Ecosystem Research, James Cook University, PO Box 6811, Cairns, QLD 4870, Australia

## ARTICLE INFO

## Article history:

Received 22 July 2015

Received in revised form 20 September 2015

Accepted 22 September 2015

Available online 28 September 2015

## Keywords:

Blue carbon

Carbon stock

Patchiness

Marine sediment

*Zostera muelleri*

Climate change

## ABSTRACT

Seagrass ecosystems, considered among the most efficient carbon sinks worldwide, encompass a wide variety of spatial configurations in the coastal landscape. Here we evaluated the influence of the spatial configuration of seagrass meadows at small scales (metres) on carbon storage in seagrass sediments. We intensively sampled carbon stocks and other geochemical properties ( $\delta^{13}\text{C}$ , particle size, depositional fluxes) across seagrass–sand edges in a *Zostera muelleri* patchy seagrass landscape. Carbon stocks were significantly higher (ca. 20%) inside seagrass patches than at seagrass–sand edges and bare sediments. Deposition was similar among all positions and most of the carbon was from allochthonous sources. Patch level attributes (e.g. edge distance) represent important determinants of the spatial heterogeneity of carbon stocks within seagrass ecosystems. Our findings indicate that carbon stocks of seagrass areas have likely been overestimated by not considering the influence of meadow landscapes, and have important relevance for the design of seagrass carbon stock assessments.

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The necessity to reduce  $\text{CO}_2$  fluxes to mitigate climate change has generated considerable interest in quantifying the capacity of natural ecosystems to trap and sequester carbon (McLeod et al., 2011). Most efforts have focussed on quantifying carbon sinks in terrestrial ecosystems, but recently vegetated coastal habitats have been highlighted for their carbon storage potential (Pendleton et al., 2012; Duarte et al., 2013a).

The carbon sequestered by vegetated coastal habitats, referred commonly as “blue carbon”, provides long-term organic carbon ( $\text{C}_{\text{org}}$ ) storage once it has become bound within sediments. The large amount of  $\text{C}_{\text{org}}$  stored in coastal sediments, combined with their high carbon sequestration rates, highlights the important role that coastal ecosystems play as natural carbon sinks (Macreadie et al., 2014a,b). These ecosystems, particularly mangroves, saltmarshes and seagrasses, have a disproportionately large  $\text{C}_{\text{org}}$  storage potential relative to their global area (Duarte et al., 2005, 2013a; McLeod et al., 2011).

Seagrasses develop organic-rich soils derived from both autochthonous (produced internally — e.g. seagrass detritus) and allochthonous (of external origin — e.g. sestonic particles) sources (Gacia and Duarte, 2001; Kennedy et al., 2010). Seagrass carbon storage capacity is a result of a high primary productivity, the refractory nature of seagrass tissues and its capacity to trap particles from the water column and incorporate them into the sediment (Hendriks et al., 2008; Kennedy et al., 2010).

These facts, in combination with high sedimentation rates and the anaerobic nature of these soils, that promotes slow microbial decomposition rates, provide environments where carbon can be buried and preserved over long time frames, such as the millennial carbon deposits found in temperate seagrass meadows formed by persistent, long-lived species (Mateo et al., 1997, 2006).

The relevance of seagrasses as a potential mitigating agent of  $\text{CO}_2$  emissions was already suggested in the last decades (Smith, 1981), although, the paucity of data on the carbon budgets from seagrass ecosystems hindered the inclusion of seagrasses in models of global carbon transfer and global green-house gas abatement schemes (Macreadie et al., 2014a). Over the past five years, however, there has been a major increase in the accuracy of regional and global estimates of carbon stocks and accumulation rates in seagrass ecosystems (Kennedy et al., 2010; Duarte et al., 2010; Fourqurean et al., 2012; Serrano et al., 2012, 2014; Greiner et al., 2013; Lavery et al., 2013).

Seagrass ecosystems encompass a wide variety of species across a range of depositional environments and depths (Carruthers et al., 2007). Species identity as well as abiotic habitat characteristics have been highlighted as factors driving variability of sedimentary carbon stocks of seagrass meadows (Lavery et al., 2013; Serrano et al., 2014). However, our understanding of the factors regulating this variability is still limited (Duarte et al., 2010; Nellemann et al., 2009; Serrano et al., 2014).

Moreover, seagrass meadows present high spatial heterogeneity (Jackson et al., 2006). They can occur either as large, continuous

\* Corresponding author.

E-mail address: [auroramartinez@ub.edu](mailto:auroramartinez@ub.edu) (A.M. Ricart).



meadows or in the form of patches of various shapes and sizes with unvegetated or macroalgal regions interspersed among more homogenous seagrass areas (Robbins and Bell, 1994). Patchiness is an intrinsic feature of most seagrass meadows, especially in shallow and estuarine areas where natural and anthropogenic forcing is severe (Montefalcone et al., 2010). Patchiness increases the amount of edge associated to the habitat (Smith et al., 2008), which constitutes an important transitional gradient from one structural type to another. Habitat edges influence patterns in biological and physical activity by promoting interactions with the surrounding landscape (Puth and Wilson, 2001; Macreadie et al., 2010a,b). In particular for seagrasses, the edge has been defined as a boundary that stops the water flow, increasing turbulence on the edge of the canopy (Granata et al., 2001) and enhancing deposition of particles inside the seagrass meadow (Macreadie et al., 2010a; Zong and Nepf, 2011). Over time, these processes could potentially generate spatial heterogeneity in  $C_{org}$  accumulation, both in the seagrass patches and in adjacent bare sediments.

Carbon burial rates on seagrasses can be as much as threefold higher when compared to bare sediments (Duarte et al., 2005), as seagrass canopies promote sedimentation and reduce particle resuspension (Gacia and Duarte, 2001; Gacia et al., 1999). But this is unlikely to be uniform inside such seagrass meadows, where significant differences in burial rates due to the meadow architecture and spatial configuration occurs (Granata et al., 2001; Gruber and Kemp, 2010). Small-scale variability (e.g. one seagrass patch might be denser than another patch) could influence carbon sequestration at the patch level thus affecting carbon stocks estimations at the habitat and landscape scale.

Despite previous works quantifying seagrass carbon sinks, little is known about the within-meadow variability on sediment carbon storage. In this study we selected a patchy seagrass distribution to evaluate the small-scale spatial variability on carbon storage within seagrass ecosystems. We evaluated horizontal spatial variability by quantifying carbon stocks and sources both in seagrass patches and adjacent bare sediments at increasing distances from the seagrass–sand edge. We also evaluated vertical variability by sampling at different depth sections in the sediment. We hypothesized that (1) sediment carbon stocks will be highly variable across seagrass patches, with carbon stocks increasing across the transition from bare sediments to seagrass patch edges and to seagrass patch interiors; and (2) there will be greater contribution of autochthonous (plant-produced) carbon to the carbon stocks within seagrass patches than in bare sediments.

The study was conducted in the middle of the growing season (September 2013), well before the seasonal dieback, on Pelican Banks inside the entrance to Port Curtis Harbour (23°46′57″S; 151°18′0″E). Port Curtis is a macro-tidal estuary in central Queensland (Australia) characterized by the presence of *Zostera muelleri* Irmsch ex Asch. beds in most of its intertidal areas. The selected area for this study represented a patchy landscape characterized by a mosaic of large seagrass patches (diameter about hundreds of metres) interspersed with naturally occurring unvegetated (bare) sediments.

To test for variability in carbon storage, four different locations on the boundary among seagrass patches and bare sediments were selected haphazardly at the same water depth. The seagrass patches selected for the study were determined to be persistent for at least the last 10 years by using satellite imagery (Google Earth, 2013). At each location, cores were taken at 6 different positions along a transect perpendicular to a seagrass–bare sediment edge: at 0.5 m, 10 m and 20 m into seagrass (S0.5, S10 and S20) and at 0.5 m, 10 m and 20 m into bare sediment (B0.5, B10 and B20). Four replicated transects were sampled, resulting in a total of 24 (6 positions  $\times$  4 transects) sampling points. Seagrass cover, measured in three replicate 50  $\times$  50 cm quadrates at each seagrass position, was similar among all positions ( $p > 0.05$ ) with an overall mean of  $22.5\% \pm 3.06$  SE.

Sediments were collected manually by inserting open-barrel PVC pipes (20 cm length, 5 cm internal diameter) into sediments to a depth of 10 cm, and using a piston to provide suction as cores were

withdrawn. Compaction during coring was low ( $<10\%$ ). Once extracted, cores were capped at both ends and transported to the laboratory. In the laboratory, the sediments were extruded, sliced into 10 sections at 0–0.5, 0.5–1, 1–1.5, 1.5–2, 2–3, 3–4, 4–5, 5–6, 6–8, and 8–10 cm intervals, dried at 60 °C and weighed in order to calculate dry bulk density (Howard et al., 2015).

Each sediment section was split into two sub-samples, with grain size particle distribution analysed from the first subsample using a Malvern Mastersizer 2000 laser microgranulometer. Organic matter (OM) was removed from the subsample by addition of hydrogen peroxide 10%, while large organic material was removed by hand. Particle size distribution was expressed as % volume for particle diameters from 0 to 2000  $\mu$ m. The  $d_{50}$  (i.e. the diameter corresponding to the median of particle volumes assuming that all particles are spherical) and skewness (Folk and Ward, 1957) were used as an indicator of the particle size distribution.

The second sub-sample was used for  $C_{org}$  elemental and isotopic analysis after being sieved through a 1 mm mesh to remove living plant material and coarse inorganic particles (i.e. carbonate material). Samples were then ground and acidified with HCl 1 M to remove any carbonates that were too small to be sieved. After drying, samples were re-ground and then analysed for carbon elemental and isotopic composition.

The apparent depositional flux (considered as the result of both deposition and resuspension processes) was measured in each position sampled along the transects using sediment traps consisting of cylindrical centrifuge tubes (20.5 ml) with a height versus diameter ratio of 5, with the aperture of the tube positioned at 5 cm above the sediment surface. Sediment traps were removed after 7 days of deployment. These short-term measurements were used only to capture relative spatial differences, and not to elaborate annual budgets. In the laboratory contents of the sediment traps were filtered through pre-combusted (450 °C for 4 h) 25 mm GF/F filters and dried (60 °C for 48 h) to obtain dry weight. Samples were analysed for  $C_{org}$  elemental and isotopic composition, after acidification, using the method described above.

Measurements of carbon elemental composition and stable isotope ratios (as a tracer of carbon sources) were performed using a continuous-flow isotope-ratio mass spectrometer MAT253 (Thermo Finnigan) coupled to an elemental analyser EA1108 (Carlo Erba Instruments) through a ConFlo III interface (Thermo Finnigan). Carbon isotope ratios are expressed as  $\delta$  values in parts per thousand (‰) relative to VPDV (Vienna Pee Dee Belemnite) according to standard notation ( $\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 1000$ , where  $R$  is the ratio  $^{13}C/^{12}C$ ). Standing carbon stocks per volume unit were calculated using dry bulk density data and  $C_{org}$  content and expressed as  $mg C_{org} cm^{-3}$ .

Differences in dry bulk density, particle size median diameter ( $d_{50}$ ), sedimentary  $C_{org}$  elemental and isotopic composition, and carbon stocks were analysed using two-way Analysis of Variance (ANOVA) with position along the transects and depth section as fixed factors. The apparent depositional flux and the carbon elemental and isotopic composition of the material collected in the sediment traps were compared using a one-way ANOVA with position along the transects as a fixed factor. Where a significant ( $p < 0.05$ ) difference occurred, post hoc Tukey's HSD tests were used to distinguish differences among groups. When necessary, data were fourth root transformed to meet ANOVA assumptions. Non-transformed values (means  $\pm$  SE) are shown in figures and tables.

Dry bulk density of sediments was similar across all the positions along the transects and depth sections (Table 1) with an overall mean of  $1.57 \pm 0.08 g cm^{-3}$ . The median diameter of sediment particles ( $d_{50}$ ) was similar among all positions along the transects and depth sections (Table 1), ranging from 103  $\mu$ m to 120  $\mu$ m, while skewness was positive (overall mean  $0.83 \pm 0.01$ ) indicating that sediments can be characterized as fine sands.

**Table 1**  
Results of the two-way ANOVA's, with position (P) and depth (D) as fixed factors.

		df	SS	F	p
<i>Sediment cores variables<sup>a</sup></i>					
Dry bulk density (g cm <sup>-3</sup> )	P	5	0.24	1.09	0.37
	D	9	0.76	1.90	0.06
	P*D	45	2.01	0.01	0.48
	Residuals	180	8.05		
Particle size median diameter (d <sub>50</sub> )	P	5	14.10	0.30	0.91
	D	9	133.10	1.56	0.13
	P*D	45	327.50	0.77	0.84
	Residuals	1	1116.10		
Carbon content (%C <sub>org</sub> )	P	5	4.31	13.45	<0.01
	D	9	0.45	0.78	0.63
	P*D	45	1.71	0.59	0.98
	Residuals	180	11.53		
Carbon stocks (mg C <sub>org</sub> cm <sup>-3</sup> )	P	5	1059.00	6.93	<0.01
	D	9	113.00	0.41	0.93
	P*D	45	9.77	0.71	0.91
	Residuals	180	5503.00		
δ <sup>13</sup> C (‰)	P	5	48.85	19.38	<0.01
	D	9	4.76	1.05	0.40
	P*D	45	7.73	0.34	0.01
	Residuals	180	90.74		
<i>Sediment traps variables<sup>b</sup></i>					
Deposition rates (mg m <sup>-2</sup> day <sup>-1</sup> ) <sup>c</sup>	P	5	0.06	1.90	0.15
	Residuals	18	0.11		
Carbon content (%C <sub>org</sub> ) <sup>c</sup>	P	5	0.61	11.82	<0.01
	Residuals	14	0.15		
δ <sup>13</sup> C (‰)	P	5	0.64	0.10	0.99
	Residuals	14	18.76		

<sup>a</sup> Comparisons for sediment cores variables.

<sup>b</sup> Comparisons for sediment traps variables.

<sup>c</sup> Data were fourth root transformed to meet ANOVA assumptions.

The C<sub>org</sub> content, carbon stocks and δ<sup>13</sup>C showed significant differences among positions but not among depth sections, and a significant interaction between both factors was found for δ<sup>13</sup>C (Table 1). The profile values of C<sub>org</sub> content and δ<sup>13</sup>C through the top 10 cm of the sediment cores remain fairly constant through depth in the innermost seagrass positions, and showed more variability in all other positions, although this variability neither followed any trend nor provided any indication of past seagrass presence (Fig. 1a and b). The C<sub>org</sub> content in the sediment was significantly higher in the innermost seagrass positions than in seagrass edges. The lowest C<sub>org</sub> content was found in bare sediment positions (Fig. 2a). Carbon stocks showed a similar pattern as C<sub>org</sub> content, with higher values in seagrass positions than in bare sediments positions (Fig. 2b). The δ<sup>13</sup>C values were significantly more negative in bare sediments than in the rest of positions. Inside seagrass positions, δ<sup>13</sup>C values were more negative in the seagrass edge than in the other two innermost seagrass positions (Fig. 2c).

The apparent depositional flux showed high variability in bare sediment positions compared to seagrass positions resulting in the lack of significant differences among positions (Table 1, Fig. 3a). The C<sub>org</sub> content in the material deposited was significantly lower in bare sediments than in seagrass patches. Within seagrass positions, the seagrass edge showed values ca. 3-fold higher relative to bare sediments and the innermost seagrass positions showed values ca. 5-fold higher relative to bare sediments (Table 1, Fig. 3b). The δ<sup>13</sup>C values of the material deposited did not show differences among positions (Table 1, Fig. 3c).

Our results show that seagrass carbon storage varied at small scales (metres). Seagrass patches showed a clear pattern of variation with lower carbon stocks at seagrass edges than in the innermost parts. Carbon stocks in bare sediments were lower than in seagrass vegetated areas, but higher than expected because of the influence of the seagrasses. Patch level attributes (distance to the edge) represent an important determinant of the spatial heterogeneity of carbon storage within seagrass ecosystems. Variability on spatial distribution of carbon

stocks needs to be taken into account for the accurate estimations on blue carbon assessments for seagrasses.

The present work confirms that *Z. muelleri* meadows have a high capacity for C<sub>org</sub> storage in the sediments. Areal estimates of carbon stocks in the upper 10 cm (the depth of the cores we used in this study) give values as high as 600 g C m<sup>-2</sup> in the innermost parts of seagrass patches. The values of seagrass patches are higher to those reported elsewhere for this same species (Lavery et al., 2013), and are among the highest standing stocks of total carbon, on a unit-area basis, reported in tropical seagrasses (Lavery et al., 2013).

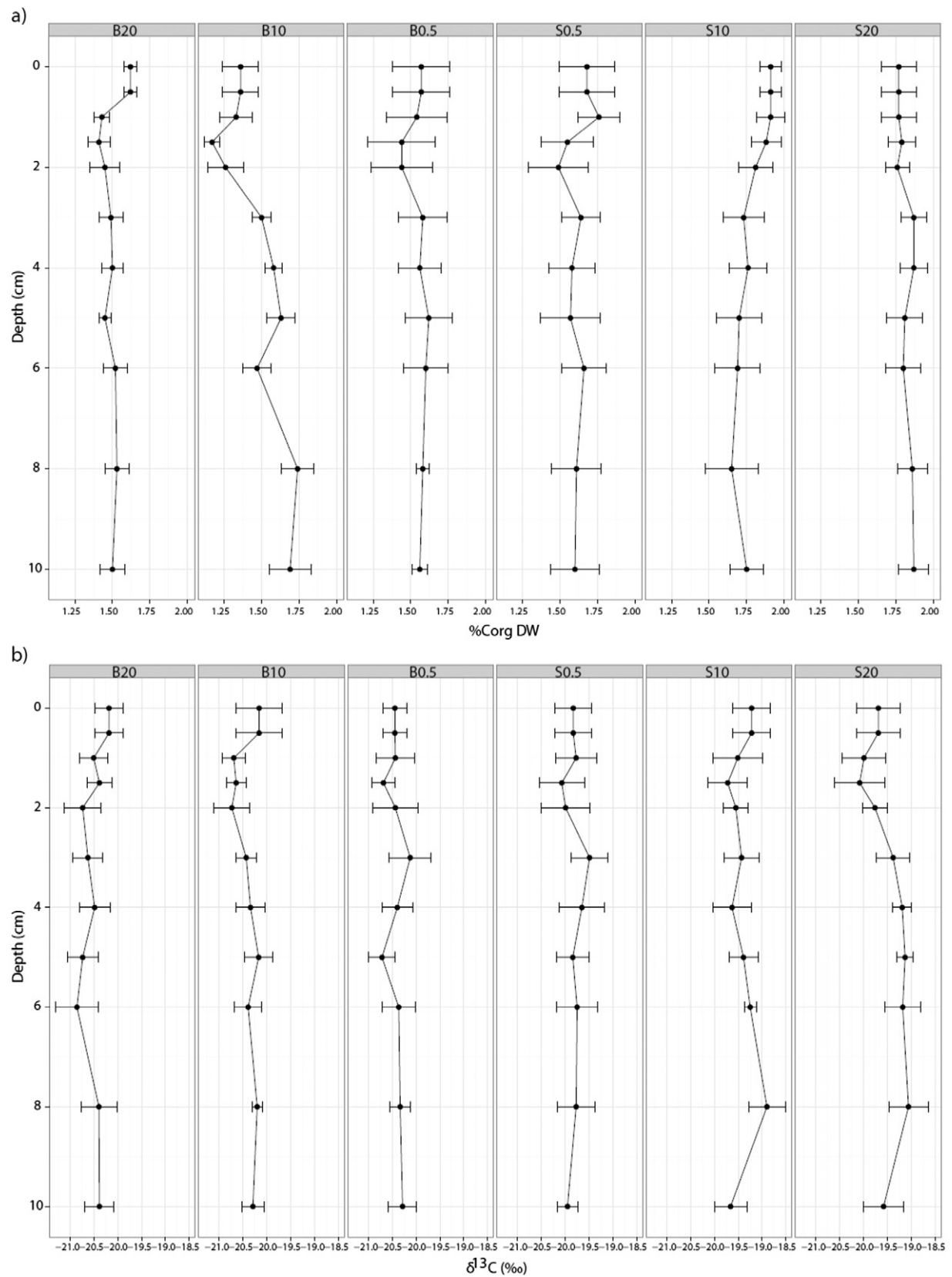
The lower carbon stocks found in the edges of seagrass patches, relative to the innermost parts, could be caused by several processes related with habitat boundaries. Habitat boundaries are recognized to have patterns differing from the main part of the habitat (Puth and Wilson, 2001; Smith et al., 2011) and these patterns will be influenced by the nature of the adjacent habitats (Sheaves, 2009). The boundary from sand to dense seagrass cover enhances flow acceleration over the canopy (Folkard, 2005), while producing a secondary circulation cell at the seagrass meadow edge (Granata et al., 2001). Thus, turbulence increases at the interface between sand and seagrass, likely promoting higher resuspension of sediments and export of seagrass derived materials in the seagrass edge, and also less deposition of suspended particles, reducing carbon storage capacity when compared with the innermost parts of the seagrass patches.

On the other hand, the lower carbon stocks found in the seagrass edge could be related with the development stage of the seagrass patches. As far as we know from satellite imagery, the seagrass patches studied in this work have been stable for the past decade, however, the history of each seagrass patch sampled (i.e. the permanency of the boundary) is not known prior to this study. Different stages of patch development could affect carbon storage (Duarte et al., 2013b; Greiner et al., 2013; Marbà et al., 2015). If the patch is growing, the edge is younger than the central part of the patch and is formed by fast-growing rhizomes and new leaf clusters (Duarte and Sand-Jensen, 1990). During the first years after patch establishment, most of the carbon is stored as aboveground biomass, however, during the middle-aged and old stages of development, the importance of below-ground biomass and below-ground detrital mass as carbon stores increase (Cebrián et al., 2000). Thus, the seagrass-derived carbon that is available for burial in the edge is likely to be lower if the plant is still colonizing the sediments.

It is also possible that the meadow could be in regression and the lower carbon stocks at seagrass edges were related with a prior loss of seagrass habitat cover in the adjacent bare sediments. Carbon stocks in the sediments could be lost after the regression of the seagrass habitat by physical removal, promoting a higher resuspension of sediments or even remineralisation (Fourqurean et al., 2012). However, evidence of a reduction in stored C<sub>org</sub> from sediment after seagrass loss is inconclusive (Macreadie et al., 2014b).

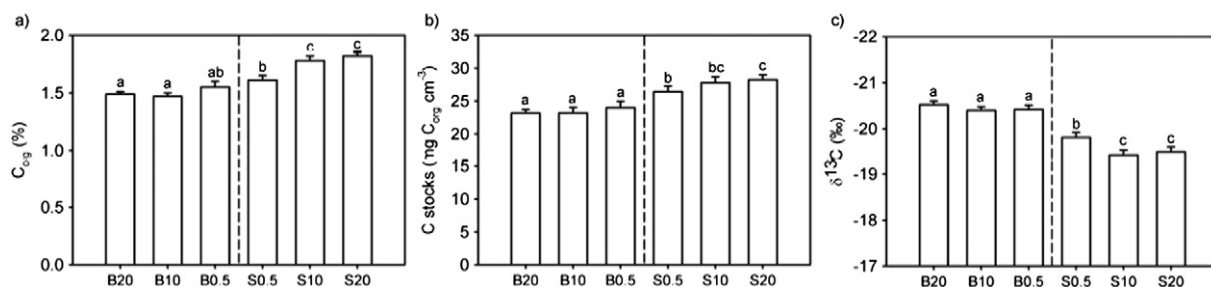
In this study, bare sediments on the patchy seagrass landscape showed less carbon storage than seagrass patches, however, their carbon stocks were higher than those previously reported for continuous *Z. muelleri* meadows approximately 1000 km to the north of our study in Queensland, Australia (Lavery et al., 2013). Seagrass beds are known to modify the C<sub>org</sub> content of nearby sediments (Miyajima et al., 1998) influencing its C<sub>org</sub> content in two different ways; by direct supply of materials detached from the plants in a mechanism of seagrass-derived carbon spill over (Miyajima et al., 1998); and also by reducing the velocity of the water flow, because of the structural influence of the seagrass canopy, thus depositing suspended particulate materials not only within the seagrass but also in adjacent bare sediments areas (Fonseca and Koehl, 2006; Pujol and Nepf, 2012).

Resuspension, transport and resettlement of seagrass-derived organic matter have been suggested as factors controlling the horizontal distribution of C<sub>org</sub> in the sediment (Miyajima et al., 1998). Evidence for export of the organic matter produced within the seagrass meadow



**Fig. 1.** Depth profiles of the top 10 cm of sediment cores from the different positions along the transects (mean and SE). (a) Carbon organic content ( $C_{org}$ ) expressed as percentage of the total sample dry weight (%); (b) carbon isotopic ratios. Labels: S0.5, S10, and S20 positions at 0.5 m, 10 m and 20 m into seagrass patches and B0.5, B10, and B20 positions at 0.5 m, 10 m and 20 m into bare sediment.





**Fig. 2.** Results from sediment variables (mean and SE) in the top 10 cm of sediment cores from the different positions along the transects. (a) Carbon organic content ( $C_{org}$ ) expressed as percentage of the total sample dry weight (%); (b) carbon stocks; (c) carbon isotopic ratios. Labels: S0.5, S10, and S20 positions at 0.5 m, 10 m and 20 m into seagrass patches and B0.5, B10, and B20 positions at 0.5 m, 10 m and 20 m into bare sediment. Dotted line represents the seagrass/sand boundary. Bars labelled with the same letter do not differ significantly according to a Tukey's HSD post hoc test.

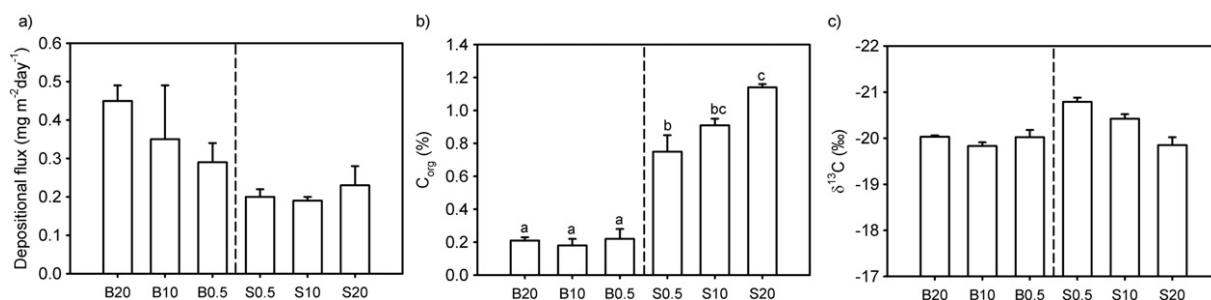
to adjacent bare sediments has been reported in several studies (Kennedy et al., 2010; Miyajima et al., 1998). However, in our case, values of  $\delta^{13}C$  presented in both habitats were extremely  $^{13}C$  depleted, suggesting that most of the  $C_{org}$  was from an allochthonous origin. Potential allochthonous sources of  $C_{org}$  like seston (ca.  $\delta^{13}C - 20\%$ ), epiphytes, microphytobenthos and macroalgae (all with typical ca.  $\delta^{13}C - 17\%$ ), and terrestrial organic matter (ca.  $\delta^{13}C - 27\%$ ) are all isotopically light relative to  $\delta^{13}C$  seagrass (ca.  $\delta^{13}C - 12\%$  for *Z. muelleri*) (Moncreiff and Sullivan, 2001; Leduc et al., 2009; Kennedy et al., 2010; Dubois et al., 2012; Oakes and Eyre, 2014; Ricart et al., 2015). It is likely that the high  $C_{org}$  content found on the bare sediments on the seagrass patchy landscape may be due to the seagrass canopy promoting deposition of allochthonous sources. A fact that is also suggested by the similarity in  $\delta^{13}C$  values on the trapped material in all positions along the transects. While the less negative  $\delta^{13}C$  values in sediments of seagrass patches indicated a slightly greater contribution of seagrass-produced carbon. The higher carbon content found in particles trapped inside seagrass sediments, were probably due to the higher deposition of mineral fraction in bare sediments areas. This is in accordance with previous published data which show that particles trapped and deposited in seagrass sediments are often richer in organic matter (Kennedy et al., 2010; Miyajima et al., 1998) when compared with those deposited in bare sediments.

This study provided insights on the spatial variability of sediment carbon storage of seagrasses. Patchy seagrass landscapes have a great carbon storage capacity as plants not only promote carbon storage inside the seagrass patches but also in the adjacent bare sediments. The distribution of carbon stocks within a patchy seagrass landscape could be linked to the spatial configuration of the habitats within the landscape (sand and seagrass in this study), which in time determines the carbon sources to the sediments and processes to which  $C_{org}$  accumulated in seagrass sediments could be subjected (e.g. less resuspension). Tropical areas, as the one in this study, could be subjected to temporal and spatial fluctuations on seagrass abundance both

seasonally and between years (Rasheed et al., 2014; York et al., 2015), although this study did not address the issue of temporal variability of carbon storage, due the short term of the measurements.

Total carbon storage of seagrass ecosystems will depend in part on the amount of seagrass vegetated coverage and the amount of edges found. This fact has to be taken into account when attempting global blue carbon estimates, especially in naturally patchy meadows or in human induced fragmented ones. The major issue in carbon stocks assessments at large scales come down to how researchers scale up their data. Usually sediment samples are taken in core areas of the seagrass meadows and then extrapolated to the landscape scale based on the measured seagrass vegetated areas. This could lead to overestimation of carbon stocks, because, as shown in this study, seagrass edges stored less carbon than the innermost parts of seagrass meadows. To avoid overestimation in continuous seagrass meadows researchers should not be taking into account seagrass edges (e.g. first 10 m to the interior of seagrass areas) when scaling up the data. While for patchy seagrass landscapes a correct estimation of the surface of vegetated areas is required. We recommend the use of high resolution cartography to localize core areas of seagrass meadows, seagrass edges and bare areas interspersed, and the sampling of sediment cores in these three parts to scale up the data for each part separately. Landscape configuration and patch dynamics are factors that are often overlooked by managers when assessing the level of ecosystem function provided by seagrasses, as happens with meadow form, habitat and life history of each species (Kilminster et al., 2015). Detailed seagrass mapping needs to be used, and seagrass ecology and functional aspects of these ecosystems need to be taken into account in environmental decision making, management and policy.

Findings reported in this study demonstrated that spatial heterogeneity of the seagrass meadows affects its sediment carbon storage. These results also highlight concerns for future changes to seagrass ecosystems in the face of increasing anthropogenic impacts and seagrass decline (Waycott et al., 2009). The increasing rate of



**Fig. 3.** Results from sediment traps on the different positions (mean and SE). (a) Apparent depositional flux; (b) carbon organic content ( $C_{org}$ ) in deposited materials expressed as percentage of the total sample dry weight (%); (c) carbon isotopic ratios of deposited material. Labels: S0.5, S10, and S20 positions at 0.5 m, 10 m and 20 m into seagrass patches and B0.5, B10, and B20 positions at 0.5 m, 10 m and 20 m into bare sediment. Dotted line represents the seagrass/sand boundary. Bars labelled with the same letter do not differ significantly according to a Tukey's HSD post hoc test.

degradation of seagrass ecosystems will promote habitat fragmentation and increase the patchiness, which in turn will influence seagrass carbon storage. Gaining insights on the variability of carbon storage of seagrasses is important to properly manage and protect carbon stocks of these valuable coastal ecosystems.

## Acknowledgements

We thank Jessie Jarvis for her assistance in the field. PM was supported by an Australian Research Council DECRA Fellowship (DE130101084) and program CSIRO Coastal Climate Cluster. AMR was supported by the Spanish Government (fellowships BES-2011-046849 and EEBB-I-13-06916 and project CTM2013-48027-C3-1-R).

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