UNIVERSITAT DE BARCELONA

Biodiversity patterns of coralligenous outcrops over broad spatial and temporal scales in the NW Mediterranean Sea: baseline data for conservation

Edgar Casas i Güell

Tesi doctoral 2016

Tesi Doctoral

Universitat de Barcelona
Facultat de Biologia
Programa de doctorat en Ciències del Mar

Biodiversity patterns of coralligenous outcrops over broad spatial and temporal scales in the NW Mediterranean Sea: baseline data for conservation

Memòria presentada per **Edgar Casas i Güell** per optar al Grau de Doctor per la

Universitat de Barcelona

Universitat de Barcelona (UB)
Institut de Ciències del Mar (ICM-CSIC)
Consejo Superior de Investigaciones Científicas
Barcelona, Abril de 2016

Doctorand: **Edgar Casas i Güell** (Institut de Ciències del Mar – CSIC)

Directores:
Dr. Núria Teixidó Ullod (Institut de Ciències del Mar –(ICM-CSIC) –i Stazione Zoologica Anton Dohrn, Nàpols, Itàlia)

Dr. Emma Cebrian i Pujol (Universitat de Girona)

Tutor: **Dr. Joan Lluís Pretus Real (**Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona).

Casas-Güell, E. (2016) Biodiversity patterns of coralligenous outcrops over broad spatial and temporal scales in the NW Mediterranean Sea: baseline data for conservation.

Dedicada al lector i ...

... y para Mer, por todo.

Agraïments / Acknowledgements

En primer lloc, els primers agraïments van dirigits als supervisors d'aquesta tesi: la Núria, en Quim i l'Emma. A la Núria per ser qui em va donar una gran quantitat de fotografies per analitzar sota un disseny en l'espai i el temps i donar-me així la temàtica central d'aquesta tesi, per fer-me participar en cursos de formació, assistir a xerrades i en definitiva, per fer-me participar activament en recerca: gràcies al seu enorme esforç, a la seva paciència, rectitud, exigència i constant control aquesta tesi finalment ha estat possible. Al Quim per coordinar els diferents aspectes de la tesi, llegir-se, corregir i comentar els diferents manuscrits de la mateixa, permetre'm poder tenir un lloc físic a l'ICM per poder fer la tesi (taula i el "super-computer") i facilitar-me tots els papeleos "habidos y por haber" durant tot el doctorat. A l'Emma per moltes coses, però bàsicament pel seu optimisme (vital de necessitat per poder acabar una tesi!), concreció i claredat en els missatges científics i eficiència en les correccions dels manuscrits: aquesta tesi s'ha pogut iniciar i acabar en molt bona part gràcies a la seva constància, dedicació i paciència. Eternament agraït als tres.

Agraïr també als responsables del programa de doctorat en Ciències del Mar la seva professionalitat i ajuda que han mostrat en tot moment, en especial a en Miquel Canals i a en Toni Calafat. També al meu tutor del departament d'Ecologia de la facultat de Biologia de la UB, en Joan Pretus, per l'ajuda i signatures vàries que requereixen la fase final de dipòsit i defensa de la tesi doctoral.

Però mirant una mica més enrere, tota aquesta aventura científica va començar al iniciar el màster en Ciències del Mar i amb una "oferta" d'en Mikel Zabala d'agafar un estudiant durant les seves classes de Reserves Marines. Allà se'm van obrir els ulls com a taronges i de seguida vaig voler integrar-me en el grup de recerca de seguiment de les Illes Medes. Això va ser el curs 2007/2008 i molt ha plogut desde ençà. Varen ser un parell d'anys de proves d'anàlisi de dades de peixos amb en Toni García, busseijos i redacció de treballs fins que el Kike Ballesteros em va contractar durant un any i mig per fer el CARLIT de *Cystoseira mediterranea* i estudis bentònics de fons tous a cadascuna de les illes Balears. D'aquells temps, sempre recordaré aquell inventari sota aigua, en el sostre de la cova de la vaca a les Illes Medes, comptant, anomenant en veu alta (amb el regulador a la boca) i anotant els diferents noms d'invertebrats sèssils quan de sobte (i per manca d'aire) en Mikel va "fugir" com un coet per un forat impossible de la cova. I així em vaig quedar jo, flotant a la columna d'aigua, a les fosques de la cova dient noms d'invertebrats.

I al rescat varen venir tots els integrants del grup de recerca Medrecover. Moltíssimes gràcies a la Cristina i al Diego per haver-me deixat participar en les campanyes betòniques a la Reserva Marina de les Illes Columbretes fent feines vàries com treure i comptar multitud de reclutes de *Pinna nobilis* enganxades en xarxes, agafar mostres de *Peyssonnelia* spp. a 40 i "pico" metres en sorgències volcàniques, comptar i recolectar algues invasores i invertebrats, canviar termòmetres, ... i un llarg etc. !!. A l'Eneko i a la Laura Navarro per ser tant bons companys en tots els sentits, tan el l'humà com en el professional; al Bernat Hereu per fer de tutor a la Universitat de Barcelona en relació a diferents temes de papers i ajuts de matrícules de doctorat. També per convidar-me a participar en un curs de Màster pràctic de tècniques de mostreig amb escafandre autònoma a les Illes Medes. I a tots els companys i companyes de doctorat del grup durant el meu període FPI a l'ICM: la Silvija, Carolina, l'Andrea, Rosanna, Jordi, Stefano, Carlos, …, i al Jeanba! sobretot pel suport anímic i els bons consells a l'inici de la tesi.

A part de les campanyes al Cap de Creus i a les Illes Medes canviant termòmetres, fent "Rapid assessments" i diversos mostrejos fotogràfics, durant aquests anys de tesi el que m'ha portat més hores de feina, paciència i dedicació són les estones de lectura i redacció d'esborranys articles científics i l'anàlisi de centenars i centenars d'imatges de fons bentònics de coral·lígen per generar així grans matrius de dades de biodiversitat. Durant totes aquestes hores d'anàlisi d'imatges i dades, lectura i redacció de manuscrits, he d'agraïr la companyia dels companys i companyes de l'ICM. És d'agraïr el seu bon humor i bon caràcter, sobretot a les companyes del despatx B12 (Elisa, Miriea i Maria) per fer-me sentir en tot moment com un més de l'ICM; agraïments també a tota la gent del departament de Biologia del grups de micro, fito, zoo i acidificació (per nombrar a uns quants): Ramiro, els dos Frans, Caterina, Encarna, AnaMari, Massimo, Eva Flo, Juancho, Guillem, Sara Zamora, Sergi Martínez, Norma, Raquele, Sara-Jane, a la gent de Chile Roy i Rodrigo; també a la gent del departament de Recursos Marins Renovables (companys del màster i de despatx) com l'Anabel, l'Alba, Alfredo, Uxue, etc; als estudiants de doctorat (ja doctors) de Física: la Carmen, el Pedro i en Suso. I en general, també agraïr a tot l'staff científic i tècnic de l'ICM la seva excel·lència i en especial a Xavi Leal per compartir conversa durant molts dinars i posteriors cafès i a la Dra. Cèlia Marrasé i Dr. Rafel Coma pel seus consells en uns moments no gaire bons per a mi (i a l'Eduard Serrano també pel seu incondicional suport!!).

ah! I també gràcies als Friday-beers organitzats pel departament de Biologia que algun que altre maldecap m'han donat … i a les calçotades vàries a Vilanova!

Special mention is adressed to the scientific staff met during my stay at Massey University of New Zealand (New Zealand Institute for Advanced Study and Institute of Natural and Mathematical Sciences). The first researcher to aknowledge is Professor Marti J. Anderson because she allowed me to do the stay in a very special and important personal moment in her life, and to Dr. Mattew Pawley and Adam N. Smith because they never left me alone. With them, apart from practicing English I learned some multivariate statistics technics, discussed theorical concepts about beta diversity, and I had the great oportunity to perform several scuba diving surveys at diferent Marine Protected Areas (Leigh and Poor Knights), taking photographs of sessile macroinvertebrates on vertical walls and kelp forests. Thanks also to the members of the Statistics working group, the PhD. Student Helen L. Smith, Dr. Daniel Walsh, Dr. Barry McDonald, Dr. Beatrix Jones, and Dr. Howard Edwards. Finally, thanks also to Dr. Simon Trush who allowed me to visit the excellent marine research center NIWA at Waikato (Hamilton) and gave me the opportunity to give a short seminar about our work in the Mediterranean.

I com no, també agraïr-ho a la meva familia: als meus pares, al Josep-Maria Casas i a la Mari-Sol Güell bàsicament per tot, a les meves germanes Ivette i Gisel i també a la meva familia política uruguayo-menorquina: Guido, Charo, Pilar i Rosario.

I per anar acabant, com no podria ser d'altra manera, a nuestro pequeño (o ya no tan pequeño!) núcleo familiar: a la Mercedes i als dos peques Gerard i Júlia. Gràcies per haber sofert directament també aquesta tesi amb mi, viscuda sovint des de dins amb dolor, passió i alegria i que tan sovint ho he externalitzat: mil gràcies (i mil disculpes) per haber-me permès conviura-la íntegrament al vostre costat! Sense cap mena de dubte, la vostra energia ha empès moltíssim per finalment poder haber acabat aquesta tesi!!

I per últim, agraïr el suport econòmic: la realització d'aquesta Tesi Doctoral ha estat possible gràcies a la meva integració al grup de recerca MedRecover a nivell d'investigador predoctoral FPI BES-2010-036130 (beca/contracte lligada a un projecte de recerca del Ministerio de Ciencia e Innovación) durant el període d'Octubre de 2010

a Octubre de 2014. També gràcies a diversos projectes de recerca finançats durant l’última dècada, especialment MedChange, MedDiversa, Biorock i Smart finançats per la Total Foundation i per el Plan Nacional de I + D + I gestionat pel Ministerio de Ciencia e Innovación amb participació del Fons Social Europeu. Gràcies també a les ajudes socials per a famílies que proporciona l’Estat que m’han permès tenir un petit ajut econòmic durant l’últim any d’elaboració d’aquesta tesi.

Director's report

The supervisors of this thesis, Dr. Núria Teixidó Ullod and Dr. Emma Cebrian i Pujol, certify that Edgar Casas i Güell has actively participated in the development of the work associated to this doctoral thesis. In general, his participation included the following tasks:

- Objectives raising.
- Planning and execution of the data acquisition in the field.
- Obtained samples processing and analysing.
- Results compilation, data analysis, and interpretation.
- Chapters writing, and active involvement in their revision process.

Finally, they certify that none of the coauthors of the chapters here presented has used, neither is going to use, implicitly or explicitly, the work presented in the elaboration of another Ph.D. Thesis

Dr. Núria Teixidó Ullod
(Institut de Ciències del Mar – CSIC
and
Stazione Zoologica Anton Dohrn)

Dr. Emma Cebrian i Pujol
(Universitat de Girona)

List of articles published or in revision

Chapter 3

Kipson S[1,2], Fourt M[3], Teixidó N[1,4], Cebrian E[5], **Casas-Güell E**[1], Ballesteros E[6], Zabala M[4], Garrabou J[1,3] (2011) Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops. PLoS ONE 6(11):e27103. doi:10.1371/jour- nal.pone.0027103. **Impact factor: 3.234.**

Chapter 4

Casas-Güell E[1], Cebrian E[5,6], Garrabou J[1,7], Ledoux JB[1,8], Linares C[4], Teixidó N[9] (2016) Structure and biodiversity of coralligenous assemblages over broad spatial scales (*in revision* to *Nature Scientific Reports*). **Impact factor: 5.578.**

Chapter 5

Casas-Güell E[1], Teixidó N[1,4], Garrabou J[1,7], Cebrian E[5,6] (2015) Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales. Marine Biology Volume 162, Issue 4, pp 901-912. **Impact factor: 2.391**.

Chapter 6

Teixidó N[1,4], **Casas-Güell E**[1], Cebrian E[5,6], Linares C[4], Garrabou J[1,7] (2013) Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm. PLoS ONE 8(1):e53742. doi:10.1371/journal.pone.0053742. **Impact factor: 3.234.**

1 Institut de Ciències del Mar (ICM-CSIC), Barcelona, Catalonia, Spain

2. Division of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

3. UMR 6540 - DIMAR CNRS - Université de la Méditerranée, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Marseille, France

4. Departament d'Ecologia, Universitat de Barcelona, Barcelona, Catalonia, Spain

5. Departament de Ciències Ambientals, Facultat de Ciències, Universitat de Girona, Girona, Catalonia, Spain

6. Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Blanes, Catalonia, Spain

7. UM110, CNRS/INSU, IRD, Aix-Marseille Université, Université du Sud Toulon Var, Mediterranean Institute of Oceanography (MIO), Marseille, France

8. CIMAR/CIIMAR, Centro Interdisciplinar de Investigaçao Marinha e Ambiental, Universidade do Porto, Rua dos Bragas 177, 4050-123 Porto, Portugal

9. Stazione Zoologica Anton Dohrn, Villa Dohrn-Benthic Ecology Center, Punta San Pietro, Ischia, Naples 80077, Italy

Contents

Abstract

Increasing anthropogenic pressures and its effects on marine ecosystems urge enhanced knowledge and understanding of the current state of marine biodiversity. This baseline information is pivotal to establish the actual state, detect future changes, explore possible trends and propose adequate management actions for marine ecosystems. Coralligenous outcrops are a highly diverse and structurally complex habitat faced with major threats in the Mediterranean Sea. Despite its ecological, aesthetic and economic value, coralligenous biodiversity patterns are still poorly understood. Information on the spatial and temporal variability of the composition and structure of coralligenous assemblages is scarce for most regions. Specially, data on species diversity and structure in coralligenous outcrops dominated by *Corallium rubrum* and *Paramuricea clavata* are essentially lacking. Gathering these data is needed to build robust baselines in order to assess the responses of this highly threatened habitat to anthropogenic disturbances (e.g. mass mortality events, sedimentation and invasive species).

There is currently no single sampling method that has been demonstrated to be sufficiently representative to ensure adequate community assessment and monitoring in this habitat. In **Chapter 3**, we propose a rapid non-destructive protocol for biodiversity assessment and monitoring of coralligenous outcrops providing good estimates of its structure and species composition, based on photographic sampling and the determination of presence/absence of macrobenthic species. We followed a hierarchical sampling survey, covering more than 400 km of rocky coasts in NW Mediterranean (Catalan coast –Spain-, Provence and NW Corsica –France). This approach allowed us to determine the minimal sampling area for each assemblage (5000 cm^2 for *P. clavata* and 2500 cm^2 for *C. rubrum*). In addition, we concluded that 3 replicates provided an optimal sampling effort in order to maximize the species number and to assess the main biodiversity patterns of studied assemblages in variability studies requiring replicates.

We followed the mentioned hierarchical sampling survey in Chapter 3 to characterize the spatial and temporal variability of structure, composition, abundance and diversity of perennial species inhabiting coralligenous outcrops dominated by *C. rubrum* or *P. clavata* (Chapter 4 and Chapter 5 respectively). In **Chapter 4** spatial variability of

species composition, structure and diversity in coralligenous assemblages dominated by *C. rubrum* was assessed. We also pooled the identified species/taxa into morphofunctional groups according to their life-span and growth to characterize the structural complexity of the assemblages. Finally, we analyzed the number, size and shape of patches (landscape pattern indices) for each group in order to describe the spatial arrangement within the *C. rubrum* assemblage. Landscape pattern indices in *C. rubrum* assemblages exhibited important differences among sites in the same locality whereas localities showed similar values.

In **Chapter 5**, the spatial and temporal variability of species composition, structure and diversity in coralligenous assemblages dominated by *P. clavata* was assessed. According to ordination analyses, species composition and the structural complexity of coralligenous assemblages differed consistently across all spatial scales considered. The lowest and the highest variability were found among localities (100 km) and within sites (1-5 km), respectively. Despite the differences, the sites displayed high similarity (average similarity 55.7 %) and shared approximately 50 % of the species. Similarly, differences in diversity indices (alpha, beta and gamma) were found. Furthermore, the study of *P. clavata* addressed diversity patterns over 5-year period. Overall, no temporal changes were detected in either species composition or the morphofunctional groups considered.

In **Chapter 6**, we showed how an extreme storm event affected the dynamics of benthic coralligenous outcrops in the NW Mediterranean Sea using data acquired before (2006–2008) and after the impact (2009–2010) at four different sites. The loss of cover of benthic species resulted between 22% and 58%. The damage across these species (e.g. calcareous algae, sponges, anthozoans, bryozoans, tunicates) was uneven, and those with fragile forms were the most impacted, showing cover losses up to 50 to 100%. Interestingly, small patches survived after the storm and began to grow slightly during the following year. In contrast, sheltered sites showed no significant changes in all the studied parameters, indicating no variations due to the storm.

Overall, the results presented in this thesis furnishes a basis for the implementation of monitoring schemes of coralligenous assemblages complementing the scarce available information on assemblage composition and structure of the emblematic Mediterranean coralligenous outcrops dominated by *C. rubrum* and *P. clavata* over large spatial scales. Our results demonstrate the moderate spatial and the extremely low temporal variability in biodiversity patterns in the NW Mediterranean region, providing the first baselines for detecting potential changes due to global change effects. We also provide new insights into the responses of these assemblages to a large and rare extreme event. The development of similar sampling schemes in other Mediterranean regions will provide a global view of the biodiversity of coralligenous outcrops.

Resum

L'increment de pressions antròpiques i els seus efectes sobre els ecosistemes marins evidencien la necessitat de potenciar el coneixement i la comprenssió de l'estat actural de la biodiversitat marina. La informació de referència és esencial per tal de poder establir l'estat actual, detectar futurs canvis, explorar posibles tendències i proposar les adequades accions de gestió pels ecosistemes marins. Els afloraments de coralígen són hàbitats altament diversos i estructuralment complexos que s'enfronten amb les majors amenaces en el mar Mediterrani. Tot i els seus valors ecològics, estètics i econòmics, els patrons de biodiversitat del coralígen són encara poc entesos. Hi ha una manca d'informació sobre la variabilitat espacial i temporal en la composició i estructura de les comunitats de coralígen per la majoria de les regions. Especialment, manquen dades sobre la diversitat i estructura dels afloraments de coralígen dominats per *Corallium rubrum* i *Paramuricea clavata*. L'integració de tots aquests conjunts de dades esdevé realment un pas necessari per construir línies de referència robustes amb l'objectiu d'evaluar les respostes d'aquests hàbitats altament amenaçats degut a pertorbacions antròpiques (ex: mortalitats massives, sedimentació i espècies invasores).

Actualment no disposem d'un sol mètode unificat de mostreig que hagi estat demostrat ser suficientment representatiu per tal d'assegurar una adequada avaluació i monitoratge d'aquest hàbitat. En el **Capítol 3**, proposem un protocol ràpid i no-destructiu, basat en mostreijos fotogràfics i la determinació de la presència/absència d'espècies macrobentòniques, per a l'avaluació i monitoreig de la biodiversitat dels afloraments de coralígen resultant unes bones estimes de la seva estructura i composició d'espècies. Hem seguit un disseny de mostreig jerarquitzat en l'espai, cobrint més de 400 Km de costa rocallosa en el Mediterrani Nord Occidental (Costa Catalana – Espanya –, Provença i el NO de Còrcega – França). Aquesta aproximació metodològica ens ha permès determinar l'àrea mínima per a cadascuna de les comunitats (5000 cm^2 per *P. clavata* i 2500 cm^2 per *C. rubrum*). A més, concluïm que amb 3 rèpliques assolim un òptim esforç de mostreig en el sentit de maximitzar el número d'espècies així com per avaluar els principals patrons de biodiversitat en els estudis de variabilitat on es requereixin rèpliques.

A continucació, hem seguit el mencionat disseny jeràrquic de mostreig del **Capítol 3** per caracteritzar la variabilitat espacial i temporal en l'estructura, composició, abundància i diversitat de les espècies bentòniques sèssils i perennes que habiten en els afloraments coralígens dominats per *C. rubrum* i *P. clavata* (**Capítol 4** i **Capítol 5** respectivament). Així, en el **Capítol 4** hem avaluat la variabilitat en la composició d'espècies, estructura i diversitat en les comunitats del coralígen dominades pel corall vermell. A més, hem agrupat les espècies i/o taxons identificats en grups morfofuncionals d'acord amb la seva esperança de vida i formes de creixement per caracteritzar la complexitat structural de la comunitat. Finalment, hem calculat el número, la mida i forma de les taques (patrons d'índexs de paisatge) de cadascun dels grups amb l'objectiu de descriure la disposició en l'espai en el sí de la comunitat de *C. rubrum.* Els índexs de paissatge en la comunitat de *C. rubrum* exhibeixen importants diferències entre els llocs de la mateixa localitat mentre que les localitats mostren valors similars entre elles.

En el **Capítol 5**, hem avaluat la variabilitat espacial i temporal de la composició d'espècies, estructura i diversitat en les comunitats de coralígen dominades per *P. clavata*. D'acord amb les anàlisis d'ordenació, la composició d'espècies i la complexitat estructural de les comunitats de coralígen difereixen consistentment entre totes les escales espacials considerades. La variabilitat més baixa la varem trovar entre localitats (100 Km) mentre que la més alta la varem trovar entre sites (1-5 Km). Tot i les diferències, els sites mostraren una elevada similaritat (similaritat promig 55.7%) i comparteixen aproximadament el 50% de les espècies. Similarment, varem trovar diferències en els índexs de diversitat (alfa, beta i gamma). A més, en l'estudi de *P. clavata* també varem determiner els patrons de diversitat en un període de 5 anys. En conjunt en l'escala regional, no es van detectar canvis destacables en la composició d'espècies ni en la composició dels grups morfofuncionals considerats.

En el **Capítol 6**, mostrem com una tormenta extrema va afectar a la dinàmica dels afloraments bentònics de coralígen en el mar Mediterrani Nord Occidental emprant dades adquirides abans (2006-2008) i després de l'impacte (2009-2010) en 4 llocs diferents. La pèrdua de cobertura d'espècies bentòniques va resultar d'entre el 22% i el 58%. El dany causat entre les espècies (ex: algues calcàries, esponges, antozous, briozous i tunicats) fou desigual, i les que resultaren més impactades foren les formes fràgils, mostrant pèrdues de cobertura de entre el 50% i el 100%. També són destacables les “taques” que varen sobreviure després de la tempesta i varen començar a crèixer lleugerament durant el següent any. En contrast, els sites més reguardats no mostraren canvis en cap dels paràmetres estudiats, indicant que la tormenta no va provocar variacions en aquests sites.

En conjunt, els resultats presentats en aquesta tesi suposen els foments per a la implementació d’esquemes de monitoratge de les comunitats de coralígen complementant així l’escassesa d’informació disponible sobre la composició i estructura dels emblemàtics afloraments de coralígen Mediterrani dominats per *C. rubrum* i *P. clavata* en escales espacials grans. Els resultats en els patrons de biodiversitat en el Mediterrani Nord Occidental, demostren una variabilitat espacial moderada que contrasta amb la variabilitat extremadament baixa en el temps i suposen les primeres dades de referència per detectar potencials canvis degut als efectes del canvi global. També donem noves comprensions sobre les respostes d’aquestes comunitats a grans i poc freqüents events pertorbadors. L’implementació d’esquemes de monitoreig similars en altres regions del Mediterrani proveiran d’una visió global de la biodiversitat dels afloraments de coralígen.

Resumen

Del incremento de presiones antrópicas y sus efectos sobre los ecosistemas marinos surge la necesidad de potenciar el conocimiento y la compresión del actual estado de la biodiversidad marina. Estos datos de referencia resultan esenciales para poder determinar el estado actual de los ecosistemas marinos, así como detectar futuros cambios y explorar posibles tendencias para proponer las acciones de gestión adecuadas. Los afloramientos de coralígeno son hábitats altamente diversos y estructuralmente complejos que se enfrentan con las mayores amenazas en el mar Mediterráneo. A pesar de sus elevados valores ecológicos, estéticos y económicos, los patrones de biodiversidad del coralígeno se encuentran aún poco entendidos. Actualmente, existe una carencia de información sobre la variabilidad espacial y temporal en la composición y estructura de los afloramientos de coralígeno dominados por *Corallium rubrum* y *Paramuricea clavata*. La integración de todo este conjunto de datos resulta necesaria para construir líneas de referencia robustas con el objetivo final de evaluar las respuestas de estos hábitats altamente amenazados debido a perturbaciones antrópicas (ej. mortandades masivas, sedimentación y especies invasoras).

Actualmente no disponemos de un solo método unificado de muestreo el cual haya sido demostrado ser suficientemente representativo para asegurar una adecuada evaluación y monitoreo de este hábitat. En el **Capítulo 3**, proponemos un protocolo rápido y no-destructivo para la evaluación y monitoreo de la biodiversidad de los afloramientos del coralígeno resultando unas buenas estimas de su estructura y composición de especies, basado en muestreos fotográficos y en la determinación de la presencia/ausencia de especies macro bentónicas. Hemos seguido un diseño de muestreo jerárquico en el espacio, cubriendo más de 400 Km de costa rocosa en el Mediterráneo Norte Occidental (Costa Catalana – España –, Provenza y el Norte Occidental de Córcega – Francia). Esta aproximación metodológica nos ha permitido determinar el área mínima para cada una de las comunidades estudiadas (5000 cm^2 para *P. clavata* y 2500 cm^2 para *C. rubrum*). Además, concluimos que con 3 réplicas alcanzamos un esfuerzo de muestreo óptimo en el sentido de maximizar el número de especies así como para evaluar los principales patrones de biodiversidad en los estudios de variabilidad dónde se requieren réplicas.

A continuación, hemos seguido el mencionado diseño jerárquico de muestreo del **Capítulo 3** para caracterizar la variabilidad espacial y temporal en la estructura, composición, abundancia y diversidad de las especies bentónicas sésiles y perennes que habitan en los afloramientos coralígenos dominados por *C. rubrum* y *P. clavata* (**Capítulo 4** y **Capítulo 5** respectivamente). Así, en el **Capítulo 4** hemos evaluado la variabilidad en la composición de especies, estructura y diversidad en las comunidades de coralígeno dominadas por el coral rojo. Además, hemos agrupado las especies o los taxones identificados en grupos morfofuncionales de acuerdo con la esperanza de vida y de sus formas de crecimiento para caracterizar la complejidad estructural de la comunidad. Finalmente, hemos calculado el número, el tamaño y la forma de las "manchas" (índices de paisaje) de cada uno de los grupos con el objetivo de describir la disposición en el espacio en el sí de la comunidad de *C. rubrum*. Los índices de paisaje en la comunidad de *C. rubrum* exhiben importantes diferencias entre los sites en la misma localidad mientras que las localidades muestran valores similares.

En el **Capítulo 5**, hemos evaluado la variabilidad espacial y temporal de la composición de especies, estructura y diversidad en las comunidades de coralígeno dominadas por *P. clavata*. De acuerdo con el análisis de ordenación, la composición de especies y la complejidad estructural de las comunidades de coralígeno difieren consistentemente entre todas las escalas espaciales consideradas. La variabilidad más baja la encontramos entre localidades (100 Km) mientras que la más elevada la encontramos entre lugares (1-5 Km). A pesar de las diferencias, los sites mostraron una elevada similaridad (similaridad promedio 55.7%) i comparten aproximadamente el 50% de las especies. De manera similar, encontramos diferencias en los índices de diversidad (alfa, beta y gamma). Además, en el estudio de *P. clavata* también determinamos los patrones de diversidad en un periodo de 5 años. En el conjunto de la escala regional, no se detectaron cambios destacables en la composición de especies así como en la composición de los grupos morfo funcionales considerados.

En el **Capítulo 6**, demostramos el efecto de una tormenta extrema en la dinámica de los afloramientos bentónicos del coralígeno en el mar Mediterráneo NO utilizando datos adquiridos antes (2006-2008) y después del impacto (2009-2010) en 4 lugares diferentes. La pérdida de cobertura de especies bentónicas resultó ser de entre el 22% y el 58%. El daño causado entre las especies (ej. algas calcáreas, esponjas, antozoos, briozoos y tunicados) fue desigual, y las que resultaron más impactadas fueron las formas frágiles, mostrando pérdidas de cobertura de entre el 50% y el 100%. También son destacables las "manchas" que sobrevivieron después de la tormenta y empezaron a crecer ligeramente durante el siguiente año. En contraste, los lugares más resguardados no mostraron cambios en ninguno de los parámetros estudiados, indicando que la tormenta no provocó variaciones.

En conjunto, los resultados presentados en esta tesis suponen los fundamentos básicos para la implementación de esquemas de monitoreo de las comunidades de coralígeno complementando así la escasez de información disponible sobre la composición y estructura de los emblemáticos afloramientos de coralígeno en el Mediterráneo dominados por *C. rubrum* y *P. clavata* en escalas espaciales grandes. Los resultados en los patrones de biodiversidad en el Mediterráneo NO, demuestran una variabilidad espacial moderada que contrasta con una variabilidad extremadamente baja en el tiempo y suponen los primeros datos de referencia para detectar potenciales cambios debido a los efectos del cambio global.

También damos nuevas comprensiones sobre las respuestas de estas comunidades a grandes y poco frecuentes eventos perturbadores. La implementación de esquemas de monitoreo similares en otras regiones del Mediterráneo proporcionaran una visión global de la biodiversidad de los afloramientos de coralígeno.

1. Introduction

1.1. The Mediterranean Sea and its biodiversity loss.

General oceanographic features, biological importance and biogeographical patterns.

The Mediterranean Sea is the largest semi-enclosed sea on earth. Regarding oceanographic settings, it is an oligotrophic and seasonal sea, with a high physiographic complexity, which is in accordance with its high species richness and habitat diversity (Ros et al. 1985). It has a marked longitudinal gradient (about 30 degrees, from 5º W to 34º E) encompassing a strong nutrient gradient form Western to the Eastern, the named Eutrophia-Oligotrophia axis (Ros et al. 1985).

The Mediterranean Sea is considered a marine biodiversity hotspot, harboring approximately 10% of world's marine species and high endemism while occupying only 0.82% of the ocean surface (Bianchi and Morri 2000; Boudouresque 2004; Coll et al. 2010). The importance of the Mediterranean Sea for the human being is crucial from ancient ages: it has been the physical environment where many different cultures shared space and it is highly used for transportation by maritime navigation and recreational activities. Furthermore, it provides key and vital ecosystem services such as food provision, nutrient cycling and the modulation of climate (Micheli et al. 2013). From a biogeographic perspective, the Mediterranean Sea can be conceived as a nested system that belongs to the Temperate Northern Atlantic Realm (representing a Province) (Spalding et al. 2007). Among the 14 biogeographic units sectors (Fig. 1.1.) the northwestern Mediterranean Sea is especially biologically diverse because of the presence of both temperate and subtropical species, and also because of its complex geological history, a legacy from the Tethys Ocean (Bianchi and Morri 2000).

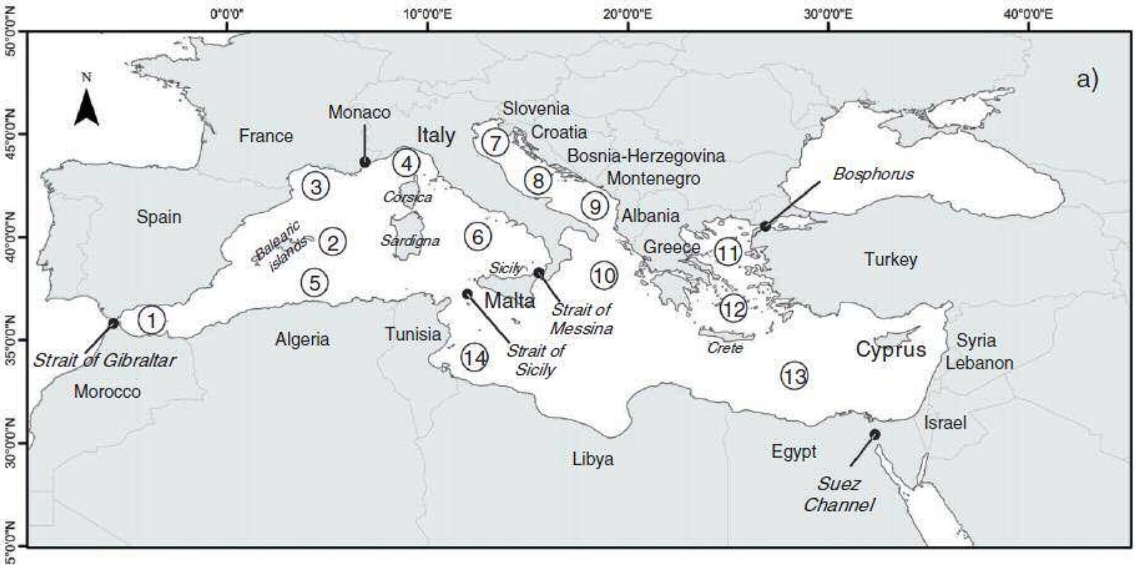


Figure 1.1. Outlined boundaries of the biogeographic sectors in the Mediterranean. 1: Alboran Sea; 2: Balearic Sea; 3: Gulf of Lions; 4: Ligurian Sea; 5: Algeria and Tunisian waters; 6: Tyrrhenian Sea; 7: North Adriatic Sea; 8: Central Adriatic Sea; 9: South Adriatic Sea; 10: Ionian Sea; 11: North Aegean Sea; 12: South Aegean Sea; 13: Levant Sea; 14: Gulf of Gabés (from Coll et al 2012).

1.2 Impacts and threats on biodiversity

Human activities have been increasing forcing the alteration, modification and change of the structure, biodiversity and functioning of marine assemblages (Halpern et al. 2008; Dornelas et al. 2014). Review studies pointed out a slow ocean defaunation and a rapid decline of native species diversity (Worm et al. 2006; McCauley et al. 2015). Nowadays, it is difficult to find any marine ecosystem and its assemblages not affected by direct or indirect human stressors, and even remote pristine areas or the deep-sea ecosystems are affected by global anthropogenic change (Sandin et al. 2008; Halpern et al. 2008; Pham et al. 2014).

The Mediterranean Sea is threatened by an increase of disturbance regimes associated to global change (Fig. 1.2) and among the main anthropogenic impacts are overfishing, water degradation and pollution, global warming, habitat destruction (e.g. construction of harbors, fishing nets) and invasive species (Bianchi and Morri 2000; Claudet and Fraschetti 2010; Coll et al. 2012; Micheli et al. 2013). The most impacted areas correspond to Gulf of Gabés, North Adriatic and Levant Sea whereas the less impacted are the Balearic Sea and the East sector of the Tyrrhenian Sea (Coll et al 2012, Micheli et al 2013).

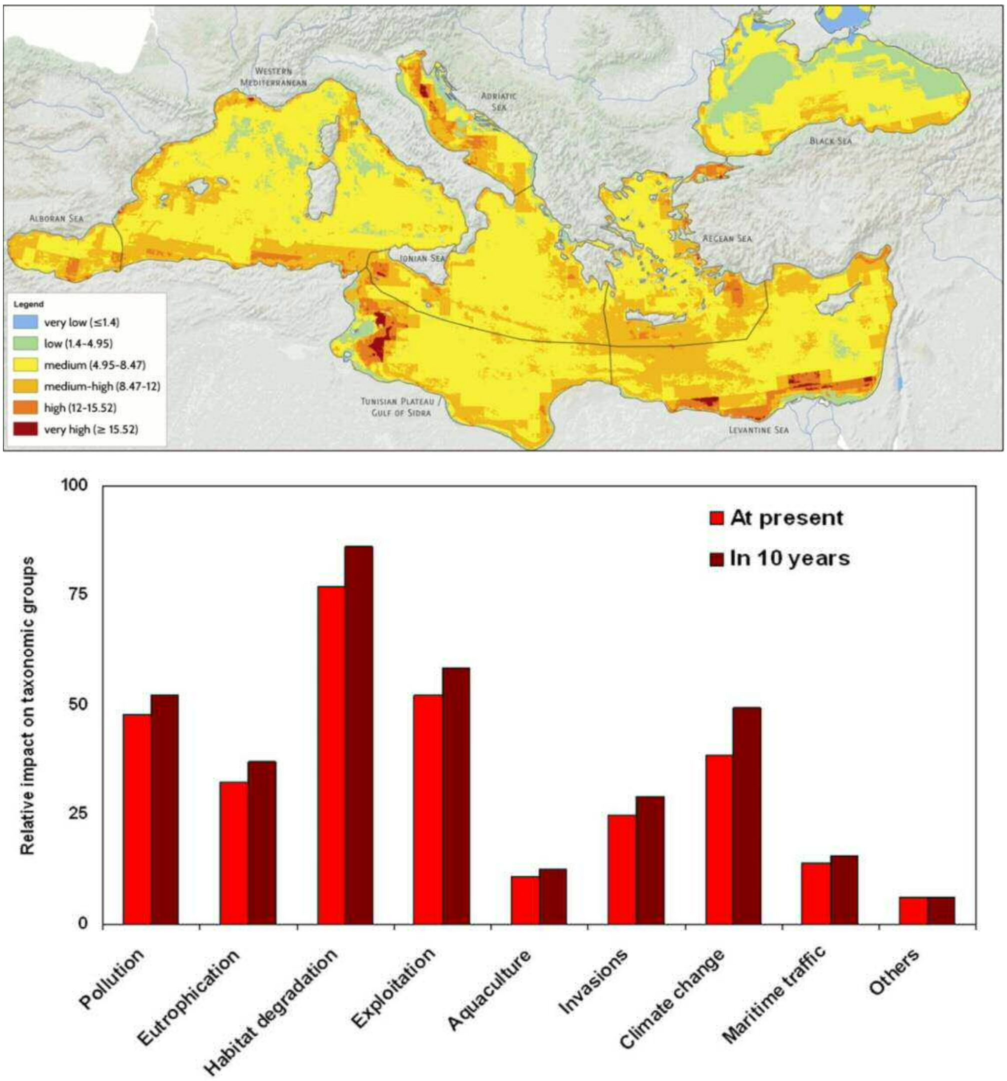


Figure 1.2. (a) Cumulative impact across global marine Mediterranean ecosystems (b) and impact agents responsible of Mediterranean degradation (from Coll et al. 2010 and Micheli et al. 2013).

1.3. Coralligenous outcrops

Composition, structure and distribution

Within the Mediterranean marine benthic subtidal assemblages, coralligenous outcrops are one of the most important, mainly because its high biodiversity, structural and functional complexity (Fig. 1.3.). Ballesteros (2006) defined coralligenous outcrops as hard substratum formations of biogenic origin that are mainly produced by the accumulation of calcareous encrusting algae growing at low irradiance levels and in relatively calm waters. Rather than a single and uniform habitat, coralligenous biogenic formations conforms a complex of different habitats whose occurrence is mainly determined by light exposure. It is largely well-known that variation in light intensity is determined by two spatial factors as depth and surface geographical orientation (Ballesteros 2006). Consequently, coralligenous outcrops develop more extensive in circalittoral zones (deeper than 30 meters depth). However, they can be also found within the range of shallower waters at the infralittoral zone (between 10 to 30 meters depth) developing on shadow surfaces (vertical walls, overhangs, crevices and deep channels). This bioherm in fact, allows the development of several kinds of habitats (Laborel 1961; Laubier 1966), including those dominated by living algae (upper part of the concretions), suspension feeders (lower part of the concretions, walls cavities and overhangs), borers (inside cavities) and even soft bottom fauna (in the sediment deposited in cavities and holes) (Ballesteros 2006). Therefore, coralligenous outcrops can be considered as a submarine landscape rather than a single habitat. Building the bio-concretion is a long-term process and age estimates varied from 8000 to 600 years before present on bio-concretions developed from 10 to 60 meters depth (Sartoretto et al. 1996).

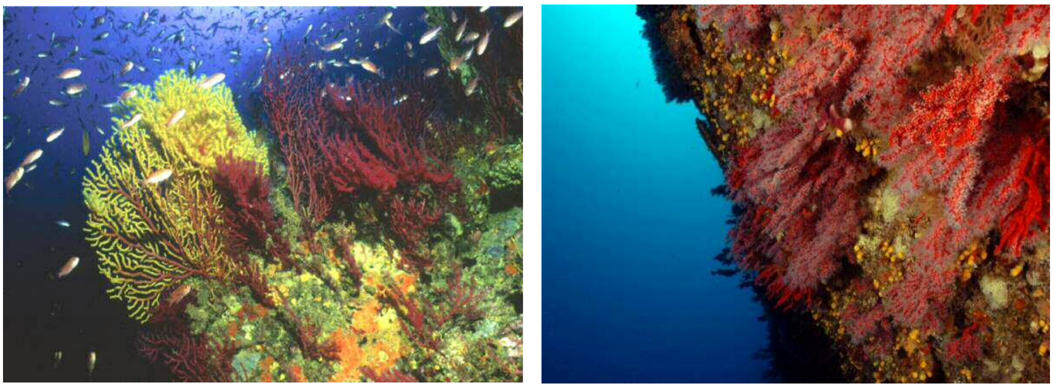


Figure 1.3. Coralligenous dominated by *Paramuricea clavata* and *Corallium rubrum* (Photos by E. Ballesteros and J. Garrabou).

Main ecological functions and ecosystem services

Ecosystem services are the benefits provided by ecosystems that contribute to make human life both possible and worth living (Díaz et al. 2006). Coralligenous outcrops are structurally complex and possess key ecological functions offering important ecosystems services.

Firstly, coralligenous outcrops presents high natural and evolutionary heritage for their biological, geological and physico-chemical features being at the same time highly vulnerable and threatened by human activities (UNEP/MAP-RAC/SPA 2014). Is a hot-spot of biological diversity and a conservative estimate of macro-species is 1666 species (315 algae, 1241 invertebrates and 110 fishes) (Ballesteros 2006). This number represent around 10% of global marine biodiversity (Coll et al. 2010) and its species richness and functional diversity is high. The main constructors and structural species of coralligenous outcrops are macro-benthic species, mainly perennial coralline algae and invertebrates (e.g. sponges, anthozoans, polychaetes, bryozoans and tunicates). Most of these species are long-lived (between 50 and 100 years), show slow growth and overall low population dynamics. Moreover, coralligenous outcrops have an important role in the carbon cycle (Piazzi et al. 2012) because organisms inhabiting within it contribute to carbonate fixation being a habitat with a net carbonate production activity. This C production is variable as a function of the environmental conditions ranging between 170 g $CaCO_3$ $m^{-2}yr^{-1}$ in deep and oligotrophic areas and up to 600 g $CaCO_3$ $m\text{-}^{2}yr^{-1}$ on highly productive and shallow regions (Ballesteros 2006). However, $CaCO_3$ production can reach up to 1000 g $CaCO_3$ $m^{-2}yr^{-1}$ at highly upwelling circalittoral waters at the Alboran Sea (Cebrian et al. 2000). Furthermore, this frameworks conforms the biogenic substratum acting as a nursery grounds for juveniles and habitat for a great number of benthic species that are economically important due the high biomass of highly appreciated commercial molluscs, crustaceans and fishes (e.g. *Ostrea edulis*, *Palinurus elephas* and *Epinephelus marginatus*).

Secondly, coralligenous outcrops are considered to have an important role within the littoral system regarding nutrient cycles (nitrogen, phosforous and silicon), water depuration and benthic-pelagic coupling (Coma et al. 1998; Maldonado et al. 2012). This is because they are mainly composed by photosynthetic (algae) and active and passive suspension-feeders invertebrates (sponges, cnidarians, bryozoans and compound ascidians). Active and passive suspension-feeders have an active role modulating seston composition and hence have an important role in pelagic-benthic coupling in the littoral ecosystem (Rossi and Gili 2009).

Other important ecosystem service is that they provide natural (pharmacological) products. Coralligenous outcrops are composed by many invertebrate taxons (e.g. molluscs, sponges, bryozoans, compound ascidians) that possess natural molecules chemically bioactive. Those molecules are used by the organisms as chemical defenses against competence and predation and have contrasted applicability in pharmacology (Turon et al. 2009; Pelay-Gimeno et al. 2013). Its potential use in pharmacology is due to its antiviral, atibiotic, analgesic and antimitotic/anticancerigen bioactivities. For instance, Cystodytes spp. (commonly found in coralligenous outcrops) has the mentioned bioactive properties (López-Gentil 2005).

Finally, coralligenous outcrops are of special concern for recreational diving. For instance, coralligenous in Medes Islands generates a total amount of 10.M € per year (Capellà 2012). This amount represents the economic expenses that visitors divers invest in their free and holidays time.

Coralligenous habitats as special concern for conservation and coastal ecosystem management

Increasing anthropogenic pressures and their consequences on water quality decline have led the European Union to engage a new strategy to conserve and recover the ecological quality of the marine environment. Since 2000, at least three different operational EU Directives: the Water Framework Directive (WFD), the Marine Strategy Framework Directive (MSFD), and the Maritime Spatial Planning Directive (MSPD) are markedly oriented to assess the water quality, good the environmental status of European marine ecosystems and sustainable use plans. The principal aim of the MSFD is to protect more effectively the marine environment across Europe achieving Good Environmental Status (GES) of the EU's marine ecosystems by 2020 and to protect the resource base upon which marine-related economic and social activities depend. The Barcelona Convention (1976) represented the agreement of 16 Mediterranean countries devoted to the protection and conservation of endangered species in the Mediterranean Sea. Although not legally binding, the Barcelona Convention's 'Action plan adopted in 2008 for the conservation of coralligenous outcrops and other calcareous bio-concretions in the Mediterranean Sea' asserts that ''coralligenous/maërl assemblages should be granted legal protection at the same level as *Posidonia oceanica* meadows' (UNEP/MAP-RAC/SPA 2008). Despite the great contribution of coralligenous communities to Mediterranean biodiversity (Ballesteros 2006) and its recognition as a natural habitat of communitarian interest, whose conservation requires the designation of Zones of Special Conservation at European level (92/43/CE Habitat Directive), at the present date, coralligenous outcrops are still defined as with the generic name "Reef habitats" (habitat code 1170-14).

Impacts and vulnerability of coralligenous outcrops

Coralligenous assemblages are highly threatened because they have already been impacted by an array of global change anthropogenic stressors such as nutrient enrichment (e.g. waste waters without nutrient-tertiary treatment, or even without any mechanical and microbiological treatment), overexploitation (e.g. fishing activities and harvesting the red coral), invasive species (e.g. by covering the seascape by permanent carpets of introduced algae turf, *Womersleyella setacea* or *Caulerpa racemosa*), increase of sedimentation and habitat destruction (e.g. construction of harbours or urbanization in general), mechanical impacts (anchoring, fishing nets and diver frequentation), acidification as well as climate change (Garrabou et al. 1998b; Balata et al. 2007; Coma et al. 2009; Garrabou et al. 2009; Linares et al. 2010; Cebrian et al. 2012; Piazzi et al. 2012; Cerrano et al. 2013; Teixidó et al. 2013b; Cecchi et al. 2014). Among all habitats that can be ascribed to coralligenous outcrops, those dominated by the emblematic Alcyonacea species *Corallium rubrum* (Linnaeus, 1758) and Paramuricea clavata (Risso, 1826) are of special interest from a conservation perspective. The main reason is because populations of both habitat-structural species are highly threatened by poaching or harvesting as well as by the drastic effects of several mortality events putatively related with unusual long-periods of high water temperature and the persistence of established invasive species (Garrabou et al. 2001; Garrabou et al. 2009; Teixidó et al. 2013b; Di Camillo and Cerrano 2015; Montero-Serra et al. 2015). All of this evidence that the loss of biodiversity and impacts have been largely studied and tested at population level (demographically and genetically) on emblematic and coralligenous-habitat-forming species as *Corallium rubrum* and *Paramuricea clavata* (Linares 2006; Coma et al. 2009; Garrabou et al. 2009; Arizmendi-Mejía et al. 2015; Montero-Serra et al. 2015). Hence, we focused this thesis on the study of these particular habitats as a whole, at the community level.

From a conservation perspective, large-scale and long-term biodiversity datasets are basic resource that furnishes the essential tools to provide information to promote sound conservation actions (Magurran et al. 2010). Hence, since the first qualitative and quantitative coralligenous species-list in the Mediterranean (Rossi 1961; Laubier 1966; True 1970; Hong 1982; Ros et al. 1985) and even followed by an increasing number of scientific publications during the last three decades, almost no studies have been conducted at regional spatial-scales and over time. Baseline data are very important to track changes and impacts on community structure. However there is a lack of high resolution of biodiversity data at regional and mid-term temporal scales, thereby hindering a proper assessment. Only with well-established and long-term monitoring programmes, we can take action detecting declining community trajectories and applying restoration measures.

Morphofunctional groups

Long-term monitoring studies of classical high resolution biodiversity data face the difficulty of taxonomic identification. The correct identification of species is a task that requires great taxonomic expertise and involves several problematic aspects, which have become increasingly evident in recent years (Rae et al 2013). Species-level identification for monitoring purposes is an expensive and time-consuming task (Fiori et al 2002). In general, taxonomic expertise on marine benthic species is increasingly in shortage,and the number of scientists able to identify correctly them is decreasing (Balata et al 2011). The necessity of taxonomists and the long-time needed to analyze samples make it difficult to process the high number of replicates required for ecological studies and monitoring surveys (Balata et al 2011; Chapman et al. 1995; Underwood and Chapman 1996; Benedetti-Cecchi et al. 2001). Therefore, the traditional taxonomic approach is today being supplemented by ecological classifications following the concepts of functional groups (FG), morpho-functional groups (MFG), and morphology-based functional groups (MBFG) (Mihaljević et al 2014). In marine ecosystems, morphological–functional groups are widely used to describe benthic assemblages (Vanderklift and Lavery 2000; Konar and Iken 2009). In a same MFG, morpho-functionally similar species are assembled together and are expected to represent a more or less defined functional trait, and thus MFG might be good indicators of ecosystems functioning and of physiological responses to global change (Balata et al. 2011). In this line, several studies have shown that trait-based approaches, which are based on the life traits of species provide general and more predictable rules for community ecology as well as a more mechanistic comprehension of biodiversity effects on ecosystem functioning and process, particularly in the context of global change (McGill et al. 2003, Mouillot et al. 2013).

Bearing in mind the difficutly of taxonomic identification of benthic species dwelling in coralligenous outcrops and the complementary information that MFG may provide, we seek to test the suitability of MFG of benthic organisms for monitoring coralligenous outcrops and to detect spacial and temporal patterns. Coralligenous outcrops configure complex habitat structures helding a variety of different morphological growth-forms and functional groups with a multilayered disposition (Garrabou et al 2002). Thus, coralligenous species can exhibit different growth forms, such as encrusting, cup, tree, massive and turf forms, which present different ecological strategies in occupying space on rocky benthic habitats and thus have been used as morphofunctional categories (Teixidó et al 2011). However, grouping organisms into categories such as morphological groups may represent an artifact, which does not reflect phylogenetic relationships and is affected by a
certain extent of subjectivity (Balata et al 2011). In the Mediterranean Sea, morpho-functional groups have been used to describe algal assemblages with results that, although interesting, remain doubtful (Mazzella et al. 1989; Sala and Boudouresque 1997; Benedetti-Cecchi et al. 2001; Piazzi et al. 2003, 2004a, b). Thus here we test the usefulness of morphofunctional groups in describing coralligenous patterns compared with the classical biodiversity studies.

2. Objectives and thesis structure

The **general objective** of this thesis is to characterize the biodiversity of sessile macrobenthic species of coralligenous outcrops across large spatial and over mid-to long-term temporal scales. The lack of a robust baseline of biodiversity structure and dynamics in coralligenous outcrops is hindering the assessment of the impacts associated with global change. The purpose of this thesis is to acquire community structure baseline data to furnish a solid basis for comparisons in terms of potential changes in species composition in response to multiple threats. To achieve this general aim, we performed photographic surveys across large spatial (hundreds of kilometers) and over mid- term temporal scales (5 to 10 years) and analyzed the corresponding images. The objectives are developed in the following four chapters, each corresponding to an independent scientific publication. The specific objectives are:

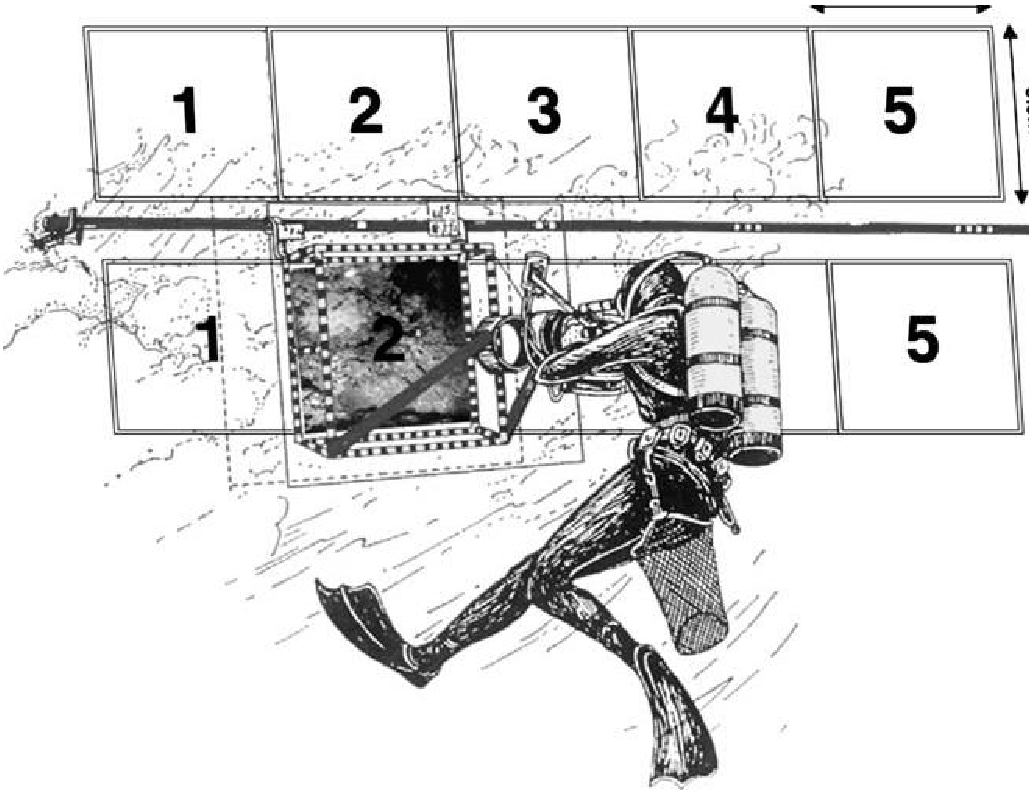
Objective 1. Evaluate the applicability of a rapid biodiversity assessment and monitoring method of coralligenous outcrops dominated by *Paramuricea clavata* and *Corallium rubrum* at regional scale (hundreds of kilometers). To reach this objective we proceeded to: *i*) determine the species composition at several hierarchical scales, *ii*) calculate the minimal sampling area for each site studied (by means of species-area relationships), *iii*) determine how many nested sites characterize each locality within the NW Mediterranean region (by means of multivariate statistics). This objective is successfully achieved in the publication of chapter 3: "*Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops*" published in PLoS ONE (impact factor in 2011= 4.092). This article represents the methodological approach that the other chapters are based.

Objective 2. Quantify patterns of the community structure, biodiversity and spatial configuration of *Corallium rubrum* dominated coralligenous outcrops over regional and decade time-scales by means of precise and high-resolution image analysis softwares. This objective is successfully achieved in the publication of chapter 4: "*Structure, biodiversity and landscape pattern indices of Corallium rubrum assemblages over broad spatial scales"* submitted to Scientific Reports (impact factor in 2014= 5.578).

Objective 3. Quantify the structure and biodiversity patterns of *Paramuricea clavata* dominated coralligenous outcrops at regional scale over 5-year period. We assessed structural complexity in terms of the specific and morphofunctional composition of the sessile perennial species. Additionally, we assessed various components of species diversity (alpha, beta, and gamma diversity) over time. This objective is successfully achieved in the publication of chapter 5: "*Structure and biodiversity of coralligenous outcrops over broad spatial scales*" published in Marine Bilogy (impact factor in 2014= 2.391).

Objective 4. Assess the effects derived from a dramatic storm occurred in the Catalan coast in 2008 by comparing changes in benthic community composition before (2006-2008) and after the storm impact (2009-2010). Storms of comparable severity have been documented to occur occasionally within periods of 50 years in the Mediterranean Sea. This objective is successfully achieved in the publication of chapter 6: "*Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm*" published in PLoS ONE (impact factor in 2013= 3.534).

3. Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops



Drawn by Beuchel and Gulliksen (2008) Temporal patterns of benthic community development in an Arctic fjord (Kongsfjorden, Svalbard): results of a 24-year manipulation study. Polar Biology 31:913-924

Kipson S, Fourt M, Teixidó N, Cebrian E, **Casas-Güell E**, Ballesteros E, Zabala M, Garrabou J (2011) Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops. **PLoS ONE** 6(11):e27103. doi:10.1371/jour- nal.pone.0027103.

Abstract

Increasing anthropogenic pressures urge enhanced knowledge and understanding of the current state of marine biodiversity. This baseline information is pivotal to explore present trends, detect future modifications and propose adequate management actions for marine ecosystems. Coralligenous outcrops are a highly diverse and structurally complex deep-water habitat faced with major threats in the Mediterranean Sea. Despite its ecological, aesthetic and economic value, coralligenous biodiversity patterns are still poorly understood. There is currently no single sampling method that has been demonstrated to be sufficiently representative to ensure adequate community assessment and monitoring in this habitat. Therefore, we propose a rapid non-destructive protocol for biodiversity assessment and monitoring of coralligenous outcrops providing good estimates of its structure and species composition, based on photographic sampling and the determination of presence/absence of macrobenthic species. We used an extensive photographic survey, covering several spatial scales (100s of m to 100s of km) within the NW Mediterranean and including 2 different coralligenous assemblages: *Paramuricea clavata* (PCA) and *Corallium rubrum* assemblage (CRA). This approach allowed us to determine the minimal sampling area for each assemblage (5000 cm^2 for PCA and 2500 cm^2 for CRA). In addition, we conclude that 3 replicates provide an optimal sampling effort in order to maximize the species number and to assess the main biodiversity patterns of studied assemblages in variability studies requiring replicates. We contend that the proposed sampling approach provides a valuable tool for management and conservation planning, monitoring and research programs focused on coralligenous outcrops, potentially also applicable in other benthic ecosystems.

3.1. Introduction

Coastal ecosystems are among the most diverse, highly productive and complex biological systems (Agardy et al. 2005). At the same time, they are highly threatened by a combination of anthropogenic impacts, such as overfishing, habitat loss, eutrophication, introductions of exotic species and climate change (Halpern et al. 2008; Rockström et al. 2009), leading to profound structural and functional changes (Jackson et al. 2001; Walther et al. 2002). However, future shifts in the species composition of assemblages cannot be evaluated without knowledge and understanding of the present state of marine biodiversity. Obtaining this baseline information represents a key step in exploring future modifications of coastal ecosystems.

The Mediterranean Sea is considered a marine biodiversity hotspot, harboring approximately 10% of world's marine species while occupying only 0.82% of the ocean surface (Bianchi and Morri 2000; Coll et al. 2010). Unfortunately, the impacts of human activities are proportionally stronger in the Mediterranean than in the other seas, raising concerns regarding threats to the conservation of the rich Mediterranean biodiversity (Coll et al. 2010). Coralligenous outcrops, which are hard bottoms of biogenic origin that thrive under dim light conditions, are among the habitats faced with major threats in the Mediterranean Sea. These outcrops are highly diverse (harboring approximately 20% of Mediterranean species) and exhibit great structural complexity (Gili and Coma 1998; Ballesteros 2006). The species that dominate coralligenous seascapes are encrusting calcareous algae, sponges, cnidarians, bryozoans and tunicates. Some of the engineering species in these environments are long-lived, and their low dynamics make coralligenous outcrops exceptionally vulnerable when faced with sources of strong disturbances, such as destructive fishing practices, pollution, invasive species or mass mortality outbreaks (Coma et al. 2004; Ballesteros 2006; UNEP/MAP-RAC/SPA 2008; Garrabou et al. 2009).

The immediate consequences and long-lasting effects of these disturbances have mostly been addressed at the population level, focusing on certain structurally important species (e.g., Bavestrello et al. 1997; Garrabou et al. 2001; Linares et al. 2005; Giuliani et al. 2005; Cupido et al. 2009; Garrabou et al. 2009). Despite the ecological, aesthetic and economic value of coralligenous outcrops, coralligenous biodiversity patterns at the community level over regional scales remain poorly understood (Ballesteros 2006;

Casellato and Stefanon 2008 and references therein). This lack of information is partially due to the complexity involved in studying these highly diverse systems with slow dynamics, coupled with general logistical constraints related to sampling at deep rocky habitats. Most of the previous studies at the assemblage level have been largely descriptive (Laubier 1966; True 1970; Hong 1982; Gili and Ros 1985). There are a few quantitative studies available, restricted to small or medium spatial scales, but their results are not comparable due to the differences in sampling methodology (e.g., scraped samples vs. photographic sampling) (Ferdeghini et al. 2000; Acunto et al. 2001; Coma et al. 2004; Piazzi et al. 2004; Balata et al. 2005; Virgilio et al. 2006). Therefore, an accurate overview of the general biodiversity patterns associated with coralligenous outcrops is lacking.

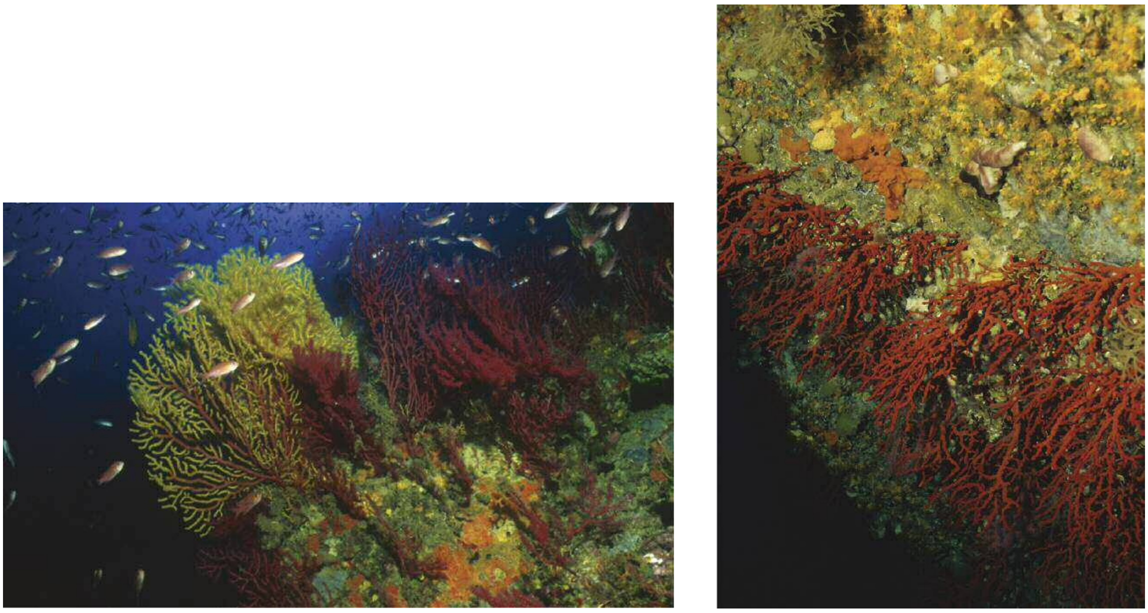


Figure 1. General aspect of 2 facies of the coralligenous outcrops considered in this study. (A) *Paramuricea clavata* dominated assemblage and (B) *Corallium rubrum* dominated assemblage (Photos by E. Ballesteros).

Ecologists, conservation practitioners, managers and policy makers highlight the need to develop cost-effective sampling methods to provide comparative measures of biodiversity and to create a platform of ‘‘biodiversity baselines’’. There is currently no single sampling method that has been demonstrated to be sufficiently representative to provide adequate community assessment and monitoring in coralligenous outcrops (Bianchi et al. 2004). To ensure the representativeness and time - and cost - efficiency of any benthic community survey, aiming to capture the original community structure and to account for its natural variability, an adequate sampling unit size and sampling effort (i.e. the number of replicates) should be determined (Kronberg 1987; Muxika et al. 2007). Therefore, when the goal is to assess the complexity of the system, a good representation of the species pool should be achieved and therefore the minimal sampling area for the assemblage should be defined, i.e. the sampling unit size over which an increase of area does not yield a significant increase in the number of species (Braun-Blanquet 1932; Cain 1938; Niell 1977). Both the sampling unit size and sampling effort will influence the representativeness of a sample data set in terms of accuracy (the ability to determine the true value) and precision (the ability to detect differences) of the estimates (Bianchi et al. 2004). While accuracy and precision generally increase with sampling effort (Bianchi et al. 2004), the high small-scale heterogeneity of coralligenous habitats additionally implies that large sampling areas are required to achieve representative results (Ballesteros 2006). However, optimization of the sampling strategy is indispensable given the considerable depths where coralligenous outcrops usually develop and the limited information that can be obtained in the restricted diving time.

Taking into account the priorities and activities defined by the Action Plan for the Conservation of the Coralligenous (UNEP/MAP-RAC/SPA 2008), we aimed to provide guidelines for the application of a rapid, non-destructive protocol for biodiversity assessment and monitoring in coralligenous habitat. The sampling procedure used in this study was designed to assess the natural spatio-temporal variability of coralligenous outcrops, which is crucial information for a posteriori assessment of the impact of anthropogenic activities.

The aims of this study were three-fold: (1) to determine the minimal sampling area required to assess the sessile macrobenthic species composition in the studied assemblages, (2) to estimate the minimal sampling effort needed to obtain a good representation of the number of species and the complexity of the overall community and (3) to explore the capacity of the proposed approach to account for assemblage composition variability on different spatial scales and among different assemblages. The application of this approach to characterizing coralligenous outcrops and detecting future changes was also assessed.

3.2. Materials and Methods

Ethics Statement

Institut de Ciències del Mar (ICM-CSIC), Centre d'Océanologie de Marseille, University of Zagreb (Faculty of Science), Universitat de Girona (Facultat de Ciències), Centre d'Estudis Avançats de Blanes-CSIC and Universitat de Barcelona approved this study.

Communities studied and study areas

Coralligenous outcrops comprise a complex of assemblages ranging from algal dominated ones to others completely dominated by macroinvertebrates with almost no algal growth (Ballesteros 2006). Here we selected two assemblages that are dominated by the long-lived gorgonians Paramuricea clavata (Risso 1826) and Corallium rubrum (L. 1758) (Fig. 1) and that displayed the same aspect at all studied sites, always thriving under dim light conditions. The P. clavata assemblage (hereafter PCA) was sampled on rocky walls at depths ranging from 17 to 24 m, whereas the C. rubrum assemblage (hereafter CRA) was sampled on overhangs and cave entrances at depths between 14 and 20 m. Further, we consider these assemblages among the most complex ones within the coralligenous outcrops, enabling us to develop a representative sampling method that would perform well in less complex coralligenous assemblages.

We studied a total of 15 sites (8 sites for PCA and 7 sites for CRA) located in three regions: northern Catalonia, Provence and Corsica, covering more than 400 km of the coastline (Fig. 2). Two to three sites per region and assemblage were sampled (sites within regions were separated by hundreds of meters to a few kilometers). The selected regions encompass a high temperature productivity gradient in the NW Mediterranean. Provence is characterized by cold, relatively eutrophic waters maintained by local upwellings. Northern Catalonia is characterized by waters largely influenced by river discharges (Flos 1985; Bensoussan et al. 2010), whereas Corsica is characterized by warmer and more oligotrophic waters (Bensoussan et al. 2010). Therefore, each region presents particular environmental conditions, thus providing a good dataset for testing the potential of the biodiversity assessment method for detecting natural inter-regional variability. In fact, along this gradient, shifts in the zonation patterns have been reported with coralligenous assemblages developing at shallower depths in the cold-eutrophic areas than in the warm-oligotrophic ones (Zabala and Ballesteros 1989). The observed depth of the coralligenous outcrops ranges from 10 to 50–55 m in Provence (Marseille area) and Catalonia (Medes Islands) (Laborel 1961; Hong 1980; Gili and Ros 1985) while in Corsica it ranges from 20 to 80 m (Laborel 1961).

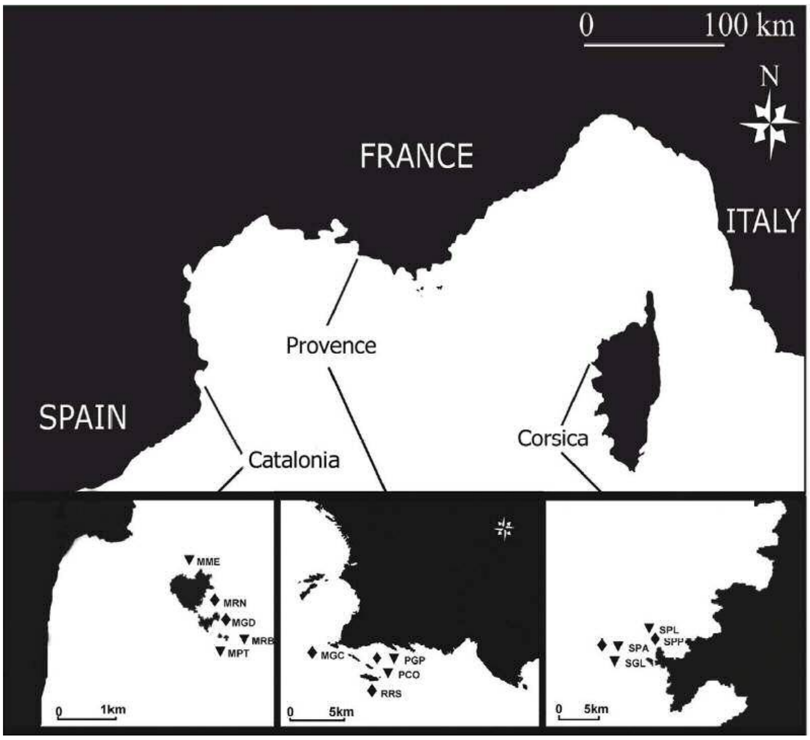


Figure 2. Map of the study area in the NW Mediterranean Sea. Three studied regions in the NW Mediterranean and sites within them (triangles = sites with *Paramuricea clavata* assemblage and diamonds = sites with *Corallium rubrum* assemblage). See Table 1 for site abbreviations.

Photographic sampling

The proposed method for biodiversity assessment was based on analysis of the presence/absence of macro-species dwelling in the understory of the selected assemblages that were identified from photographs (see below). To facilitate identification of these species, we sampled the assemblages using quadrats of 25 × 25 cm for PCA and 20 × 20 cm for CRA. The photographs were taken with a Nikon D70S digital SLR camera fitted with a Nikkor 20 mm DX lens and housed in Subal D70S housing. Lighting was provided by two electronic strobes fitted with diffusers. Sampling was conducted during spring and summer of 2006 and 2007. A total of 475 and 486 photographs were analyzed for PCA and CRA, respectively.

Species identification

Using these photographs, species were identified to the lowest possible taxonomic level. When further clarification was needed, working with marked plots (see below) allowed us to precisely track down an organism in the field and collect a voucher specimen. Thus, a total of 208 specimens were collected for further identification in the laboratory. Visually similar taxa that could not be consistently identified from photographs were grouped as indicated in Table A.1.1. Furthermore, because the time of sampling differed for different sites, the species showing clear seasonality were excluded from the subsequent analysis (see Table A.1.1).

Determination of a sampling method for biodiversity assessment in coralligenous outcrops

To determine the sampling method to be used for biodiversity assessment in coralligenous outcrops, we established the minimal sampling area (hereafter MSA) and minimal sampling effort required to provide good estimates of the species number and composition for each studied assemblage.

a) Estimation of minimal sampling areas.

To estimate MSA, we analyzed the species-area relationship (Arrhenius 1921; Braun-Blanquet 1932; Cain 1938; Connor and Mccoy 1979), taking into account the spatial arrangement of species, to obtain a good representation of the species pool, as well as the structure of the community (Boudouresque 1971; Niell 1977).

Therefore, we applied a spatially explicit design based on contiguous sampling of quadrats arrayed to cover rectangular plots. At each site, we employed plots ranging from 3.2 to 4 m^2 for PCA and from 1.76 to 3.72 m^2 for CRA. The plots were marked with screws fixed to the rock by putty, and quadrats inside the plots were sequentially positioned and photographed. Overall, 51 to 64 quadrats were photographed per site for PCA, whereas 44 to 93 quadrats were photographed per site for CRA.

For further determination of MSA, we followed the method described by Ballesteros (Ballesteros 1986). A species-area curve for each plot was produced from the subset of all possible combinations of increasing numbers of the originally ordered contiguous quadrats.

Thus, mean values of species numbers for successively larger areas were obtained and plotted vs. their respective areas. The curve was fitted to a logarithmic function (Ballesteros 1986).

$$S = z \ln A + c$$

where S is the number of species, and A is the sampling area in cm^2. To evaluate the model’s performance, r2 was used as a standard goodness-of-fit measure. Based on this equation, the parameter k was calculated, which describes the shape of the curve and provides information on the qualitative distribution of species within the community (Ballesteros 1986; Martin et al. 1993):

$$k = e^{-c/z}$$

The higher the value of k, the larger the sampling area needed to obtain a representative number of species in the community due to their more dispersed distribution (Ballesteros 1986). In this study, the qualitative minimal sampling area was determined as the point at which an increase of the sampling area by 20% yields a 5% increment in species number (Molinier point M 20/5) using the following equation:

$$A = k * e^{[\ln(1+dA)=dS]}$$

where dA and d’S are the relative increments of the surface area and species number (expressed as percentages), respectively. Hence, the Molinier point chosen in this study can be expressed as M 20/5 = Amin = 38.3 * k (Ballesteros 1986).

Table 1. Logarithmic functions fitted (goodness of fit measure, r^2) to spatially explicit species-area curves based on the original order of contiguous samples. k parameter and minimal sampling areas (A_{min}) calculated for each study site of the *Paramuricea clavata* and *Corallium rubrum* assemblages in the 3 localities of the NW Mediterranean. Site names are provided with abbreviations.

Assemblage	Locality	Site	Function	r^2	k	A_{min}/cm^2
Paramuricea clavata	Catalonia	Medallot (MME)	$y=9.26\ln(x)-45.09$	0.99	131	4999
		Tascó Petit (MPT)	$y=6.84\ln(x)-27.16$	0.973	53	2029
		Carall Bernat (MRB)	$y=8.57\ln(x)-40.83$	0.988	117	4481
	Provence	Petit Conglué (PCO)	$y=9.29\ln(x)-49.27$	0.988	202	7718
		Plane-Grotte Pérès (PGP)	$y=10.66\ln(x)-55.2$	0.992	177	6787
	Corsica	Gargallu (SGL)	$y=8.68\ln(x)-41.59$	0.996	121	4622
		Palazzino (SPL)	$y=6.85\ln(x)-29.27$	0.999	80	3050
		Palazzu (SPA)	$y=9.04\ln(x)-43.57$	0.995	124	4755
Corallium rubrum	Catalonia	Cova de la Reina (MRN)	$y=9.19\ln(x)-43.47$	0.984	113	4336
		Cova del Dofí (MGD)	$y=5.46\ln(x)-21.33$	0.997	50	1899
	Provence	Riou-Grotte Sud (RRS)	$y=5.49\ln(x)-20.39$	0.987	41	1573
		Plane-Grotte Pérès (PGP)	$y=5.9\ln(x)-19.67$	0.969	28	1079
		Maïre Grotte (MGC)	$y=5.83\ln(x)-22.92$	0.999	51	1950
	Corsica	Palazzu (SPA)	$y=7.61\ln(x)-36.51$	0.922	121	4645
		Passe Palazzu (SPP)	$y=4.48\ln(x)-18.79$	0.978	66	2530

a) Estimation of sampling effort needed to maximize species number.

In communities with a patchy distribution of species, such as coralligenous assemblages (Ballesteros 2006), combining small separate areas will usually result in a higher species count than will be obtained for a contiguous area of the same size (Hawkins and Hartnoll 1980). Therefore, we also determined the minimal number of separate quadrats required to assess the maximum number of species present at each site (hereafter random quadrats). Consequently, we produced a second set of species-area curves based on 999 permutations, ignoring the spatial arrangement of these quadrats.

Finally, we also explored the increase in the number of species associated with increasing surface area when the MSAs determined for each assemblage were considered as sampling units (replicates).

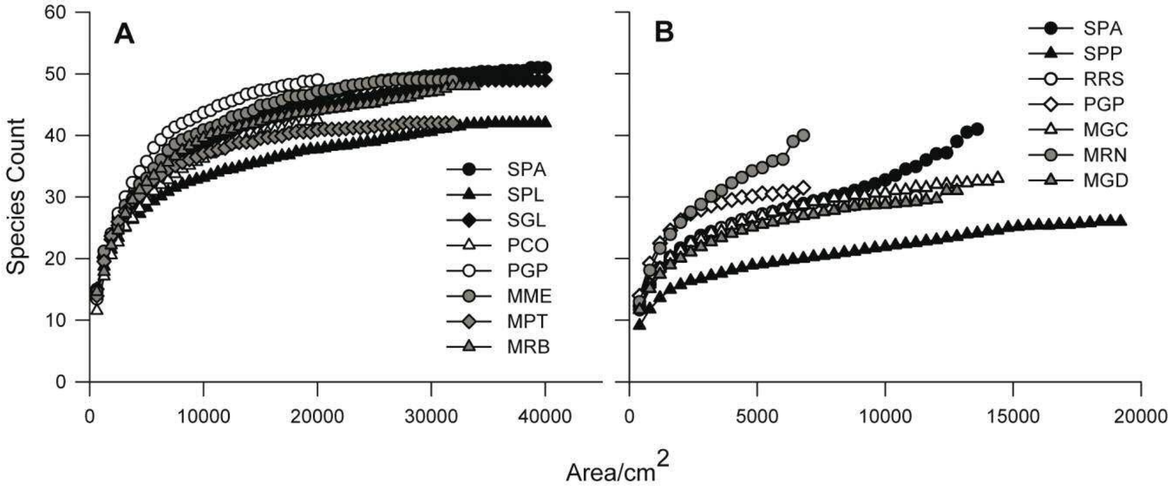


Figure 3. Spatially explicit species-area curves for each site within the 3 regions of the NW Mediterranean. (A) *Paramuricea clavata* assemblage and (B) *Corallium rubrum* assemblage (black = Corsica, white = Provence and gray = Catalonia). In a given area, each point represents multiple measures obtained from a subset of all possible combinations of increasing numbers of the originally ordered contiguous samples, with the curve based on the mean of those measures (SD not shown). See Table 1 for site abbreviations.

Tests for pattern assessment within the coralligenous outcrops

We applied multivariate analytical procedures to explore the suitability of the proposed methods for the detection of the variability of biodiversity within coralligenous outcrops on different spatial scales and among the two studied assemblages. More specifically, we explored whether the methods were able to cope with the intraregional variability (hundreds of meters to a few kilometers) and interregional variability (hundreds of kilometers) in the species composition of the two selected assemblages. Finally, we also explored the existence of differences between these assemblages.

Table 2. The local species number per unit area estimated through spatially non-explicit species-area curves (Fig. 4) for each site within each locality. Total N: total number of species recorded at each site; Species: number of species observed by analyzing a different number of random quadrats (16, 24, 32) or a combination of contiguous quadrats (368=3 replicates of 8 contiguous quadrats); % Species: percentage of species observed in comparison to the total species number recorded. For random quadrats, calculations were based on 999 permutations of replicate samples, whereas for replicates of 8 contiguous quadrats, calculations were based on a subset of all potential replicate combinations (SD not shown).

				Species				**% Species**			
Assemblage	**Locality**	**Site**	**Total N**	**16**	**24**	**32**	**3*8**	**16**	**24**	**32**	**3*8**
Paramuricea clavata	Catalonia	Medallot (MME)	52	44	47	49	44	84	90	94	85
		Tascó petit (MPT)	44	40	42	43	40	91	95	97	91
		Carall Bernat (MRB)	50	43	46	48	44	86	92	95	88
	Provence	Petit Conglué (PCO)	52	41	45	47	41	79	87	91	79
		Plane-Grotte Pérès (PGP)	58	49	53	54	48	85	91	94	83
	Corsica	Gargallu (SGL)	52	41	45	48	40	80	87	92	77
		Palazzino (SPL)	45	36	38	40	36	80	84	90	80
		Palazzu (SPA)	56	45	49	51	45	81	88	91	80
Corallium rubrum	Catalonia	Cova de la Reina (MRN)	57	40	44	47	43	71	77	82	75
		Cova del Dofí (MGD)	37	28	30	31	31	75	81	85	84
	Provence	Riou-Grotte Sud (RRS)	42	33	37	39	36	80	88	92	86
		Plane-Grotte Pérès (PGP)	35	32	33	34	32	90	94	97	91
		Maïre-Grotte (MGC)	37	32	34	35	34	85	92	95	92
	Corsica	Passe Palazzu (SPP)	49	32	36	38	34	66	73	77	69
		Palazzu (SPA)	26	21	23	24	21	81	88	92	81

Because many statistical analyses (e.g., analysis of variance) use replicate measurements to account for the amount of variation, we decided to use the MSA values obtained in this study (8 contiguous quadrats, see Results and Table 3) as replicates. Therefore, prior to analysis, presence/absence data were expressed for combinations of 8 contiguous quadrats (= replicates, measuring 50 × 100 cm for PCA and 40 × 80 cm for CRA). The total number of replicates per site ranged from 5 to 10. To determine the minimum number of replicates needed to assess biodiversity patterns, we compared the outcomes of the analysis using the overall dataset (all replicates available per site) and those using 3, 4, 5 and 6 replicates. Similarly, we explored the potential effects on biodiversity patterns when smaller sampling unit sizes were used. For this purpose, we compared the results of a multivariate analysis based on a dataset using MSA values as replicates with those based on a dataset using single quadrats as replicates (25 × 25 cm for PCA and 20 × 20 cm for CRA).

Data treatment

A Bray-Curtis similarity (Bray and Curtis 1957) matrix was constructed on the basis of presence/absence data. Non-metric multidimensional scaling (MDS) ordination (Kruskal and Wish 1978) was performed to visualize patterns of community similarities.

Non-parametric analysis of variance PERMANOVA (Anderson 2001a) was used to test for spatial variability. We applied a hierarchical design with 2 factors: Region (3 levels), as a random factor, and Site (8 and 7 levels for PCA and CRA, respectively), as a random factor nested in Region. Tests of significance were based on 9999 permutations of residuals under a reduced model (Anderson 2001b; Anderson and Ter Braak 2003). One-way PERMANOVA was applied to test for differences in species composition between the two assemblages (fixed factor). The test of significance was based on 9999 unrestricted permutations of raw data. All computations were performed using the PRIMER v6 software program with the PERMANOVA+ add-on package (Clarke and Gorley 2006; Anderson et al. 2008).

3.3. Results

Categories identified

A total of 93 macrobenthic taxa were identified: 7 macroalgae, 1 protozoan, 39 sponges, 10 anthozoans, 1 hydrozoan, 5 polychaetes, 21 bryozoans and 9 tunicates (Table A.1.1.). Following appropriate grouping and elimination of seasonal taxa (see Methods), a total of 77 taxa were retained for further analysis. Of these, 75 taxa were recorded in PCA and 72 taxa in CRA. A total of 23 taxa were present in all regions within both communities, while 5 taxa were recorded exclusively within PCA and 2 taxa within CRA (Table A.1.1). Of all identified categories (including taxa and groups), approximately 70 could be identified solely from photographs (without samples taken), upon a certain training. However, in general, the identification ability depended on the quality of photographs examined as well as whether the organisms were present in a typical morphological form or not (e.g., for the bryozoan *Turbicellepora* sp.).

Determination of sampling method

Minimal sampling area (MSA)

Spatially explicit species area curves exhibited a fairly similar shape in the case of PCA, whereas they were more variable both in their shape and relative completeness in the case of CRA (Fig. 3). A good fit of the function to the data was indicated by r^2 values higher than 0.90 in all cases (Table 1).

The mean value for the qualitative minimal sampling areas was approximately 5000 cm^2 for PCA and half the size, 2500 cm^2, for CRA (Table 1). Bearing in mind the size of the quadrats used in this study (see methods), approximately 8 contiguous quadrats (corresponding to surfaces of 50 × 100 cm for PCA and 40 × 80 cm for CRA) should be used to reach the MSAs for both assemblages as a replicate for biodiversity assessment studies. Similar inter-site differences in MSAs were observed within each assemblage (Table 1). For PCA, the estimated area varied between 2000 and 8000 cm^2, with the sites from the Provence region showing the largest MSA (around 7000 cm^2). In the case of CRA, the values obtained were slightly lower, varying between1000 and 5000 cm^2 (Table 1).

Estimation of minimum sampling effort to maximize species number.

Through analysis of all quadrats considered in this study, we determined the total number of species found at each site. For PCA, the species number ranged between 44 and 58, whereas for CRA, the number ranged between 26 and 57 (Table 2). Analysis of the species-area curves performed with random quadrats indicated that sampling efforts covering total areas of approximately 10.000 cm^2 for PCA and 5000 cm^2 for CRA would detect approximately 80% of all macrobenthic species recorded at the study sites (Fig. 4 and Table 2), whereas doubling the analyzed surface yielded more than 90% of the recorded species (Table 2). Therefore, to obtain good estimates of species number, approximately 16 to 32 random quadrats should be analyzed. When MSAs were used as sampling units, analysis of only 3 replicates of 8 contiguous quadrats provided approximately 80% of the total species found at each site (Table 2).

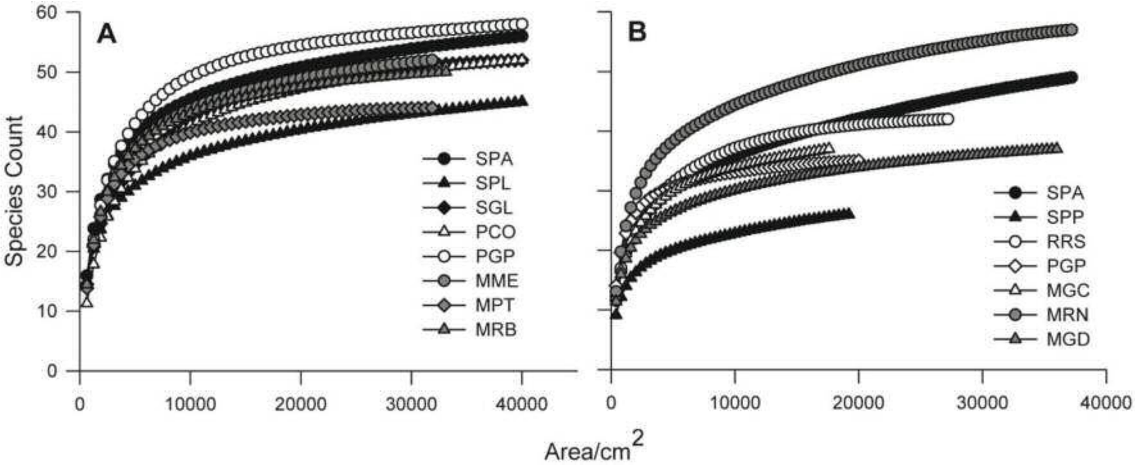


Figure 4. Spatially non-explicit species-area curves for each site within the 3 localities of the NW Mediterranean. (A) *Paramuricea clavata* assemblage and (B) *Corallium rubrum* assemblage (black = Corsica, white = Provence and gray = Catalonia). Data were based on 999 permutations of replicate samples (SD not shown). See Table 2 for site abbreviations.

Test for pattern assessment

Characterizing the regional variability of biodiversity patterns.

Disregarding the number of replicates used per site (3, 4, 5 or 6), the patterns revealed by MDS and PERMANOVA were similar to those obtained using datasets based on the maximum possible number of replicates per site (5–10). Here, only the results of the analyses based on datasets with 3 and the maximum possible number of replicates per site (5–10) are shown (Fig. 5A–5D). For both assemblages, MDS ordination revealed 3 distinct clusters, corresponding to different regions (Fig. 5A and 5B; Fig. 5C and 5D), whereas PERMANOVA indicated significant variability at spatial levels for both region and site (Table 3). In the case of PCA, the greatest variation occurred at the regional scale, followed by sites and, finally, individual quadrats, whereas in the case of CRA, the greatest variation was observed at the site level, followed by regions and individual quadrats (Table 3). Similar levels of significance and explained variability were found, independent of the number of replicates used (Table 3).

Likewise, the use of a different number of replicates did not change the outcome of comparisons of selected assemblages. In all cases, the MDS ordinations performed revealed two distinct clusters, clearly separating one assemblage from the other (Fig. 5E and 5F), while PERMANOVA indicated significant differences between them (Table 4).

Table 3. Summary of PERMANOVA analyses based on Bray-Curtis dissimilarity for macrobenthic taxa within the studied assemblages. The results were obtained from datasets based on different number of replicates of 8 contiguous quadrats and individual quadrats. VC = Variance Components; BC diss = Bray Curtis dissimilarity. P (perm) values: *<0.05. **<0.01. ***<0.001.

Sampling unit and effort	Source	A) *Paramuricea clavata* assemblage				B) *Corallium rubrum* assemblage			
		df	Pseudo-F	VC	BC diss%	df	Pseudo-F	VC	BC diss%
		A1)Sampling unit size 50 cm × 100 cm				B1)Sampling unit size 40 cm × 20 cm			
3 replicates	Locality	2	56.19**	669.28	25.87%	2	28.74*	408.70	20.22
	Site (Locality)	5	40.83***	287.16	16.95%	4	62.53***	418.79	20.46
	Residual	16		279.45	16.72%	14		239.15	15.47
	Total	23				20			
4 replicates	Locality	2	5.66**	625.67	25.01	2	2.52*	332	18.22
	Site (Locality)	5	4.91***	280.34	16.74	4	9.54***	447.64	21.16
	Residual	24		287.08	16.94	21		209.57	14.48
	Total	31				27			
5 replicates	Locality	2	6.17**	632.5	25.67	2	2.75**	363.79	19.07
	Site (Locality)	5	6.17**	308.11	16.74	4	9.36***	424.56	20.61
	Residual	32		271.23	16.47	28		253.97	15.94
	Total	39				34			
6 replicates	Locality	2	5.74**	632.5	25.15	2	2.64**	342.44	18.51
	Site (Locality)	5	8.32***	308.11	18.00	4	11.14***	434.3	20.84
	Residual	50		252.65	16.00	34		249.31	15.79
	Total	57				40			
All replicates	Locality	2	5.29**	607.82	25.00	2	2.33*	287.72	17.00
	Site (Locality)	5	9.94***	331.93	18.00	4	13.76***	440.91	21.00
	Residual	50		267.3	16.00	46		249.28	16.00
	Total	57				52			
		A2) sampling unit size 25 cm × 25 cm				B2)Sampling unit size 20 cm × 20 cm			
All quadrats	Locality	2	2.68**	529.53	23.00	2	2.52	396.64	20.00
	Site (Locality)	5	37.32***	791.64	28.00	4	39.37	548.78	23.00
	Residual	499		1367.3	37.00	34		932.88	31.00
	Total	506				40			

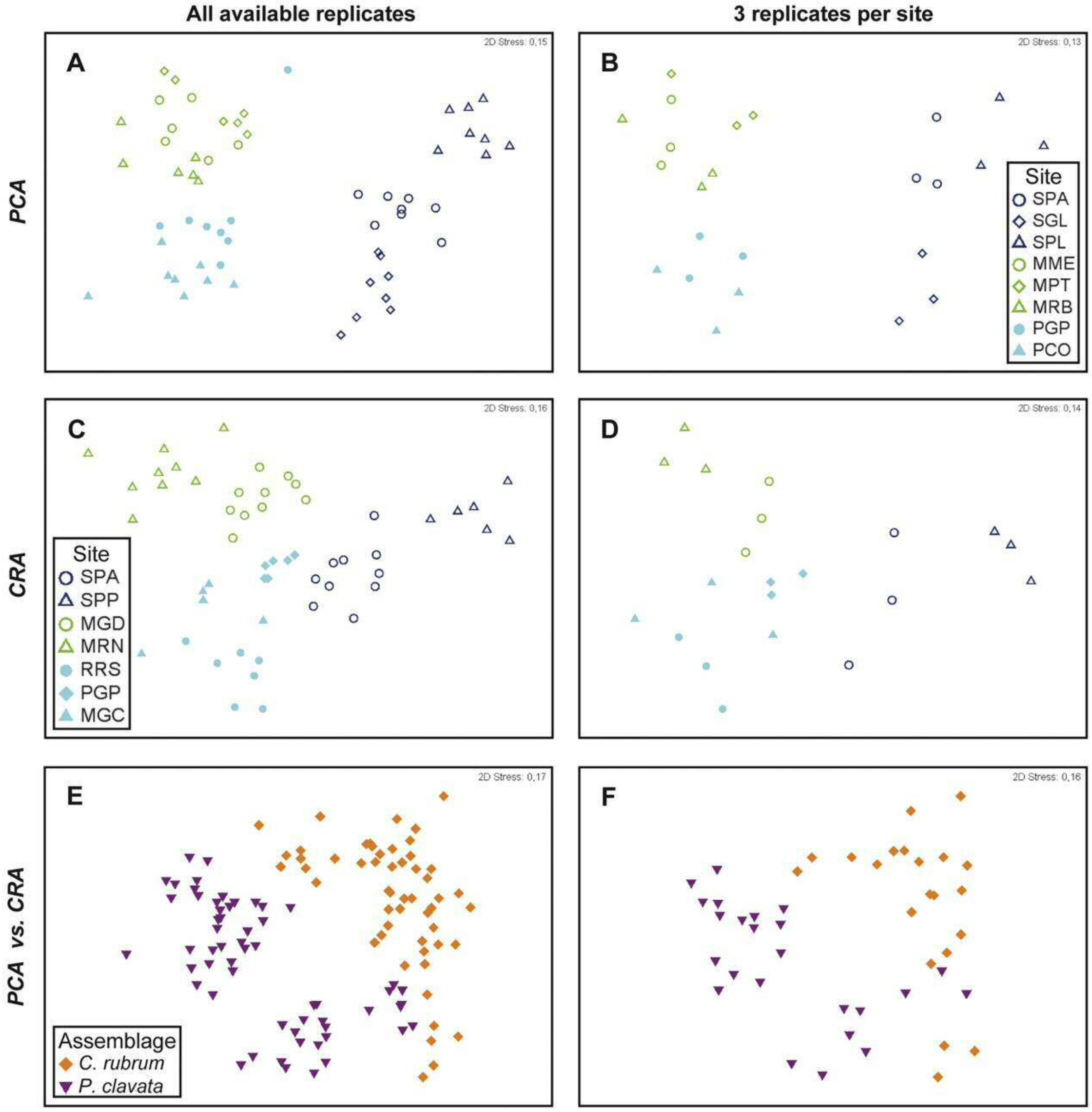


Figure 5. Non-metric multidimensional scaling (MDS) for all possible replicates and 3 replicates per site within the *Paramuricea clavata* (PCA) and *Corallium rubrum* (CRA) assemblages. Each replicate corresponds to 8 contiguous quadrats, creating a sampling unit of 50×100 cm for PCA and 40×80 cm for CRA. Three studied regions of the NW Mediterranean are depicted by colors (dark blue = Corsica, green = Catalonia and light blue = Provence). See Table 1 for site abbreviations.

Table 4. Summary of PERMANOVA analyses for the comparison of *Paramuricea clavata* (PCA) and *Corallium rubrum* (CRA) assemblages. The analyses were based on Bray-Curtis dissimilarity for macrobenthic taxa within the studied assemblages. The results were obtained from datasets based on different number of replicates of 8 contiguous quadrats and individual quadrats (25 × 25 cm for PCA and 20 × 20 cm for CRA). VC = Variance Components; BC diss = Bray Curtis dissimilarity; P (perm) values: *<0.05; **<0.01; ***<0.001.

Sampling unit and effort	Source	df	Pseudo-F	VC	BC diss (%)
3 replicates	Assemblage	1	14.03**	558.22	23.63
	Residual	43		959.82	30.98
	Total	44			
All replicates	Assemblage	1	35.58***	561.93	23.71
	Residual	109		899.97	30.00
	Total	110			
All quadrats	Assemblage	1	265.48**	1072.4	32.75
	Residual	959		2016.6	44.91
	Total	90			

b) Analyzing the effect of different sampling unit sizes on biodiversity pattern assessment.

The comparison of patterns using datasets based on individual quadrats (N = 475 for PCA and N= 486 for CRA) and 3 (or more) replicates of 8 contiguous quadrats revealed differences in the patterns and hierarchy of the spatial scales considered.

In the case of PCA, MDS ordination performed on the dataset based on individual quadrats revealed one distinct cluster corresponding to Corsica, whereas Catalonia and Provence overlapped (Fig. 5A). In the case of CRA, all clusters corresponding to different regions overlapped to a certain extent (Fig. 5B). In contrast, the MDS ordination performed on the dataset based on replicates of 8 contiguous quadrats clearly distinguished the regional clusters in both assemblages (Fig. 5A and 5C). While variability remained significant at both the region and site spatial levels, regardless of the dataset used, PERMANOVA revealed a different hierarchy of spatial scales depending on the sampling unit used. For both assemblages, in the case of datasets based on individual quadrats, the greatest component of variation was associated with the smallest spatial scale, i.e., individual quadrats (Table 4), whereas in the case of datasets based on replicates of 8 contiguous quadrats, the greatest component of variation was observed at larger spatial scales (regional level for PCA and site level for CRA). Finally, the use of smaller sampling units (individual quadrats) for comparison of selected assemblages revealed similar patterns to when larger sampling units (replicates of 8 contiguous quadrats) were used (Fig. 5C vs. Fig. 5E and 5F; Table 2), although the former method did not account for the particular structure of the assemblages because sampling unit size employed did not comply with the MSA.

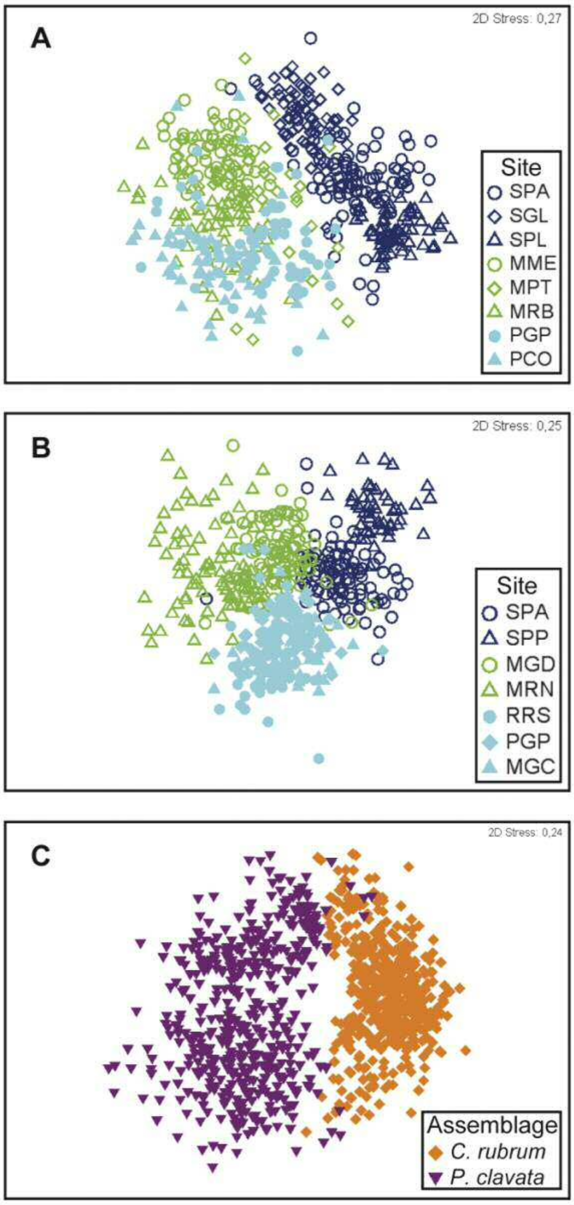


Figure 6. Non-metric multidimensional scaling (MDS) for the studied assemblages and their comparison. (A) *Paramuricea clavata* assemblage (sampling unit of 25 × 25 cm), (B) *Corallium rubrum* assemblage (sampling unit of 20 × 20 cm) and (C) comparison of *P. clavata* and *C. rubrum* assemblages in the 3 regions of the NW Mediterranean (dark blue = Corsica, green = Catalonia and light blue = Provence). See Table 2 for site abbreviations.

3.4. Discussion

Here, we propose, for the first time, a standardized biodiversity assessment method for coralligenous assemblages that provides good estimates of assemblage structure and species composition based on photographic sampling and determination of the presence/absence of macrobenthic species. We used an extensive photographic survey (almost 1000 photographs) covering several spatial scales (hundreds of meters to hundreds of kilometers) and including 2 different coralligenous assemblages (PCA and CRA), which allowed us to determine the MSA for each assemblage and optimize the sampling effort to assess biodiversity patterns and provide estimates of species number. Furthermore, we propose MSAs as unitary sampling units for variability studies requiring replicates. Three replicates measuring 5000 cm^2 for PCA and 2500 cm^2 for CRA were found to be sufficient to maximize the species number and to assess the main biodiversity patterns present (Tables 2 and 3). To ensure species identification and to facilitate the sampling procedures, we propose that photographs of smaller quadrats than the MSA arrayed to cover MSA surfaces should be obtained (e.g., 8 quadrats of 25 × 25 cm for PCA and 8 quadrats of 20 × 20 cm for CRA).

By combining a photographic survey and data acquired at the presence-absence level, the proposed method allows a large number of samples to be obtained during the limited diving time periods that are possible in deep water habitats (down to 50 m) (Bohnsack 1979; Parravicini et al. 2009) and thus, to cope with the high spatial heterogeneity of coralligenous assemblages, while greatly reducing image time processing, which is one of the main constrains of photosampling.

Recent studies comparing commonly used sampling methods in hard bottom communities also advocate the use of photo-quadrats attaining adequate sampling areas in change/impact studies or whenever a large number of replicates are needed (Leujak and Ormond 2007; Parravicini et al. 2009). Additionally, the proposed protocol enables obtaining permanent objective records of both qualitative and quantitative data that can be further analyzed. For instance, analysis of species presence/absence datasets allows identifying the determinant species for such assemblages (SIMPER analysis, Primer (Clarke 1993)), which can be further used to focus the quantitative (cover area) studies on these determinant species and thus optimize the image processing involved, alongside other methods that improve time efficiency in quantitative studies, such as recording frequencies instead of estimating cover (Parravicini et al. 2010) and/or applying an automated software (Teixidó et al. 2011). Likewise, analysis of species presence/absence datasets allows establishment of species area relationships (SARs), which have been recently proposed as indicators of community-level changes in biodiversity and may be useful in quantifying human impact (Tittensor et al. 2007).

One of the key aspects of the proposed method is the determination of MSAs as sampling units for the characterization of the coralligenous assemblages. To our knowledge, MSAs had only previously been estimated for studying cnidarian species dwelling in coralligenous assemblages (Weinberg 1978; Gili and Ballesteros 1991). Interestingly, both studies determined comparable values for areas required to reach at least 80% of species: approximately 5000 cm2 for PCA and 4000cm2 for CRA. In the present study, use of the MSA as a sampling unit was crucial for the assessment of biodiversity patterns. Comparison of the patterns obtained using MSA and smaller individual quadrats (used in the photo sampling) as replicates clearly showed a shift in the hierarchy of the estimates of variance components from large to small spatial scales. In general, the variation in the observed similarities among samples increases as the size of the sampling unit decreases (Nekola and White 1999). Thus, using sampling units smaller than the MSA may have resulted in increased stochastic variability in the species composition at the smallest spatial scale. Similar effects have been reported previously in different habitats (e.g., (Steinitz et al. 2006; Parravicini et al. 2009; Rocchini et al. 2010)). However, previous studies on coralligenous outcrops adopted sampling units ranging between 240 and 600 cm2 (e.g., (True 1970; Hong 1983; Ferdeghini et al. 2000; Acunto et al. 2001; Cocito et al. 2002; Piazzi et al. 2004; Balata et al. 2005; Virgilio et al. 2006; Piazzi et al. 2010), which were therefore much lower than MSA values, and found the highest variability at the replicate scale (e.g., (Ferdeghini et al. 2000; Acunto et al. 2001). Hence, we emphasize the necessity to determine MSAs and use them as sampling units in the assessment of biodiversity patterns within coralligenous (and other) assemblages.

Although coralligenous assemblages harbor a significant proportion of the biodiversity that exists in the Mediterranean Sea (Ballesteros 2006), little is known about the biodiversity patterns within them. Bearing in mind the current pressures on coralligenous habitats (Ballesteros 2006), methods are urgently needed to assess prevailing patterns, evaluate impacts to which they are subjected and provide baseline data to explore future trajectories of these high diversity assemblages. We contend that the adoption of the method proposed in this study could furnish the required data to address these timely issues. In our opinion, three main research domains could be easily addressed using this method in a reasonable time framework to facilitate the development of meaningful management and conservation plans for coralligenous assemblages.

First, the method displayed potential for the characterization of biodiversity patterns. Its application to the analysis of spatial patterns at different scales (1 to 103 km), including areas with differential environmental conditions and anthropogenic pressures, could help to establish conservation status baselines for coralligenous assemblages and, consequently, identify potential management actions needed for the recovery of areas with a low conservation status. Additionally, the method developed in this study could be used to address rarely surveyed deep coralligenous banks (extending from 60 down to 120 m, depending on the geographical position and local light conditions (Ballesteros 2006)), as ROVs (remotely operated vehicles) or research submersibles have the operational capability to collect high-resolution digital photographs that we contend are compatible with the proposed method. However, it has to be emphasized that the application of the proposed method for the assessment of deep coralligenous banks would be comparatively more difficult, since in our study scuba divers could manage to obtain the images even in coralligenous assemblages displaying high structural complexity (e.g. high density of vertical stratum) and/or developing on complex substrates such as overhangs or vaults. Obtaining the required sets of images with remote devices can be more challenging in deep coralligenous banks due to operational difficulties. Despite of this, we emphasize that the applicability of our approach is already suitable here by adapting the process of image acquisition. For instance, to ensure acquisition of spatially contiguous photographs of a standard size in these conditions of reduced operability at depth, individual still photographs could be obtained from a high resolution video transect. Besides, we strongly recommend to verify the actual number and size of replicates during the preliminary assessment, as the knowledge on the structure of deep coralligenous banks is very scarce. Finally, we believe that future technical advancements and improved operating abilities of ROVs/submersibles ensure the interest for developing biodiversity assessment methods based on the acquisition of images.

Second, the method could be applied to the evaluation of temporal changes in coralligenous assemblages, which would allow identification of impacts on the monitored assemblages. In this sense, it is crucial to establish temporal baselines to properly evaluate the significance of observed changes. Our results detected significant differences at the intra-regional scale, indicating that a reliable assessment of temporal trends should be carried out at the site level.

Finally, the proposed method proved to be sufficiently sensitive to detect significant differences between the studied coralligenous assemblages at both the community and geographic levels. Considering that coralligenous outcrops are regarded as a complex of assemblages (Ballesteros 2006), this approach may help to provide an objective basis to identify assemblages within coralligenous outcrops.

Application of unified sampling approaches over different regions, depths and times will allow tremendous progress to be made in our understanding of the biodiversity patterns of coralligenous outcrops. In this study, we developed a robust method for biodiversity assessment with the intention of providing a useful tool for management and conservation planning, monitoring and research programs focused on one of the most highly valued and emblematic Mediterranean habitats. We further contend that this method is potentially applicable in other benthic ecosystems.

Supporting Information

Table A.1.1. List of the taxa identified in this study. List of the taxa identified within the assemblages dominated by the red gorgonian *Paramuricea clavata* and the red coral *Corallium rubrum* in three regions of the NW Mediterranean.

Acknowledgments

The authors are very grateful for the helpful assistance during the photographic surveys and/or biological data collection of J.M. Dominici (Réserve Naturelle de Scandola, Parc Naturel Régional de Corse), B. de Ligondes, R. Graille and F. Zuberer (Diving staff, Centre d'Océanologie de Marseille), C. Marschal, O. Torrents, J.B. Ledoux, O. Bianchimani (UMR 6540 DIMAR), C. Linares (Universitat de Barcelona) and the Medes Marine Reserve Staff. We further thank J.G. Harmelin, H. Zibrowius, J. Vacelet and N. Boury- Esnault for taxonomic assistance.

Author Contributions

Analyzed the data: SK MF NT E. Cebrian E. Casas EB MZ JG. Wrote the paper: SK NT E. Cebrian E. Casas EB JG. Designed the study: MZ JG. Collected the data: SK MF NT E. Cebrian E. Casas EB MZ JG.

3.5. References

Acunto S, Balata D, Cinelli F (2001) Variabilita spaziale nel coralligeno e considerazioni sul metodo di campionamento. Biol Mar Medit 8:191–200.

Agardy T, Alder J, Dayton P, et al (2005) Coastal Systems. In: Baker J, Moreno Casasola P, Lugo A, et al. (eds) Ecosystems and Human Well-being: Current Status and Trends. Washington: Island Press, pp 513–550

Anderson MJ (2001a) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46.

Anderson MJ (2001b) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58:626–639. doi: 10.1139/f01-004

Anderson MJ, Gorley R, Clarke K (2008) PERMANOVA + for PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK

Anderson MJ, Ter Braak CJ (2003) Permutation tests for multi-factorial analysis of variance. J Stat Comput Simul 73:85–113. doi: 10.1080/00949650215733

Arrhenius O (1921) Species and Area. J Ecol 76:91–104.

Balata D, Piazzi L, Cecchi E, Cinelli F (2005) Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposition. Mar Environ Res 60:403–21. doi: 10.1016/j.marenvres.2004.12.005

Ballesteros E (1986) Métodos de análisis estructural en comunidades naturales, en particular del fitobentos. Oecol aquat 8:117–131.

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr Mar Biol an Annu Rev 123–195.

Bavestrello G, Cerrano C, Zanzi D, Cattaneo-Vietti R (1997) Damage by fishing activities to the Gorgonian coral Paramuricea clavata in the Ligurian Sea. Aquat Conserv Mar Freshw Ecosyst 7:253–262. doi: 10.1002/(SICI)1099-0755(199709)7:3<253::AID-AQC243>3.0.CO;2-1

Bensoussan N, Romano J-C, Harmelin J-G, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: To better understand responses of benthic communities to climate change. Estuar Coast Shelf Sci 87:431–441. doi: 10.1016/j.ecss.2010.01.008

Bianchi CN, Morri C (2000) Marine Biodiversity of the Mediterranean Sea: Situation, Problems and Prospects for Future Research. Mar Pollut Bull 40:367–376.

Bianchi CN, Pronzato R, Cattaneo-Vietti R, et al (2004) CHAPTER 6: Hard Bottoms. Biol Mar Mediterr 11:185–215.

Bohnsack JA (1979) Photographic quantitative sampling of hard-bottom benthic communities. Bull Mar Sci 29:242–252.

Boudouresque C-F (1971) Methodes detude qualitative et quantitative du benthos. Tethys 3:79–104.

Braun-Blanquet J (1932) Plant sociology; the study of plant communities; New York and London, McGraw-Hill book company, inc.

Bray RJ, Curtis JT (1957) An ordination of the upland forest communities of southern wisconsin. Ecol Monogr 27:325–349.

Cain S (1938) The species-area curve. Am Midl Nat 19:573–581.

Casellato S, Stefanon A (2008) Coralligenous habitat in the northern Adriatic Sea: an overview. Mar Ecol 29:321–341. doi: 10.1111/j.1439-0485.2008.00236.x

Clarke K, Gorley R (2006) PRIMER v6: User Manual/Tutorial. Plymouth: PRIMER E

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x

Cocito S, Bedulli D, Sgorbini S (2002) Distribution patterns of the sublittoral epibenthic assemblages on a rocky shoal in the Ligurian Sea (NW Mediterranean). Sci Mar 66:175–181.

Coll M, Piroddi C, Steenbeek J, et al (2010) The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. PLoS One 5:e11842. doi: 10.1371/journal.pone.0011842

Coma R, Pola E, Ribes M, Zabala M (2004) Long-Term Assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecol Appl 14:1466–1478.

Connor EF, Mccoy ED (1979) The Statistics and Biology of the Species-Area Relationship. Am Nat 113:791–833.

Cupido R, Cocito S, Barsanti M, et al (2009) Unexpected long-term population dynamics in a canopy-forming gorgonian coral following mass mortality. Mar Ecol Prog Ser 394:195–200. doi: 10.3354/meps08260

Ferdeghini F, Acunto S, Cocito S, Cinelli F (2000) Variability at different spatial scales of a coralligenous assemblage at Giannutri Island (Tuscan Archipelago , northwest Mediterranean). Hydrobiologia 440:27–36.

Flos J (1985) The driving machine. In: Margalef R (ed) Western Mediterranean: key environments. Oxford: Pergamon, pp 60–99

Garrabou J, Coma R, Bensoussan N, et al (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol 15:1090–1103. doi: 10.1111/j.1365-2486.2008.01823.x

Garrabou J, Perez T, Sartoretto S, Harmelin J (2001) Mass mortality event in red coral Corallium rubrum populations in the Provence region (France, NW Mediterranean). Mar Ecol Prog Ser 217:263–272. doi: 10.3354/meps217263

Gili J-M, Ballesteros E (1991) Structure of cnidarian populations in Mediterranean sublittoral benthic communities as a result of adaptation to different environmental conditions. Oecologia Aquat 10:243–254.

Gili J-M, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–21.

Gili J-M, Ros J (1985) Estudio cuantitativo de tres poblaciones ciralitorales de Cnidarios bentónicos. Investig Pesq 49:323–352.

Giuliani S, Virno Lamberti C, Sonni C, Pellegrini D (2005) Mucilage impact on gorgonians in the Tyrrhenian sea. Sci Total Environ 353:340–9. doi: 10.1016/j.scitotenv.2005.09.023

Halpern BS, Walbridge S, Selkoe K a, et al (2008) A global map of human impact on marine ecosystems. Science 319:948–52. doi: 10.1126/science.1149345

Hawkins SJ, Hartnoll RG (1980) A study of the small-scale relationship between species number and area on a rocky shore. Estuar Coast Mar Sci 10:201–214. doi: http://dx.doi.org/10.1016/S0302-3524(80)80058-2

Hong J-S (1982) Contribution à l’étude des peuplements d'un fond de Concrétionnement Coralligène dans la région marseillaise en Méditerranée Nord-occidentale. Bull KORDI 4:27–51.

Hong J-S (1980) Etude faunistique dun fond de concretionnement de type coralligene. Universite d’Aix-Marseille II

Hong J-S (1983) Impact of the pollution on the benthic community: environmental impact of the pollution on the benthic coralligenous community in the Gulf of Fos, northwestern Mediterranean. Bull Korean Fish Soc 16:273–290.

Jackson JBC, Kirby MX, Berger WH, et al (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629–37. doi: 10.1126/science.1059199

Kronberg I (1987) Accuracy of species and abundance minimal areas determined by similarity area curves. Mar Biol 96:555–561.

Kruskal JB, Wish M (1978) Multidimensional scaling. Sage publications, Beverly Hills

Laborel J (1961) Le concretionment algal coralligene et son importance geomorphologique en mediterranee. Rec Trav St Mar End 23: 37-60.

Laubier L (1966) Le coralligène des Albères: monographie biocénotique. Ann Inst Océanogr Monaco 43:139–316.

Leujak W, Ormond RFG (2007) Comparative accuracy and efficiency of six coral community survey methods. J Exp Mar Bio Ecol 351:168–187. doi: http://dx.doi.org/10.1016/j.jembe.2007.06.028

Linares C, Coma R, Diaz D, et al (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Mar Ecol Prog Ser 305:127–137. doi: 10.3354/meps305127

Martin D, Ballesteros E, Gili JM, Palacín C (1993) Small-scale Structure of Infaunal Polychaete Communities in an Estuarine Environment: Methodological Approach. Estuar Coast Shelf Sci 36:47–58. doi: 10.1006/ecss.1993.1004

Muxika I, Ibaibarriaga L, Saiz J, Borja a (2007) Minimal sampling requirements for a precise assessment of soft-bottom macrobenthic communities, using AMBI. J Exp Mar Bio Ecol 349:323–333. doi: 10.1016/j.jembe.2007.05.026

Nekola JC, White PS (1999) The distance decay of similarity in Biogeography and Ecology. J Biogeogr 26:867–878.

Niell FX (1977) Método de recoleccion y área mínima de muestreo en estudios estructurales de macrofitobentos rocoso intermareal de la ria de Vigo. Inv. Pesq. 41:509–521.

Parravicini V, Micheli F, Montefalcone M, et al (2010) Rapid assessment of epibenthic communities: A comparison between two visual sampling techniques. J Exp Mar Bio Ecol 395:21–29. doi: 10.1016/j.jembe.2010.08.005

Parravicini V, Morri C, Ciribilli G, et al (2009) Size matters more than method: Visual quadrats vs photography in measuring human impact on Mediterranean rocky reef communities. Estuar Coast Shelf Sci 81:359–367. doi: 10.1016/j.ecss.2008.11.007

Piazzi L, Balata D, Cecchi E, et al (2010) Species composition and patterns of diversity of macroalgal coralligenous assemblages in the north-western Mediterranean Sea. J Nat Hist 44:1–22. doi: 10.1080/00222930903377547

Piazzi L, Balata D, Pertusati M, Cinelli F (2004) Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substratum inclination. Bot Mar 47:105–115. doi: 10.1515/BOT.2004.010

Rocchini D, He KS, Oldeland J, et al (2010) Spectral variation versus species beta-diversity at different spatial scales: a test in African highland savannas. J Environ Monit 12:825–31. doi: 10.1039/b921835a

Rockström J, Steffen W, Noone K, et al (2009) A safe operating space for humanity. Nature 461:472–475. doi: 10.1016/j.visres.2011.12.003

Steinitz O, Heller J, Tsoar A, et al (2006) Environment, dispersal and patterns of species similarity. J Biogeogr 33:1044–1054. doi: 10.1111/j.1365-2699.2006.01473.x

Teixidó N, Albajes-Eizagirre A, Bolbo D, et al (2011) Hierarchical segmentation-based software for cover classification analyses of seabed images (Seascape). Mar Ecol Prog Ser 431:45–53. doi: 10.3354/meps09127

Tittensor DP, Micheli F, Nyström M, Worm B (2007) Human impacts on the species-area relationship in reef fish assemblages. Ecol Lett 10:760–772. doi: 10.1111/j.1461-0248.2007.01076.x

True M (1970) Étude quantitative de quatre peuplements sciaphiles sur substrat rocheurs dans la région marseillaise. Bull Inst Oceànogr Monaco 60:1–41.

UNEP/MAP-RAC/SPA (2008) UNEP-MAP RAC/SPA. In: Action Plan for the Conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean sea.

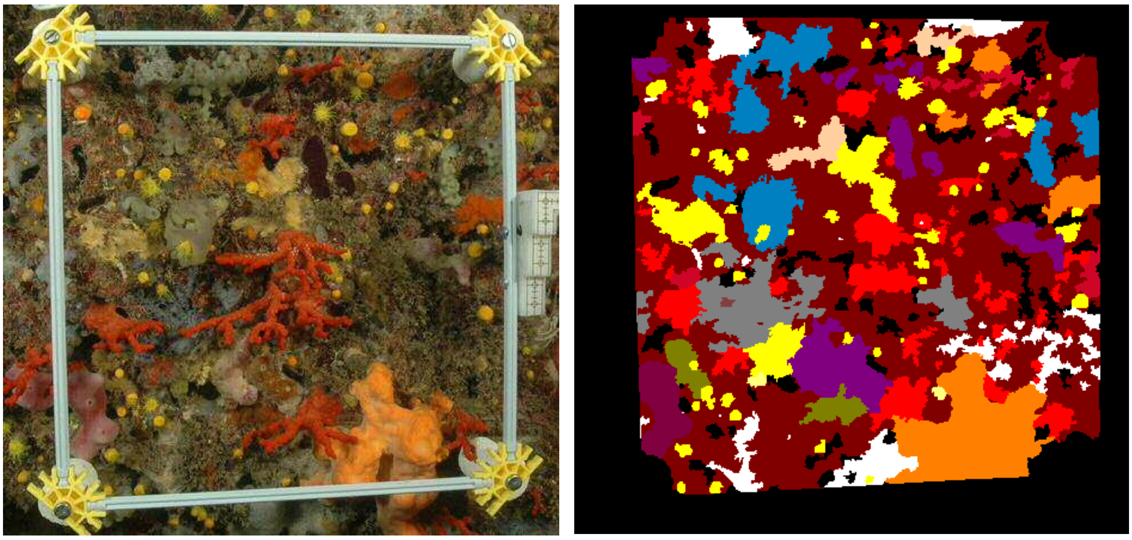
Virgilio M, Airoldi L, Abbiati M (2006) Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. Coral Reefs 25:265–272. doi: 10.1007/s00338-006-0100-2

Walther G, Post E, Convey P, et al (2002) Ecological responses to recent climate change. Nature 416:389–395. doi: doi:10.1038/416389a

Weinberg S (1978) The minimal area problem in invertebrate communities of Mediterranean rocky substrata. Mar Biol 49:33–40. doi: 10.1007/BF00390728

Zabala M, Ballesteros E (1989) Surface-dependent strategies and energy flux in benthic marine communities or, why corals don't exist in the Mediterranean. Sci Mar 53:3–17.

4. Structure, biodiversity and landscape pattern indices of *Corallium rubrum* assemblages over broad spatial scales



Casas-Güell E, Cebrian E, Garrabou J, Ledoux JB, Linares C, Teixidó N (2016) Structure and biodiversity of coralligenous assemblages over broad spatial scales (submitted to *Nature Scientific Reports*)

Abstract

Data on species diversity and structure in coralligenous outcrops dominated by *Corallium rubrum* are lacking. Gathering these data is needed to build robust baselines in order to assess the responses of this habitat highly threatened by anthropogenic disturbances (e.g. mass mortality events, sedimentation and invasive species). A hierarchical sampling including 3 localities and 9 sites covering more than 400 km of rocky coasts in NW Mediterranean (Catalan coast -Spain-, Provence and NW Corsica -France-), was designed to characterize the spatial variability of structure, composition and diversity of perennial species inhabiting coralligenous outcrops dominated by *C. rubrum*. From photographic surveys, we estimated species/taxa composition and abundance. We pooled the identified species/taxa into eight morpho-functional groups according to their life span and growth to characterize the structural complexity of the outcrops. We analyzed the number, size and shape of patches for each morpho-functional group to describe their spatial arrangement in the outcrop. According to ordination analyses, the species composition and structural complexity of red coral assemblages differed consistently across all spatial scales considered. The lowest and the highest variability were found among localities (separated by 100 km) and within sites (separated by 1-5 km), respectively supporting differences in diversity indices (alpha, beta and gamma). The morpho-functional groups displayed a consistent spatial arrangement across study sites. These results contribute to filling the gap on the understanding of assemblage composition and structure in the emblematic Mediterranean coralligenous outcrops dominated by *C. rubrum* over large spatial scales.

4.1. Introduction

Fine-scale and high-resolution knowledge on the variability of relevant habitats, such as the coralligenous benthic assemblages, covering a range of spatial scales is important for effective management and conservation of coastal marine habitats (Ballesteros 2006; Giakoumi et al. 2013; Martin et al. 2014; Gatti et al. 2015a). The EU Habitats Directive (92/43/CE) classified coralligenous outcrops as a key habitats and foster some of the richest assemblages found in Mediterranean, harboring approximately 10% of marine Mediterranean species (Pérès and Picard 1964; Laubier 1966; Ros et al. 1985; Boudouresque 2004; Ballesteros 2006). Most of the species that characterize these assemblages are long-lived algae and sessile invertebrates, exhibit low dynamics and belong to various taxonomic groups (Garrabou et al. 2002; Ballesteros 2006; Teixidó et al. 2011b). Coralligenous outcrops are being affected by several impacts such as nutrient enrichment, overexploitation, invasive species, increase of sedimentation, mechanical impacts as well as climate change, resulting in dramatic consequences for many species (Balata et al. 2007; Garrabou et al. 2009; Cebrian et al. 2012; Piazzi et al. 2012; Teixidó et al. 2013; Cecchi et al. 2014).

The precious Mediterranean red coral *Corallium rubrum* (L. 1758) is considered as one of the engineering species inhabiting coralligenous outcrops (Teixidó et al. 2011b; Kipson et al. 2011). This species is typically associated with animal-dominated communities growing in dimly lit habitats, such as caves and smaller cavities, vertical cliffs and overhangs, from 10 to 200 m in depth (Carpine and Grasshoff 1975; Weinberg 1978). *C. rubrum* is a slow-growing and long-lived species, can play a paramount role in the structure and functioning of coralligenous habitats, through their trophic activity, biomass and perennial biogenic structure (Gili and Coma 1998; Garrabou and Harmelin 2002; Tsounis et al. 2010). Mature populations comprising large-sized colonies of *C. rubrum* enhances habitat complexity (Garrabou and Harmelin 2002), which it has been related to influence the maturity of ecosystems by increasing the ratio between biomass and productivity (Eriksson et al. 2006), as well as biodiversity and ecosystem functioning (Levin et al. 2001). This species is threatened mainly by intensive historical harvesting, causing an overall shift in the population structure with a decrease in biomass and colony size (Bramanti et al. 2014). Warming and the potential effects of ocean acidification are also major threats affecting shallow populations (Garrabou et al. 2001; Garrabou et al. 2009; Cerrano et al. 2013; Bramanti et al. 2013). In general, it has been demonstrated that a decrease in the abundance of habitat-forming species can lead to a rapid fragmentation in community structure and loss of species benefiting from structural complexity provided by habitat-forming species (Hughes 1994; Cerrano et al. 2013; Ponti et al. 2014; Valls et al. 2015). Despite the key ecological role of *C. rubrum* in the structure and functioning of coralligenous outcrops, there are few studies dealing with the characterization and variability at fine scale of the whole assemblage over large regional scales (but see (Giannini et al. 2003; Virgilio et al. 2006)).

Landscape ecology approach has been successfully applied to marine benthic communities and provided new insights on the structural and ecological processes (Garrabou et al. 1998; Teixidó et al. 2002; Wedding et al. 2011). Landscape ecology emphasizes the interaction between spatial patterns and ecological processes across a range of spatial and temporal scales (Forman and Godron 1986; Turner et al. 2002; Turner 2008). This analysis is based on the notion that communities can be observed as patch mosaics, where patches correspond to different categories (e.g. species). From this perspective, community spatial patterns and dynamics can be analyzed focusing on the characteristics of the patch mosaics (e.g. number, size and complexity of patches). Within this context, approaches that provide new perspectives on how environmental conditions and biological interactions affect the structural pattern are highly needed in marine habitats.

To our knowledge, few studies addressing coralligenous assemblages have extended to larger spatial scales up to 200 km of coastline (Casas-Güell et al. 2015), and mostly of them focused on the phytobenthic component (Piazzi et al. 2004; Piazzi et al. 2010). Thus, the structure of coralligenous outcrops has been poorly understood because there are no spatial studies or baseline data at the assemblage level over large scales. This situation has prevented a proper assessment of the current state of biodiversity and potential impact assessments in this valuable assemblage (but see (Gatti et al. 2015a)). Precise, high-resolution and quantitative biodiversity data dealing to encompass regional spatial patterns are required to furnish and implement monitoring schemes for conservation facing with global change. As coralligenous assemblages are dominated by long-lived species, declining or recovery trajectories are more difficult to detect due to the slow pace of their population dynamics (Teixidó et al. 2011b; Teixidó et al. 2013; Gatti et al. 2015b). In this context, high quality and large scale biodiversity datasets are a basic resource that furnishes the essential tools to provide information to promote sound conservation actions (Magurran et al. 2010). Thus, high-resolution quantitative data is very important in order to obtain an estimate of the abundance for the whole community and the present study provides fine-scale and high-resolution quantification in the different components of biodiversity of coralligenous assemblages on a regional scale. Moreover, we also characterized the spatial configuration by analyzing the number, the size and the shape of patches.

4.2. Materials and methods

Study sites and sampling design

This study was conducted in three localities in the NW Mediterranean region (Catalonia; Provence and Corsica), covering more than 400 Km^2 (5º E-W) and more than 200 Km of the coastline (Fig. 1).

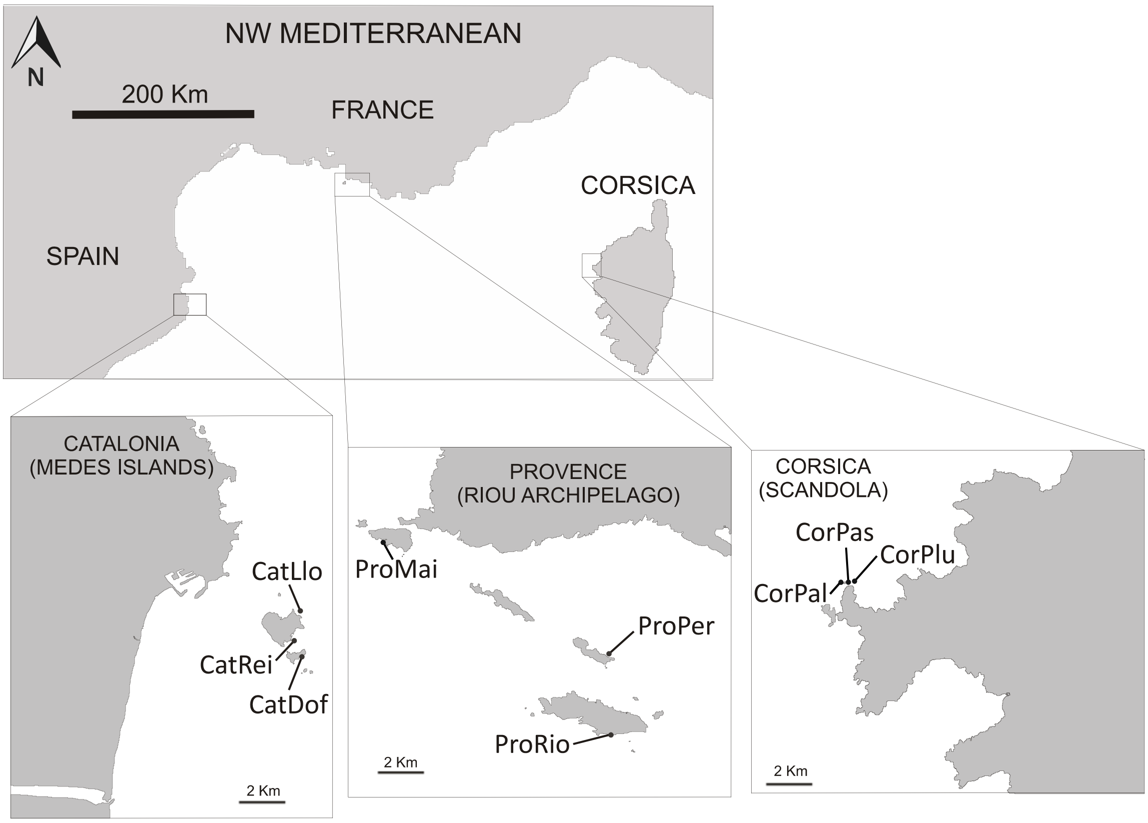


Figure 1. Map showing the Mediterranean geographic area where the photographic surveys were conducted.

We used a hierarchical, nested sampling design to characterize and quantify structure and diversity patterns of coralligenous outcrops dominated by the red coral *Corallium rubrum*. At each locality, three sites (separated by approximately 1 km) were sampled as follows: in Catalonia, Cova del Dofí (CatDof), Cova de la Reina (CatRei) and Pota de Llop (CatLlo) located in the Natural Park of Montgrí, Medes Islands and Baix Ter; in Provence, Ille Plane-Grotte Pérès (ProPer), Riou Sud (ProRio) and Maïre Grotte (ProMai), located in Riou Archipelago in the National Park des Calanques; and in Corsica, Palazzu (CorPal), Palazzinu (CorPlu) and Passe Palazzu (CorPas), located in the Scandola Natural Reserve (see Appendix I for latitude/longitude coordinates).

At each site, three transects approximately 0.32 m^2 in size (80 cm long and 40 cm wide) were set up between depths of 15-40 m. Transects area was selected based on the results obtained from a previous study addressing the minimal sampling area (MSA) for this habitat (Kipson et al. 2011). Transects from all sites were sampled from 2007 to 2013. Each transect was monitored photographically using quadrats of 20 × 20 cm (400 cm2) to facilitate species identification being a set of 8 contiguous quadrats the replicate sampling unit used (0.32 m^2). A total of 212 photographs (8 photos × 3 replicates × 9 sites) were analyzed. The photographs were taken with a Nikon D70S digital SLR camera fitted with a Nikkor 20 mm DX lens (3000 × 2000 pixel resolutions) and housed in a Subal D70S housing. Lighting was achieved by two electronic strobes fitted with diffusers.

Analysis of photographs

From each photograph, all individuals of sessile macrobenthic species were identified at the lowest taxonomic level and classified within 8 morphofunctional groups (hereafter MFG) based on their taxonomy, life-span and growth form (Table 1) (Casas-Güell et al. 2015). We recorded the number (NP) and measured the size of each individual species present, which were designed as "patch" using the SEASCAPE software (Teixidó et al. 2011a). For perennial, long-lived and slow-growing species, hereafter "perennial species" (see Appendix A.2.2), we also calculated the mean patch size (MPS: mm2) and the mean shape index (MSI) for all individual patch species. MSI was calculated applying the formula (Cebrian and Uriz 2007):

$$MSI = Api \,/\, Ac_{\mathrm{i}}$$

Were Ap is the area of each patch and Ac is the area of a perfect circle with perimeter equivalent to the patch. This indices measures patch circularity and a value of 1 represents a perfect circle, while 0 is approached as the patch outline becomes more irregular. NP, MPS and MSI have been evaluated to perform well detecting spatial seascape patterns along a depth gradient of subtidal Mediterranean rocky communities (Garrabou et al. 1998; Garrabou et al. 2002).

To characterize the structural complexity, for each MFG we calculated (1) the number of perennial species (2) their abundance as a % cover and number of patches (NP) and (3) the MPS and MSI. For the *Animal tree* morphofunctional group (e.g.

Corallium rubrum, Paramuricea clavata), cover was measured as the surface attached to the substrate as the photographs were taken to capture the cover of the whole assemblage and not the three-dimensionality of these species.

Table 1. Biological categories used in this study combining taxonomy, life span and morphological growth forms (adapted from Garrabou et al. 2002 and Teixidó et al. 2011).

Biological categories	Description
1- Seasonal algal turf	Annual erect or semi-erect fleshy algal species, with one or multiple zones of attachment to the substratum; generally constitutes algal cushions or thin sheets with mixtures of algal species.
2- Seasonal animal turf	Small seasonal animal species, mainly bryozoans and hydrozoans; usually is forming animal cushions or thin sheets with mixtures
3- Seasonal mixture complex turf	Small seasonal algae and animal species (mainly bryozoans and hydrozoans), sediment, detritus and fragments; normally forming cushions or thin sheets with mixtures of species.
4- Perennial algal encrusting	Species growing mainly as two dimensional sheets; more or less completely attached to the substratum.
5- Perennial algal erect	Species attached to the substratum usually with a unique zone (visible even in winter) of basal attachment to the substratum.
6- Perennial algal turf – invasive	Perennial dense thick filamentous turf algae with the ability to maintain permanent carpets (e.g. the invasive species *Womersleyella setacea*).
7- Perennial animal encrusting	Species of sponges, cnidarians, bryozoans and tunicates growing as two dimensional sheets; more or less completely attached to the substratum.
8- Perennial animal massive	Mound species of sponges and cnidarians with vertical and lateral growth; normally attached to the substratum all along their basal area.
9- Perennial animal tree	Erect species of cnidarians and bryozoans, more or less branched; usually with a single point of attachment to the substratum.
10- Perennial animal cup	Solitary corals attached to the substratum all along their basal area.
11- Perennial animal boring	Excavating organisms living into the rock (e.g. *Cliona viridis*).
12- Perennial animal epibiont	Species growing over other invertebrates or calcareous algae (mainly polychaetes e.g. *Salmacina dysteri* or *Filograna implexa* and bryozoans e.g. *Chartella tenella*).

Spatial patterns of diversity metrics for perennial species were assessed by quantifying the number of species (average number of species per sampling unit) and beta diversity (multivariate distance between group-centroids determined with the PERMDISP procedure). PERMDISP is an approach used to compare the degree of multivariate dispersion of different groups of samples based on a distance matrix. When PERMDISP is used on a Jaccard distance presence/absence matrix, it is directly interpretable as a test for similarity in beta diversity among groups (Anderson et al.

2006; Anderson et al. 2011). Additionally, the pool of species at locality spatial level or gamma diversity (the number of species observed within the sites at each locality studied) was calculated.

Statistical analyses

The variability of structural complexity of assemblages, both in terms of morphofunctional groups and specific composition of perennial species assemblages over spatial scales was tested based on the hierarchical sampling design. It included 2 spatial factors: Locality (random factor, 3 levels), Site (random factor, 3 levels, nested in Locality). A non-parametric analysis of variance, PERMANOVA (Anderson and Robinson 2001; Anderson 2001; Anderson et al. 2008), was applied using Bray-Curtis and Euclidean distances for multivariate and univariate analyses, respectively.

To visualize similarity patterns at different spatial scales, a non-metric multi-dimensional scaling (nMDS) ordination analysis was performed based on the Bray–Curtis similarity measure for presence/absence and abundance (cover %) data. Furthermore, a similarity percentage procedure analysis (SIMPER, Clarke and Warwick 1994) was performed to identify the percentage contribution of taxa and MFG to the significant dissimilarities among sites and localities.

Numerical analyses were computed using the program Primer v6 with the PERMANOVA + add-on package.

4.3. Results

Species composition

A total of 112 macrobenthic taxa were identified across the region studied: 20 macroalgae, 1 protozoan, 41 sponges, 6 hydrozoans, 11 anthozoans, 1 mollusk, 3 polychaetes, 21 bryozoans and 8 tunicates (see Appendix II for the species list and the assignment to the various morphofunctional groups). Of these taxa, 81 were perennial and 31 seasonal. Perennial taxa represented between the 30 and 55 % of cover, whereas seasonal species hardly reached 10 % cover in all sites (Table A.2.3).

Structural complexity - morphofunctional groups

Number of species and cover %

The number of species and % cover for each MFG was similar among *Locality* but showed significant differences among *Sites* (Figs. 2A and B, Table 2).

Table 2. PERMANOVA analysis on the number and % of cover of perennial species of the morpho-functional groups on the basis of Bray-Curtis dissimilarity measure.

	Source of variation	df	SS	MS	Pseudo-F	P(perm)
Number of species	Locality	2	236.07	118.04	1.6344	0.2492
	Site (Locality)	6	433.33	72.222	8.945	0.0001
	Residual	18	145.33	8.0741		
	Total	26	814.74			
Cover %	Locality	2	3884.4	1942.2	1.4787	0.2275
	Site (Locality)	6	7880.7	1313.4	12.538	0.0001
	Residual	18	1885.6	104.76		
	Total	26	13651			

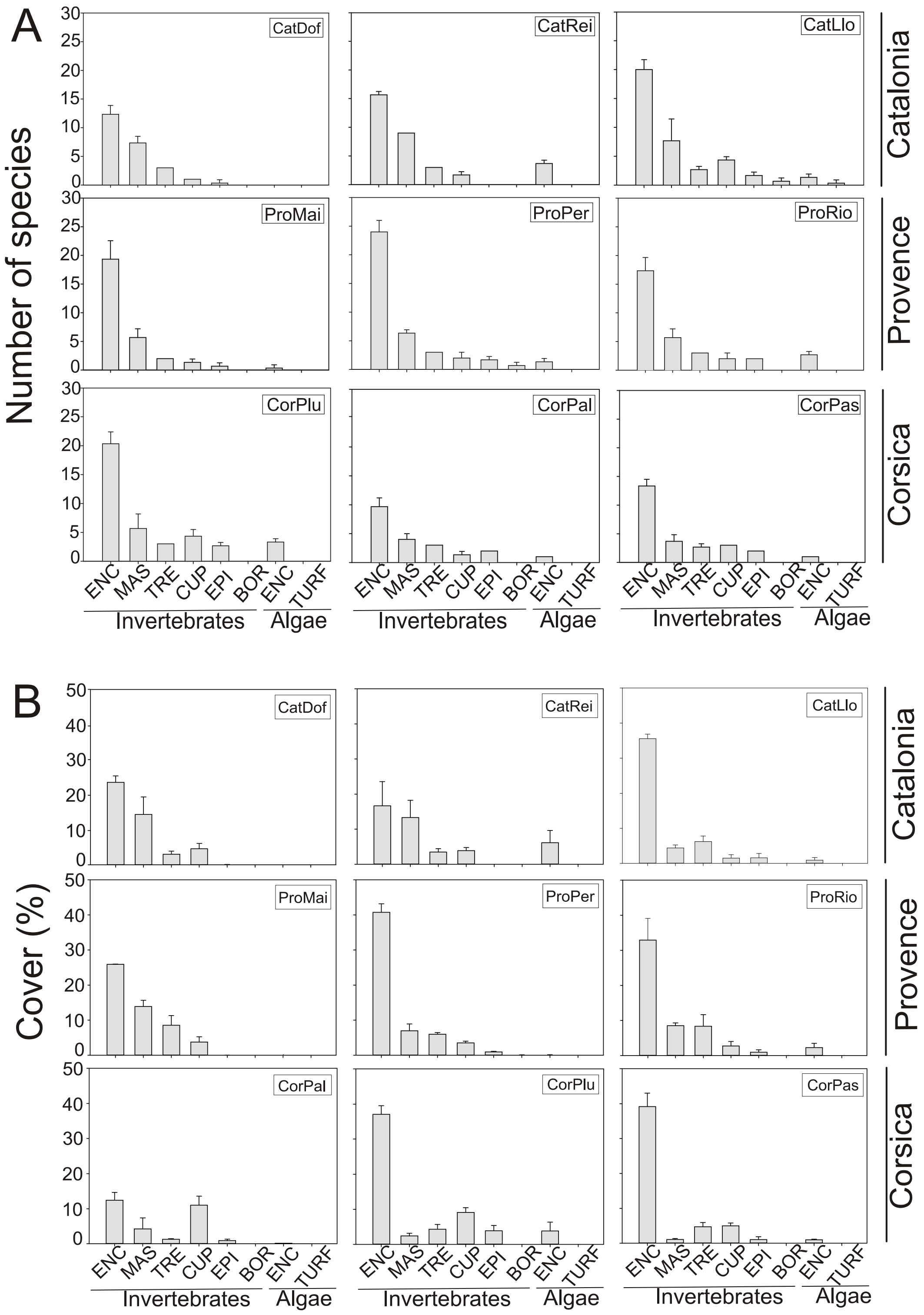


Figure 2. Number of perennial species ± SD (A) and % of cover ± SD (B) of each morpho-functional group and study site.

The principal group in terms of number of species and % cover was the *Animal encrusting*, with values ranging from 10 ± 2 to 24 ± 2.00 species in CorPal and ProPer, respectively and % cover ranging from 10.91% ± 1.81 to 39.81% ± 3.26 in CorPal and CorPas, respectively. The second MFG characterizing the assemblage of Catalonia and Provence in terms of number of species and % cover was *Animal massive* with % cover ranging from 3.96% ± 0.47 to 14.76% ± 4.21 in CorPas and CatDof, respectively. In contrast, although the *Animal massive* was the second group in terms of number of species in Corsica, the second most abundant group in terms of % cover was *Animal cup*. *Animal tree* was, in general, the less abundant MFG in terms of species number (ranging from 1 ± 0 to 4 ± 1 species) and moderate to low % cover (ranging from 1.58% ± 0.48 to 8.68% ± 2.21 in CorPal and ProMai). The remaining categories *Algae encrusting, Algae turf* and *Animal epibiont* were represented by very few species (Fig 2A). *Animal boring* and *epibionts, Algal encrusting* and *turf* also showed the lowest % of cover values and exhibited a higher degree of variability.

Structural complexity – specific composition

The structure and specific composition of perennial species assemblages differed significantly among *Sites* as well as among *Localities* regardless of the measure analyzed (presence/absence and % cover, Fig. 3; Table 3). Accordingly, the variability explained for each of the spatial factors showed a consistent pattern: the highest percentage of variation was found at *Site* spatial level (values ranging from 21.14% to 35.07%) followed by *Locality* (values from 15.85% to 27.43%) and finally the residuals, at the sampling unit level, which showed the lowest source of variability (values from 14.92% to 23.32%). The estimates of variance components presented higher values for % cover than for presence/absence (Table 3).

Table 3. PERMANOVA analyses of community structure and specific composition on the basis of Bray-Curtis dissimilarity measure.

Abundance	Source of variation	df	SS	MS	Pseudo-F	P(perm)	ECV (Sq. root)
Cover (%)	Locality	2	22015	11007	2.5998	0.0116	27.434
	Site (Locality)	6	25403	4233.9	7.7889	0.0001	35.073
	Residual	18	9784.5	543.58			23.315
	Total	26	57202				
Presence and absence	Locality	2	7645.5	3822.7	2.4464	0.0105	15.847
	Site (Locality)	6	9375.6	1562.6	7.0219	0.0001	21.135
	Residual	18	4005.6	222.53			14.917
	Total	26	21027				

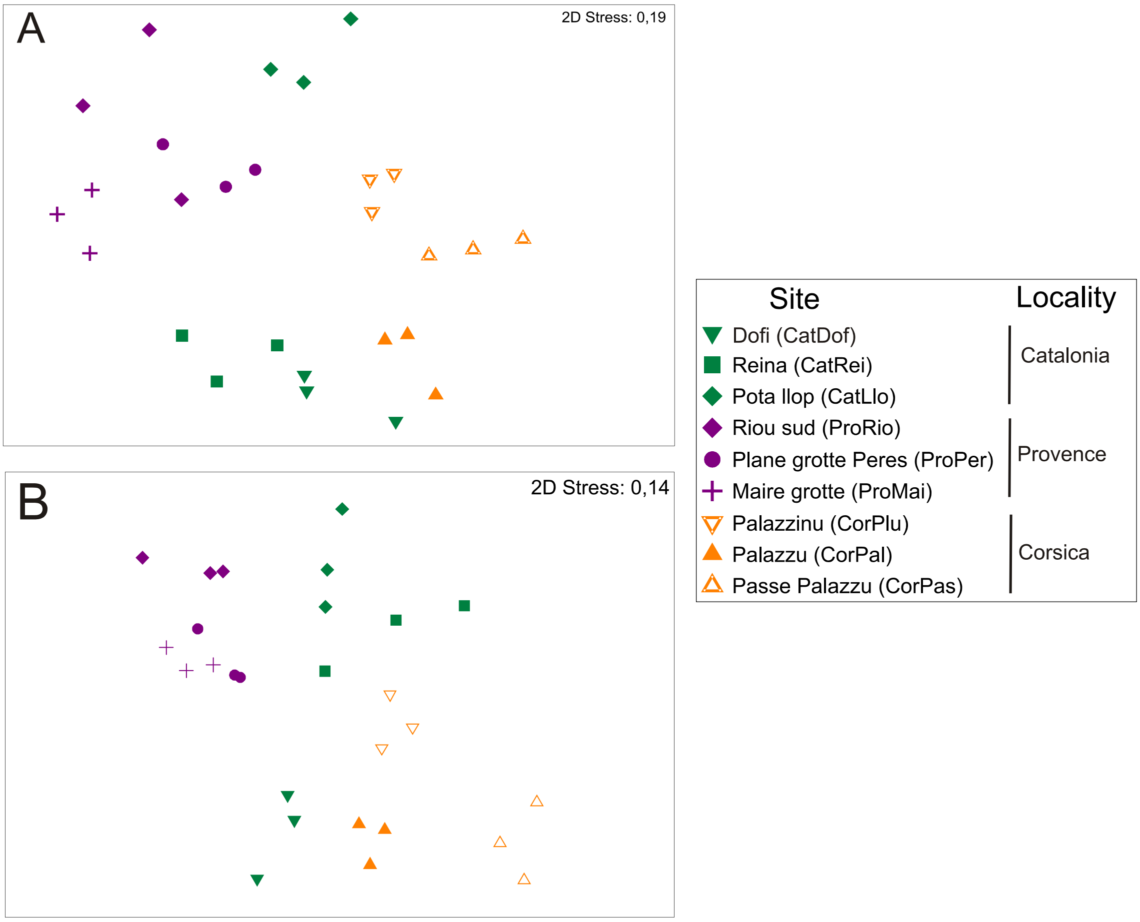


Figure 3. Non-metric multidimensional scaling (nMDS) ordination plot of perennial macrobenthic species in the three localities of the NW Mediterranean Sea. Analysis performed on Bray-Curtis dissimilarities for (A) presence-absence data and (B) for % of cover. For each locality (orange = Corsica; purple = Provence; green = Catalonia), the three sites are shown by different shapes.

Parallel to these results, the SIMPER analysis showed an overall average dissimilarity (up to 75%) in % cover across localities (Table 4a,b shows the number of species contributing to 50% of the similarity and dissimilarity) was approximately 76.78% between Provence and Corsica, 68.38% between Corsica and Catalonia and 66.01% dissimilarity between Provence and Catalonia. Focusing in the *Animal encrusting* differences on the relative abundance of the sponges *Crella (Grayella) pulvinar*, *Pleraplyssilla spinifera*, *Dendroxea lenis* and the bryozoan *Gregarinidra gregaria* mainly explained the dissimilarities among localities. Regarding *Animal massive*, dissimilarity among *Locality* were mainly explained by differences on the relative abundance of *Oscarella* sp. and *Petrosia ficiformis*.

Table 4a. Species by morpho-functional groups contributing more than 50% to the similarity of studied regions. The underlined species are those contributing to the similarity of most of the sites. Similarity analysis based on % cover dataset. The average similarity for the NW Mediterranean was 35.45%

	Similarity		
	Provence (61.51%)	Corsica (66.01%)	Catalonia (68.38%)
Animal encrusting	*Pleraplysilla spinifera* (35.35%) *Crella (Grayella) pulvinar* (8.65%) Encrusting sponge n.idd. (4.36%) *Scalarispongia scalaris* (4.36%) Serpulidae (1.69%)	*Pleraplysilla spinifera* (9.36%) Encrusting Bryozoans (15.35%) Encrusting sponge n.idd. (7.01%) *Gregarinidra gregaria* (6.17%)	*Pleraplysilla spinifera* (9.97%) Encrusting sponge n.idd. (13.50%) Encrusting Bryozoans (6.28%)
Animal massive	*Oscarella* sp. (5.88%) *Petrosia ficiformis* (2.71%) *Ircinia variabilis* (2.40%) *Aplysina cavernicola* (2.27%)	*Haliclona mucosa* (4.78%)	*Petrosia ficiformis* (5.27%)
Animal tree	*Corallium rubrum* (17.71%)	*Corallium rubrum* (3.91%) *Reteporella grimaldii* (3.75%)	*Corallium rubrum* (11.89%)
Animal cup	*Caryophyllia inornata* (2.89%) *Hoplangia durotrix* (2.45%)	*Leptopsammia pruvoti* (30.78%)	*Leptopsammia pruvoti* (8.87%)

Table 4b. Species by morphofunctional groups contributing more than 50% to the dissimilarity of studied regions. The underlined species are those contributing to the similarity of most of the sites. Analysis based on cover (%) dataset. n.idd. = non identified.

	Dissimilarity		
	Provence VS Corsica (76.78%)	Provence VS Catalonia (66.01%)	Catalonia VS Corsica (68.38%)
Animal encrusting	*Pleraplysilla spinifera* (12.57%) *Gregarinidra gregaria* (9.04%) Encrusting Bryozoan n.idd. (4.61%) *Crella (Grayella) pulvinar* (4.55%)	*Pleraplysilla spinifera* (14.60%) *Scalarispongia scalaris* (4.31%) *Crella (Grayella) pulvinar*(3.89%) *Dendroxea lenis* (3.84%) *Parazoanthus axinellidae* (3.35%)	*Gregarinidra gregaria* (11.32%) *Pleraplysilla spinifera*(6.19%) Encrusting Bryozoans (4.81%) *Haliclona (Sostella) mucosa* (3.62%) Sponge n.idd. (3.87%) *Parazoanthus axinellae* (3.56%) *Crambe crambe* (3.36%) *Dendroxea lenis* (3.33%)
Animal massive	*Oscarella sp.* (4.59%)	*Petrosia ficiformis* (5.24%) *Oscarella spp.* (4.54%)	*Petrosia ficiformis* (5.18%)
Animal cup	*Leptopsammia pruvoti* (10.02%)	-	*Leptopsammia pruvoti* (9.42%)

Diversity indices

The mean ± SD values of alpha diversity remained similar for all *Localities* but showed significant differences among *Sites* (Fig. 4a; Table 5).

Table 5. Univariate analysis of variance for alpha diversity with permutation tests (PERMANOVA) based on Euclidian distances. Beta diversity (% unshared species) tested with PERMDISP routine.

Diversity metrics	Source	df	SS	MS	Pseudo-F	P-value
Species number	Locality	2	156.22	78.11	0.39591	0.6709
	Site (Locality)	6	1183.8	197.3	18.626	0.0001
	Residual	18	190.67	10.60		
	Total	26	1530.7			
Beta diversity (% unshared species)	Source	df_1	df_2	F	P-value	
	Locality	2	24	1.2974	0.3316	
	Site	8	18	2.7976	0.2285	

Alpha diversity showed the highest variability in Catalonia and Corsica (Fig. 4a) with mean values ranging from 24 ± 2 to 41± 5.3 and 21 ± 4.2 and 40 ± 3.1, respectively. The variability in Corsica was due to CorPlu that showed the highest values for alpha compared with CorPal and CorPas. Provence presented consistent mean alpha diversity values at all *Sites* ranging from 30 ± 5.8 to 39 ± 2.5 (Fig. 4a). The percentage of unshared species (beta diversity) was similar at both spatial levels (Fig. 4b; Table 5). A similar pattern of beta diversity was found at all study *Sites* with average percentage of unshared species ranging from 14.93% ± 1.5 to 24% ± 0.38 (Fig. 4b). Gamma diversity showed similar values among the three *Locality* studied with 57 species in Corsica, 68 in Catalonia and 72 species in Provence (Fig. 4c).

Values of all diversity indices for all *Localities* and *Sites* can be found at Table A.2.4.

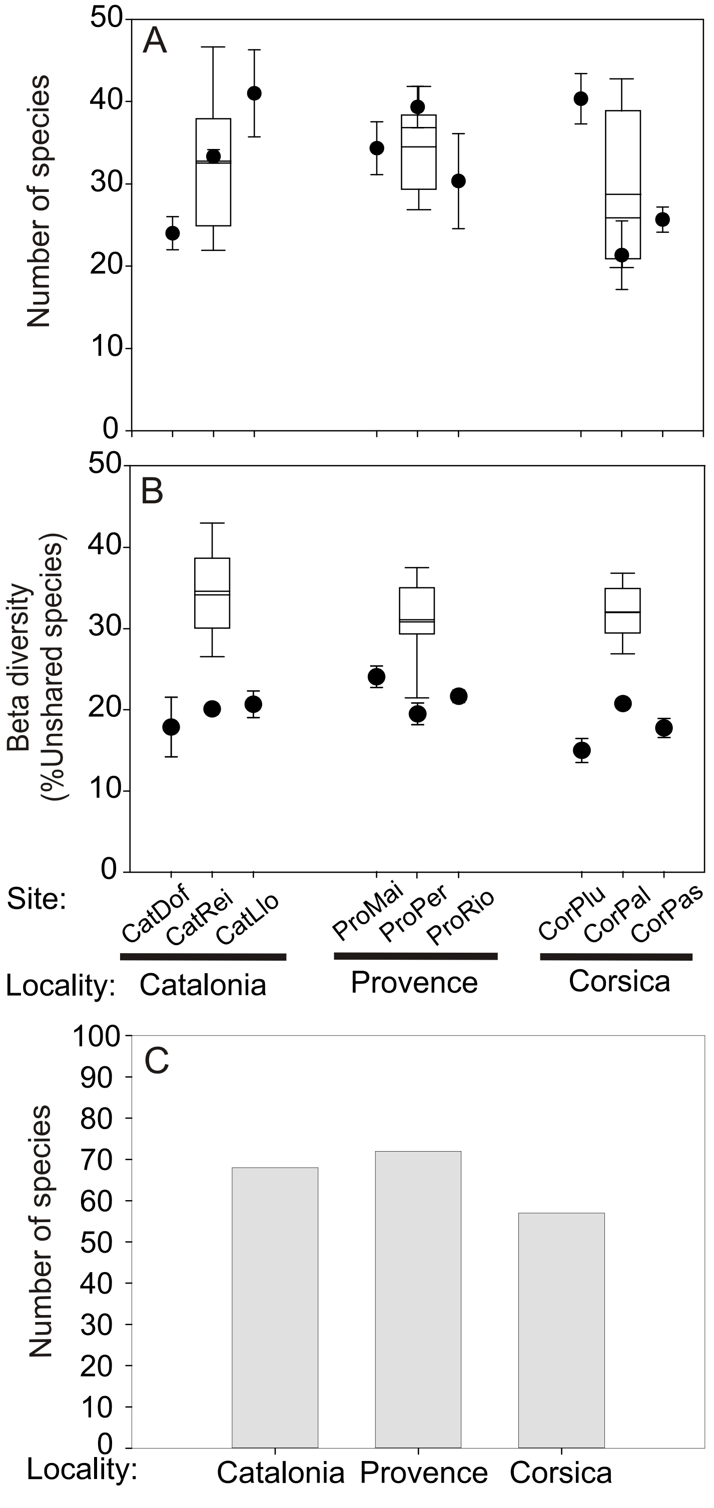


Figure 4. Diversity indices for all study sites and localities. (A) Number of species (alpha diversity), (B) % of unshared species (β-diversity) and (C) local number of species (gamma diversity).

Spatial configuration of perennial species

Overall, the mean perennial species cover was quite different between sites and across localities except for the locality CorPal with the lowest value of 30% ± 1.82. Overall, values ranged from 60% ± 2.65 in CorPlu to 30% ± 1.82 in CorPal (see Table A.2.3).

For perennial species as a whole, NP, MPS and MSI were similar among *Localities* and *Sites* (Fig 5; Table 6). The number of patches range from 802 to 1491 patches in Catalonia, from 826 to 1876 patches in Provence and from 914 to 1695 patches in Corsica. The MPS showed relatively small values with range values from 83 to 172 mm^2 in Catalonia, from 87 to 196 mm^2 in Provence and from 80 to 165 mm^2 in Corsica. The MSI showed a narrow range with values from 0.37 to 0.57 over all the localities.

Table 6. Univariate PERMANOVA analyses of Number of patches (NP), Mean patch size (MPS) and Mean shape index (MSI) for overall set of perennial species on the basis of Euclidian distances

Seascape pattern indices	Source of variation	df	SS	MS	Pseudo-F	P(perm)	ECV (Sq. root)
Number of patches	Locality	2	2.27e5	1.14e5	1.79260	0.2930	38.612
	Site (Locality)	7	4.40e5	62805	0.63582	0.7130	-58.421
	Residual	18	9.68e6	98778			314.29
	Total	98	1.02e7				
Mean patch size	Locality	2	1.6588	0.82938	1.1936	0.343	6.3167e-2
	Site (Locality)	6	4.6673	0.66668	0.2837	0.952	-0.39972
	Residual	98	230.37	2.3507			1.5332
	Total	107	237.19				
Mean shape index	Locality	2	1.28e-2	6.38e-3	0.6365	0.557	-1.0401e-2
	Site (Locality)	6	7e-2	1e-2	0.8455	0.556	-1.3169e-2
	Residual	98	1.1591	1.18e-2			0.10876
	Total	107	1.2507				

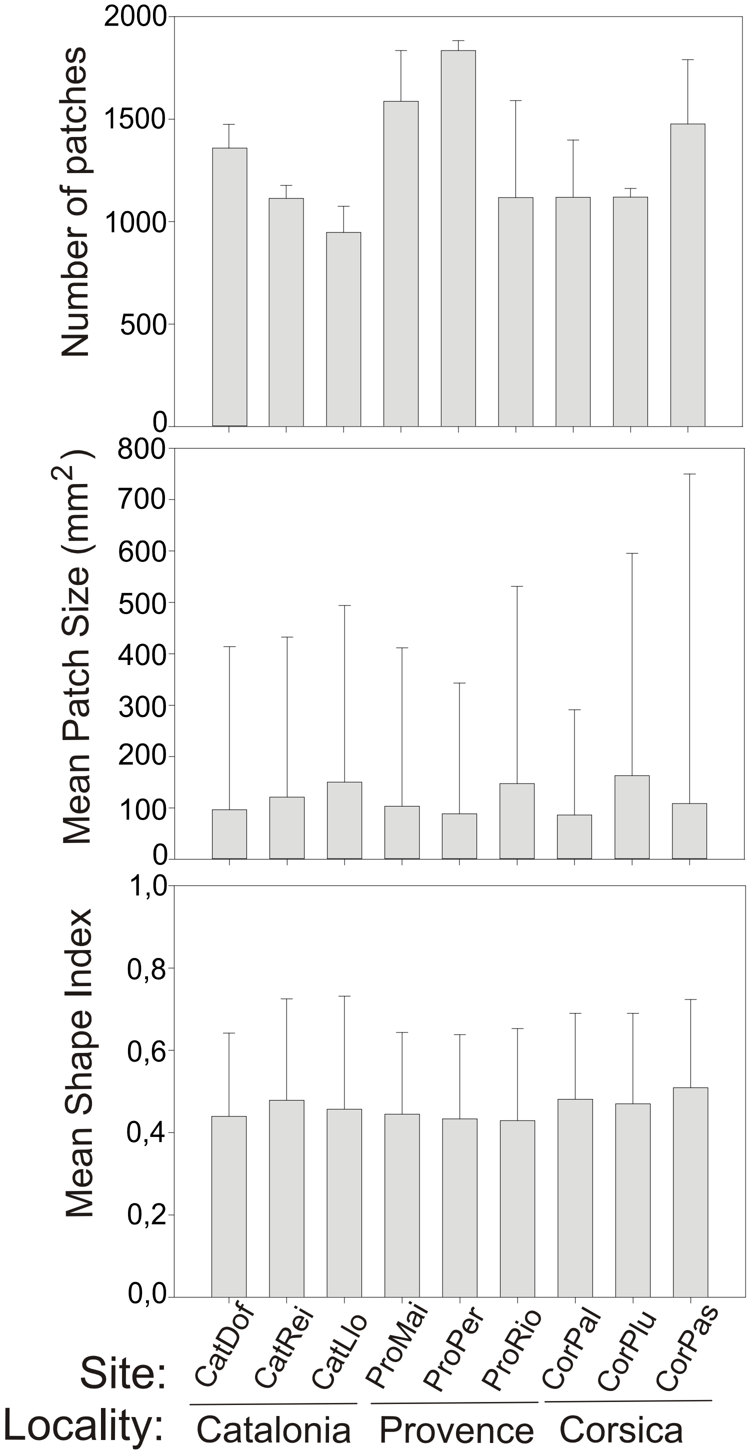


Figure 5. Number of patches (NP ± SD), Mean Patch Size (MPS ± SD) and Mean Shape Index (MSI ± SD) for the overall set of perennial species.

Within the perennial species, benthic seascape was characterized by a high NP of *Animal encrusting*, the most important group also in terms of % cover, with values ranging from 353.3 ± 92.90 patches to 1240.33 ± 10.65 patches and a relatively small MPS (values ranging from 84.60 mm^2 ± 13.83 to 240.61 mm^2 ± 25.08) at all *Locality* and *Site* (Fig. 6; Table 7). *Animal encrusting* patches were quite irregular with MSI values close to 0.4 at all *Locality* and *Site* (Fig. 6; Table 7). *Animal cup* was the second most abundant MFG in terms of NP at all *Locality* and *Site* (except in CorPal where was the most abundant) with values ranging from 96 patches ± 46.78 to 664.33 patches ± 72.04. This MFG exhibited the smallest MPS (values ranging from 23.10 mm^2± 0.66 to 84.60 mm^2± 13.83) and a higher MSI (close to 0.6 - 0.7) (Fig. 6; Table 7). *Animal massive* showed comparatively lower NP than *Animal cup* and *Animal encrusting* at all *Locality* and *Site* with values ranging from 11 patches ± 2.94 to 153.67 patches ± 23.63 but presented the biggest MPS with values ranging from 227.46 mm^2 ± 144.98 to 520.40 mm^2 ± 159.70. MSI of *Animal encrusting* was characterized by some irregular shapes with overall values not reaching the 0.5. *Animal tree* showed lower NP ranging from 25.67 patches ± 10.69 to 180.33 patches ± 13.04 with MPS ranging from 94.97 ± 17.53 to 304.58 ± 51.59 mm^2. Data showed that *Animal tree* was the MFG with more irregular forms (Fig. 6; Table 7).

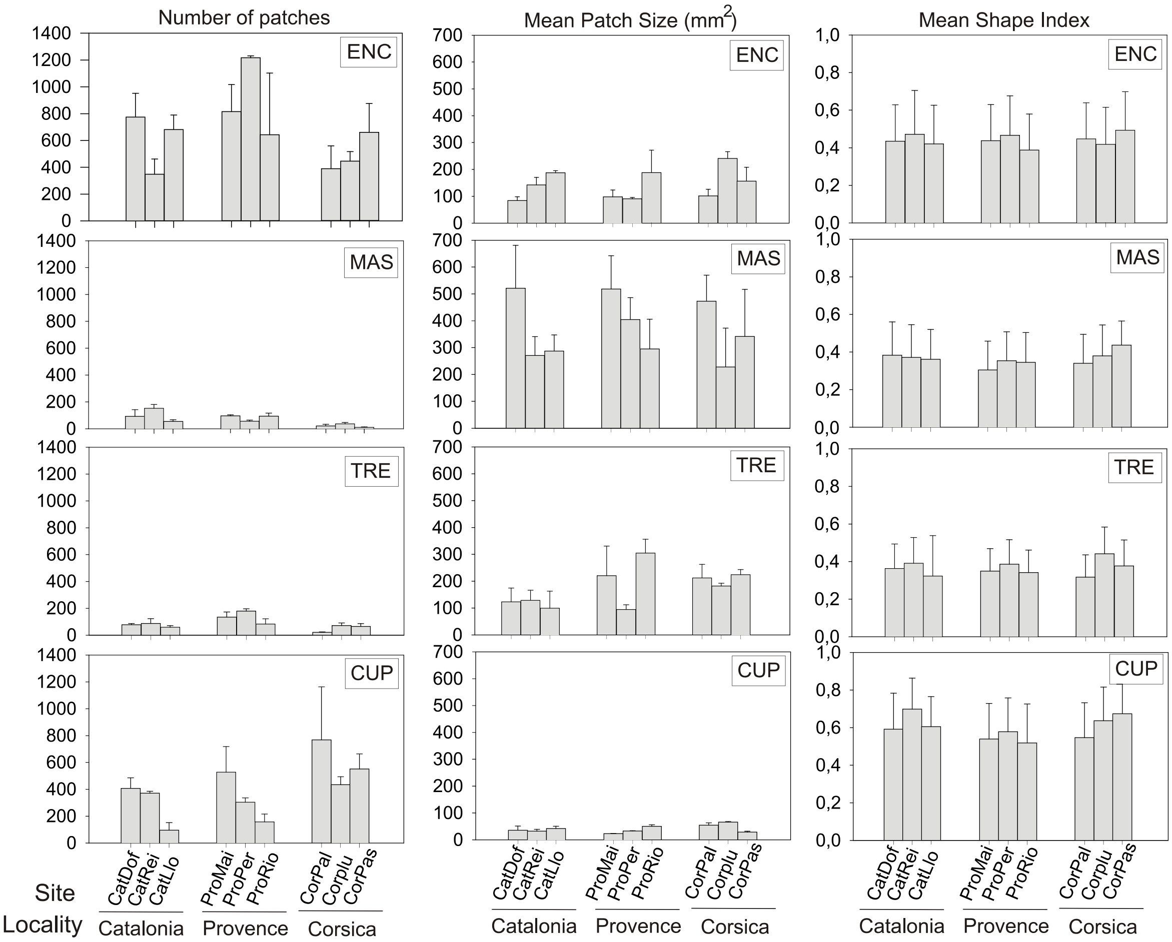


Figure 6. Number of patches, Mean patch size and Mean shape index of each invertebrate morphofunctional group at each site studied.

Table 7. Univariate PERMANOVA analyses of Number of patches (NP), Mean patch size (MPS) and Mean shape index (MSI) for each morphofunctional group at *Site* and *Locality* spatial levels.

Seascape Pattern indices	Morfofunctional group	Source of variation	*df*	SS	MS	*Pseudo-F*	*p-value*	ECV (Sq.root)
NP	Encrusting	Locality	2	9.82e5	4.91e5	2.972	0.1215	196.61
		Site (Locality)	7	1.17e6	1.67e5	3.821	0.0102	216.47
		Residual	17	7.44e5	4377			209.21
		Total	26	2.64e6				
								31.201
	Massive	Locality	2	22783	1139	3.574	0.0782	31.785
		Site (Locality)	7	22624	3232	5.671	0.0011	23.872
		Residual	17	9688	569.9			
		Total	26	56275				37.691
								45.884
	Tree	Locality	2	37520	18760	2.764	0.1355	36.513
		Site (Locality)	7	48164	880.6	5.161	0.004	
		Residual	17	22665	333.2			113.79
		Total	26					147.48
								93.13
	Cup	Locality	2	3.48e5	1.74e5	2.678	0.1442	
		Site (Locality)	7	4.61e5	65986	7.608	0.0004	
		Residual	17	1.47e5	8673.1			
		Total	26					
MPS	Encrusting	Locality	2	0.986	0.493	0.558	0.5823	-0.2154
		Site (Locality)	7	6.276	0.897	6.063	0.0014	0.5331
		Residual	17	2.514	0.148			0.3846
		Total	26	9.563				
	Massive	Locality	2	1.5170.758		0.191	0.8099	-0.617
		Site(Locality)	7	28.124.018		2.634	0.0474	0.9726
		Residual	17	25.931.523				1.2351
		Total	26	55.80				
						1.868		0.3228
	Tree	Locality	2	3.7811.891		3.375	0.221	0.5231
		Site(Locality)	7	7.1711.024			0.018	0.5509
		Residual	17	5.1600.304				
		Total	26	17.12				
						1.1048		0.0025
	Cup	Locality	2	1.1070.0053		8.5358	0.4038	0.1287
		Site(Locality)	7	0.3460.0049			0.0002	0.0076
		Residual	17	0.0100.0006				
		Total	26	0.552				
MSI	Encrusting	Locality	2	3.3e-4	1.6e-4	6.3e-2	0.9315	-1.70e-2
		Site (Locality)	7	1.8e-2	2.6e-3	2.79	0.0394	2.528e-2
		Residual	17	1.6e-2	9.4e-4			3.071e-2

	Total	26	3.5e-2				
					1.57		
Massive	Locality	2	8.1e-3	4.07e-3	2.71	0.302	1.3e-2
	Site(Locality)	6	1.8e-2	2.63e-3		0.045	2.5e-2
	Residual	18	1.6e-2	9.69e-4	0.15		3.1e-2
	Total	26	4.9e-2		6.63		
						0.8763	
Tree	Locality	2	1.38e-3	6.9e-4	1.27	0.0006	-2.13e-2
	Site(Locality)	6	3.20e-2	4.6e-3	11.49		3.84e-2
	Residual	18	1.17e-2	6.9e-4		0.3518	2.62e-2
	Total	26	4.50e-2			0.0001	
			1.78e-2	8.9e-3			1.50e-2
Cup	Locality	2	4.96e-2	7.1e-3			4.96e-2
	Site(Locality)	6	1.05e-2	6.2e-4			2.48e-2
	Residual	18	8.21e-2				
	Total	26					

4.4. Discussion

Within coralligenous outcrops, habitats dominated by red coral *Corallium rubrum* are of special interest from a conservation perspective. The main reason is because the red coral populations are highly threatened by poaching and harvesting and by the drastic effects of several mortality events putatively related with climate change (Garrabou et al. 2001; Linares et al. 2010; Montero-Serra et al. 2015). The effects of these disturbances at red coral population level have been assessed, however the effects at community level have rarely addressed.

The present study provides the first base line information at community level on diversity, structure and composition patterns of red coral dominated coralligenous outcrops highlighting habitat homogeneity at regional scale (among localities). Although, strong differences in specific composition and species abundances distributions were found within the assemblages at site level, these differences were clearly smoothed at locality level (Fig. 2 and Fig. 3). Interestingly, using the similar regional scale patterns, Casas-Güell et al. 2015 found high variability and beta diversity among sites of *Paramuricea clavata* dominated-assemblages. The processes behind these multi-scale patterns in structuring assemblages are difficult to discern, but it's accepted that species composition across localities does not appear to be determined primarily by the differences in physico-chemical conditions (Bensoussan et al. 2010) and/or the differential impact of major disturbances as mass mortality events (Garrabou et al. 2009). Instead, biological factors (growth rates, recruitment, competition, successional patterns) should be the major driver of patterns found at the site spatial level (or, alternatively, should explain most of the variability found at the site level). Species driving coralligenous outcrops generally display a limited dispersal capacity (Uriz 1998; Duran 2003; Abbiati et al. 2009; Ledoux et al. 2010b) that may shape the high heterogeneity observed at small scale. This may imply that local persistence will be enhanced once the populations are established (Costantini et al. 2007; Ledoux et al. 2010a). Our results are in agreement with other studies on coralligenous assemblages, where variability observed at small scale (replicates or patches) is considerably high (Ferdeghini et al. 2000; Balata 2006; Casas-Güell et al. 2015). Diversity values found in this study were similar (from 24 to 41 species at site level and from 57 to 72 species at locality level) than that found in other coralligenous studies dominated by Paramuricea clavata (from 23 to 42 and from 52 to 66 species at locality level) (Casas-Güell et al.

2015) but markedly higher than other studies of *Corallium rubrum* outcrops (18 species or taxonomic groups) (Virgilio et al. 2006). Coralligenous reefs may encompass a number of markedly different biogenic formations and hence, its regional diversity is relatively high compared with other assemblages (Ballesteros 2006; Kipson et al. 2011; Giakoumi et al. 2013; Martin et al. 2014). The assemblages of our study were sampled at dim light conditions at a range of deeps from 15 to 45 meters, at vertical walls, semi-dark and dark caves. In all sites and localities, perennial and long-lived macro-invertebrates were the most abundant species and within the perennial functional group, the precious red coral was the dominant canopy-forming stratum, whereas the basal stratum was dominated mainly by small cup corals and encrusting sponges (Fig. 2 and 6). These results can be contrasted with that obtained by Ponti et al. 2011 where turf and encrusting algae where the dominant groups in North Adriatic coralligenous outcrops. At regional scale, some environmental parameters, in terms of nutrient availability and dim light conditions, may structure and drive the differences detected on different studies of the same habitat but at different depths (Bonacorsi et al. 2012). Overall, we cannot discard, however that differences can also be enhanced by anthropogenic stressors affecting these assemblages at local, regional and the whole Mediterranean basin scales.

This study showed for the first time that morphofunctional groups tended toward common patterns when comparing their abundances at a range of regional spatial scales. As mentioned above, the canopy was dominated by *Corallium rubrum* whereas basal layers were generally fully covered by encrusting and massive invertebrates together with mixture-complex matrix. The encrusting sponge was the group which contained more species and the most abundant (highest % of cover) for almost all sites and localities (Fig. 2 and 3) except in Palazzu (Corsica), with high dominance of the cup coral *Leptopsammia pruvoti*. High dominance and diversity of sponges have been previously reported for coralligenous outcrops (Ponti et al. 2011; Kipson et al. 2011; Bertolino et al. 2013; Ponti et al. 2014).

Overall, our results showed high spatial complexity and diversity in the coralligenous outcrops dominated by the red coral. Different aspects of spatial patterns (patch number, size and shape) were consistently repeated for the overall set of perennial species along the sites and localities studied (Fig. 5, Table 6). The results presented here reflect that the assemblages were mainly characterized by intermediated coverage of perennial species (e.g. sponges, anthozoans, bryozoans, and tunicates), which showed high NP, intermediated MPS and with complex shapes MSI (Fig. 5). Interestingly, macro-invertebrate groups showed significant differences at site level (Fig. 6, Table 7), representing a seascape mainly composed of encrusting and cup forms with high NP. However, the biggest sizes corresponded to massive forms followed by encrusting and tree forms (Fig. 6). The MSI was quite irregular for all groups except for the cup who displayed the most regular shapes (Fig. 6). This finding may indicate that irregular forms of encrusting, tree-like, massive species were the most abundant in characterizing the assemblages and these showed more regular (circular) shapes, when coral cup form was dominant group (Fig. 6). Garrabou et al. (2002) using LPI to study spatial patterns along a depth gradient, found that coralligenous outcrops exhibited the greatest spatial pattern complexity. The authors argued that a decrease in dynamics (% of area changed) might enhance high diversity and thus complex spatial patterns. In light of the key habitat that represents coralligenous outcrops, we believe that seascape indices may be also excellent proxies to estimate the health of coralligenous outcrops (see below). For example, one of the most evident phenomena after disturbances in terrestrial and marine ecosystems is the significant changes in number, size and forms of patches (Turner et al. 2002; Teixidó et al. 2007; Wedding et al. 2011). We may predict that after a large disturbance, major shifts may be observed on these parameters, from high NP, intermediated MPS and irregular forms of well-mixed groups of invertebrates to lower values of these indices and more circular forms. However, this approach should be followed over time to avoid potential misunderstanding of the local variability (e.g. high natural abundance of the scleractinan cup forms with circular shape in Palazzu, Corsica).

Coralligenous outcrops are key habitats within the Mediterranean coastal ecosystems mainly because its high biodiversity and complex structure (Ballesteros 2006). Furthermore, coralligenous habitats dominated by the canopy-forming species Corallium rubrum are exceptionally important for conservation because their populations are highly threatened (Montero-Serra et al. 2015). The fine-scale and high resolution data presented in this study represents a step forward due to the scarcity of studies dealing with these habitats and the need of baseline information for management plans. Concretely, this data is crucial in order to establish baselines with the final objective of synthesize all the information within indices that defines the ecological state of coralligenous assemblages to fulfill the requirements of the European Marine Strategy Framework Directive (e.g. see COARSE index in Gatti et al. 2015a). We believe that biotic metrics as species diversity, growth forms and abundances measures of key species or taxonomic groups (% cover or number of colonies) are an excellent metrics to measure the health of the assemblages. However, we think that other metrics as the seascape indices presented here (NP, MPS and MSI) could be integrated at regional scale to better copse the highly complexity of these assemblages.

4.5. References

Abbiati M, Airoldi L, Costantini F, et al (2009) Spatial and temporal variation of assemblages in Mediterranean coralligneous reefs. In: UNEP-RAC/SPA.

Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58:626–639. doi: 10.1139/f01-004

Anderson MJ, Crist TO, Chase JM, et al (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol Lett 14:19–28. doi: 10.1111/j.1461-0248.2010.01552.x

Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. Ecol Lett 9:683–93. doi: 10.1111/j.1461-0248.2006.00926.x

Anderson MJ, Gorley R, Clarke K (2008) PERMANOVA + for PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK

Anderson MJ, Robinson J (2001) Permutation tests for linear models. Aust N Z J Stat 43:75–88.

Balata D (2006) Spatio-temporal variability and vertical distribution of a low rocky subtidal assemblage in the north-west Mediterranean. Estuar Coast Shelf Sci 67:553–561.

Balata D, Piazzi L, Benedetti-Cecchi L (2007) Sediment disturbance and loss of beta diversity on subtidal rocky reefs. Ecology 88:2455–61.

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr Mar Biol an Annu Rev 123–195.

Bensoussan N, Romano J-C, Harmelin J-G, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: To better understand responses of benthic communities to climate change. Estuar Coast Shelf Sci 87:431–441. doi: 10.1016/j.ecss.2010.01.008

Bertolino M, Cerrano C, Bavestrello G, et al (2013) Diversity of Porifera in the Mediterranean coralligenous accretions, with description of a new species. Zookeys 37:1–37. doi: 10.3897/zookeys.336.5139

Bonacorsi M, Pergent-Martini C, Clabaut P, Pergent G (2012) Coralligenous “atolls”: discovery of a new morphotype in the Western Mediterranean Sea. C R Biol 335:668–72. doi: 10.1016/j.crvi.2012.10.005

Boudouresque CF (2004) Marine biodiversity in the mediterranean: status of spicies, populations and communities. Sci Rep Port-Cros natl Park Fr 20:97–146.

Bramanti L, Movilla J, Guron M, et al (2013) Detrimental effects of ocean acidification on the economically important Mediterranean red coral (Corallium rubrum). Glob Chang Biol 19:1897–908. doi: 10.1111/gcb.12171

Bramanti L, Vielmini I, Rossi S, et al (2014) Demographic parameters of two populations of red coral (Corallium rubrum L. 1758) in the North Western Mediterranean. Mar Biol 161:1015–1026. doi: 10.1007/s00227-013-2383-5

Carpine C, Grasshoff M (1975) Les gorgonaires de la Mediterranee. Bull l'Institut Océanographique Monaco 71:1–140.

Casas-Güell E, Teixidó N, Garrabou J, Cebrian E (2015) Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales. Mar Biol. doi: 10.1007/s00227-015-2635-7

Cebrian E, Linares C, Marschal C, Garrabou J (2012) Exploring the effects of invasive algae on the persistence of gorgonian populations. Biol Invasions 14:2647–2656. doi: 10.1007/s10530-012-0261-6

Cebrian E, Uriz MJ (2007) Do heavy metals play an active role in sponge cell behaviour in the absence of calcium? Consequences in larval settlement. J Exp Mar Bio Ecol 346:60–65. doi: http://dx.doi.org/10.1016/j.jembe.2007.02.010

Cecchi E, Gennaro P, Piazzi L, et al (2014) Development of a new biotic index for ecological status assessment of Italian coastal waters based on coralligenous macroalgal assemblages. Eur J Phycol 49:298–312. doi: 10.1080/09670262.2014.918657

Cerrano C, Cardini U, Bianchelli S, et al (2013) Red coral extinction risk enhanced by ocean acidification. Sci Rep 3:1457. doi: 10.1038/srep01457

Clarke KR, Warwick RM (1994) Change in Marine Communities: an approach to statistical analysis and interpretation. Bourne Press Limited, Bournemouth, UK

Costantini F, Fauvelot C, Abbiati M (2007) Genetic structuring of the temperate gorgonian coral (Corallium rubrum) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. Mol Ecol 16:5168–82. doi: 10.1111/j.1365-294X.2007.03579.x

Duran S (2003) Phylogeography, gene flow and population structure of Crambe crambe (Porifera : Poecilosclerida). PhD. Thesis. Universitat de Barcelona.

Eriksson BK, Rubach A, Hillebrand H (2006) Biotic Habitat Complexity Controls Species Diversity and Nutrient Effects on Net Biomass Production. Ecology 87:246–254. doi: 10.1890/05-0090

Ferdeghini F, Acunto S, Cocito S, Cinelli F (2000) Variability at different spatial scales of a coralligenous assemblage at Giannutri Island (Tuscan Archipelago , northwest Mediterranean). Hydrobiologia 440:27–36.

Forman R, Godron M (1986) Landscape ecology. John Wiley & Sons, Ltd, New York

Garrabou J (2002) Structure and Dynamics of North-western Mediterranean Rocky Benthic Communities along a Depth Gradient. Estuar Coast Shelf Sci 55:493–508. doi: 10.1006/ecss.2001.0920

Garrabou J, Ballesteros E, Zabala M (2002) Structure and Dynamics of North-western Mediterranean Rocky Benthic Communities along a Depth Gradient. Estuar Coast Shelf Sci 55:493–508. doi: 10.1006/ecss.2001.0920

Garrabou J, Coma R, Bensoussan N, et al (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol 15:1090–1103. doi: 10.1111/j.1365-2486.2008.01823.x

Garrabou J, Harmelin J-G (2002) A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean : and management needs insights into conservation and management needs. J Anim Ecol 71:966–978.

Garrabou J, Perez T, Sartoretto S, Harmelin J (2001) Mass mortality event in red coral Corallium rubrum populations in the Provence region (France, NW Mediterranean). Mar Ecol Prog Ser 217:263–272. doi: 10.3354/meps217263

Garrabou J, Riera J, Zabala M (1998) Landscape pattern indices applied to Mediterranean subtidal rocky benthic communities. Landsc Ecol 225–247.

Gatti G, Bianchi CN, Morri C, et al (2015a) Coralligenous reefs state along anthropized coasts: Application and validation of the COARSE index, based on a rapid visual assessment (RVA) approach. Ecol Indic 52:567–576. doi: 10.1016/j.ecolind.2014.12.026

Gatti G, Bianchi CN, Parravicini V, et al (2015b) Ecological change, sliding baselines and the importance of historical data: lessons from combing observational and quantitative data on a temperate reef over 70 years. PLoS One 10:e0118581. doi: 10.1371/journal.pone.0118581

Giakoumi S, Sini M, Gerovasileiou V, et al (2013) Ecoregion-based conservation planning in the Mediterranean: dealing with large-scale heterogeneity. PLoS One 8:e76449. doi: 10.1371/journal.pone.0076449

Giannini F, Gili J, Santangelo G (2003) Relationships between the spatial distribution of red coral Corallium rubrum and coexisting suspension feeders at Medas Islands Marine Protected Area (Spain). Ital J Zool 70:233–239. doi: 10.1080/11250000309356523

Gili J-M, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–21.

Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science (80-) 265:1547–1550.

Kipson S, Fourt M, Teixidó N, et al (2011) Rapid Biodiversity Assessment and Monitoring Method for Highly Diverse Benthic Communities: A Case Study of Mediterranean Coralligenous Outcrops. PLoS One 6:e27103. doi: 10.1371/journal.pone.0027103

Laubier L (1966) Le coralligène des Albères: monographie biocénotique. Ann Inst Océanogr Monaco 43:139–316.

Ledoux J-B, Garrabou J, Bianchimani O, et al (2010a) Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, Corallium rubrum. Mol Ecol 4204–4216. doi: 10.1111/j.1365-294X.2010.04814.x

Ledoux J-B, Mokhtar-Jamaï K, Roby C, et al (2010b) Genetic survey of shallow populations of the Mediterranean red coral [Corallium rubrum (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. Mol Ecol 19:675–90. doi: 10.1111/j.1365-294X.2009.04516.x

Levin LA, Etter RJ, Rex MA, et al (2001) Environmental influences on regional deep-sea species diversity. Annu Rev Ecol Syst 32:51–93.

Linares C, Bianchimani O, Torrents O, et al (2010) Marine Protected Areas and the conservation of long-lived marine invertebrates: the Mediterranean red coral. Mar Ecol Prog Ser 402:69–79. doi: 10.3354/meps08436

Magurran AE, Baillie SR, Buckland ST, et al (2010) Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. Trends Ecol Evol 25:574–82. doi: 10.1016/j.tree.2010.06.016

Martin CS, Giannoulaki M, De Leo F, et al (2014) Coralligenous and maërl habitats: predictive modelling to identify their spatial distributions across the Mediterranean Sea. Sci Rep 1–8. doi: 10.1038/srep05073

Montero-Serra I, Linares C, García M, et al (2015) Harvesting effects, recovery mechanisms, and management strategies for a long-lived and structural precious coral. PLoS One 10:e0117250. doi: 10.1371/journal.pone.0117250

Pérès JM, Picard J (1964) Nouveau Manuel de Bionome benthique de la Mer Mediterranee. Recl des Trav la Stn Mar l'Endoume 47:5–137.

Piazzi L, Balata D, Cecchi E, et al (2010) Species composition and patterns of diversity of macroalgal coralligenous assemblages in the north-western Mediterranean Sea. J Nat Hist 44:1–22. doi: 10.1080/00222930903377547

Piazzi L, Balata D, Pertusati M, Cinelli F (2004) Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substratum inclination. Bot Mar 47:105–115. doi: 10.1515/BOT.2004.010

Piazzi L, Gennaro P, Balata D (2012) Threats to macroalgal coralligenous assemblages in the Mediterranean Sea. Mar Pollut Bull 64:2623–9. doi: 10.1016/j.marpolbul.2012.07.027

Ponti M, Fava F, Abbiati M (2011) Spatial–temporal variability of epibenthic assemblages on subtidal biogenic reefs in the northern Adriatic Sea. Mar Biol 158:1447–1459. doi: 10.1007/s00227-011-1661-3

Ponti M, Perlini RA, Ventra V, et al (2014) Ecological shifts in mediterranean coralligenous assemblages related to gorgonian forest loss. PLoS One 9:e102782. doi: 10.1371/journal.pone.0102782

Ros J, Romero J, Ballesteros E, Gili J-M (1985) Chapter 8. The benthos. In: Diving in the blue water.

Teixidó N, Albajes-Eizagirre A, Bolbo D, et al (2011a) Hierarchical segmentation-based software for cover classification analyses of seabed images (Seascape). Mar Ecol Prog Ser 431:45–53. doi: 10.3354/meps09127

Teixidó N, Casas E, Cebrian E, et al (2013) Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm. PLoS One 8:e53742. doi: 10.1371/journal.pone.0053742

Teixidó N, Garrabou J, Arntz WE (2002) Spatial pattern quantification of Antarctic benthic communities using landscape indices. Mar Ecol Prog Ser 242:1–14.

Teixidó N, Garrabou J, Gutt J, Arntz WE (2007) Iceberg disturbance and successional spatial patterns: the case of the shelf Antarctic benthic communities. Ecosystems 10:143–158. doi: 10.1007/s10021-006-9012-9

Teixidó N, Garrabou J, Harmelin J-G (2011b) Low dynamics, high longevity and persistence of sessile structural species dwelling on Mediterranean coralligenous outcrops. PLoS One 6:e23744. doi: 10.1371/journal.pone.0023744

Tsounis G, Rossi S, Grigg R, et al (2010) The Exploitation and Conservation of Precious Corals. Oceanogr Mar Biol an Annu Rev 161–212.

Turner MG (2008) Landscape Ecology: The Effect of Pattern on Process. Annu Rev Ecol Syst 20:171–197.

Turner MG, Tinker DBT, Gergel SEG, Chapin FS (2002) Landscape disturbance Location, pattern, and dynamics. In: Turner SEG and MG (ed) Learning landscape ecology: a practical guide to concepts and techniques. Springer-Verlag, New York, pp 147–165

Uriz M-J (1998) How do reproductive output, larval behaviour, and recruitment contribute to adult spatial patterns in Mediterranean encrusting sponges ? Mar Ecol Prog Ser 167:137–148.

Valls A, Coll M, Christensen V (2015) Keystone species: toward an operational concept for marine biodiversity conservation. Ecol Monogr 85:29–47.

Virgilio M, Airoldi L, Abbiati M (2006) Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. Coral Reefs 25:265–272. doi: 10.1007/s00338-006-0100-2

Wedding L, Lepczyk C, Pittman S, et al (2011) Quantifying seascape structure: extending terrestrial spatial pattern metrics to the marine realm. Mar Ecol Prog Ser 427:219–232. doi: 10.3354/meps09119

Weinberg S (1978) Mediterranean octocorallian communities and the abiotic environment. Mar Biol 49:41–57. doi: 10.1007/BF00390729

5. Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales

Casas-Güell E, Teixidó N, Garrabou J, Cebrian E (2015) Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales. **Marine Biology** Volume 162, Issue 4, pp 901-912.

Abstract

Coralligenous outcrops rank among the most important ecosystems in the Mediterranean Sea, primarily because of their biodiversity. Information on the spatial and temporal variability of the composition and structure of coralligenous assemblages is essentially lacking for most regions. We explored the variability of the biodiversity patterns of coralligenous outcrops dominated by the red gorgonian *Paramuricea clavata* in the NW Mediterranean region using a hierarchical sampling design. The study addressed two overlooked spatial and temporal scales: from 1 to > 100 km and a 5-year period. Overall, no temporal changes were detected in either species composition or the 12 morphofunctional groups considered. Significant differences in species composition were found at the various spatial scales. However, variation in composition at the locality level (>100 km apart) showed the lowest values in comparison with the differences found at the site level (<1 km apart). Despite the differences, the sites displayed high similarity (average similarity 55.7%) and shared approximately 50% of the species. Similarly, the patterns of diversity at different scales, the alpha (site) and gamma (locality) were consistent with the specific composition trends, whereas the beta diversity showed the greatest differences among sites. Our results demonstrate the moderate spatial variability in biodiversity in the NW Mediterranean region and an extremely low temporal variability. This study provides baselines for detecting potential effects due to global change, and it furnishes a basis for the implementation of monitoring schemes of coralligenous assemblages. The development of similar sampling schemes in other Mediterranean regions will provide a global view of the biodiversity of coralligenous outcrops.

Key-words: Biodiversity, *Paramuricea clavata,* coralligenous, NW Mediterranean region, spatial and temporal scales, conservation.

5.1. Introduction

Subtidal reefs foster a high biodiversity of animal and plant species (Knowlton and Jackson 2001; Witman and Dayton 2001), representing one of the most productive habitats in marine ecosystems and offering substantial commercial, recreational and cultural value to society. During recent decades, however, an increasing proportion of the world's reefs have been reported to be severely impacted (Halpern et al. 2008; Crain et al. 2009; Jackson 2010). From a conservation perspective, the collection of data on species composition and assemblage structure over a variety of spatial scales is indispensable for understanding the variation of biodiversity at local and regional scales (Lourie and Vincent 2004). Additionally, these baseline datasets are valuable for assessing changes associated with several anthropogenic threats and the outcome of management actions.

Knowing and understanding patterns of variability of benthic assemblages at a range of spatial and temporal scales are crucial for effective management and conservation of coastal marine habitats (Ferdeghini et al. 2000; Piazzi et al. 2004; Virgilio et al. 2006). This is even more relevant in key habitats such as the coralligenous outcrops, which are considered one of the most important ecosystems in the Mediterranean Sea (Ballesteros 2006; Piazzi et al. 2012). Coralligenous outcrops have been identified as Mediterranean priority habitats by the EU Habitats Directive (92/43/CE) and foster some of the richest assemblages found in Mediterranean, harboring approximately 10% of marine Mediterranean species (Pérès and Picard 1964; Laubier 1966; Ros et al. 1985; Boudouresque 2004; Ballesteros 2006). The species that characterize such assemblages belong to various taxonomic groups, primarily encrusting calcareous algae, sponges, cnidarians, polychaetes, bryozoans and tunicates (Garrabou et al. 2002; Ballesteros 2006). Most of these species are long-lived and exhibit low dynamics, making them vulnerable to various types of threats (Garrabou et al. 1998; Teixidó et al. 2011). In fact, many coralligenous outcrops have already been affected by various stressors such as nutrient enrichment, overexploitation, invasive species, increase of sedimentation, mechanical impacts as well as climate change, resulting in dramatic consequences for many species and even for the structure of the whole assemblages (Balata et al. 2007; Garrabou et al. 2009; Piazzi and Balata 2011; Cebrian et al. 2012; Teixidó et al. 2013; Cecchi et al. 2014). However, much of our understanding of the composition and structure of coralligenous outcrops is still limited and based mainly on descriptive studies restricted to smaller or medium spatial scales - from a few meters to a few km (10s of km) (reviews by Ballesteros 2006, Giaccone 2007, Casellato & Stefanon 2008 and references therein) and some experimental studies (e.g. Balata et al. 2007; Gennaro and Piazzi 2011). To our knowledge, few studies addressing coralligenous assemblages have extended to larger spatial scales (up to 200 km of coastline), and mostly of them focused on the phytobenthic component (Piazzi et al. 2004; Piazzi et al. 2010). Thus, the structure of coralligenous outcrops has been poorly understood because there are no spatio-temporal studies or baseline data at the assemblage level over large scales. This situation has prevented a proper assessment of the current state of biodiversity and future changes in this valuable assemblage. Difficulties related to the study of these highly diverse and structurally complex systems, coupled with general logistic constraints related to the sampling of intermediate-deep rocky habitats, have hindered progress in our knowledge of this topic (Parravicini et al. 2010).

The aim of this study was to quantify the variability of the structure and dynamics of coralligenous assemblages dominated by the red gorgonian *Paramuricea clavata* across different localities in the NW Mediterranean separated by hundreds of km and over a 5-year period. The long-lived gorgonian *P. clavata* is considered a key species in Mediterranean coralligenous assemblages (Gili & Coma 1998), provides biomass and biogenic substrata that significantly shapes the assemblage (Harmelin & Marinopoulos 1994; Gili & Coma 1998). It contributes greatly to the aesthetic value of the Mediterranean sublittoral seascape (Bianchi et al. 1995) and attracts high numbers of recreational divers (Coma et al. 2004). We assessed structural complexity in terms of the specific and morphofunctional composition of the sessile perennial species associated to *P. clavata*. Additionally, we assessed various components of species diversity (alpha, beta, and gamma diversity) over time. The ultimate goal of this study is to provide assemblage structure baseline data to furnish a solid basis for comparisons in terms of potential changes in species composition changes in response to multiple threats.

5.2. Materials and methods

Study sites and sampling design

A hierarchical sampling design was used to assess spatial and temporal patterns of coralligenous assemblages dominated by the red gorgonian *Paramuricea clavata*. Three localities were selected in the NW Mediterranean region (Catalonia, Provence and Corsica), covering more than 400 Km (5º E-W) and more than 200 Km (1º N-S) of the coastline (Fig. 1). All localities were located in marine protected areas. At each locality, two sites (separated by approximately 1 km) were sampled: in Catalonia, Tascó Petit (MPT) and Medallot (MME), located in the Natural Park of Montgrí, Medes Islands and Baix Ter; in Provence, Petit Conglué (PCO) and Plane-Grotte Pérès (PGP), located in Riou Archipelago in the National Park of Calanques; and in Corsica, Palazzu (SPA) and Gargallu (SGL), located in the Scandola Natural Reserve (Fig. 1).

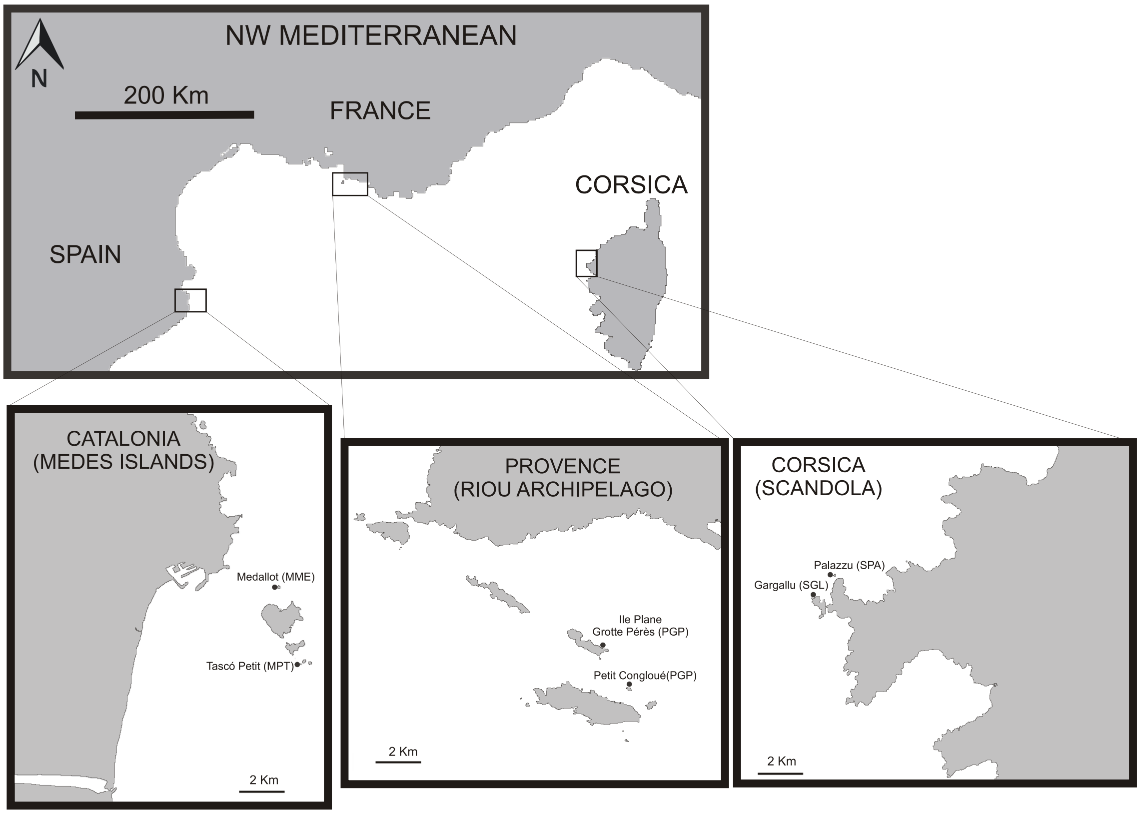


Figure 1. Map showing the Mediterranean geographic area where the photographic surveys were conducted: region, localities and sites.

At each site between depths of 17-25 m, one or two permanent plots were setup to cover a total area of approximately 4 m2. The plots were photographically sampled during 5 years (2006-2010) using 25 × 25 cm (625 cm2) to facilitate species identification. For this study only 3 years were considered within a 5-year period. From each site and year considered, 3 different groups of 8 contiguous quadrats of 25 × 25 cm (625 cm^2) that corresponded to the minimum sampling area (hereafter MSA; 8 quadrats = 5000 cm^2, for more information see (Kipson et al. 2011) were randomly chosen within the permanent plots, resulting in a total of 432 photographs (24 photos × 6 sites × 3 years). The photographs were taken with a Nikon D70S digital SLR camera fitted with a Nikkor 20 mm DX lens (3000 × 2000 pixel resolutions) and housed in a Subal D70S housing. Lighting was achieved by two electronic strobes fitted with diffusers. Specimens were also collected in order to facilitate species identification by photographic samples. Photographic sampling was chosen as it provides a good compromise between optimal resolution (Bohnsack 1979; Foster et al. 1991; Deter et al. 2012) and conservation of the habitat, although could probably underestimate small and cryptic species. This study did not involve sampling or damage of any endangered or protected species. Furthermore, the non-destructive methodological approach obtaining biodiversity data through the analysis of images is suitable to study marine benthic communities in Marine Protected Areas.

Analysis of photographs

From each photograph, sessile macrobenthic species were identified at the lowest taxonomic level (whenever possible). The species lists were crosschecked with the World Register of Marine Species database (Worms, available at: http://www.marinespecies.org). Sessile macrobenthic species were classified into 12 morpho-functional groups based on their taxonomy and growth form (3 seasonal and 9 perennial groups) (see Table 1) (adapted from Garrabou et al. 2002 and Teixidó et al. 2011).

We calculated (1) the percentage of species belonging to the various morphofunctional groups found in each site and year and (2) a semi-quantitative abundance for each morphofunctional group estimated from the species presence/absence data in each of the MSA (group of 8 contiguous 25 × 25 cm photographs) sampled at each site. For instance, a presence in only one photograph corresponded to 12.5% of cover [(1 presence / 8 photographs) × 100]. Finally, the values for each MSA were averaged to obtain site estimates. We focused on perennial species for specific composition and diversity indices analyses (see below).

Table 1. Biological categories used in this study combining taxonomy, life span and morphological growth forms (adapted from Garrabou et al. 2002 and Teixidó et al. 2011).

Biological categories		Description
1-	Seasonal algal turf	Annual erect or semi-erect fleshy algal species, with one or multiple zones of attachment to the substratum; generally constitutes algal cushions or thin sheets with mixtures of algal species.
2-	Seasonal animal turf	Small seasonal animal species, mainly bryozoans and hydrozoans; usually is forming animal cushions or thin sheets with mixtures species.
3-	Seasonal mixture complex turf	Small seasonal algae and animal species (mainly bryozoans and hydrozoans), sediment, detritus and fragments; normally forming cushions or thin sheets with mixtures of species.
4-	Perennial algal encrusting	Species growing mainly as two dimensional sheets; more or less completely attached to the substratum.
5-	Perennial algal erect	Species attached to the substratum usually with a unique zone (visible even in winter) of basal attachment to the substratum.
6-	Perennial algal turf – invasive	Perennial dense thick filamentous turf algae with the ability to maintain permanent carpets (e.g. the invasive species *Womersleyella setacea*).
7-	Perennial animal encrusting	Species of sponges, cnidarians, bryozoans and tunicates growing as two dimensional sheets; more or less completely attached to the substratum.
8-	Perennial animal massive	Mound species of sponges and cnidarians with vertical and lateral growth; normally attached to the substratum all along their basal area.
9-	Perennial animal tree	Erect species of cnidarians and bryozoans, more or less branched; usually with a single point of attachment to the substratum.
10-	Perennial animal cup	Solitary corals attached to the substratum all along their basal area.
11-	Perennial animal boring	Excavating organisms living into the rock (e.g. *Cliona viridis*).
12-	Perennial animal epibiont	Species growing over other invertebrates or calcareous algae (mainly polychaetes e.g. *Salmacina dysteri* or *Filograna implexa* and bryozoans e.g. *Chartella tenella*).

Statistical analyses

Presence/absence specific data were analyzed by a non-metric multi-dimensional scaling (nMDS) ordination analysis to visualize spatial and temporal patterns based on the Bray-Curtis similarity measure. Furthermore, a similarity percentage procedure analysis (SIMPER, Clarke and Warwick 1994) was performed to identify the percentage contribution of taxa and morphological groups to the significant dissimilarities among sites and localities.

Species composition, morphofunctional groups and diversity indices were analyzed by a nonparametric analysis of variance, PERMANOVA (Anderson 2001a; Anderson 2001b; Anderson et al. 2008), applied using Bray-Curtis and Euclidean distances for multivariate and univariate analyses, respectively. The model included 3 factors: Location “Lo” (random factor, 3 levels), Site “Si” (random factor, 2 levels, nested in Location), and Time (random factor, 3 levels, crossed to Location and Site).

Spatial and temporal patterns of diversity measures were assessed by quantifying alpha diversity (average number of species per sampling unit), gamma diversity (the total number of species within a locality), and beta diversity (multivariate distance between group-centroids determined with the PERMDISP procedure). PERMDISP is an approach used to compare the degree of sample dispersion of different groups based on a distance matrix. When PERMDISP is used on a Jaccard distance presence/absence matrix, it is directly interpretable as a test for similarity in beta diversity among groups (Anderson et al. 2006; Anderson et al. 2011).

The PERMANOVA and PERMDISP analyses were computed using the program Primer v6 with the PERMANOVA + add-on package.

5.3. Results

Species composition

A total of 106 macrobenthic taxa were identified across the region studied: 13 macroalgae, 1 protozoan, 42 sponges, 3 hydrozoans, 10 anthozoans, 1 mollusk, 4 polychaetes, 22 bryozoans and 10 tunicates (see A.3.1 for the species list and the assignment to the various morphofunctional groups). Of these taxa, 88 were perennial and 18 seasonal.

Structural complexity - morphofunctional groups

Overall, the structure of morphofunctional groups showed a consistent pattern at spatial and temporal scales in terms of either percentages of species per group or their abundance (Figs. 2 and 3). The principal group that characterized the outcrops in terms of species was the *Animal encrusting*, with values of approximately 30 to 40% of the species found in each site, followed by *Animal massive* and *Animal tree*, which encompassed 10 to 20% of the species in most of the sites. The remaining categories, *Algal encrusting*, *Algal erect*, *Animal cup* and others, represented approximately 0-10% of species (Fig. 2).

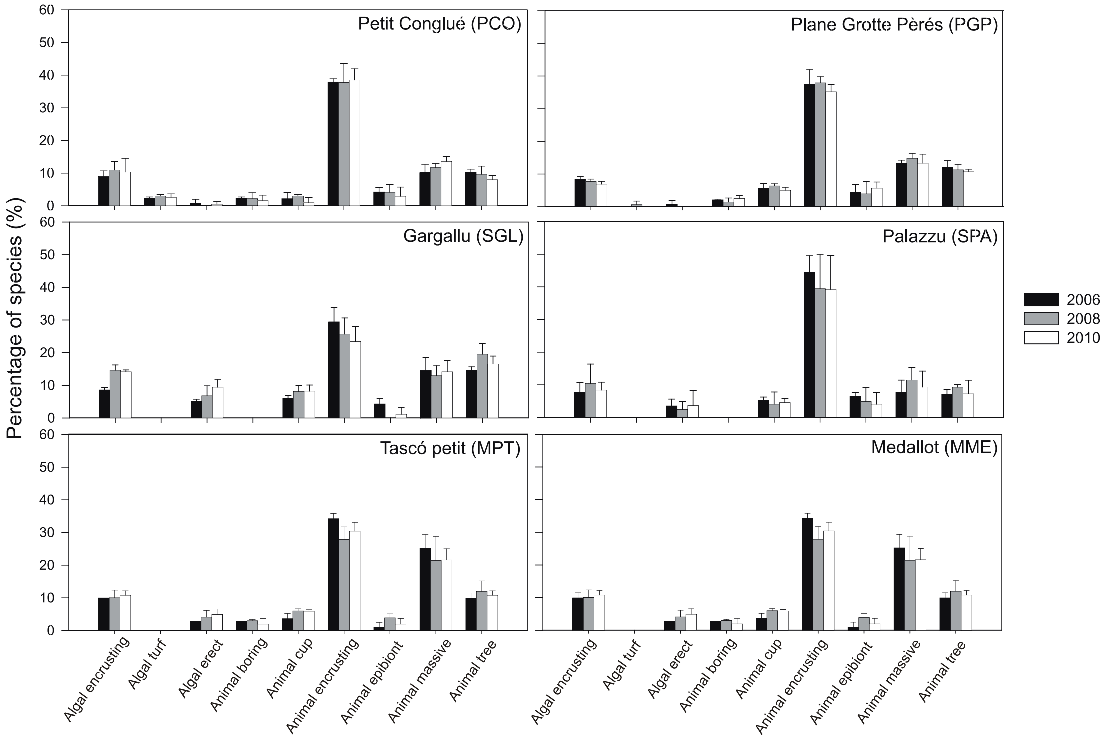


Figure 2. Percentage of perennial species relative to morphological growth forms for each site and year studied.

The morphofunctional groups displaying the highest constant abundances (> 50% of cover) at all sites and over time were *Algal encrusting*, *Animal encrusting*, *Animal massive* and *Animal tree* (Fig. 3). *Animal cup* and *Animal boring* showed moderate to high abundances (ranging between 20 to 100% cover), with a degree of temporal variability depending on the site (Fig. 3). Finally, the categories *Algal turf*, *Algae erect* and *Animal epibionts* exhibited a higher degree of variability (ranging from 0 to 100%).

Table 2. PERMANOVA analysis testing the significance level of the different factors for the abundance of the different perennial morphofunctional groups.

Source of variation	df	SS	MS	Pseudo-F	P(perm)
Locality	2	3420.6	1710.3	1.2566	0.3514
Site (Locality)	3	4077.8	1359.3	13.51	0.0001
Time	2	103.26	51.631	0.63095	0.6842
Locality * Time	4	327.32	81.831	0.81334	0.6058
Site(Locality) * Time	6	603.67	100.61	1.129	0.3498
Residual	36	3208.3	89.119		
Total	53	117.1			

The abundance of the different morphofunctional groups was similar among localities ($F_{2,53} = 1.19$, $p>0.05$, Table 2.2.2) and time ($F_{3,53} = 1.94$, $p>0.05$, Table 2.2.2) but showed differences among sites ($F_{3,53} = 17.49$, $p<0.05$, Table 2). Variability among sites correspond to a comprehensive variability in the contribution of *Algal* and *Animal encrusting*, *Animal Massive, Animal cup* and *Animal Tree* within sites except for PCO in Provence, where exceptional high abundances (up to 100%) of *Algal turf* were present (Fig. 3).

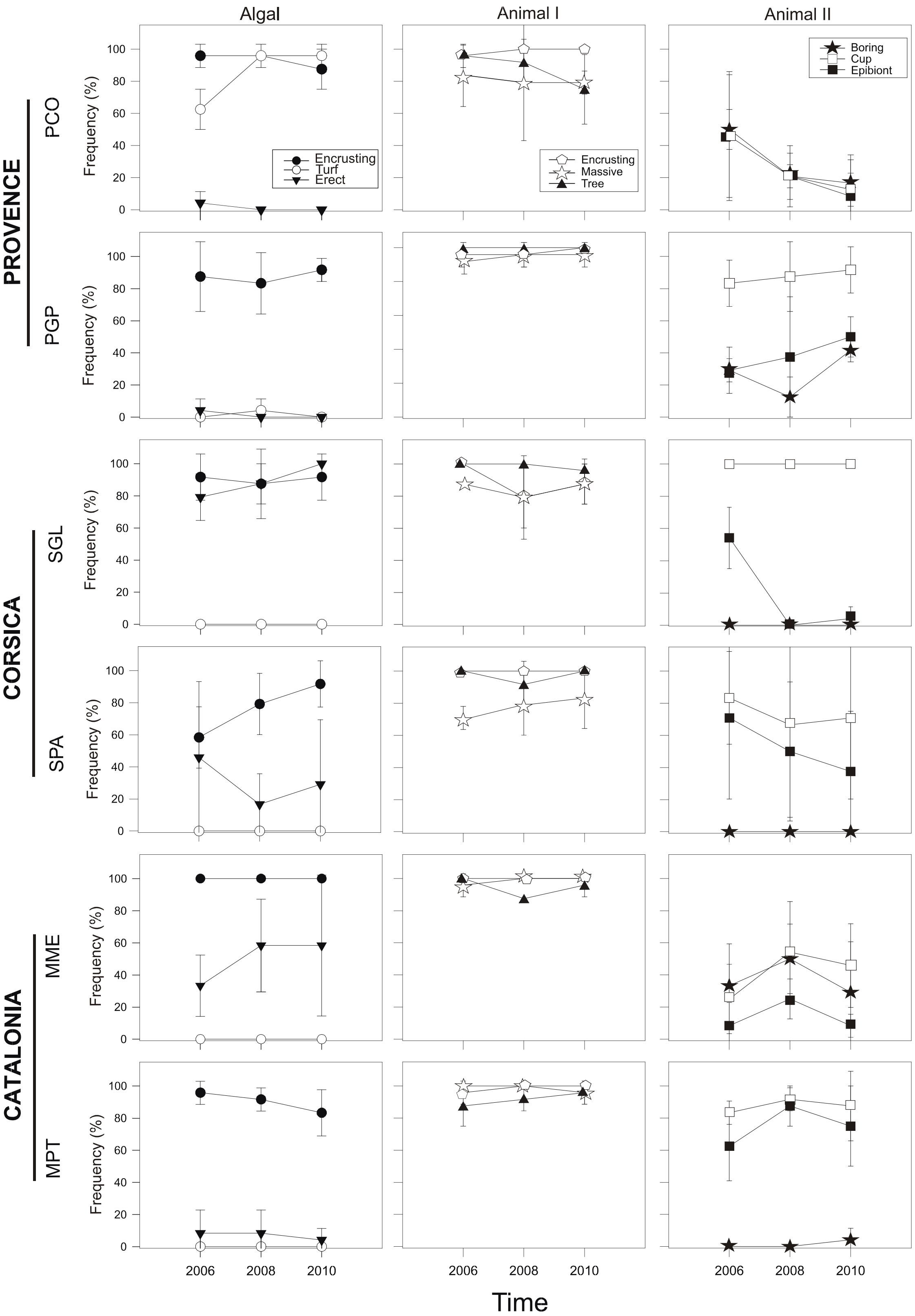


Figure 3. Abundance (expressed as %) of each morphofunctional group for each site and year studied.

Structural complexity – specific composition

The specific composition of the perennial macrobenthic species was constant over time for all localities and sites (Fig. 4, Table 3) but differed significantly among localities and sites (Table 3). The *Locality* and *Site* nested in *Locality* factors explained a high percentage of the variation with 18.49% and 20.56%, respectively (Table 3). Variability among replicate units explained the 20.65% indicating a high spatial variability at the smallest (replicate) scale.

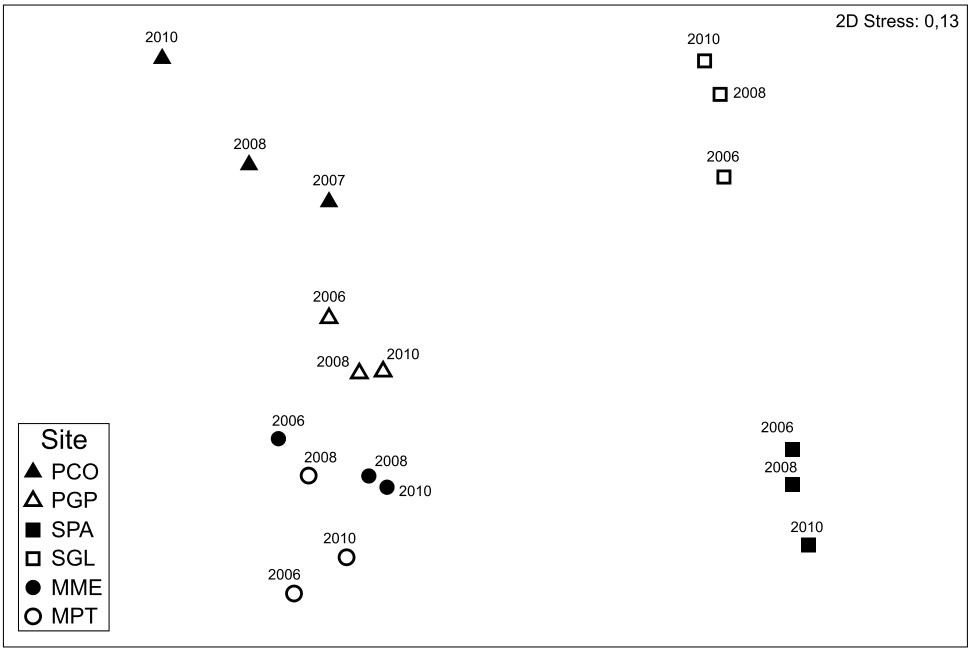


Figure 4. Non-metric multidimensional scaling (nMDS) ordination plot of macrobenthic species in the three regions of the NW Mediterranean Sea over time (2006, 2008 and 2010). Analysis performed on Bray-Curtis dissimilarities of presence-absence data. For each locality, the two sites are shown by open or closed dots (orange = Catalonia; dark = Provence; green = Corsica). See “Materials and Methods” for site abbreviations.

Parallel to these results, the SIMPER analysis showed an overall average similarity in specific composition, up to 55.74% across localities and time. The number of species contributing to 50% of the similarity for each site ranged between 10 and 16 species (Table 4). Overall, 33 different species contributed to the similarity for each site. Most of the species belonged to the morphofunctional groups displaying the highest number of species and abundance, i.e., *Algae encrusting*, *Animal encrusting*, *Animal massive* and *Animal tree* (Table 4). For these groups, certain species played determining roles at most sites, such as *Mesophyllum alternans* and *Peysonnelia* sp. for *Encrusting* *algae*; *Parazoanthus axinellae*, *Crambe crambe* and Serpulidae for *Animal encrusting*, *Paramuricea clavata* for *Animal tree* and *Leptopsammia pruvoti* for *Animal cup*.

Table 3. PERMANOVA analysis testing the significance level of the different factors in the specific composition on the basis of Bray-Curtis dissimilarity measure.

Source	df	SS	MS	Pseudo-*F*	*P*(perm)	ECV (Sq. root)
Locality	2	20802	10401	2.3016	0.0088	18.491
Site (Locality)	3	12855	4284.9	8.8866	0.0001	20.555
Time	2	1410.3	705.14	1.5897	0.1937	3.8121
Locality * Time	4	1774.3	443.56	0.9199	0.5714	-2.5368
Site(Locality) * Time	6	2893.1	482.18	1.1304	0.2797	-4.3066
Res	36	15355	426.54			20.653
Total	53	55089				

Table 4. Species by morpho-functional groups contributing more than 50% to the similarity of studied sites. The underlined species are those contributing to the similarity of most of the sites.

	Provence		Corsica		Catalonia	
	PCO	*PGP*	*SPA*	*SGL*	*MME*	*MPT*
Algal encrusting	*Lithophyllum* spp. *Palmophyllum crassum* *Mesophyllum alternans* Peyssonnelia sp.	*Mesophyllum alternans* *Peyssonnelia* sp.	*Mesophyllum alternans* *Peyssonnelia* sp.	*Lithophyllum* spp. *Mesophyllum alternans* *Peyssonnelia* sp.	*Mesophyllum alternans* *Peyssonnelia* sp.	*Mesophyllum alternans* *Peyssonnelia sp.*
Algal erect		-	-	*Flabellia petiolata*	-	-
Algal turf	*Womersleyella_type*		-	-	-	-
Animal encrusting	*Parazoanthus axinellae* *Crambe crambe*	*Crella pulvinar* *Rhynchozoon sp.* *Haliclona mucosa* *Oscarella sp.* *Pleraplysilla spinifera* *Crambe crambe* Serpulidae	*Cacospongia sp.* *Crella pulvinar* *Miniacina miniacea* Serpulidae	Serpulidae	*Pleraplysilla spinifera* *Parazoanthus axinellae* *Crambe crambe* Serpulidae	*Oscarella sp.* *Dictyonella sp.* *Parazoanthus axinellae* *Crambe crambe*
Animal massive	*Axinella damicornis*	*Acanthella acuta* *Agelas oroides* *Axinella damicornis*	*Ircinia variabilis* *Spongia officinalis*	-	*Agelas oroïdes* *Axinella damicornis* *Cystodites dellechiajei*	*Acanthella acuta* *Axinella damicornis* *Cystodites dellechiajei*
Animal tree	*Eunicella cavolinii* *Paramuricea clavata*	*Adeonella/Smittina* *Paramuricea clavata*	*Reteporella grimaldii* *Paramuricea clavata*	*Myriapora truncata* *Adeonella/Smittina* *Paramuricea clavata*	*Margaretta cereoides* *Adeonella/Smittina* *Paramuricea clavata*	*Adeonella/Smittina* *Paramuricea clavata*
Animal cup	-	*Caryophyllia inornata* *Leptopsammia pruvoti*	*Leptopsammia pruvoti*	*Caryophyllia inornata* *Leptopsammia pruvoti*	*Leptopsammia pruvoti*	*Leptopsammia pruvoti*
Animal epibiont	-	-	-	-	-	*Chartella* sp.

Diversity indices

Alpha diversity (mean ± SD) remained constant over time at almost all the studied sites (Fig. 5) and similar for all localities (Table 5), but it showed significant differences among sites (Table 5). The values of alpha diversity ranged from 23 ± 2 species in PCO to 42 ± 3 species in PGP. A constant pattern of beta diversity was found over time (Fig. 5; Table 5b). The sites located in Catalonia showed lower mean values (ranged from 12.7% ± 0.6 to 15.7% ± 0.8) compared with the sites in Corsica (ranged from 13.2% ± 0.8 to 23.3% ± 3.0) and in Provence (ranged from 16.0% ± 0.5 to 20.8% ± 2.6) (Table 5). Gamma diversity showed similar and constant values among the three localities studied (Fig. 5; Appendix A.3.2).

Table 5. Univariate tests based on Euclidean distances for alpha and gamma diversities and PERMDISP test for beta diversity.

a) Alpha diversity (PERMANOVA)

Source	df	SS	MS	Pseudo-*F*	*P*(perm)
Locality	2	337.04	168.52	0.7275	0.6453
Site (Locality)	3	725.50	241.83	8.0018	0.0180
Time	2	306.81	153.41	4.8931	0.0922
Locality × Time	4	125.41	31.352	1.0374	0.4588
Site(Locality) × Time	6	181.33	30.222	1.753	0.1409
Residual	36	620.67	17.241		
Total	53	2296.8			

b) Beta diversity (PERMDISP)

Source	df1	df2	F	P
Locality	2	51	11.4792	0.0005
Site	5	48	6.7646	0.0001
Time	2	51	4.8931	0.4123

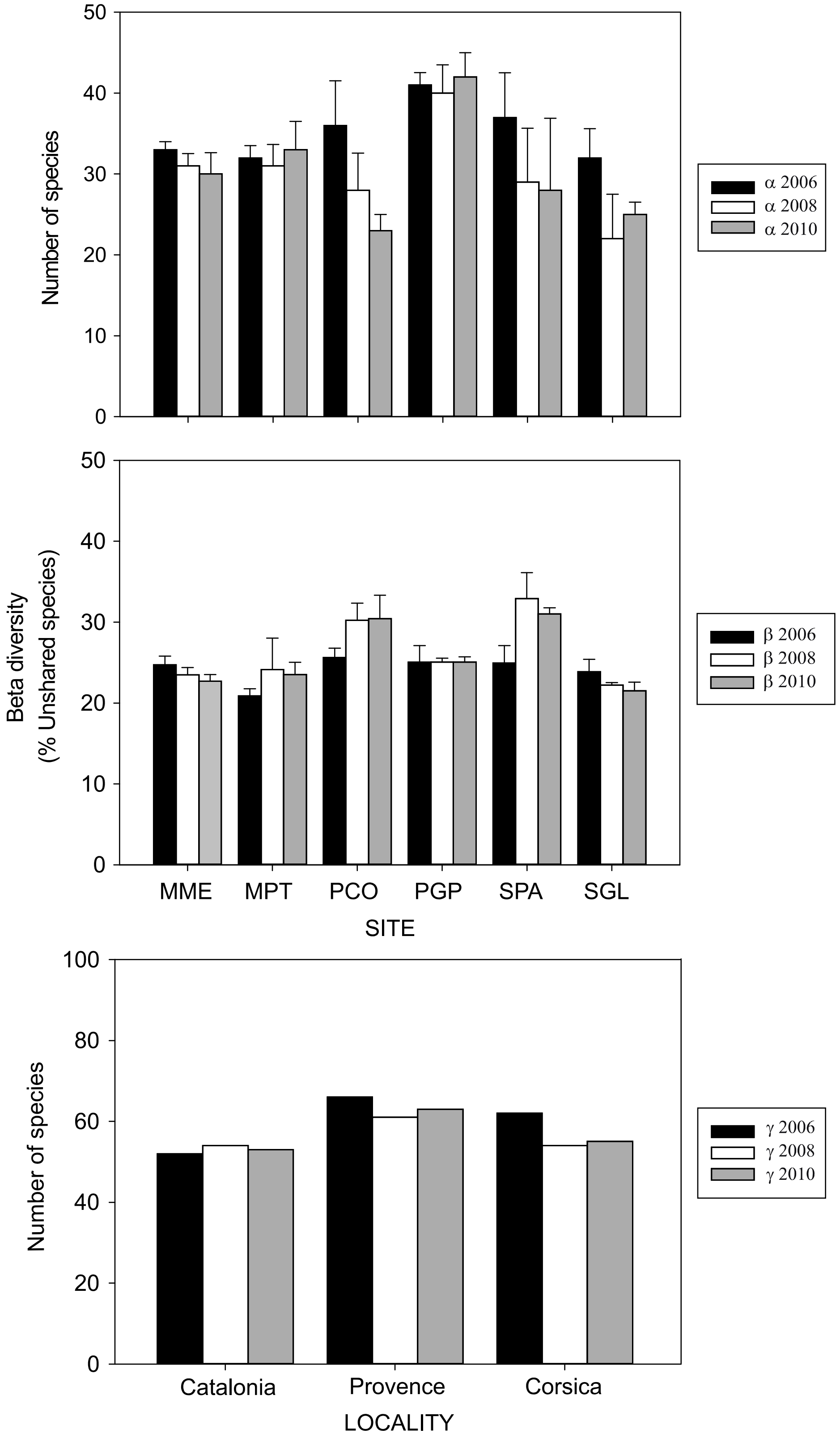


Figure 5. Diversity metrics (α-diversity, β-diversity and γ-diversity) over time.

5.4. Discussion

Studies addressing spatial and temporal patterns of biodiversity are urgently needed to identify the most effective monitoring schemes for in situ conservation and sustainable use (Gaston 2000). In assemblages dominated by long-lived assemblages (such as the coralligenous ones) the challenge is even greater since declining or recovery trajectories are more difficult to detect due to the slow pace of their population dynamics (Hughes et al. 2013). In this context, large scale long-term biodiversity datasets are a basic resource that furnishes the essential tools to provide information to promote sound conservation actions (Magurran et al. 2010). The present study provides the first baseline information on temporal and spatial patterns of coralligenous assemblages, highlighting no temporal changes in the patterns in the different components of biodiversity over the study period and most of the spatial variation in species composition among sites distant appreciatively 1 km.

Interestingly, the morphofunctional groups tended toward common patterns at spatial and temporal scales. This result supports the hypothesis that coralligenous outcrops dominated by the red gorgonian *P. clavata*, despite differences in species composition, displayed similar structural complexity, at least within the NW Mediterranean. This common composition of the assemblages was complemented by the abundance of other morphofunctional groups, which displayed a greater abundance and/or a higher degree of temporal variability such as *Algal turf*, *Algae erect* and *Animal epibionts.* In fact, these groups could be related to dysfunctions in the assemblages since most are characterized by faster growth rates (especially in comparison with other perennial species included in the other groups). For instance *algal turf* abundance resulted from a recent introduction, such as the *Womersleyella*-type, which only showed high abundance at one site (Petit Conglué), highlights the suitability and interest of using the considered morphofunctional groups.

The present study found differences in specific composition among the three localities studied (see below). However, it is important to note the relatively high similarity found among them (overall average similarity 55.7%). In addition, 51% of the perennial species were always found at the three localities (see Appendix A.3.1). Interestingly, the greatest variability was found at the Site and Replicate level. Other studies in coralligenous assemblages have also found the highest variability at the smallest scales investigated (e.g. among replicates) (Ferdeghini et al. 2000; Virgilio et al. 2006; Terlizzi et al. 2007; Abbiati et al. 2009; Ponti et al. 2011). Similarly, patterns of diversity at different scales, the alpha (site) and gamma (locality) values were congruent with the specific composition trends.

In short, significant differences in species composition were found at the different spatial scales analyzed. However, variation in composition at the large scale considered here (at the locality level) showed the lowest values compared with the differences found at the small scale. This general finding was also consistent with the beta diversity values, where the highest differences were found among sites. The processes behind these multi-scale patterns in assemblages are difficult to discern. In any case, species composition across localities does not appear to be determined primarily by the differences in physico-chemical conditions (Bensoussan et al. 2010) and/or the differential impact of major disturbances (e.g. mass mortality) (Garrabou et al. 2009). Instead, biological factors (growth rates, recruitment, competition, successional patterns) should be the major driver of patterns found at the site level (or, alternatively, should explain most of the variability found at the site level). As mentioned previously, coralligenous assemblages are typically dominated by long-lived species characterized by slow population dynamics (Garrabou 1999; Linares et al. 2007; Teixidó et al. 2009; Teixidó et al. 2011). Additionally, these species generally display a limited dispersal capacity (Uriz 1998; Duran 2003; Abbiati et al. 2009; Ledoux et al. 2010b). These life-history traits may shape the high heterogeneity observed at a small scale. For instance, the recruitment rates for most species are low (Garrabou 1999; Mariani et al. 2006; Teixidó et al. 2011) but can also show low-frequency pulses (e.g., Garrabou and Harmelin 2002). The arrival of pulses of new offspring can shape the assemblage for long periods because of the longevity of the coralligenous species. Besides, the limited dispersal ability, based either on sexual or asexual reproduction, implies that local persistence is enhanced once the populations are established (Constantini et al. 2007; Ledoux et al. 2010). Finally, the slow growth rates hinder competition displacement between species, which could result in the dominance of competitive (Huston 1979; Garrabou et al. 2002). Overall, we argue that the interaction of these different biological processes appears to shape the species composition and abundance at each site (or even patches within sites). Thus, the observed patterns would result from the contribution of each factor over contemporary (several decades) or historical scales (hundreds of years) to the species pool and to the abundance of the species at each site. In fact, in agreement with other studies on coralligenous assemblages, that variability observed at the smallest scale (replicates or patches) is considerably high showing a patch distribution of organisms linked both to the heterogeneity of substrate and to the interactions among sessile organisms (Ferdeghini et al. 2000; Acunto et al. 2001; Balata et al. 2005). The observation that characteristic facies occur within the coralligenous assemblages (i.e., patches within the assemblages with a high abundance of certain species –for example, *Parazoanthus axinellae* or *Leptopsamia pruvoti*) appears to corroborate this hypothesis (Pérès and Picard 1964).

Coralligenous outcrops are reported to occur primarily in the northern part of the Mediterranean Basin, with few records from the eastern or southern basin (Martin et al. 2014). However, the datasets reviewed contained information for 30% of the total coastline of the Mediterranean Basin, indicating an extremely limited basic knowledge of these bioconstructions (Martin et al. 2014). Thus, the identification of similar structure in the coralligenous outcrops is important for the effective management and conservation of this sensitive habitat (Mokhtar-Jamaï et al. 2011; Giakoumi et al. 2013). Fine-scale knowledge on the distribution of species, such as that produced by this study, is crucial to furnish data for studies on marine conservation planning and the implementation of conservation plans (Giakoumi et al. 2013; Martin et al. 2014). In this context, a large-scale study on spatial variation on coral reefs composition and abundance revealed substantial changes at regional scales along the Great Barrier Reef, with profound implications for future projections of regional-scale impacts of climate change (Hughes et al. 2012). We believe that the development of this type of fine-scale study in various regions of the Mediterranean Basin will provide a global perspective on and characterization of the distribution of the biodiversity of the area and will support critical data to protect the full range of biodiversity (e.g., genes, species, life history stages), with implications for predictions of climate change impacts on coralligenous outcrops. Finally, the results obtained in this study could already help in the definition of monitoring schemes (periodicity and spatial coverage) required for the assessment of conservation status of Mediterranean marine habitats. This kind of information is especially timely to fulfill the requirements of the European Marine Strategy Framework Directive (Piazzi et al. 2014). Overall, thus, pursuing the characterization through the adoption of standardized methods could provide to the coastal management authorities (from local to international level) enormous benefits for the design of effective management and conservation plans.

Acknowledgments

The authors thank C. Linares, J.B. Ledoux, and S. Kipson for field assistance. The study has been funded by the Spanish Ministry of Science and Innovation to E. Casas-Güell, a doctoral fellowship linked to the Biorock Project (CTM2009–08045, http://biorock.medrecover.org/) and the Total Foundation (medDiversa project, http://meddiversa.medrecover.org/). ECG thanks helpful and valuable comments on earlier drafts of the manuscript to Dr. M.J. Anderson during a research visit fellow (EEBB-I-13-06897) at NZIAS-INMS (Massey University), and to Dr. M. Pawley and A. N. H. Smith for made my stay especially comfortable. The authors are members of the Marine Conservation Research Group (http://medrecover.org) of the Generalitat de Catalunya.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

5.5. References

Abbiati M, Airoldi L, Costantini F, et al (2009) Spatial and temporal variation of assemblages in Mediterranean coralligneous reefs. In: UNEP-RAC/SPA.

Acunto S, Balata D, Cinelli F (2001) Variabilita spaziale nel coralligeno e considerazioni sul metodo di campionamento. Biol Mar Medit 8:191–200.

Anderson MJ (2001a) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46.

Anderson MJ (2001b) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58:626–639. doi: 10.1139/f01-004

Anderson MJ, Crist TO, Chase JM, et al (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol Lett 14:19–28. doi: 10.1111/j.1461-0248.2010.01552.x

Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. Ecol Lett 9:683–93. doi: 10.1111/j.1461-0248.2006.00926.x

Anderson MJ, Gorley R, Clarke K (2008) PERMANOVA + for PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK

Balata D, Piazzi L, Benedetti-Cecchi L (2007) Sediment disturbance and loss of beta diversity on subtidal rocky reefs. Ecology 88:2455–61.

Balata D, Piazzi L, Cecchi E, Cinelli F (2005) Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposition. Mar Environ Res 60:403–21. doi: 10.1016/j.marenvres.2004.12.005

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr Mar Biol an Annu Rev 123–195.

Bensoussan N, Romano J-C, Harmelin J-G, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: To better understand responses of benthic communities to climate change. Estuar Coast Shelf Sci 87:431–441. doi: 10.1016/j.ecss.2010.01.008

Bianchi CN, Dore G, Morri C (1995) Guida del subacqueo naturalista: Mediterraneo e tropici. Editrice Archivio Fotografico Sardo, Nuoro

Bohnsack JA (1979) Photographic quantitative sampling of hard-bottom benthic communities. Bull Mar Sci 29:242–252.

Boudouresque CF (2004) Marine biodiversity in the mediterranean: status of spicies, populations and communities. Sci Rep Port-Cros natl Park Fr 20:97–146.

Casellato S, Stefanon A (2008) Coralligenous habitat in the northern Adriatic Sea: an overview. Mar Ecol 29:321–341. doi: 10.1111/j.1439-0485.2008.00236.x

Cebrian E, Linares C, Marschal C, Garrabou J (2012) Exploring the effects of invasive algae on the persistence of gorgonian populations. Biol Invasions 14:2647–2656. doi: 10.1007/s10530-012-0261-6

Cecchi E, Gennaro P, Piazzi L, et al (2014) Development of a new biotic index for ecological status assessment of Italian coastal waters based on coralligenous macroalgal assemblages. Eur J Phycol 49:298–312. doi: 10.1080/09670262.2014.918657

Clarke KR, Warwick RM (1994) Change in Marine Communities: an approach to statistical analysis and interpretation. Bourne Press Limited, Bournemouth, UK

Coma R, Pola E, Ribes M, Zabala M (2004) Long-Term Assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecol Appl 14:1466–1478.

Costantini F, Fauvelot C, Abbiati M (2007) Genetic structuring of the temperate gorgonian coral (Corallium rubrum) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. Mol Ecol 16:5168–82. doi: 10.1111/j.1365-294X.2007.03579.x

Crain CM, Halpern BS, Beck MW, Kappel C V (2009) Understanding and managing human threats to the coastal marine environment. Ann N Y Acad Sci 1162:39–62. doi: 10.1111/j.1749-6632.2009.04496.x

Deter J, Descamp P, Boissery P, et al (2012) A rapid photographic method detects depth gradient in coralligenous assemblages. J Exp Mar Bio Ecol 418-419:75–82. doi: 10.1016/j.jembe.2012.03.006

Duran S (2003) Phylogeography, gene flow and population structure of Crambe crambe (Porifera : Poecilosclerida). PhD. Thesis. Universitat de Barcelona.

Ferdeghini F, Acunto S, Cocito S, Cinelli F (2000) Variability at different spatial scales of a coralligenous assemblage at Giannutri Island (Tuscan Archipelago , northwest Mediterranean). Hydrobiologia 440:27–36.

Foster MS, Harrold C, Hardin DD (1991) Point vs. photo quadrat estimates of the cover of sessile marine organisms. J Exp Mar Bio Ecol 146:193–203. doi: 10.1016/0022-0981(91)90025-R

Garrabou J (1999) Life-history traits of Alcyonium acaule and Parazoanth us axinellae (Cnidaria , Anthozoa), with emphasis on growth. 178:193–204.

Garrabou J, Ballesteros E, Zabala M (2002) Structure and Dynamics of North-western Mediterranean Rocky Benthic Communities along a Depth Gradient. Estuar Coast Shelf Sci 55:493–508. doi: 10.1006/ecss.2001.0920

Garrabou J, Coma R, Bensoussan N, et al (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol 15:1090–1103. doi: 10.1111/j.1365-2486.2008.01823.x

Garrabou J, Harmelin J-G (2002) A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean : and management needs insights into conservation and management needs. J Anim Ecol 71:966–978.

Garrabou J, Sala E, Arcas A, Zabala M (1998) The Impact of Diving on Rocky Sublittoral Communities : A Case Study of a Bryozoan Population. Conserv Biol 12:302–312.

Gaston KJ (2000) Global patterns in biodiversity. Nature 405:220–7. doi: 10.1038/35012228

Gennaro P, Piazzi L (2011) Synergism between two anthropic impacts: Caulerpa racemosa var. cylindracea invasion and seawater nutrient enrichment. Mar Ecol Prog Ser 427:59–70. doi: 10.3354/meps09053

Giaccone G (2007) Coralligenous assemblage as underwater seascape: distribution off Italian coasts. Biol Mar Mediterr 14:124–141.

Giakoumi S, Sini M, Gerovasileiou V, et al (2013) Ecoregion-based conservation planning in the Mediterranean: dealing with large-scale heterogeneity. PLoS One 8:e76449. doi: 10.1371/journal.pone.0076449

Gili J-M, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–21.

Halpern BS, Walbridge S, Selkoe KA, et al (2008) A global map of human impact on marine ecosystems. Science 319:948–52. doi: 10.1126/science.1149345

Harmelin J-G, Marinopoulus J (1994) Population structure and partial mortality of the gorgonian Paramuricea clavata (Risso) in the north-western Mediterranean (France, Port-Cros Island). Mar Life 4:5–13.

Hughes TP, Baird AH, Dinsdale E a, et al (2012) Assembly rules of reef corals are flexible along a steep climatic gradient. Elsevier Ltd

Hughes TP, Linares C, Dakos V, et al (2013) Living dangerously on borrowed time during slow, unrecognized regime shifts. Trends Ecol Evol 28:149–55. doi: 10.1016/j.tree.2012.08.022

Huston M (1979) A General Hypothesis of Species Diversity. Am Nat 113:81–101.

Jackson JBC (2010) The future of the oceans past. Philos Trans R Soc Lond B Biol Sci 365:3765–78. doi: 10.1098/rstb.2010.0278

Kipson S, Fourt M, Teixidó N, et al (2011) Rapid Biodiversity Assessment and Monitoring Method for Highly Diverse Benthic Communities: A Case Study of Mediterranean Coralligenous Outcrops. PLoS One 6:e27103. doi: 10.1371/journal.pone.0027103

Knowlton N, Jackson JBC (2001) The ecology of coral reefs. In: Bertness MD, Gaines SD, Mark EH (eds) Marine community ecology. Sinauer Associates, Inc., Sunderland, USA,

Laubier L (1966) Le coralligène des Albères: monographie biocénotique. Ann Inst Océanogr Monaco 43:139–316.

Ledoux J-B, Garrabou J, Bianchimani O, et al (2010a) Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, Corallium rubrum. Mol Ecol 4204–4216. doi: 10.1111/j.1365-294X.2010.04814.x

Ledoux J-B, Mokhtar-Jamaï K, Roby C, et al (2010b) Genetic survey of shallow populations of the Mediterranean red coral [Corallium rubrum (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. Mol Ecol 19:675–90. doi: 10.1111/j.1365-294X.2009.04516.x

Linares C, Doak DF, Coma R, et al (2007) Life history and viability of a long-lived marine invertebrate: the octocoral Paramuricea clavata. Ecology 88:918–28.

Lourie SA, Vincent ACJ (2004) Using biogeography to help set priorities in marine conservation. Conserv Biol 18:1004–1020.

Magurran AE, Baillie SR, Buckland ST, et al (2010) Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. Trends Ecol Evol 25:574–82. doi: 10.1016/j.tree.2010.06.016

Mariani S, Uriz M-J, Turon X, Alcoverro T (2006) Dispersal strategies in sponge larvae: integrating the life history of larvae and the hydrologic component. Oecologia 149:174–84. doi: 10.1007/s00442-006-0429-9

Martin CS, Giannoulaki M, De Leo F, et al (2014) Coralligenous and maërl habitats: predictive modelling to identify their spatial distributions across the Mediterranean Sea. Sci Rep 1–8. doi: 10.1038/srep05073

Mokhtar-Jamaï K, Pascual M, Ledoux J-B, et al (2011) From global to local genetic structuring in the red gorgonian Paramuricea clavata: the interplay between oceanographic conditions and limited larval dispersal. Mol Ecol 20:3291–305. doi: 10.1111/j.1365-294X.2011.05176.x

Parravicini V, Micheli F, Montefalcone M, et al (2010) Rapid assessment of epibenthic communities: A comparison between two visual sampling techniques. J Exp Mar Bio Ecol 395:21–29. doi: 10.1016/j.jembe.2010.08.005

Pérès JM, Picard J (1964) Nouveau Manuel de Bionome benthique de la Mer Mediterranee. Recl des Trav la Stn Mar l'Endoume 47:5–137.

Piazzi L, Balata D (2011) Coralligenous habitat: patterns of vertical distribution of macroalgal assemblages. Sci Mar 75:399–406. doi: 10.3989/scimar.2011.75n2399

Piazzi L, Balata D, Cecchi E, et al (2010) Species composition and patterns of diversity of macroalgal coralligenous assemblages in the north-western Mediterranean Sea. J Nat Hist 44:1–22. doi: 10.1080/00222930903377547

Piazzi L, Balata D, Cecchi E, et al (2014) Effectiveness of different investigation procedures in detecting anthropo- genic impacts on coralligenous assemblages. Sci Mar 78:319–328. doi: 10.3989/scimar.03989.28A

Piazzi L, Balata D, Pertusati M, Cinelli F (2004) Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substratum inclination. Bot Mar 47:105–115. doi: 10.1515/BOT.2004.010

Piazzi L, Gennaro P, Balata D (2012) Threats to macroalgal coralligenous assemblages in the Mediterranean Sea. Mar Pollut Bull 64:2623–9. doi: 10.1016/j.marpolbul.2012.07.027

Ponti M, Fava F, Abbiati M (2011) Spatial–temporal variability of epibenthic assemblages on subtidal biogenic reefs in the northern Adriatic Sea. Mar Biol 158:1447–1459. doi: 10.1007/s00227-011-1661-3

Ros J, Romero J, Ballesteros E, Gili J-M (1985) Chapter 8. The benthos. In: Diving in the blue water.

Teixidó N, Casas E, Cebrian E, et al (2013) Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm. PLoS One 8:e53742. doi: 10.1371/journal.pone.0053742

Teixidó N, Garrabou J, Harmelin J-G (2011) Low dynamics, high longevity and persistence of sessile structural species dwelling on Mediterranean coralligenous outcrops. PLoS One 6:e23744. doi: 10.1371/journal.pone.0023744

Teixidó N, Pineda M-C, Garrabou J (2009) Decadal demographic trends of a long-lived temperate encrusting sponge. Mar Ecol Prog Ser 375:113–124. doi: 10.3354/meps07757

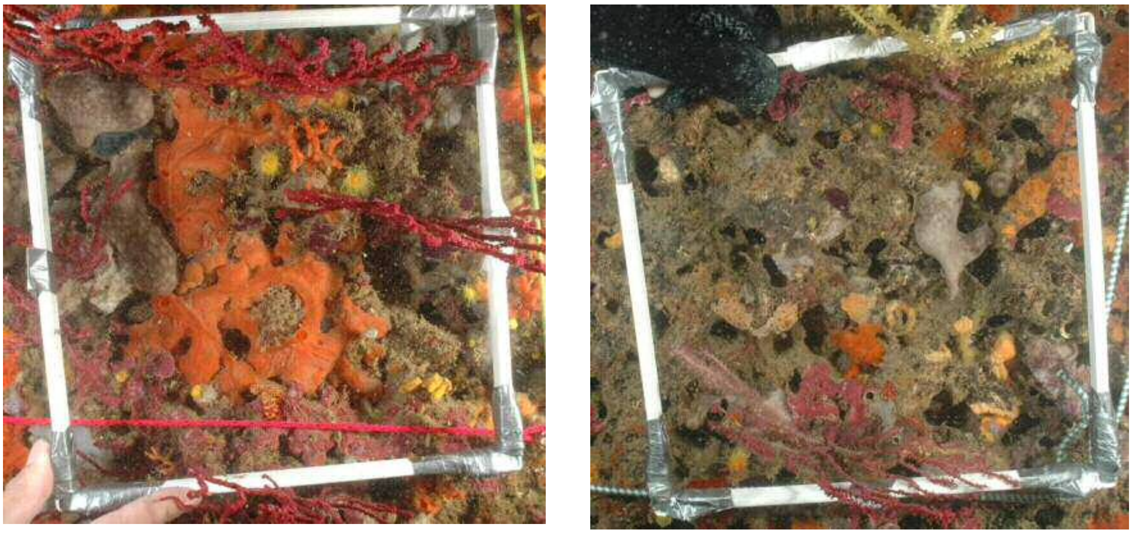
Terlizzi A, Anderson MJ, Fraschetti S, Benedetti-Cecchi L (2007) Scales of spatial variation in Mediterranean subtidal sessile assemblages at different depths. Mar Ecol Prog Ser 332:25–39. doi: 10.3354/meps332025

Uriz M-J (1998) How do reproductive output , larval behaviour , and recruitment contribute to adult spatial patterns in Mediterranean encrusting sponges ? Mar Ecol Prog Ser 167:137–148.

Virgilio M, Airoldi L, Abbiati M (2006) Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. Coral Reefs 25:265–272. doi: 10.1007/s00338-006-0100-2

Witman JD, Dayton PK (2001) Rocky subtidal communities. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Associates, Inc. Sunderland, USA.

6. Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm



Teixidó N, **Casas-Güell E**, Cebrian E, Linares C, Garrabou J (2013) Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm. PLoS ONE 8(1):e53742. doi:10.1371/journal.pone.0053742.

Abstract

Extreme events are rare, stochastic perturbations that can cause abrupt and dramatic ecological change within a short period of time relative to the lifespan of organisms. Studies over time provide exceptional opportunities to detect the effects of extreme climatic events and to measure their impacts by quantifying rates of change at population and community levels. In this study, we show how an extreme storm event affected the dynamics of benthic coralligenous outcrops in the NW Mediterranean Sea using data acquired before (2006-2008) and after the impact (2009-2010) at four different sites. Storms of comparable severity have been documented to occur occasionally within periods of 50 years in the Mediterranean Sea. We assessed the effects derived from the storm comparing changes in benthic community composition at sites exposed to and sheltered from this extreme event. The sites analyzed showed different damage from severe to negligible. The most exposed and impacted site experienced a major shift immediately after the storm, represented by changes in the species richness and beta diversity of benthic species. This site also showed higher compositional variability immediately after the storm and over the following year. The loss of cover of benthic species resulted between 22% and 58%. The damage across these species (*e.g.* calcareous algae, sponges, anthozoans, bryozoans, tunicates) was uneven, and those with fragile forms were the most impacted, showing cover losses up to 50 to 100%. Interestingly, small patches survived after the storm and began to grow slightly during the following year. In contrast, sheltered sites showed no significant changes in all the studied parameters, indicating no variations due to the storm. This study provides new insights into the responses to large and rare extreme events of Mediterranean communities with low dynamics and long-lived species, which are among the most threatened by the effects of global change.

6.1. Introduction

Extreme events are rare, stochastic perturbations that can cause abrupt and dramatic ecological change within a short period of time relative to the lifespan of organisms (Turner et al. 1998; Easterling et al. 2000; Jentsch et al. 2007). Extreme events are also considered rapid drivers with the potential to alter the state and trajectory of community structure and dynamics at wide spatial scales (White 1979; Petraitis et al. 1989; Sousa 2001), quickly forcing the system away from its equilibrium state and shaping its dynamics far into the future (Holling 1973; Gunderson 2000; Hughes et al. 2010). When ecosystems are forced beyond a threshold, regime shifts occur and the system enters into alternate stable states with a structure and function that are fundamentally different from the previous regime (Scheffer et al. 2001; Folke et al. 2004). Thus, understanding the community dynamics affected by extreme events is crucial for ecology and conservation research in a climatically changing world. As a consequence, interest in large phase-shifts and ecosystem resilience related to extreme events has increased considerably during recent decades due to the high level of disturbances that both terrestrial and marine ecosystems are suffering (Scheffer et al. 2001; Folke et al. 2004; Carpenter et al. 2006).

Studies characterizing marine ecosystem responses to anthropogenic climate change have revealed decreases in ocean productivity, alterations in food web dynamics, changes in physiology, increases in disease incidence, shifts in species distributions, and reduced abundance of habitat-forming species (Harley et al. 2006; Rosenzweig et al. 2007; Hoegh-Guldberg & Bruno 2010). In contrast, little is known about how extreme events affect marine communities. Under the conditions of ongoing climate change, observations and global change models predict increases in the frequency and intensity of extreme weather and climatic events, including heat waves, droughts, and intense tropical and mid-latitude storms (Easterling et al. 2000; IPCC 2007). Extreme storms, such as hurricanes and severe storms in the tropics and mid-latitude storms in temperate areas abruptly alter ecological processes and structure and severely affect marine littoral communities (Dayton 1971; Underwood 1999; Woodley et al. 1981; Sousa 2001). In comparison with our understanding regarding the effects of hurricanes and tropical storms affecting coral reefs (*e.g.* Knowlton et al. 1981; Harmelin-Vivien et al. 1994; Hughes 1994; Gardner et al. 2005; Walker et al. 2008), there is little knowledge about how extreme storms affect rocky benthic communities in temperate regions such as the Mediterranean Sea. This lack of knowledge may partially be explained by the rarity and stochastic nature of extreme storms in the Mediterranean Sea, combined with the scarcity of baseline data and long-term studies, making it difficult to study the effects of these events. Nevertheless, analyzing the impacts of these events may provide new insights into processes that shape the structure of benthic communities in this region.

The Mediterranean Sea is considered a hotspot of marine biodiversity, harboring approximately 10% of the world's marine species while covering less than 1% of the world ocean surface (Bianchi & Morri; Coll et al. 2010). This region has a long history of modification of natural ecosystems by human activities (Coll et al. 2010). In the Mediterranean Sea, coralligenous outcrops are of special concern, as they represent one of the most important hotspots for biological diversity (harboring approximately 20% of Mediterranean species), exhibit great structural complexity, and are among the habitats facing major threats (Gili & Coma 1998; Ballesteros 2006). The species that characterize coralligenous seascapes are encrusting calcareous algae, sponges, cnidarians, bryozoans, and tunicates. Some of the engineering species in these environments are long-lived; hence, their low dynamics make coralligenous outcrops exceptionally vulnerable to anthropogenic disturbances, such as destructive fishing practices, pollution, invasive species or mass mortality outbreaks linked to climate change (Cerrano et al. 2000; Coma et al. 2004; Garrabou et al. 2009; Teixidó et al. 2011; Cebrian et al. 2012). Moreover, the Mediterranean basin is also considered to represent a climate change hotspot and will undergo one of the largest changes in climate worldwide, with an increase in the frequency of hot wave extremes of 200 to 500% predicted at the end of the twenty-first century (Giorgi et al. 2008; Dequé 2007; Diffenbaugh et al. 2007; IPCC 2007).

Studies over time provide exceptional opportunities to reveal the effects of extreme climatic events and to measure their impacts by quantifying rates of change at population and community levels. These studies are even more valuable when addressing slow-growing, long-lived species, which do not often undergo marked declines and in which adult mortality is rarely observed (Linares et al. 2007; Miriti et al. 2007; Teixidó et al. 2011). Since 2006, we have annually surveyed coralligenous outcrops in the Medes Islands Marine Reserve in the western Mediterranean, and we were able to detect the impact of a dramatic coastal storm in December 2008 (Jimenez

2012; Navarro 2011; Sánchez-Vidal 2012) that shifted the community composition and structure of the most common long-lived benthic species in the area. Storms of comparable severity have been documented to occur irregularly within 50 year periods in the Mediterranean Sea (Sánchez-Vidal 2012; Bolanos 2009). Here, we provide evidence of the immediate impact of this severe coastal storm on the coralligenous outcrops and their responses over the following year. We assessed changes in the dynamics of the benthic community structure using data from before (2006-2008) and after the impact (2009-2010) and by analyzing: i) the community composition, species richness and beta diversity of sessile benthic perennial species with low dynamics, ii) community cover dynamics, and iii) the sensitivity of representative benthic species to the effects of the storm by quantifying cover changes. The final aim of the study is to identify the responses of communities with low dynamics and long-lived species to large and rare extreme events, providing new insights to understand and predict how present and future impacts affect these communities.

Extreme storm event on December 26th 2008

The December 26th 2008 storm was an extreme event considered to be one of the strongest impacting the Catalan coast in the last 50 years (Mateo & García-Rubies 2012; Sanchez-Vidal et al. 2012, Bolanos et al. 2009). Storms of an equivalent intensity were reported for the same area in the early (31/01/1911) (Meteorological Service of Catalonia, http://www20.gencat.cat/docs/meteocat/Continguts/Noticies/2011/Gener/pdf/31degenerde1911.pdf), (Ansell *et al.* 2006) and mid-twentieth century (22/02/1948) (La Vanguardia newspaper archives, http://hemeroteca.lavanguardia.com/preview/1948/02/22/pagina-4/34354259/pdf.html); but there are no instrumental wave records of these storms. On December 25th 2008, a strong high pressure system developed over northern Europe (1047 hPa) blocking the western atmospheric circulation and forcing northern cold air and a deep cyclone to flow towards the NW Mediterranean Basin (Sanchez-Vidal et al. 2012; Jimenez 2012). This convergence caused maritime eastern winds and stormy seas to reach the Catalan coast. The storm reached category 5 (Mendoza et al. 2009) as it moved from the Gulf of Genoa to the Catalan coast, where it hit the shore on December 26th, with wind gusts up to 20 m s^{-1}, wave heights of 8 m with peaks of 14.4 m, and wave periods of 14 s (Jimenez 2012; Sanchez-Vidal et al. 2012). The damage caused by the intense waves was accentuated by the dislodged material that they carried, scouring sand and the displacement of large rocks (Sanchez-Vidal et al. 2012), [N. Teixidó *pers. observ*]. Shallow sublittoral communities (5-10 m) in the Natural Park of Montgri, Medes Islands and Baix Ter and adjacent areas showed high abrasion, with density reductions of 50-80 % of sea urchin populations and loss of algal cover up to 90% within a depth range of 5-10 m (Hereu et al. 2012; Hereu et al. 2012). Although these shallow habitats were the most impacted, damage was also registered in deeper habitats (20 m depth), with 80% of mortality of the brown alga *Cystoseira zosteroides* (Navarro et al. 2011). The most damaged communities were dwelling on surfaces facing the East. An exploratory dive immediately after the storm at a depth of approximately 16-20 m (one week afterward, 03/01/2009) revealed detached living colonies and fragments of gorgonians (*Paramuricea clavata* and *Eunicella singularis*) and massive sponges (*Ircinia oros*) on the sea floor, torn skeleton bases with living tissue of *P. clavata,* rhizomes of *P. oceanica*, and displacement of large rocks with compressed sessile organisms (N. Teixidó *pers. observ.*). Additionally, the storm caused a significant decline of the sea bass population (*Dicentrarchus labrax*), the burial of 20% of *Posidonia oceanica* meadows and affected the deep-sea environment (300-1500 m) through increases of current speed, sediment transport, and the grain size of particles (Mateo & García-Rubies; Sanchez-Vidal et al. 2012).

6.2. Material and methods

Study area

We assessed the impact of this dramatic storm on coralligenous outcrops in the Natural Park of Montgrí, Medes Islands and Baix Ter of the NW Mediterranean Sea (42º 3'N 3º 13'E, NE Spain). This area harbors well-developed coralligenous outcrops with a depth distribution of 15-70 m (Gili & Ros 1985). Due to their beauty and aesthetic value, these outcrops are among the most attractive areas for recreational scuba diving and are subjected to diving impacts (Garrabou *et al.* 1998, Coma *et al.* 2004).

Field activity and data collecting

We quantified the immediate impact of the storm on the benthic community and the following year using before-and-after data (Fig. 1). Sampling site locations had different exposure orientation, where the most exposed face the East and the most sheltered the North-West: Carall Bernat faces the NE, Medallot the SW, Tascó Petit the NW, and Punta Salines the N. The sites are separated by few hundreds of meters to 3 kilometers. Carall Bernat was the site most exposed, whereas Tascó Petit and Punta Salines were the most sheltered; thus used as controls. We present data from surveys that were performed annually before the storm event (July –August 2006, 2007, and 2008), shortly after the storm (February 2009) and one year later (August 2010). Data available from Punta Salines cover only 2008 and February 2009. However, the Punta Salines data set has a meaningful ecologic value because it covers the most relevant time span of the analyzed temporal variation (before and immediately after); thus, we considered as a valid control site. This severe storm was a natural experiment affecting sublittoral communities with differences in exposure among sites and offered the possibility to reveal the effects produced after this severe meteorological event.

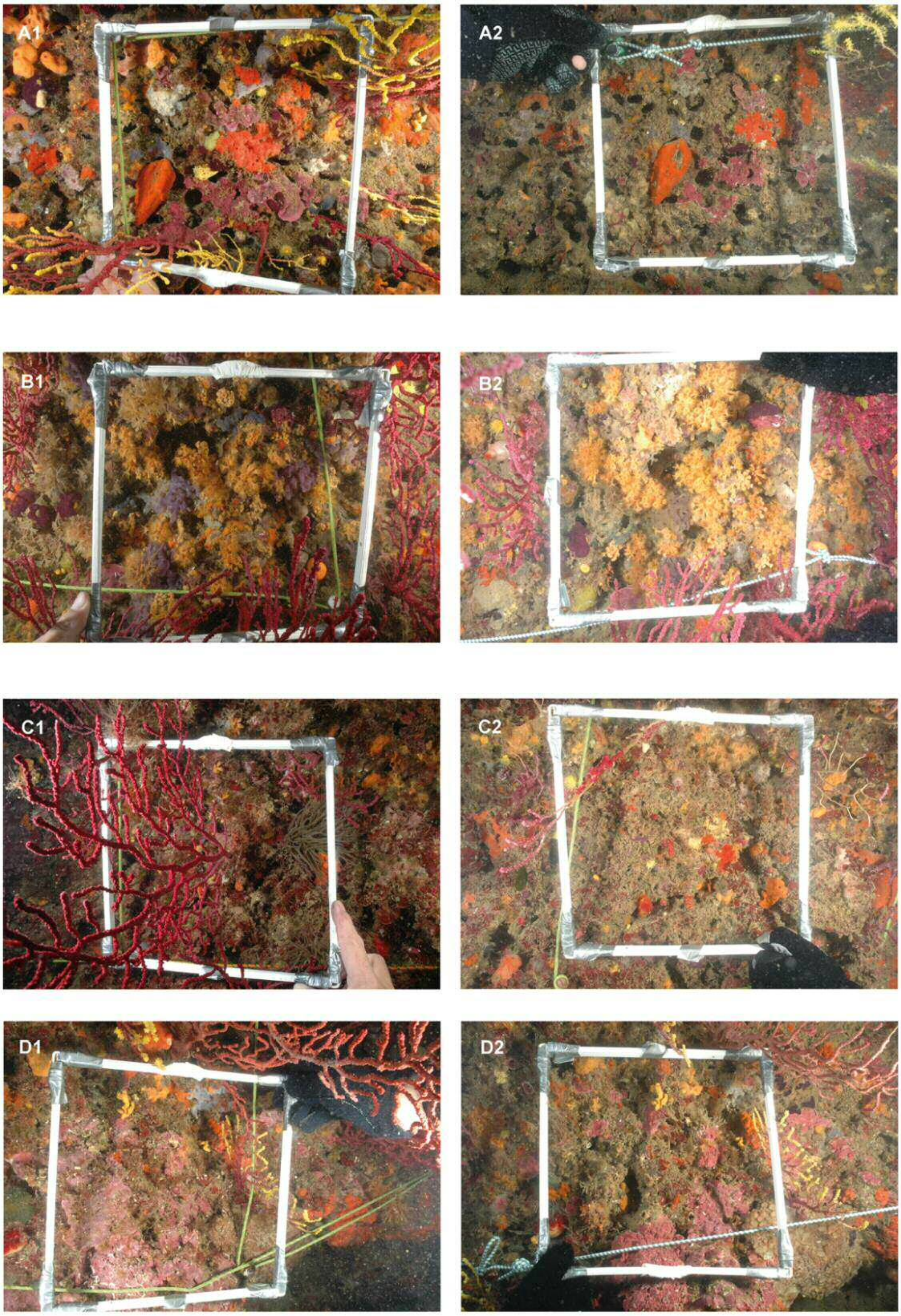


Figure 1. Photographs showing the impact of the coastal storm on coralligenous outcrops. These photographs show the same quadrat (25 × 25 cm) before (2008) and after the storm event (2009). A: Carall Bernat, dates A1: 23/06/2008, A2: 10/02/2009; B: Tascó Petit dates B1: 24/06/2008, B2 16/02/2009; C: Medallot, dates C1: 22/06/2008, C2: 09/02/09, D: Punta Salines dates: D1: 25/06/2008, D2: 09/02/2009.

We used data from 4 permanent plots (4 m long × 0.8 m wide, total area = 3.2 m^2) located haphazardly at a depth of ~ 20 m at four different sites. The corners of each plot were marked with PVC screws fixed in holes in the rocky substratum with two-component putty (Linares et al. 2007). During each survey, elastic bands were placed around the corners to facilitate the recognition of plot borders. Then, each plot was monitored photographically using quadrats of 25 × 25 cm to facilitate species identification (Kipson et al. 2011). The photographs were taken with a Nikon D70S digital SLR camera fitted with a Nikkor 20 mm DX lens (3000 × 2000 pixel resolution) and housed in Subal D70S housing. Lighting was achieved using two electronic strobes fitted with diffusers. Approximately 64 quadrats covered the entire surface of the permanent plot. In each permanent plot, we analyzed 3 replicates of 8 photographic quadrats (5000 cm^2) as a minimal sampling area as the optimal sampling effort (Kipson *et al.* 2011). These 3 replicates per site (n=51 in total: 3 sites × 3 replicates × 5 years = 45; 1 site × 3 replicates × 2 years= 6) allowed replication and further statistical comparisons. A total of 404 photographs were analyzed.

All necessary permits for the described field studies were obtained from the authority responsible for this Protected Area. The locations are not privately-owned. This study did not involve endangered or protected species. Moreover, we did not perform any disturbance to species during our fieldwork. Our data were based on the analysis of images, a non-destructive technique to study marine benthic communities.

Benthic communities

Natural variability of coralligenous outcrops shows little changes over time (Garrabou et al. 2002, Teixidó et al. 2011a, Teixidó et al. 2011b). Changes in the benthic coralligenous outcrops due to the severe storm were evaluated in three ways:

i) Changes in community composition, species richness, and beta diversity were measured based on the presence-absence of perennial sessile macro-species. Overall, these perennial species are characterized by slow growth and low dynamics (hereafter referred to as SG, see Table A.4.1 for the species list) (Coma et al. 1998; Garrabou & Ballesteros 2000; Teixidó et al. 2009; Teixidó et al. 2011). They mainly consisted of macroalgae and encrusting red algae, sponges, anthozoans, bryozoans and tunicates. A total of 64 SG species were identified at the lowest taxonomic level from photographs.

Additional dives were performed for species identification (see Kipson et al. 2011 for further details). This approach based on presence-absence analysis is an optimal method for coralligenous biodiversity assessment and monitoring, providing good estimates of the composition and structure of these communities (Kipson et al. 2011).

ii) The percent cover of functional groups of sessile organisms and substrates was measured. These groups were classified as slow growing species (hereafter SG, i.e., the 64 species mentioned above with low dynamics); fast growing species (hereafter FG, including small, filamentous and seasonal hydrozoans and bryozoans with high dynamics); turf of algae (hereafter TA, corresponding to a multispecific assemblage of small and filamentous algae); detritic matrix (hereafter DM, consisting of conglomerates of detritus and microalgae); and bare substrate (hereafter BS).

iii) The percent cover of the representative slow growing species was determined by the similarity percentage procedure (SIMPER analysis) (see below). Then, the 37 representative species were grouped into 6 different morphological forms: Boring (BOR), Cup (CUP), Encrusting (ENC), Encrusting algae (ENA), Massive (MAS), and Tree (TREE). Furthermore, we measured the sensitivity of these 37 representative species by comparing the change in the percentage of cover before and after the storm (see Table A.4.2 for cover values). The sensitivity values ranged from -100% (total disappearance of cover after the storm) to 0 % (no cover change) to positive values (increased cover). To perform the cover analyses, each photograph was projected onto a grid of 25 squares (5 cm × 5 cm), and abundances were quantified by counting the number of squares filled in the grid by either each functional group or representative species and expressing the final values as percentages (Sala et al. 1997, Balata et al. 2005). For the red gorgonian *Paramuricea clavata,* which exhibits an arborescent form, cover was calculated as the area occupied by its base. Percent cover of functional (ii) and morphological (iii) groups over 5 years were calculated for sites where all temporal range was available.

Statistical analysis

Changes in community composition were investigated using non-metric multidimensional scaling (MDS) on the basis of the Bray-Curtis dissimilarities of the presence-absence of 64 perennial macro-species as well as the presence-absence of the functional groups described above (FG, TA, DM, BS). The null hypothesis of no structure in the data was tested using the similarity profile test (SIMPROF) (with 9999 permutations and a 0.1% significance level (Clarke et al. 2008) on the Bray-Curtis matrix). This technique is a permutation-based ranking procedure aimed at testing genuine clusters in samples with no *a priori* assumptions about group membership. Differences in beta-diversity (% of unshared perennial macro-species) among sites and before and after the impact were analyzed using the PERMDISP routine. This is a routine for comparing the degree of dispersion of different groups of samples based on a distance matrix. We tested for similarity in the beta-diversity among groups on a Jaccard distance matrix (Anderson et al. 2006). The representative taxa for each site before the storm were determined using the similarity percentage procedure (SIMPER) (Clarke et al. 1994). Then, we measured the sensitivity of these taxa by comparing the percentage of cover change before and after the storm.

Non-parametric analysis of variance PERMANOVA (Anderson 2001) was used to examine the changes generated by the storm. The sampling design included 2 factors: *Site*, which was random with 4 (changes in community composition) or 3 (cover of functional and morphologic groups) levels; and *Before/After*, which was fixed with 2 levels. Differences between samples were quantified using i) Bray-Curtis dissimilarities for the multivariate perennial macro-species data matrix and ii) Euclidean distances for univariate analyses. Analyses were performed with 9999 unrestricted random permutations of the raw data. Pair-wise comparisons for all combinations of *Site x Before/After* were also carried out using t-tests and 9999 permutations of the raw data. Chi-squared tests were carried out to test for differences in the frequency of sensitivity among the sites and taxonomic as well as morphological form groups. The analyses were computed using the program Primer v6 with the PERMANOVA + add-on package (Anderson et al. 2008) and Statistica (version 8.0 StatSoft).

6.3. Results

The community composition of sessile macro-species showed a major shift after the immediate impact of the storm (Fig. 2). The most exposed site Carall Bernat was the most impacted and underwent a change in benthic structure, resulting in a distinct cluster (SIMPROF test p<0.01) containing the immediate post-storm (2009) data and those of the following year (2010).

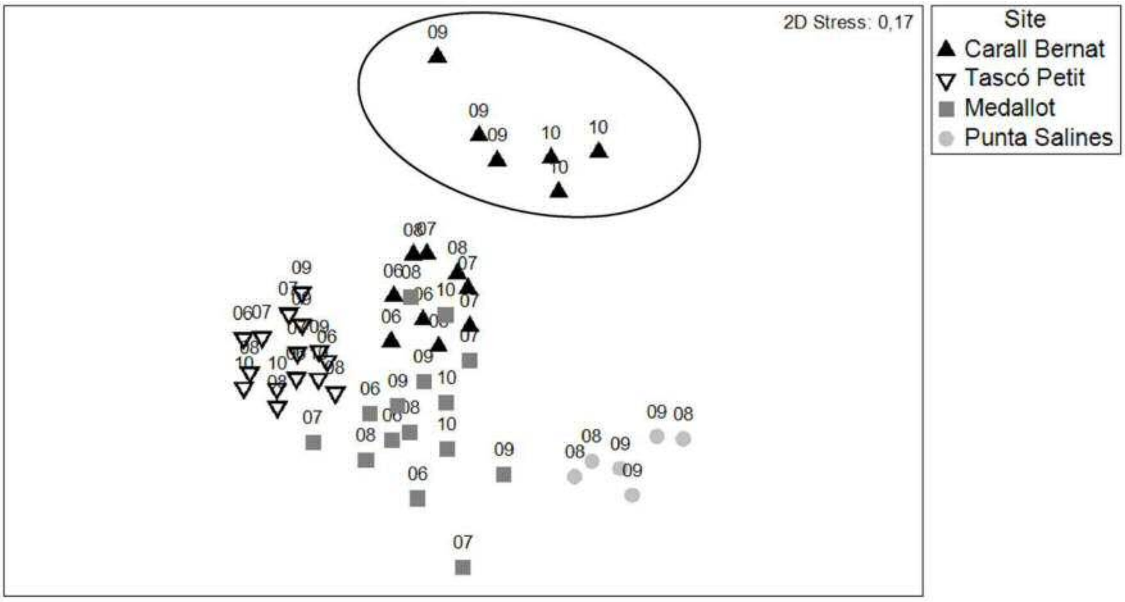


Figure 2. Shift in the structure of the perennial benthic species in coralligenous outcrops in the Medes Islands in response to the 2008 dramatic storm episode. Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis resemblance measure for species presence/absence data from 2006 to 2010. The circle indicates a SIMPROF group containing the immediate post-storm.

This post-storm group showed a higher dissimilarity and larger multivariate dispersion than the pre-storm data (2006-2008) and those from the other three sampling sites; no significant changes were observed (Fig. 2). There was a significant interaction between sites and before-after the storm impact ($F_{3,43}$ = 2.96, p<0.0001) (see Table A.4.3 for 2-way PERMANOVA and pair-wise tests).

Considering the pair-wise comparisons, only Carall Bernat showed a significant difference before (85% similarity) and after the storm (74% similarity) (t=2.98, p<0.0001, Table A.4.3). This shifting pattern was corroborated by a significant decrease in the mean species number ($F_{3,43}$ = 8.91, p<0.001), from the mean values of 35.5 ± 0.57 before to 27 ± 0.62 sessile species after the storm, representing a decline of 24% (pair-wise comparisons t=5.99, p<0.0001) (Fig. 3) (see Table A.4.4 for 2-way PERMANOVA and pair-wise tests).

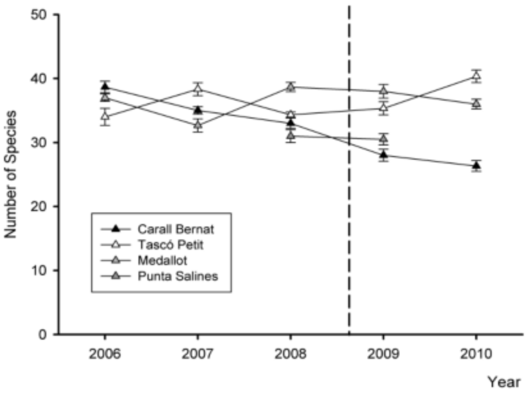


Figure 3. Mean number of sessile species (± SE) over time in the Medes Islands. The dotted line represents the impact of the unusual storm in December 2008.

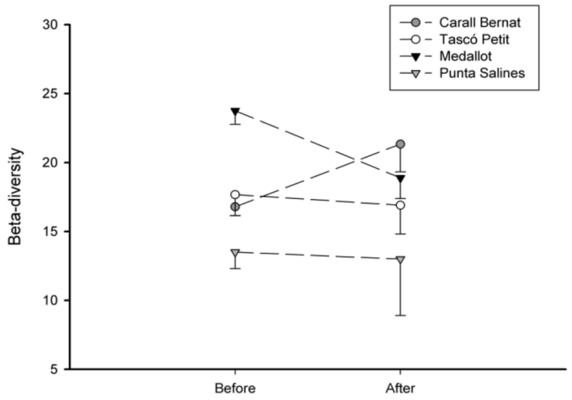


Figure 4. Mean (±1 SE) beta-diversity (as the percentage of unshared species) before and after the storm for each site. The results of PERMDISP analyses are shown.

The clear shift in the community composition was also evident based on the beta-diversity analysis ($F_{7,43} = 5.8$, $p<0.001$) (Fig. 4). Carall Bernat showed a significant increase of beta-diversity after the storm (18.2% ± 0.7 before vs. 22.33% ± 1.4 after) (t= 1.78, p<0.01), indicating higher variation in the benthic composition, whereas Medallot exhibited a decrease, which was not significant (24.4% ± 1.2 before vs 18.7% ± 1.4 after) (t= 2.97, p>0.05). Regarding the non-impacted sites, no changes were observed in the community structure (Fig. 4, Table A.4.3), mean species number (before: Tascó Petit 34.3 ± 0.43, Punta Salines 31.1 ± 1.1; after: Tascó Petit 35.3 ± 1.0, Punta Salines 30.5 ± 0.8) (Fig 3, Table A.4.4) and beta diversity (Fig 4) (t=0.9 p>0.05 for Tascó Petit, t=0.9, p>0.05 for Punta Salines).

Before the storm event (2006-2008), the coralligenous outcrops were characterized by a high cover of perennial and slow growing species (SG) (mean values of 87% ± 0.9 Carall Bernat, 88% ± 0.8 Tascó Petit, and 84% ± 0.7 Medallot) (Fig. 5 & 6), such as encrusting and fragile calcareous algae, encrusting sponges, tree bryozoans and gorgonians, massive sponges and tunicates, and an overall high structural complexity. These patterns were constant over the three years and reflected the low natural variability of coralligenous outcrops (Fig. 5). There was a significant change in the percentage of cover of the principal functional groups among the three sites and before-after the storm ($F_{2,39}$= 10.7, $p<0.0001$) (Fig. 5), and these differences were significant for the pair-wise comparisons of the interaction term at Carall Bernat and Medallot ($p<0.001$, see Table A.4.5). The cover of SG at Carall Bernat was by far the most severely damaged, showing a decrease to 37% ± 3.9 of the total area immediately after the impact (2009), but increased to 46% ± 4.7 in the following year (2010) (Fig. 5). The distribution of damage also depended on small-scale position effects at Carall Bernat, where 16% and 25% of the area analyzed in 2009 showed values as low as10% and 50% of SG cover, respectively.

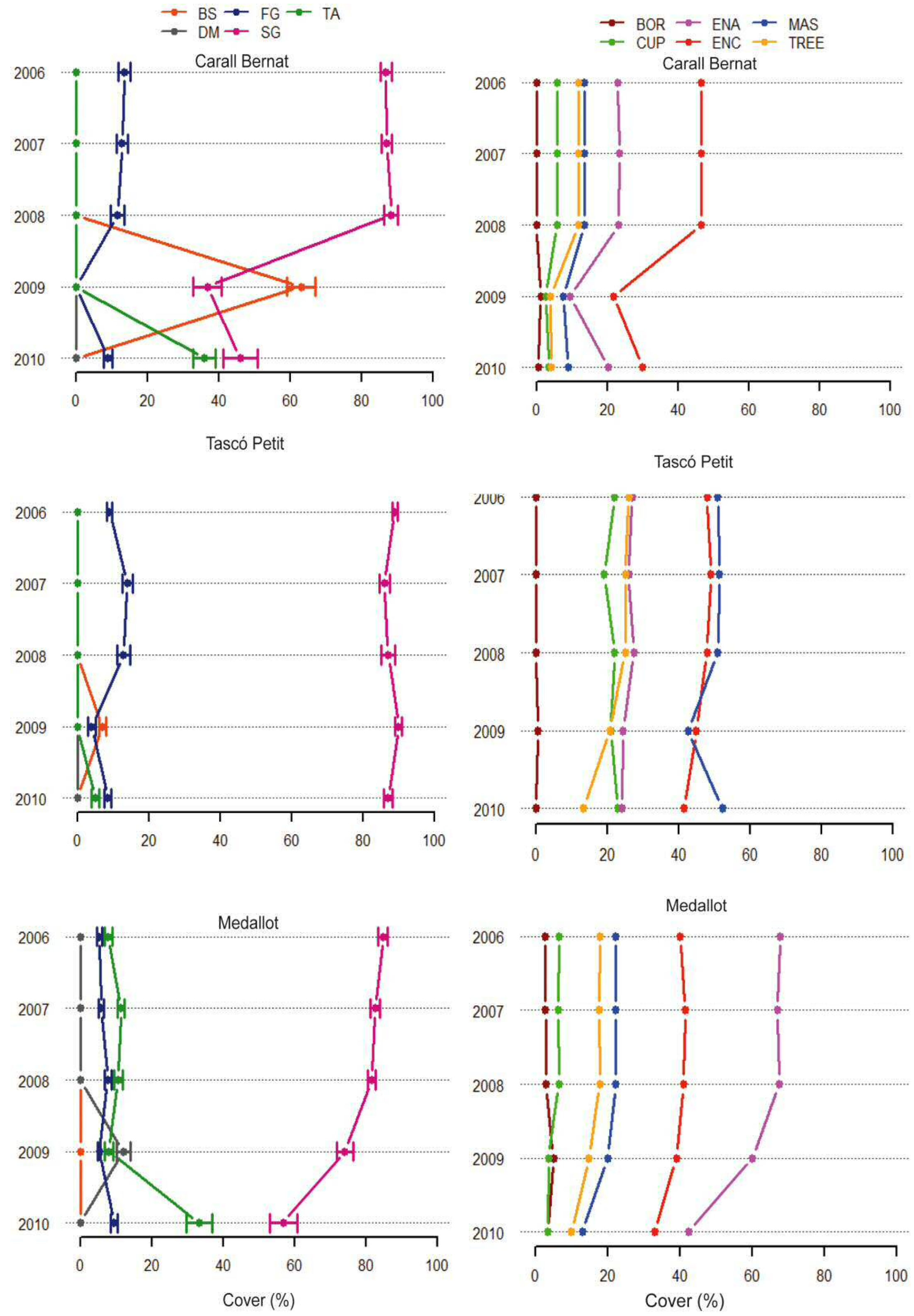


Figure 5. Response to storm episode regarding the cover area of the principal groups of sessile organisms and bare substrate: Bare substrate (BS), Detritic Matrix (DM), Turf algae (TA), Fast growing species (FG) and Slow growing species (SG). SIMPER taxa (90% of the average similarity within each site) are represented as 5 morphological forms: Boring (BOR), Cup (CUP), Encrusting algae (ENA), Encrusting (ENC), Massive (MAS), and Tree (TREE).

The scouring effect of the storm was evident in the peak of bare substrate (BS) (mean value 63% ± 4) observed immediately after the storm (2009) at Carall Bernat, which was replaced by turf algae (TA) in 2010 (mean value 44% ± 3). Interestingly, surveys at Medallot showed a moderate decrease of SG, with a reduction from 84% ± 0.7 (before) to 74% ± 2.3 (2009), followed by a further decrease down to 56% ± 3.8 (2010), suggesting a delayed loss of SG cover (Fig. 5). Furthermore, there was an increase of detritic matrix (DM) in 2009 (mean value 12 % ± 0.2) and a 3-fold increment from the pre-storm cover valueof TA (mean value 33.3% ± 3.6in 2010). In contrast, Tascó Petit was almost not affected after the storm, exhibiting a discrete peak of 5% BA and a reduction of 66% in fast growing species (FG) (12% ± 1.8 before vs. 4% ± 1 after) (Fig. 5). No significant change in cover percentage of the principal functional groups was observed before and after the storm (pair-wise comparisons t=2.63; p>0.05, see Table A.4.5).

The damage to the cover of different growth forms differed significantly among localities and before-after the storm (Fig. 5) ($F_{2,9}$= 3.7, p<0.01). The cover loss of massive (MAS) and encrusting (ENC) sponges and tunicates, scleractinian corals (CUP), encrusting algae (ENA), and arborescent gorgonians and bryozoans (TREE) ranged from 45% to 66% in relation to the pre-storm cover at Carall Bernat (Fig. 5). The damage to ENC and ENA was particularly striking due to the high pre-storm cover (approximately 46.5% and 23% before and 21.8% and 9.5% after the storm, respectively), as well as for the TREE category, which despite its low cover before the storm (approximately 12%) declined considerably to 4% (Fig. 5). Medallot and Tascó Petit did not show significant differences before and after the storm (pair-wise comparisons t=2.13 and t=2.45, p>0.05, see Table A.4.6).

The sensitivity of the representative species most affected by the storm was significantly different across sites, with 95% of species being affected at Carall Bernat (n=24), 38% at Tascó Petit (n=31), 34% at Medallot (n=32), and less than 1% at Punta Salines (n=19) (X^2= 54.2, df=3, p<0.0001) (Fig.6).

The alga *Peyssonnelia sp*., the encrusting and delicate sponges *Hemimycale columella* and *Pleraplysilla spinifera*, the massive-ropy fragile sponge *Clathrina clathrus* and the bryozoans *Adeonella calveti* and *Myriapora truncata* were reduced by up to 100% at Carall Bernat (Fig.6, Table A.4.2). In addition, among the species that exhibited high coverage before the storm (each species showing a cover value of approximately 10%), *Lithophyllum stictaeforme* (ENC) was reduced to 85%, *Parazoanthus axinellae* (ENC) to 72%, *Paramuricea clavata* (TREE) to 70%, *Disydea avara* (ENC) to 64%, *Phorbas tenacior* (ENC) to 49%, and *Crambe crambe* (ENC) to 20%. Similar patterns of damage were not found at the other sites. For example, at Medallot, the reduction was 100% only for *F. implexa* and for *M. truncata* (TREE*),* and other species showed values lower than 50% such as *D. avara* (ENC)*, Reteporella spp.* (TREE), and *P. clavata* (TREE). Only 3 species out of 31 showed high to moderate values of cover loss at Tascó Petit: 100% for *Halocynthia papillosa* (MAS), 67% for *C .clathrus* (MAS), and 50% for *Filograna implexa* (TREE). No evident changes of cover loss were detected in Punta Salines (Fig. 6). Overall, there was no significant difference regarding taxonomic groups (X^2= 6.9, df=5, p>0.05) or morphological forms (X^2= 9.8, df=5, p>0.05). The massive and robust sponges *Chondrosia reniformis* and *Agelas oroides* appeared to be less affected, showing approximately 5% cover loss at Carall Bernat and Tascó Petit and no change at Medallot. After the storm, the removal of sessile organisms on the boring sponge *Cliona sp.* during the storm increased the exposed area by 45% at Carall Bernat and +20% at Medallot, respectively (Fig. 6).

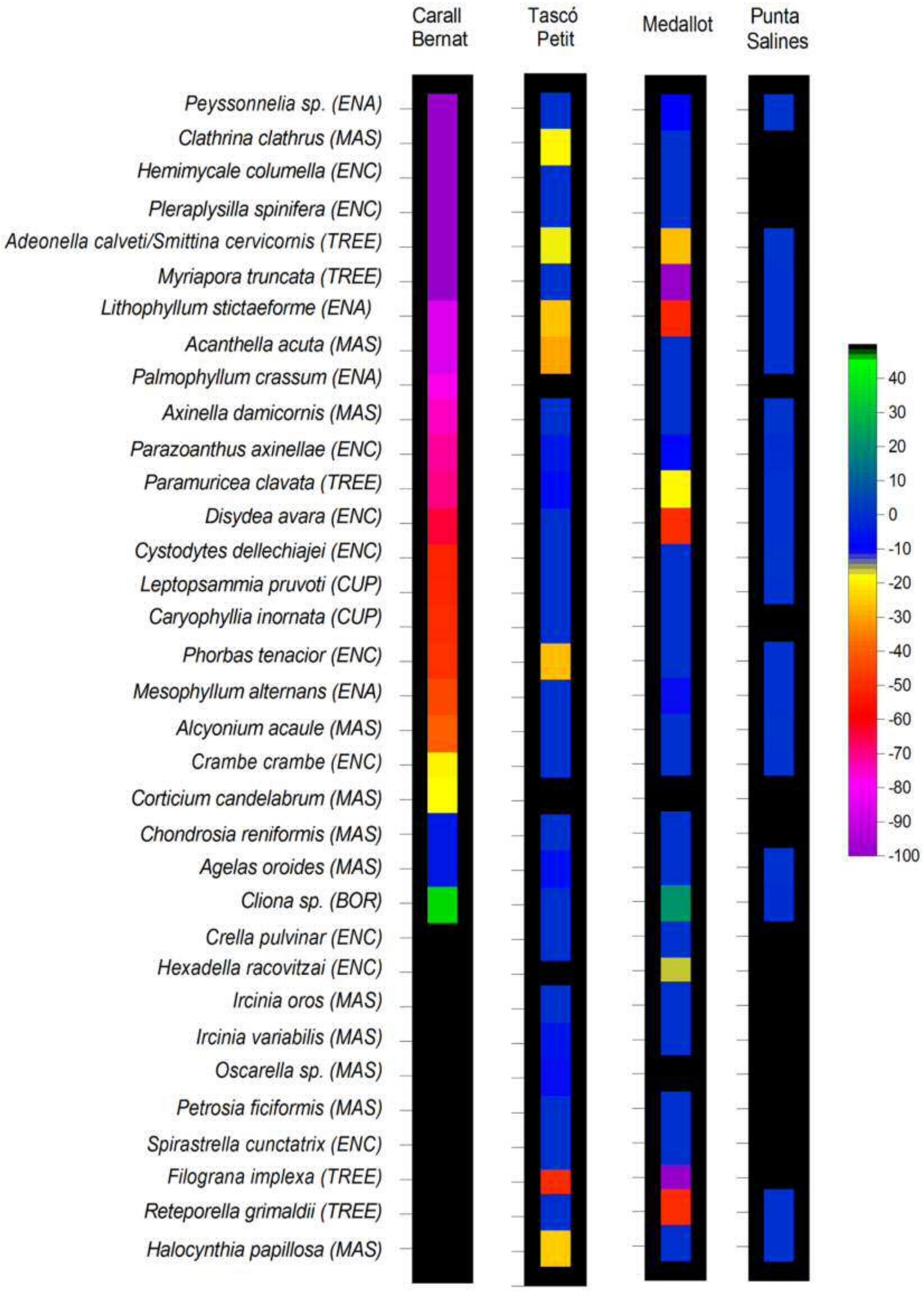


Figure 6. Sensitivity (as a % of cover change) of the representative macrobenthic taxa to the physical disturbance generated by the storm. Cover change (from highest, 2100%, to lowest, 0%) at Carall Bernat defines the order of the taxa at the other sites. Representative macrobenthic taxa were chosen from SIMPER analysis. Black areas indicate that taxa were not representative for the specific site.

6.4. Discussion

The storm of December 26th 2008 was considered to be the strongest recorded in the last 50 years in the northern part of the Catalan coast (41ºN - 42º30'N), with the greatest wave power, the highest wave heights, and the longest duration (Sánchez-Vidal et al.2012; Jiménez 2012; Mendoza et al. 2009). Its impact, including abrasion of sediment particles, severely affected the littoral communities in the region, causing mortality of sessile benthic organisms, including some long-lived species (mainly sponges and anthozoans) estimated to be more than 50 years old (Coma et al. 1998; Teixidó et al. 2011). Coralligenous outcrops exhibit low dynamics and few changes over time at population and community level in absence of large disturbances (Garrabou et al. 2002a, Garrabou et al. 2002b; Ballesteros 2006; Teixidó et al. 2011), which makes the impact of extreme events very important for community dynamics. This is even more important in the Mediterranean Sea, which is considered a hotspot of climate change, where exceptional events such as this storm or heat-waves in summers are predicted to increase (Giorgi 2008; Déqué 2007; Diffenbaugh et al. 2007, IPCC 2007). Overall, our results quantified the different effects of this rare, extreme event on the community structure dynamics of long-standing coralligenous outcrops. This study is unique in using high-resolution sampling over time to reveal how extreme events can shift coralligenous outcrops characterized by long-lived species and may be of general interest regarding ecological responses to extreme and unusual climatic events.

Spatial patterns of the storm impacts

The loss of cover of slow growing benthic species was between 58% and 22% immediately after the storm at two of the studied sites, Carall Bernat and Medallot, respectively (Fig. 5). The damage caused by the storm (with an eastern wind direction) was influenced by aspects of orientation, local habitat profiles, depth, and the presence of boulders. The most impacted site, Carall Bernat (wall facing NE), is located within a narrow channel surrounded by large stone blocks, whereas Medallot (wall facing SW) is located in the most westerly part of an archipelago and is better protected from easterly wave swells. Our data showed that approximately 18 out of 24 species at Carall Bernat were severely affected, showing cover loss values higher than 50% (Fig. 6), accompanied by a significant decrease of perennial species richness (24%) (Fig. 3).

Although the impact was local and restricted to the northernmost part of the Catalan coast, the damage was among the highest registered for coralligenous outcrops of the NW Mediterranean Sea. High mortality rates of gorgonian populations have been reported to be up to 10-60 % after major episodes of mass mortality, such as those related to positive temperature anomalies in the summers of 1999 and 2003 in the NW Mediterranean Sea (Cerrano et al. 2000; Linares et al. 2005; Garrabou et al. 2009). However, these studies did not explore overall community shifts (including changes in calcareous algae, sponges, anthozoans, bryozoans, and tunicates). Other studies quantifying the impacts of severe hurricanes and cyclones on coral reefs have also focused on primary framework corals (e.g., Edmunds et al. 1991; Knowlton et al. 1981) and have reported reef losses, with values ranging from 17% to 60% (Stoddart 1974; Wooley 1981; Hughes et al. 1999; Gardner et al. 2005; Álvarez-Filip et al. 2009), there by highlighting the importance in terms of the broader community changes.

In this study, the effects of the storm were not found to be uniform and synchronous. Although Carall Bernat was the most impacted site, it showed a low recovery of perennial species after one year (see below), whereas Medallot exhibited little cover loss immediately after the storm (cover loss of approximately 8%) but showed a further decline in the following year (23% cover loss of slow growing species), accompanied by a considerable increase in turf algae (~33%) (Fig. 5). We acknowledge the different responses of benthic community dynamics, which integrate different complex history processes and disturbances, and highlight the complexity of identifying unique, combined and/or synergetic effects of disturbance when most coastal habitats are exposed to multiple stressors (Hughes et al. 1999; Jackson et al. 2001; Wilson et al. 2006). Based on this complexity, our results showed that benthic communities dwelling in rather small areas (less than ~ 10 km^2) can exhibit significantly different responses to sudden disturbances.

An abrupt shift in the multivariate structure of coralligenous outcrops after the storm was only observed at the most exposed and impacted site, Carall Bernat, which showed the highest compositional variability in response to the disturbance (Fig. 2). Tascó Petit and Punta Salines did not show any significant change on community structure before and after the storm, indicating no major effects of this severe storm at the sheltered sites. This pattern of greater variability was corroborated by an increase of beta diversity in the perennial species composition after the disturbance (immediately after and in the following year) (Fig. 4). After extreme events such as this storm, post-disturbance variability is expected to be elevated and to persist for a longer period of time relative to pre-disturbance conditions, and this variability will be stabilized more gradually, only after the disturbed state has returned to the baseline condition (Collins 2000; Fraterrigo et al. 2008). Our results demonstrate that this unusual storm produced a mosaic of small remaining survivor patches in the most impacted site and reduced the structural complexity of perennial slow-growing benthic species, creating a seascape habitat exhibiting higher fluctuations in the presence/absence of component species, accompanied by a reduction in the number of species, thus reducing the species pool (a decrease of 8 perennial species) (Fig. 2 and 3). Interestingly, this severe storm appeared to have opposite effects on beta diversity (Fig. 4), such that it increased significantly in Carall Bernat due to a high variability in composition, whereas it declined significantly at Medallot. This increased similarity at Medallot between pre-and post-storm conditions may be explained as a consequence of the change in the relative cover of perennial-slow benthic species (decreasing) and turf algae (increasing).

Overall, this change in beta diversity (increase or decrease) was accompanied by a loss of functional groups in Carall Bernat and Medallot (Fig. 5 and 6), with a shift in dominance from encrusting algae and perennial animal species to turf-forming algae. Encrusting calcareous algae are the major contributors to coralligenous outcrops and, together with sponges, cnidarians, bryozoans, and tunicates, are the species that characterize this habitat (Ballesteros 2006). Thus, their replacement by turf-forming algae may increase the sensibility to invasion, as some of these algae belong to the most invasive species in the Mediterranean Sea, triggering substantial changes in the structure and dynamics of rocky communities and rendering surfaces inhospitable to the recruitment of native invertebrates (Airoldi 1998; Piazzi et al. 2001, Linares et al. 2012).

Species sensitivity

Species sensitivity showed a gradient regarding the site exposure: from high through intermediate to low values of cover loss at Carall Bernat, Medallot, Tascó Petit, and Punta Salines (Fig. 6). Our findings indicated that the damage across perennial species was uneven and those with fragile forms, irrespective of their morphology, were the most impacted, showing cover losses between 50 and 100 % (Fig. 6). These results agree with the general observation that fragile branching and foliose corals are the most susceptible to hurricane damage to coral reefs (Woodley et al. 1981; Hughes et al. 1999), as well as in the NW Mediterranean Sea, a severe winter storm caused high mortality of the fragile bryozoan *Pentapora facialis* (Cocito 1998). In the present study, the species ranged from short-lived perennial species with estimated ages of 2-5 years (e.g., the crustose coralline alga *Peyssonnelia sp*., the massive-ropy fragile sponge *Clathrina clathrus*, and the delicate tree-like bryozoans *Adeonella calveti* and *Myriapora truncata*) to persistent and long-lived perennial species with estimated longevities of 50-100 years (e.g., the gorgonian *Paramuricea clavata*, the scleractinians *Leptopsammia pruvoti* and *Caryophyllia inornata*, and the alcyonacean *Alcyonium acaule*) and encrusting calcareous algae (e.g. *Lithophyllum stictaeforme* and *Mesophyllum alternans*) with low natural adult mortality (Coma et al. 1998; Ballesteros 2006; Linares et al. 2007; Teixidó et al. 2011). Thus, this unusual event produced high episodic mortality of adults in a community in which this rarely occurs under natural conditions.

These observations are in agreement with the fact that large and infrequent disturbances such as this storm are considered to drive species interactions and community dynamics, which cause long-term effects on both marine and terrestrial communities (Sousa 2001; Pickett 1985; Petraitis 1989; Hughes et al. 1999).

Patterns of surviving patches

The strong abrasive effect of the storm did not completely homogenize the available space by creating a seascape of bare substrate at the most impacted site; rather, it produced a mosaic of small remaining surviving patches of perennial benthic species (with values of perennial-slow growing species cover ranging from 10% to 50%), associated with a decrease of habitat complexity and heterogeneity. Spatial heterogeneity following large disturbances has been widely documented in both marine and terrestrial ecosystems (Picket 1985; Foster et al. 1998; Turner 2010), and it has been recognized that biotic residuals (e.g., surviving roots and rhizomes of plants, as well as fragments of corals and sponges) are regularly available, even following a large disturbance (Connell et al. 1977; Highsmith 1982; Teixidó et al. 2007).

In the present study, the existence of small patches after the storm (mainly encrusting algae and clonal animals such as encrusting sponges, anthozoans, and tunicates) at the most impacted site was fundamental for slight recovery, with a minor increase of cover being observed during the following year. This increase of perennial-slow growing species represented an increase of 10% (37% ± 3.9) immediately after the impact (2009) to 46% ± 4.7 (2010) (Fig. 5). We hypothesize that these surviving colonies and fragments favored faster recovery via vegetative regrowth and this partial recovery occurred more rapidly than could take place through the growth of new recruits via larvae. Our results showed that one year is not enough to re-establish the community to its prior state before the storm (Figs. 2–6). Only two encrusting calcified algae (*Lithophyllum stictaeforme* and *Mesophyllum alternans)* and six clonal species (the sponges *Crambe crambe*, *Dysidea avara, Corticium candelabrum, Phorbas tenacior*, the anthozoans *Parazoanthus axinellae*, and the tunicate *Cystodytes dellechiajei*) contributed to regrowth from the remnant tissue. The finding of small remaining surviving patches is of special interest to understand community responses due to the overall low dynamics of coralligenous species combined with the infrequent or unsuccessful recruitment events recorded for sexually produced larvae of clonal organisms (Hughes et al. 2000; Linares et al. 2007; Teixidó et al. 2011).

Conclusion

With the increasing threat to coastal habitats due to global warming and other interacting factors, there is growing concern about the capacity of ecosystems to absorb multiple disturbances occurring over short time periods (Gunderson 2000; Scheffer et al. 2001; Folke et al. 2004). Global warming is predicted to increase the frequency and magnitude of extreme climate and weather events (Easterling 2000; IPCC 2007). For the western Mediterranean Sea, a decrease in the total number of cyclones has been predicted (Ulbrich et al. 2009), but an increase of wind and wave intensity (Young et al. 2011; Marcos et al. 2011). Consequently, the observed damage makes it evident that recurrent severe storms will seriously affect coralligenous outcrops, posing threats to their resilience. Based on the complex responses to disturbance, efforts to acquire and analyze data over time are fundamental to quantify these changes and evaluate the ecological mechanisms behind them, which will ultimately allow us to develop our capacity to predict long-term and larger scale community shifts.

The effects of this storm were difficult to predict, but now that they have been registered, they provide new insights into population and community dynamics. Consequently, under the present warming scenario and due to the high diversity that the Mediterranean Sea harbors (Somot et al. 2008; Coll et al. 2010), we emphasize the need for long-time empirical and modeling studies on sublittoral benthic communities.

This information is crucial not only for understanding the mechanisms underlying the dynamics of these communities and the ecological consequences of global climate change but also for determining effective management and conservation approaches to maintain the biodiversity of the Mediterranean.

Acknowledgments

Special recognition is given to R. Coma, R. Graille, E. Isla, S. Kipson, M. Zabala, and F. Zuberer for field assistance. The authors are very grateful to E. Ballesteros and MJ. Uriz for taxonomic advices. N. Teixidó thanks J.LL. Riera and J. Santiago for help with graphic and image editing and W. Arntz for critical reading. An anonymous reviewer and a former Academic Editor, Tasman Crowe, provided valuable comments on an earlier draft. The authors are members of the Marine Conservation Research Group (http://medrecover.org) of the Generalitat de Catalunya.

6.5. References

Airoldi L, Virgilio M (1998) Responses of turf-forming algae to spatial variations in the deposition of sediments. Mar Ecol Prog Ser 165:271–282.

Álvarez-Filip L, Millet-Encalada M, Reyes-Bonilla H (2009) Impact of Hurricanes Emily and Wilma on the Coral Community of Cozumel Island, Mexico. Bull Mar Sci 84:295–306.

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46.

Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. Ecol Lett 9:683–93. doi: 10.1111/j.1461-0248.2006.00926.x

Anderson MJ, Gorley R, Clarke K (2008) PERMANOVA + for PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK

Ansell T, Jones P, Allan R, et al (2006) Daily mean sea level pressure reconstructions for the European-North Atlantic region for the period 1850-2003. J Clim 19:2717–2742.

Balata D, Piazzi L, Cecchi E, Cinelli F (2005) Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposition. Mar Environ Res 60:403–21. doi: 10.1016/j.marenvres.2004.12.005

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr Mar Biol an Annu Rev 123–195.

Bianchi CN, Morri C (2000) Marine biodiversity of the Mediterranean Sea : situation, problems and prospects for future research. Mar Pollut Bull 40:367–376.

Bolanos R, Jorda G, Cateura J, et al (2009) The XIOM: 20 years of a regional coastal observatory in the Spanish Catalan coast. J Mar Syst 77:237–260.

Carpenter S, Brock W (2006) Rising variance: a leading indicator of ecological transition. Ecol Lett 9:311–318.

Cebrian E, Linares C, Marschal C, Garrabou J (2012) Exploring the effects of invasive algae on the persistence of gorgonian populations. Biol Invasions 14:2647–2656. doi: 10.1007/s10530-012-0261-6

Cerrano C, Bianchi CN, Cattaneo-Vietti R, et al (2000) A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North- western Mediterranean), summer 1999. Ecol Lett 3:284–293.
Clarke KR, Warwick RM (1994) Change in Marine Communities: an approach to statistical analysis and interpretation. Bourne Press Limited, Bournemouth, UK

Cocito S, Sgorbini S, Bianchi CN (1998) Aspects of the biology of the bryozoan *Pentapora fascialis* in the northwestern Mediterranean. Mar Biol 131:73–82. doi: 10.1007/s002270050298

Coll M, Piroddi C, Steenbeek J, et al (2010) The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. PLoS One 5:e11842. doi: 10.1371/journal.pone.0011842

Collins S (2000) Disturbance Frequency and Community Stability in Native Tallgrass Prairie. Am Nat 155:311–325. doi: 10.1086/303326

Coma R, Pola E, Ribes M, Zabala M (2004) Long-Term Assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecol Appl 14:1466–1478.

Coma R, Ribes M, Zabala M, Gili J-M (1998) Growth in a modular colonial marine invertebrate. Estuar Coast Shelf Sci 47:459–470. doi: 10.1006/ecss.1998.0375

Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. Am Nat 111:1119–1144.

Dayton PK (1971) Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecol Monogr 41:351–389.

Déqué M (2007) Frequency of precipitation and temperature extremes over France in an anthropogenic scenario: model results and statistical correction according to observed values. Glob Planet Chang 57:16–26.

Diffenbaugh NS, Pal JS, Giorgi F, Gao X (2007) Heat stress intensification in the Mediterranean climate change hotspot. Geophys Res Lett 34:L11706. doi: 10.1029/2007GL030000

Easterling D, Meehl G, Parmesan C, et al (2000) Climate extremes: observations, modelling, and impacts. Science 289:2068–2074.

Edmunds PJ, Witman JD (1991) Effect of Hurricane Hugo on the primary framework of a reef along the south shore of St. John, US Virgin Islands. 78:201–204.

Folke C, Carpenter S, Walker B, et al (2004) Regime shifts, resilience, and biodiversity in ecosystem management. Annu Rev Ecol Evol Syst 557–581.

Foster DR, Knight DH, Franklin JF (1998) Landscape patterns and legacies resulting from large, infrequent forest disturbances. Ecosystems 1:497–510.

Fraterrigo JM, Rusak JA (2008) Disturbance-driven changes in the variability of ecological patterns and processes. Ecol Lett 11:756–770. doi: 10.1111/j.1461-0248.2008.01191.x

Gardner T A, Côté IM, Gill J A, et al (2005) Hurricanes and Caribbean Coral Reefs: Impacts, Recovery Patterns, and Role in Long-Term Decline. Ecology 86:174–184. doi: 10.1890/04-0141

Garrabou J, Ballesteros E (2000) Growth of Mesophyllum alternans and Lithophyllum frondosum (Corallinales, Rhodophyta) in the northwestern Mediterranean. Eur J Phycol 35:1–10. doi: 10.1080/09670260010001735571

Garrabou J, Ballesteros E, Zabala M (2002) Structure and Dynamics of North-western Mediterranean Rocky Benthic Communities along a Depth Gradient. Estuar Coast Shelf Sci 55:493–508. doi: 10.1006/ecss.2001.0920

Garrabou J, Coma R, Bensoussan N, et al (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol 15:1090–1103. doi: 10.1111/j.1365-2486.2008.01823.x

Garrabou J, Harmelin J-G (2002) A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean : and management needs insights into conservation and management needs. J Anim Ecol 71:966–978.

Garrabou J, Sala E, Arcas A, Zabala M (1998) The impact of diving on rocky sublittoral communities: a case study of a bryozoan population. Conserv Biol 12:302–312.

Gili J-M, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–21.

Gili J-M, Ros J (1985) Estudio cuantitativo de tres poblaciones ciralitorales de Cnidarios bentónicos. Investig Pesq 49:323–352.

Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Glob Planet Change 63:90–104. doi: 10.1016/j.gloplacha.2007.09.005

Gunderson L (2000) Ecological resilience–in theory and application. Annu Rev Ecol Evol Syst 425–439.

Harley CDG, Randall Hughes A, Hultgren K, et al (2006) The impacts of climate change in coastal marine systems. Ecol Lett 9:228–241.

Harmelin-Vivien M (1994) The effects of storms and cyclones on coral reefs: a review. J Coast Res 211–231.

Hereu B, Garcia-Rubies A, Linares C, et al (2012a) Impact of the Sant Esteve's storm (2008) on the algal cover in infralittoral rocky photophilic communities. In: Assessment of the ecological impact of the extreme storm of Sant Esteve's Day (26 December 2008) on the littoral ecosystems of the north Mediterranean Spanish coasts Final Report (PIEC 200430E599). Centro Estudios Avanzados de Blanes, Consejo Superior Investigaciones Científicas, Blanes, Spain, pp 123–143

Hereu B, Linares C, Sala E, et al (2012b) Multiple Processes Regulate Long-Term Population Dynamics of Sea Urchins on Mediterranean Rocky Reefs. PLoS One. doi: e36901–e36901

Highsmith RC (1982) Reproduction by Fragmentation in Corals. Mar Ecol Prog Ser 7:207–226. doi: 10.3354/meps007207

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328:1523–1528.

Holling C (1973) Resilience and stability of ecological systems. Annu Rev Ecol Evol Syst 4:1–23.

Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science (80-) 265:1547–1550.

Hughes TP, Baird AH, Dinsdale EA, et al (2000) Supply-Side Ecology Works Both Ways: The Link between Benthic Adults, Fecundity, and Larval Recruits. Ecology

81:2241–2249. doi: 10.2307/177111

Hughes TP, Connell JH (1999) Multiple stressors on coral reefs: A long-term perspective. Limnol Oceanogr 44:932–940. doi: 10.4319/lo.1999.44.3_part_2.0932

Hughes TP, Graham N, Jackson J, et al (2010) Rising to the challenge of sustaining coral reef resilience. Trends Ecol Evol 25:633–642.

IPCC (2007) The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jackson JBC, Kirby MX, Berger WH, et al (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629–37. doi: 10.1126/science.1059199

Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate-change experiments: events, not trends. Front Ecol Env 5:365–374.

Jimenez J (2012) Characterizing Sant Esteve’s storm (26th December 2008) along the Catalan coast (NW Mediterranean). In: Assessment of the ecological impact of the extreme storm of Sant Esteve’s Day (26 December 2008) on the littoral ecosystems of the north Mediterranean Spanish coasts Final Report (PIEC 200430E599). Centro de Estudios Avanzados de Blanes, Consejo Superior de Investigaciones Científicas, Blanes, Spain, pp 31–44

Kipson S, Fourt M, Teixidó N, et al (2011) Rapid Biodiversity Assessment and Monitoring Method for Highly Diverse Benthic Communities: A Case Study of Mediterranean Coralligenous Outcrops. PLoS One 6:e27103. doi: 10.1371/journal.pone.0027103

Knowlton N, Lang JC, Christine Rooney M, Clifford P (1981) Evidence for delayed mortality in hurricane-damaged Jamaican staghorn corals. Nature 294:251–252.

Linares C, Cebrian E, Coma R (2012) Effects of turf algae on recruitment and juvenile survival of gorgonian corals. Mar Ecol Prog Ser 452:81–88. doi: 10.3354/meps09586

Linares C, Coma R, Diaz D, et al (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Mar Ecol Prog Ser 305:127–137. doi: 10.3354/meps305127

Linares C, Doak DF, Coma R, et al (2007) Life history and viability of a long-lived marine invertebrate: the octocoral Paramuricea clavata. Ecology 88:918–28.

Marcos M, Jordā G, Gomis D, Pérez B (2011) Changes in storm surges in southern Europe from a regional model under climate change scenarios. Glob Planet Change 77:116–128.

Mateo M, Garcia-Rubies A (2012) Assessment of the ecological impact of the extreme storm of Sant Esteve's Day (26 December 2008) on the littoral ecosystems of the north Mediterranean Spanish coasts. Final Report (PIEC 200430E599). Blanes

Mendoza Ponce E, Jiménez Quintana J (2009) Regional vulnerability analysis of Catalan beaches to storms. Inst Civ Eng 162:127–135.

Miriti M, Rodríguez-Buriticá S, Wright S, Howe H (2007) Episodic death across species of desert shrubs. Ecology 88:32–36.

Navarro L, Ballesteros E, Linares C, Hereu B (2011) Spatial and temporal variability of deep-water algal assemblages in the Northwestern Mediterranean: The effects of an exceptional storm. Estuar Coast Shelf Sci 95:52–58. doi: 10.1016/j.ecss.2011.08.002

Petraitis PS, Latham RE, Niesenbaum R a. (1989) The maintenance of species diversity by disturbance. Q Rev Biol 64:393. doi: 10.1086/416457

Piazzi L, Ceccherelli G, Cinelli F (2001) Threat to macroalgal diversity: effects of the introduced green alga. 210:149–159.

Pickett S, White PS (1985) The ecology of natural disturbance and patch dynamics. Academic Press, New York

Rosenzweig C, Casassa G, Karoly D, et al (2007) Assessment of observed changes and responses in natural and managed systems. In: Climate change 2007: Impacts, Adaptation, and Vulnerability Contribution of Working Group II to the Fouth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, pp 79–131

Sala E, Boudouresque C-F (1997) The role of fishes on the organization of a Mediterranean sublittoral community. I: Algal communities. J Exp Mar Bio Ecol 212:25–44.

Sanchez-Vidal A, Canals M, Calafat AM, et al (2012) Impacts on the Deep-Sea Ecosystem by a Severe Coastal. doi: 10.1371/journal.pone.0030395

Scheffer M, Carpenter S, Foley JA, et al (2001) Catastrophic shifts in ecosystems. Nature 413:591–596.

Somot S, Sevault F, Déqué M, Crépon M (2008) 21st century climate change scenario for the Mediterranean using a coupled atmosphere–ocean regional climate model. Glob Planet Change 63:112–126.

Sousa W (2001) Natural disturbance and the dynamics of marine benthic communities. In: Bertness MD, Gaines SD, Hay M (eds) Marine community ecology. Massachusetts: Sinauer Associates, pp 85–130

Stoddart D (1974) Post-hurricane changes on the British Honduras reefs: re-survey of 1972. In: Proc Second Int Coral Reef Symp 2. Great Barrier Reef Committee, Brisbane, pp 473–483

Teixidó N, Casas-Güell E, Cebrian E, et al (2011a) Integrating life-history traits and biodiversity patterns of coralligenous communities in the NW Mediterranean Sea. In: World Conference on Marine Biodiversity. Aberdeen, Scotland (UK).

Teixidó N, Garrabou J, Gutt J, Arntz WE (2007) Iceberg Disturbance and Successional Spatial Patterns: The Case of the Shelf Antarctic Benthic Communities. Ecosystems 10:143–158. doi: 10.1007/s10021-006-9012-9

Teixidó N, Garrabou J, Harmelin J-G (2011b) Low dynamics, high longevity and persistence of sessile structural species dwelling on Mediterranean coralligenous outcrops. PLoS One 6:e23744. doi: 10.1371/journal.pone.0023744

Teixidó N, Pineda M-C, Garrabou J (2009) Decadal demographic trends of a long-lived temperate encrusting sponge. Mar Ecol Prog Ser 375:113–124. doi: 10.3354/meps07757

Turner MG (2010) Disturbance and landscape dynamics in a changing world. Ecology 91:2833–2849. doi: 10.1890/10-0097.1

Turner MG, Baker WL, Peterson CJ, Peet RK (1998) Factors influencing succession: lessons from large, infrequent natural disturbances. Ecosystems 1:511–523.

Ulbrich U, Leckebusch G, Pinto J (2009) Extra-tropical cyclones in the present and future climate: a review. Theor Appl Climatol 96:117–131.

Underwood A (1999) Physical disturbances and their direct effect on an indirect effect: responses of an intertidal assemblage to a severe storm. J Exp Mar Biol Ecol 232:125–140.

Walker S, Degnan B, Hooper J, Skilleter G (2008) Will increased storm disturbance affect the biodiversity of intertidal, nonscleractinian sessile fauna on coral reefs? Glob Chang Biol 14:2755–2770.

White PS (1979) Pattern, process, and natural disturbance in vegetation. Bot Rev 45:229–299.

Wilson SK, Graham NAJ, Pratchett MS, et al (2006) Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? Glob Chang Biol 12:2220–2234. doi: 10.1111/j.1365-2486.2006.01252.x

Woodley JD, Chornesky EA, Clifford PA, et al (1981) Hurricane Allen's impact on Jamaican coral reefs. Science 214:749–755.

Young I, Zieger S, Babanin A (2011) Global trends in wind speed and wave height. Science 332:451–455.

7. Summary of Results

This thesis used an extensive photographic survey, covering several spatial scales (100s of m to 100s of km) within the NW Mediterranean and including 2 different coralligenous assemblages: *Paramuricea clavata* and *Corallium rubrum* assemblage. A rapid non-destructive protocol based on photographic sampling for biodiversity assessment and monitoring of coralligenous outcrops provided good estimates of its species composition, by analyzing presence/absence of macrobenthic species. This approach allowed us to determine the minimal sampling area for each assemblage (5000 cm^2 for *P. clavata* assemblages CA and 2500 cm^2 for *C. rubrum* assemblages). In addition, we concluded that 3 replicates provided an optimal sampling effort in order to maximize the species number and to assess the main biodiversity patterns of studied assemblages.

The spatial variability of biodiversity patterns on coralligenous outcrops dominated by *C. rubrum* assemblages and *P. clavata* differed consistently with spatial scale, presenting higher variability at medium (sites) spatial scales and lower variability at the largest ones (localities). Similar patterns were also found for the morphofunctional groups, diversity and abundance; highlighting the highest % cover and number of species corresponding to encrusting invertebrates (while algal group was almost negligible). Besides, evidence of differences was detected at site level regarding alfa diversity of perennial species, whereas beta and gamma diversity showed more consistent values at locality level. Finally, several seascape indexes (NP, MPS and MSI) regarding the spatial configuration of patches belonging to perennial species were also studied for *C. rubrum* assemblages, which confirmed the same robust patterns at the three spatial scales studied.

The thesis also addressed biodiversity patterns over time for coralligenous outcrops dominated by the red gorgonian *P. clavata*. Overall, no temporal changes over 5 years were detected in either species composition or the 12 morphofunctional groups considered.

This thesis assessed the effects derived from an extreme storm event comparing changes in benthic community composition using data acquired before (2006-2008) and after the impact (2009-2010) at four different sites in the NW Mediterranean Sea. The sites analyzed showed different damage from severe to negligible. The most exposed and impacted site experienced a major shift, represented by changes in the species richness and beta diversity of benthic species. The loss of cover of benthic species resulted between 22% and 58%. The damage across these species (*e.g.* calcareous algae, sponges, anthozoans, bryozoans, tunicates) was uneven, and those with fragile forms were the most impacted, showing cover losses up to 50 to 100%. Interestingly, small patches survived after the storm and began to grow slightly during the following year. In contrast, sheltered sites showed no significant changes in all the studied parameters, indicating no variations due to the storm.

8. General discussion

8.1. Coralligenous and the lack of quantitative data for a proper conservation status assessment

Large scale long-term biodiversity datasets are a basic resource that furnishes the essential tools to provide information to guide conservation actions (Magurran et al. 2010) as well as to evaluate the effectiveness of management plans (Gaston 2000). In assemblages dominated by long-lived species, such as the coralligenous ones (Teixidó et al. 2011), the challenge is even greater since declining or recovery trajectories are more difficult to detect due to the slow pace of their population dynamics (Hughes et al. 2013). At our knowledge, most biodiversity assessments on the coralligenous encompassed small spatial and short temporal scales (Cocito et al. 2002; Piazzi et al. 2004; but see Gatti et al. 2015 and Doxa et al. 2016). In this context, the main goal of this thesis was to provide information on coralligenous composition and structure over regional spatial (>100 km's) and long-term temporal (five to ten years) scales.

8.2. Biodiversity monitoring method for coralligenous outcrops

During last years, different biodiversity and health assessments have been developed to assess the status of coralligenous outcrops. Based on the necessity of standardized and comparable biodiversity assessment methods, the first objective of the thesis was to determine the minimal sampling area (MSA) and the optimum sampling effort to assess biodiversity patterns (Chapter 3). Three replicates measuring 5000 cm^2 for *Paramuricea clavata* and 2500 cm^2 for *Corallium rubrum* were found to be sufficient to maximize the species number and to assess the main biodiversity patterns present (Chapter 3, Tables 2 and 3). To our knowledge, MSAs had only previously been estimated for studying cnidarian species dwelling in coralligenous assemblages (Weinberg 1978a; Gili and Ros 1985). Interestingly, both studies determined comparable values for areas required to reach at least 80% of species: approximately 5000 cm^2 for the gorgonian and 4000 cm^2 for red coral. In the present thesis, the use of the MSA as a sampling unit was crucial for the assessment of biodiversity patterns. Comparison of the patterns obtained using MSA and smaller individual quadrats (used in the photo sampling) as replicates clearly showed a shift in the hierarchy of the estimates of variance components from large to small spatial scales. In general, the variation in the observed similarities among samples increases as the size of the sampling unit decreases (Nekola and White 1999). Thus, using sampling units smaller than the MSA may have resulted in increased stochastic variability in the species composition at the smallest spatial scale. Similar effects have been reported previously in different habitats (Steinitz et al. 2006; Parravicini et al. 2009; Rocchini et al. 2010). However, previous studies on coralligenous outcrops adopted sampling units ranging between 240 and 600 cm^2 (True 1970; Hong 1982; Ferdeghini et al. 2000; Piazzi et al. 2004; Balata 2006; Virgilio et al. 2006; Piazzi et al. 2010), as expected since the sampling units were much lower than MSA values, these studies found the highest variability at the replicate scale (Ferdeghini et al. 2000; Acunto et al. 2001). Hence, we emphasize the necessity to determine MSAs and use them as sampling units in the assessment of biodiversity patterns within coralligenous (and other) assemblages.

The methodological approach developed in this thesis could be also used to study rarely surveyed deep coralligenous banks (extending from 60 down to 120 m, depending on the geographical position and local light conditions (Ballesteros 2006), as ROVs (remotely operated vehicles) or research submersibles have the operational capability to collect high-resolution digital photographs.

8.3. Description and quantification of structural parameters of coralligenous outcrops at regional and decadal spatio-temporal scales.

Studies on the red coral *Corallium rubrum* and red gorgonian *Paramuricea clavata* communities have been mainly focused at the population level (Garrabou and Harmelin 2002; Linares 2006; Ledoux et al. 2010; Arizmendi-Mejía et al. 2015) and just few studies addressed the assessment at the community level, focusing on the structure and dynamics (Weinberg 1978b; Deter et al. 2012; Ponti et al. 2014). Both species are threatened by disturbances associated to global changes such as climate-driven mass-mortality events, sedimentation, and invasive species (Cerrano et al. 2000; Cebrian et al. 2012). Besides red coral is affected by overharvesting (Linares et al. 2010; Montero-Serra et al. 2015). Since both gorgonian species coral are key engineering species of coralligenous systems, changes in the population abundance may be affecting the entire community. However the lack of baseline information and specific studies is hindering to explore the impact of global change at community level.

Hence, the first/second aim of this thesis was furnishing the first baseline information on spatial and temporal patterns of coralligenous assemblages dominated by red coral *Corallium rubrum* (Chapter 4) and red gorgonian *Paramuricea clavata* (Chapter 5). In this thesis, we showed for first time that diversity and structure of morphofunctional groups (hereafter MFG) are constant at regional spatial and temporal scales considered. This result supports the hypothesis that in general, coralligenous outcrops dominated by the red gorgonian (Chapter 5) and the red coral (Chapter 4), displayed similar structural complexity, especially for perennial and slow growing species. However, other MFGs displayed higher degree of spatial and temporal variability such as *Algal turf*, *Algal erect* and *Animal epibionts*. In fact, these groups could be good indicators of the impact of different disturbances since most of them are characterized by faster growth rates. For instance, a sudden increase of *Algal turf* in Petit Conglué resulted from a recent introduction of the red filamentous algae *Womersleyella setacea*.

Focusing on the similarities in species composition, the results of this thesis showed differences in specific composition at site and locality level for both assemblages, being the highest variability at site level (Chapter 4 and Chapter 5) and clearly reduced at locality level (Chapter 4 Fig. 2 and 3, and Chapter 5 Fig. 3). Interestingly, the greatest variability was almost equally found at the site and replicate (MSA) levels. Other studies in coralligenous assemblages have also found the highest variability at the smallest scales investigated (e.g. among replicates) (Ferdeghini et al. 2000; Virgilio et al. 2006; Terlizzi et al. 2007; Abbiati et al. 2009; Ponti et al. 2011). However, despite this variability, it is important to note the relatively high similarity found among NW Mediterranean sites (overall average similarity 55.7% for *P.clavata* assemblages whereas for *C. rubrum* the average similarity was 60.70%).

Diversity metrics

Similar patterns of diversity, the alpha (site) and gamma (locality) values were found for both assemblages. Diversity values found for *C. rubrum* assemblages were similar (from 24 to 41 species at site level and from 57 to 72 species at locality level) than those found in coralligenous dominated by P. clavata (from 23 to 42 and from 52 to 66 species at locality level). The diversity values of this thesis were markedly higher than other studies specially performed on *C. rubrum* outcrops (18 species or taxonomic groups, Virgilio et al. 2006). The reason can be due because *C. rubrum*'s sites of this thesis where placed at different habitats such as crevices, overhangs, caves and depth vertical walls (>40 m.) and covering an extension of more than 400 km whereas other studies (e.g. Virgilio et al. 2006) focused at small spatial scale (< 5km).

Landscape pattern indices

Overall, our results highlighted the high spatial complexity and diversity previously reported for coralligenous outcrops (Ponti et al. 2011; Kipson et al. 2011; Bertolino et al. 2013; Ponti et al. 2014). Focusing on the spatial arrangement addressed in the study of the red coral assemblage, the indices used to characterize different aspects of spatial patterns - patch number (NP), size (MPS) and shape (MSI) - showed consistent range of values across sites and localities (Chapter 3, Figures 5, Table 6). Garrabou et al. 2002 using LPI to study spatial patterns along a depth gradient, found that coralligenous outcrops exhibited the greatest spatial pattern complexity. The authors argued that a decrease in dynamics (% of area changed) might enhance high diversity and thus complex spatial patterns. The combination of the use of morphofunctional groups with spatial arrangement indices may be also excellent proxies to estimate the health of coralligenous outcrops (see below). For example, one of the most evident phenomena after disturbances in terrestrial and marine ecosystems is the significant changes in number, size and forms of patches in the main morphological groups (Turner et al. 2002; Teixidó et al. 2007; Wedding et al. 2011). We may predict that the impact of a large disturbance could result in a major shift implying a decrease in indices used in this study: higher NP, intermediated MPS and irregular forms indicating that well-mixed groups of invertebrates become less complex.

The processes behind the multi-scale observed patterns in assemblages are difficult to discern. In any case, species composition across localities does not appear to be determined primarily by the differences in physico-chemical conditions at regional level (Bensoussan et al. 2010) and/or the differential impact of major disturbances (e.g. mass mortality) (Garrabou et al. 2009). Instead, we contend that biological factors (growth rates, recruitment, competition, successional patterns) should be the major driver of patterns found at the site level (or, alternatively, should explain most of the variability found at the site level). As mentioned previously, coralligenous assemblages are typically dominated by long-lived species characterized by slow population dynamics (Garrabou 1999; Linares 2006; Teixidó et al. 2009; Teixidó et al. 2011). Additionally, these species generally display a limited dispersal capacity (Uriz 1998; Duran 2003; Abbiati et al. 2009; Ledoux et al. 2010). These life-history traits may shape the high heterogeneity observed at a small scale. For instance, the recruitment rates for most species are low (Garrabou 1999; Mariani et al. 2006; Teixidó et al. 2011) and probably most species show low-frequency recruitment pulses (e.g., Garrabou and Harmelin 2002). The arrival of pulses of new offspring can shape the assemblage for long periods because of the longevity of the coralligenous species. Besides, the limited dispersal ability, based either on sexual or asexual reproduction, implies that local persistence is enhanced once the populations are established (Costantini et al. 2007; Ledoux et al. 2010). Finally, the slow growth rates hinder competition displacement between species, which could result in the dominance of competitive (Huston 1979; Garrabou and Harmelin 2002). Overall, we argue that the interaction of these different biological processes appears to shape the species composition and abundance at each site (or even patches within sites). Thus, the observed patterns would result from the contribution of each factor over contemporary (several decades) or historical scales (hundreds of years) to the species pool and to the abundance of the species at each site. In addition, this thesis remarks the high constancy and low dynamics of coralligenous assemblages at decadal time-scale over regional spatial scales (Casas-Güell et al. 2015).

8.4. Impacts on coralligenous outcrops at local scale: the effects of an extreme storm event at the community level

Finally, we addressed the analysis of the impact of a dramatic storm occurred in the Catalan coast in 2008 to evidence changes on the coralligenous structure. This storm was considered to be the strongest recorded in the last 50 years in the northern part of the Catalan coast, with the greatest wave power, the highest wave heights, and the longest duration (Sanchez-Vidal et al. 2012). In this thesis, we benefited from the availability of photographic series from sites previous to the storm, that allowed to investigate the effects of this rare, extreme event on the community structure dynamics of long-standing coralligenous outcrops. The occurrence of this storm provided an exceptional opportunity to reveal the effects of extreme climatic events and to measure their impacts by quantifying rates of change at population and community levels. This study is even more valuable because coralligenous outcrops are dominated by slow-growing, long-lived species, which do not often undergo marked declines and in which mortality rates are rarely high.

.High mortality rates of gorgonian populations have been reported to be up to 10–60% after major episodes of mass mortality, such as those related to positive temperature anomalies in the summers of 1999 and 2003 in the NW Mediterranean Sea (Cerrano et al. 2000; Linares et al. 2005; Garrabou et al. 2009). After this severe storm, Coma et al. 2012 reported a decrease in colony density of *P. clavata* ranging from 6.1 to 20.6% (mean value of 13.4 ± 2.7%) at the Medes Islands. Other studies quantifying the impacts of severe hurricanes and cyclones on coral reefs have also focused on primary framework corals (e.g., Knowlton et al. 1981; Edmunds and Witman 1991) and have reported reef losses, with values ranging from 17% to 60% (Woodley et al. 1981; Hughes and Connell 1999; Gardner et al. 2005), thereby highlighting the importance in terms of the broader community changes.

Species sensitivity showed a gradient regarding the site exposure: from high through intermediate to low values of cover loss at Carall Bernat, Medallot, Tascó Petit, and Punta Salines (Chapter 4 Fig. 7). Our findings indicated that the damage across perennial species was uneven and those with fragile growth forms, irrespective of their morphology such as *Adeonella calveti* or *Myriapora truncata*, were the most impacted, showing cover losses between 50 and 100% (Chapter 4 Fig. 7). In the present study, the affected species ranged from short-lived perennial species with estimated ages of 2–5 years to persistent and long-lived perennial species with estimated longevities of 50–100 years and encrusting calcareous algae. Thus, this unusual event produced high episodic mortality of adults in a community in which this rarely occurs under natural conditions (Coma et al. 1998; Ballesteros 2006; Linares et al. 2007; Teixidó et al. 2011b).

The storm did not completely homogenize the available space by creating a seascape with large patches of bare substrate at the most impacted site; rather, it produced a mosaic of small remaining surviving patches of perennial benthic species (with values of perennial-slow growing species cover ranging from 10% to 50%), associated with a decrease of habitat complexity and heterogeneity. Spatial heterogeneity following large disturbances has been widely documented in both marine and terrestrial ecosystems (Pickett and White 1985; Foster et al. 1998; Turner 2010) and it has been recognized that biotic residuals (e.g., surviving roots and rhizomes of plants, as well as fragments of corals and sponges) are regularly available, even following a large disturbance (Connell and Slatyer 1977; Highsmith 1982; Teixidó et al. 2007). In the present study, the existence of small patches after the storm (mainly encrusting algae and clonal animals such as encrusting sponges, anthozoans, and tunicates) at the most impacted site was fundamental for slight recovery, with a minor increase of cover being observed during the following year. This increase of perennial-slow growing species represented an increase of 10% immediately after the impact (2009) to 46% (2010) (Fig. 5). It was hypothesized that these surviving colonies and fragments favored faster recovery via vegetative regrowth and this partial recovery occurred more rapidly than could take place through the growth of new recruits via larvae. The finding of small remaining surviving patches is of special interest to understand community responses due to the overall low dynamics of coralligenous species combined with the infrequent or unsuccessful recruitment events recorded for sexually produced larvae of clonal organisms (Hughes et al. 2000; Linares et al. 2007; Teixidó et al. 2011).

9. Conclusions and future directions

9.1. Conclusions

- A biodiversity rapid assessment method that has been developed and proved to be sufficiently sensitive to study species diversity, structure and composition of coralligenous assemblages, dominated by *Corallium rubrum* and *Paramuricea clavata*. This method is useful for the evaluation of temporal changes in coralligenous assemblages, allowing the identification of impacts on the monitored assemblages. Consequently this method could be adopted to fulfill one the main goals of the Marine Strategy Framework Directive (MSFD): "establishment of innovative cost effective monitoring programs and protocols for status assessment, effective management and protection measures of these habitats".
- Consistent patterns have been found for both assemblages at the different hierarchical spatial and temporal scales. Regarding species composition and diversity, abundance and landscape patterns for the different morpho-functional groups analyzed,it was found a great spatial variability at small spatial scales (site, few Km's) with a smoothing trend at greater spatial scales (locality, hundreds of Km's).
- Diversity metrics of perennial species at different spatial scales (alpha, beta and gamma) showed similar patterns: alpha and beta diversity variability was the highest among sites whereas gamma was similar among localities. Thus, species composition across localities did not appear to be determined primarily by the differences in physicochemical conditions and/or the differential impact of major disturbances (e.g., mass mortality), but due to biological factors (growth rates, recruitment, competition and successional patterns) determining patterns found at the site level.
- The abundance (% cover) of the different morphofunctional groups was similar at local and regional scales. At site level, some groups displayed important differences offering the opportunity to use them as suitable indicators in monitoring schemes.
- Landscape pattern indices (NP, MPS, MSI) of morphofunctional groups were useful to describe differences among sites in the same locality. Interestingly, localities showed similar patterns within the NW Mediterranean.
- For a conservation perspective, it will be important to ensure adequate

conservation at small spatial scales *(i.e.* site level) including the different habitat conditions but obviously ensuring a large geographical extension.

- Temporal comparisons (5 year period) in the structure of coralligenous assemblages dominated by *Paramuricea clavata* did not depict significant differences. Thereby highlighting the parsimony of these assemblages and therefore the vulnerability to disturbances.

The occurrence of exceptional (secular) storm allowed to evaluate the impacts of a severe disturbances at community level. The storm affected significantly coralligenous outcrops. The community shifted to a mosaic of small remaining surviving patches of perennial benthic species, associated with a decrease of habitat complexity and heterogeneity.The study of the response of the community affected by the storm offered an exceptional opportunity to understand community recovery pathways in coralligenous outcrops.

9.2. Future directions

The results obtained in this thesis are an important contribution for designing research programs and monitoring schemes (spatial and temporal scales) required for the assessment of conservation status of one of the most highly valued and emblematic Mediterranean habitats. The application of this robust method to other unstudied regions of the Mediterranean basin, will be crucial to furnish data on composition and structure of coralligenous outcrops dwelling in contrasting environmental conditions (e.g. Eastern and Southern Mediterranean) and subjected to different global and local stressors (e.g. global warming, fisheries, harvesting).

Contributions from these approaches combined with habitat/species distribution models will substantially advance the knowledge about the relation of environmental data and coralligenous (habitat and species) at different geographic scales. These models estimate the response of species to environmental factors, projecting them into the geographical space to assess the probability of the presence in the areas under consideration. These techniques can help in marine spatial planning, with effective management and conservation plans. More specifically, they can be applied as a first step to i) identify the importance of environmental variables in structuring coralligenous habitats and therefore to forecast changes of coralligenous outcrops distribution in relation with climate, ii) to assess which species and regions are likely to be most affected by climate change, and iii) to help in the assessment of habitat distributions in areas that, due to their complexity and/or depth, are difficult to evaluate and show limited data access.

10. Bibliography

Bibliography

References from introduction and general discussion

Abbiati M, Airoldi L, Costantini F, et al (2009) Conservation of coralligenous reefs: effective larval dispersal, scales of connectivity and resilience. In: First Mediterranean Symposium on Coralligenous conservation and other calcareous bio-concretions. TUNIS, UNEP-MAP-RAC/SPA, 2009, pp. 28 - 33

Acunto S, Balata D, Cinelli F (2001) Variabilità spaziale nel coralligeno e considerazioni sul metodo di campionamento. Biol Mar Medit 8:191–200.

Arizmendi-Mejía R, Linares C, Garrabou J, et al (2015) Combining genetic and demographic data for the conservation of a mediterranean marine habitat-forming species. PLoS One 10:e0119585. doi: 10.1371/journal.pone.0119585

Balata D (2006) Spatio-temporal variability and vertical distribution of a low rocky subtidal assemblage in the north-west Mediterranean. Estuar Coast Shelf Sci 67:553–561.

Balata D, Piazzi L, Benedetti-Cecchi L (2007) Sediment disturbance and loss of beta diversity on subtidal rocky reefs. Ecology 88:2455–61.

Balata D, Piazzi L, Rindi F (2011) Testing a new classification of morphofunctional functional groups of marine macroalgae for the detection of responses to stress. Mar Biol 158:2459-2469.

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr Mar Biol an Annu Rev 123–195.

Benedetti-Cecchi L, Pannacciulli F, Bulleri F, et al (2001) Predicting the consequences of anthropogenic disturbance: large-scale effects of loss of canopyalgae on rocky shores. Mar Ecol Prog Ser 214:137–150

Bensoussan N, Romano J-C, Harmelin J-G, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: To better understand responses of benthic communities to climate change. Estuar Coast Shelf Sci 87:431–441. doi: 10.1016/j.ecss.2010.01.008

Bertolino M, Cerrano C, Bavestrello G, et al (2013) Diversity of Porifera in the Mediterranean coralligenous accretions, with description of a new species. Zookeys 37:1–37. doi: 10.3897/zookeys.336.5139

Bianchi CN, Morri C (2000) Marine Biodiversity of the Mediterranean Sea: Situation, Problems and Prospects for Future Research. Mar Pollut Bull 40:367–376.

Boudouresque CF (2004) Marine biodiversity in the mediterranean: status of spicies, populations and communities. Sci Rep Port-Cros natl Park Fr 20:97–146.

Capellà J (2012) La protecció i valorització del medi natural, una estrategia de regeneració de destins turístics consolidats. In: Hereu B, Quintana X (eds) El fons marí de les Illes Medes i el Montgrí, Recerca i Territori. pp 177–194.

Casas-Güell E, Teixidó N, Garrabou J, Cebrian E (2015) Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales. Mar Biol. doi: 10.1007/s00227-015-2635-7

Cebrian E, Ballesteros E, Canals M (2000) Shallow rocky bottom benthic assemblages as calcium carbonate producers in the Alboran Sea (southwestern Mediterranean). Oceanol Acta 23:311–322.

Cebrian E, Linares C, Marschal C, Garrabou J (2012) Exploring the effects of invasive algae on the persistence of gorgonian populations. Biol Invasions 14:2647–2656. doi: 10.1007/s10530-012-0261-6

Cecchi E, Gennaro P, Piazzi L, et al (2014) Development of a new biotic index for ecological status assessment of Italian coastal waters based on coralligenous macroalgal assemblages. Eur J Phycol 49:298–312. doi: 10.1080/09670262.2014.918657

Cerrano C, Bianchi CN, Cattaneo-Vietti R, et al (2000) A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North- western Mediterranean), summer 1999. Ecol Lett 3:284–293.

Cerrano C, Cardini U, Bianchelli S, et al (2013) Red coral extinction risk enhanced by ocean acidification. Sci Rep 3:1457. doi: 10.1038/srep01457

Chapman MG, Underwood AJ, Skilleter GA (1995) Variability atdifferent spatial scales between a subtidal assemblage exposed tothe discharge of sewage and two control assemblages. J Exp MarBiol Ecol 189:103–122

Claudet J, Fraschetti S (2010) Human-driven impacts on marine habitats: A regional meta-analysis in the Mediterranean Sea. Biol Conserv 143:2195–2206. doi: 10.1016/j.biocon.2010.06.004

Cocito S, Bedulli D, Sgorbini S (2002) Distribution patterns of the sublittoral epibenthic assemblages on a rocky shoal in the Ligurian Sea (NW Mediterranean). Sci Mar 66:175–181.

Coll M, Piroddi C, Albouy C, et al (2012) The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. Glob Ecol Biogeogr 21:465–480. doi: 10.1111/j.1466-8238.2011.00697.x

Coll M, Piroddi C, Steenbeek J, et al (2010) The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. PLoS One 5:e11842. doi: 10.1371/journal.pone.0011842

Coma R, Ribes M, Serrano E, et al (2009) Global warming-enhanced stratification and mass mortality events in the Mediterranean. Proc Natl Acad Sci U S A 106:6176–6181.

Coma R, Ribes M, Zabala M, Gili J-M (1998) Growth in a Modular Colonial Marine Invertebrate. Estuar Coast Shelf Sci 47:459–470. doi: 10.1006/ecss.1998.0375

Coma R, Serrano E, Linares C, et al (2012) Effect of a severe storm event on the mortality rate of the gorgonian Paramuricea clavata on the Medes Islands Marine Reserve and the nearby Montgrí coast. In: Mateo MA, Garcia-Rubies T (eds) Assessment of the ecological impact of the extreme storm of Sant Esteve’s Day (26 December 2008) on the littoral ecosystems of the north Mediterranean Spanish coasts. Final Report (PIEC 200430E599). Centro de Estudios Avanzados de Blanes, Consejo Superior de Investigaciones Científicas, pp 67–78

Costantini F, Fauvelot C, Abbiati M (2007) Genetic structuring of the temperate gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. Mol Ecol 16:5168–82. doi: 10.1111/j.1365-294X.2007.03579.x

Deter J, Descamp P, Ballesta L, et al (2012) A preliminary study toward an index based on coralligenous assemblages for the ecological status assessment of Mediterranean

French coastal waters. Ecol Indic 20:345–352. doi: 10.1016/j.ecolind.2012.03.001

Di Camillo CG, Cerrano C (2015) Mass mortality events in the NW Adriatic Sea: phase shift from slow- to fast-growing organisms. PLoS One 10:e0126689. doi: 10.1371/journal.pone.0126689

Díaz S, Fargione J, Chapin FS, Tilman D (2006) Biodiversity loss threatens human well-being. PLoS Biol 4:e277. doi: 10.1371/journal.pbio.0040277

Dornelas M, Gotelli NJ, McGill B, et al (2014) Assemblage time series reveal biodiversity change but not systematic loss. Science 344:296–9. doi: 10.1126/science.1248484

Doxa A, Holon F, Deter J, et al (2016) Mapping biodiversity in three-dimensions challenges marine conservation strategies: The example of coralligenous assemblages in North-Western Mediterranean Sea. Ecol Indic 61:1042–1054. doi: 10.1016/j.ecolind.2015.10.062

Duran S (2003) Phylogeography, gene flow and population structure of Crambe crambe (Porifera : Poecilosclerida). PhD. Thesis. Universitat de Barcelona.

Edmunds PJ, Witman JD (1991) Effect of Hurricane Hugo on the primary framework of a reef along the south shore of St. John, US Virgin Islands. 78:201–204.

Ferdeghini F, Acunto S, Cocito S, Cinelli F (2000) Variability at different spatial scales of a coralligenous assemblage at Giannutri Island (Tuscan Archipelago, northwest Mediterranean). Hydrobiologia 440:27–36.

Fiori CS, Soares-Gomes A (2002) Taxonomic sufficiency for a monitoring program in a tropical continental shelf, Rio de Janeiro, Brazil. In: Brebbia CA (eds) Oil and hydrocarbon Spills III, pp. 339-349

Gardner T , Côté IM, Gill J, et al (2005) Hurricanes and Caribbean coral reefs: impacts, recovery patterns, and role in long-term decline. Ecology 86:174–184. doi: 10.1890/04-0141

Garrabou J (1999) Life-history traits of Alcyonium acaule and Parazoanth us axinellae (Cnidaria, Anthozoa), with emphasis on growth. Mar Ecol Prog Ser 178:193–204.

Garrabou J, Ballesteros E, Zabala M (2002) Structure and dynamics of North-western

Mediterranean rocky benthic communities along a depth gradient. Estuar Coast Shelf Sci 55:493–508. doi: 10.1006/ecss.2001.0920

Garrabou J, Coma R, Bensoussan N, et al (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol 15:1090–1103. doi: 10.1111/j.1365-2486.2008.01823.x

Garrabou J, Harmelin J-G (2002) A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean : and management needs insights into conservation and management needs. J Anim Ecol 71:966–978.

Garrabou J, Perez T, Sartoretto S, Harmelin J (2001) Mass mortality event in red coral *Corallium rubrum* populations in the Provence region (France, NW Mediterranean). Mar Ecol Prog Ser 217:263–272. doi: 10.3354/meps217263

Garrabou J, Riera J, Zabala M (1998a) Landscape pattern indices applied to Mediterranean subtidal rocky benthic communities. Landsc Ecol 225–247.

Garrabou J, Sala E, Arcas A, Zabala M (1998b) The impact of diving on rocky sublittoral communities: a case study of a bryozoan population. Conserv Biol 12:302–312.

Gaston KJ (2000) Global patterns in biodiversity. Nature 405:220–7. doi: 10.1038/35012228

Gatti G, Bianchi CN, Morri C, et al (2015) Coralligenous reefs state along anthropized coasts: Application and validation of the COARSE index, based on a rapid visual assessment (RVA) approach. Ecol Indic 52:567–576. doi: 10.1016/j.ecolind.2014.12.026

Gili J-M, Ros J (1985) Estudio cuantitativo de tres poblaciones ciralitorales de Cnidarios bentónicos. Investig Pesq 49:323–352.

Halpern BS, Walbridge S, Selkoe KA, et al (2008) A global map of human impact on marine ecosystems. Science 319:948–52. doi: 10.1126/science.1149345

Hong J-S (1982) Contribution à l’étude des peuplements d'un fond de Concrétionnement Coralligène dans la région marseillaise en Méditerranée Nord-occidentale. Bull KORDI 4:27–51.

Hughes TP, Connell JH (1999) Multiple stressors on coral reefs: A long-term perspective. Limnol Oceanogr 44:932–940. doi: 10.4319/lo.1999.44.3_part_2.0932

Hughes TP, Linares C, Dakos V, et al (2013) Living dangerously on borrowed time during slow, unrecognized regime shifts. Trends Ecol Evol 28:149–55. doi: 10.1016/j.tree.2012.08.022

Hughes TP, Rodrigues MJ, Bellwood DR, et al (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol 17:360–5. doi: 10.1016/j.cub.2006.12.049

Huston M (1979) A General Hypothesis of Species Diversity. Am Nat 113:81–101.

Jackson JBC (1979) Morphological strategies in sessile animals. In: Jackson JBC, Buss LW CR (ed) Population biology and evolution of clonal organisms. Yale University Press New Haven, pp 499–555.

Kipson S, Fourt M, Teixidó N, et al (2011) Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops. PLoS One 6:e27103. doi: 10.1371/journal.pone.0027103

Knowlton N, Lang JC, Christine Rooney M, Clifford P (1981) Evidence for delayed mortality in hurricane-damaged Jamaican staghorn corals. Nature 294:251–252.

Konar B, Iken K, Edwards M (2009) Vertical patterns of community structure on rocky shores in the Gulf of Alaska. Mar Ecol 30: 63–73.

Laborel J (1961) Le concrétionment algal "coralligène" et son importance géomorphologique en Méditerranée. Rec Trav St Mar End 23: 37-60.

Laubier L (1966) Le coralligène des Albères: monographie biocénotique. Ann Inst Océanogr Monaco 43:139–316.

Ledoux J-B, Mokhtar-Jamaï K, Roby C, et al (2010) Genetic survey of shallow populations of the Mediterranean red coral [*Corallium rubrum* (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. Mol Ecol 19:675–90. doi: 10.1111/j.1365-294X.2009.04516.x

Linares C (2006) Population ecology and conservation of a long-lived marine species:

the red gorgonian Paramuricea clavata. PhD. Thesis. Universitat de Barcelona.

Linares C, Bianchimani O, Torrents O, et al (2010) Marine Protected Areas and the conservation of long-lived marine invertebrates: the Mediterranean red coral. Mar Ecol Prog Ser 402:69–79. doi: 10.3354/meps08436

Linares C, Coma R, Diaz D, et al (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Mar Ecol Prog Ser 305:127–137. doi: 10.3354/meps305127

Ling SD, Scheibling RE, Rassweiler A, et al (2014) Global regime shift dynamics of catastrophic sea urchin overgrazing. Proc Natl Acad Sci U S A. doi: http://dx.doi.org/10.1098/rstb.2013.0269

López-legentil S (2005) Multidisciplinary studies of the genus Cystodytes (Ascidiacea): from molecules to species. PhD. Thesis. Universitat de Barcelona

Magurran AE, Baillie SR, Buckland ST, et al (2010) Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. Trends Ecol Evol 25:574–82. doi: 10.1016/j.tree.2010.06.016

Maldonado M, Ribes M, van Duyl FC (2012) Nutrient fluxes through sponges: biology, budgets, and ecological implications. In: Becerro MA, Uriz MJ, Maldonado M, Turon X (eds) Advances in Marine Biology. Elsevier Vol. 62 pp. 113-182.

Mariani S, Uriz M-J, Turon X, Alcoverro T (2006) Dispersal strategies in sponge larvae: integrating the life history of larvae and the hydrologic component. Oecologia 149:174–84. doi: 10.1007/s00442-006-0429-9

Mazzella L, Scipione MB, Buia MC (1989) Spatio-temporal distri-bution of algal and animal communities in a Posidonia oceanica (L.) Delile meadow. PSZNI Mar Ecol 10:107–131

McCauley DJ, Pinsky ML, Palumbi SR, et al (2015) Marine defaunation: Animal loss in the global ocean. Science (80) 347:1255641–1255641. doi: 10.1126/science.1255641

McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol 21: 178–185

Micheli F, Halpern BS, Walbridge S, et al (2013) Cumulative human impacts on Mediterranean and Black Sea marine ecosystems: assessing current pressures and opportunities. PLoS One 8:e79889. doi: 10.1371/journal.pone.0079889

Mihaljević M, Stević F, Špoljarić D, Pfeiffer TZ (2014) Application of Morpho-Functional classifications in the evaluation of phytoplankton changes in the Danube river. Acta zool. bulg., Suppl. 7: 153-158

Montero-Serra I, Linares C, García M, et al (2015) Harvesting effects, recovery mechanisms, and management strategies for a long-lived and structural precious coral. PLoS One 10:e0117250. doi: 10.1371/journal.pone.0117250

Mouillot D, Graham NAJ, Villéger S, et al (2013) A functional approach reveals community responses to disturbances. Trends Ecol Evol 28 (3): 167-177

Nekola JC, White PS (1999) The distance decay of similarity in Biogeography and Ecology. J Biogeogr 26:867–878.

Rae M, Folch H, Moniz MBJ, Wolff CW, McCormack GP, Rindi F, Johnson MP (2013) Marine bioactivity in Irish waters. Phytochem Rev 12:555-565

Parravicini V, Morri C, Ciribilli G, et al (2009) Size matters more than method: Visual quadrats vs photography in measuring human impact on Mediterranean rocky reef communities. Estuar Coast Shelf Sci 81:359–367. doi: 10.1016/j.ecss.2008.11.007

Pelay-Gimeno M, García-Ramos Y, Jesús Martin M, et al (2013) The first total synthesis of the cyclodepsipeptide pipecolidepsin A. Nat Commun 4:1–10. doi: 10.1038/ncomms3352

Pham CK, Ramirez-Llodra E, Alt CHS, et al (2014) Marine litter distribution and density in European seas, from the shelves to deep basins. PLoS One 9:e95839. doi: 10.1371/journal.pone.0095839

Piazzi L, Balata D, Cecchi E, Cinelli F (2003) Co-occurrence of *Caulerpa taxifolia* and *C. racemosa* in the Mediterranean Sea: interspecific interactions and influence on native macroalgal assemblages. Cryptogamie Algol 24:233–243

Piazzi L, Balata D, Cecchi E, et al (2010) Species composition and patterns of diversity of macroalgal coralligenous assemblages in the north-western Mediterranean Sea. J Nat Hist 44:1–22. doi: 10.1080/00222930903377547

Piazzi L, Balata D, Cinelli F (2004a) Species composition and morphological groups of macroalgal assemblages around Gorgona Island (north-western Mediterranean Sea). Cryptogamie Algol 25:19–38

Piazzi L, Balata D, Pertusati M, Cinelli F (2004b) Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substratum inclination. Bot Mar 47:105–115. doi: 10.1515/BOT.2004.010

Piazzi L, Gennaro P, Balata D (2012) Threats to macroalgal coralligenous assemblages in the Mediterranean Sea. Mar Pollut Bull 64:2623–9. doi: 10.1016/j.marpolbul.2012.07.027

Piazzi L, Balata D, Cecchi E, Cinelli F (2003) Co-occurrence of *Caulerpa taxifolia* and *C. racemosa* in the Mediterranean Sea: interspecific interactions and influence on native macroalgal assemblages. Cryptogamie Algol 24:233–243

Ponti M, Fava F, Abbiati M (2011) Spatial–temporal variability of epibenthic assemblages on subtidal biogenic reefs in the northern Adriatic Sea. Mar Biol 158:1447–1459. doi: 10.1007/s00227-011-1661-3

Ponti M, Perlini RA, Ventra V, et al (2014) Ecological shifts in mediterranean coralligenous assemblages related to gorgonian forest loss. PLoS One 9:e102782. doi: 10.1371/journal.pone.0102782

Rocchini D, He KS, Oldeland J, et al (2010) Spectral variation versus species beta-diversity at different spatial scales: a test in African highland savannas. J Environ Monit 12:825–31. doi: 10.1039/b921835a

Ros JD, Romero J, Ballesteros E, Gili JM (1985) Diving in blue water. The benthos. In: Margalef R (ed) The western Mediterranean. Pergamon Press, pp 233–295

Rossi L (1961) Sur une facies à Gorgonaires de la Pointe du Mesco (Golfe de Gênes). In: Rapports et Procès-verbaux des Réunions de la C.I.E.S.M. pp 517–521

Rossi S, Gili J (2009) Near bottom phytoplankton and seston: importance in the pelagic-benthic coupling processes. In: Kersey WT, Munger SP (eds) Marine Phytoplankton. Nova Science Publishers, Inc., pp 45-85

Sanchez-Vidal A, Canals M, Calafat AM, et al (2012) Impacts on the Deep-Sea ecosystem by a severe coastal. doi: 10.1371/journal.pone.0030395

Sandin SA, Smith JE, Demartini EE, et al (2008) Baselines and degradation of coral reefs in the Northern Line Islands. PLoS One 3:e1548. doi: 10.1371/journal.pone.0001548

Sala E, Boudouresque CF (1997) The role of fishes in the organization of a Mediterranean sublittoral community. I: algal communities. J Exp Mar Biol Ecol 212:25–44

Sartoretto S, Verlaque M, Laborel J (1996) Age of settlement and accumulation rate of submarine “coralligène” (-10 to -60 m) of the northwestern Mediterranean Sea; relation to Holocene rise in sea level. Mar Geol 130:317–331.

Spalding MD, Fox HE, Allen GR, et al (2007) Marine Ecoregions of the World : A Bioregionalization of Coastal and Shelf Areas. Bioscience 57:573–583.

Steinitz O, Heller J, Tsoar A, et al (2006) Environment, dispersal and patterns of species similarity. J Biogeogr 33:1044–1054. doi: 10.1111/j.1365-2699.2006.01473.x

Teixidó N, Casas E, Cebrian E, et al (2013) Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm. PLoS One 8:e53742. doi: 10.1371/journal.pone.0053742

Teixidó N, Garrabou J, Gutt J, Arntz WE (2007) Iceberg Disturbance and Successional Spatial Patterns: The Case of the Shelf Antarctic Benthic Communities. Ecosystems 10:143–158. doi: 10.1007/s10021-006-9012-9

Teixidó N, Garrabou J, Harmelin J-G (2011) Low dynamics, high longevity and persistence of sessile structural species dwelling on Mediterranean coralligenous outcrops. PLoS One 6:e23744. doi: 10.1371/journal.pone.0023744

Teixidó N, Pineda M-C, Garrabou J (2009) Decadal demographic trends of a long-lived temperate encrusting sponge. Mar Ecol Prog Ser 375:113–124. doi: 10.3354/meps07757

Terlizzi A, Anderson MJ, Fraschetti S, Benedetti-Cecchi L (2007) Scales of spatial variation in Mediterranean subtidal sessile assemblages at different depths. Mar Ecol Prog Ser 332:25–39. doi: 10.3354/meps332025

True M (1970) Étude quantitative de quatre peuplements sciaphiles sur substrat rocheurs dans la région marseillaise. Bull Inst Oceànogr Monaco 60:1–41.

Turner MG, Tinker DBT, Gergel SEG, Chapin FS (2002) Landscape disturbance Location, pattern, and dynamics. In: Turner SEG and MG (ed) Learning landscape ecology: a practical guide to concepts and techniques. Springer-Verlag, New York, pp 147–165

Turon X, Martí R, Uriz M-J (2009) Chemical bioactivity of sponges along an environmental gradient in a Mediterranean cave. Sci Mar 73:387–397. doi: 10.3989/scimar.2009.73n2387

Underwood AJ, Chapman MG (1996) Scales of spatial patterns of distribution of intertidal invertebrates. Oecologia 107:212–224

UNEP/MAP-RAC/SPA (2015) Proceedings of the second Mediterranean Symposium on the conservation of coralligenous and other Calcareous Bio-concretions (Portorož, Slovenia, 29-30 October 2014). Bouafif C, Langar H, Ouerghi A, edits. RAC/SPA publ., Tunis: 260 pp

UNEP/MAP-RAC/SPA (2008) Action Plan for the Conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean sea. Ed. RAC/SPA, Tunis : 21 pp

Uriz M-J (1998) How do reproductive output, larval behaviour, and recruitment contribute to adult spatial patterns in Mediterranean encrusting sponges? Mar Ecol Prog Ser 167:137–148

Vanderklift M, Lavery P (2000) Patchiness in assemblages of epiphytic macroalgae on Posidonia coriacea at a hierarchy of spatial scales. Mar Ecol Prog Ser 192:127–135

Virgilio M, Airoldi L, Abbiati M (2006) Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. Coral Reefs 25:265–272. doi: 10.1007/s00338-006-0100-2

Wedding L, Lepczyk C, Pittman S, et al (2011) Quantifying seascape structure:

extending terrestrial spatial pattern metrics to the marine realm. Mar Ecol Prog Ser 427:219–232. doi: 10.3354/meps09119

Weinberg S (1978a) The minimal area problem in invertebrate communities of Mediterranean rocky substrata. Mar Biol 49:33–40. doi: 10.1007/BF00390728

Weinberg S (1978b) Mediterranean octocorallian communities and the abiotic environment. Mar Biol 49:41–57. doi: 10.1007/BF00390729

Woodley JD, Chornesky EA, Clifford PA, et al (1981) Hurricane Allen’s impact on Jamaican coral reefs. Science 214:749–755

Worm B, Barbier EB, Beaumont N, et al (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314:787–90. doi: 10.1126/science.1132294

Appendix

A Supplementary information

A.1. Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops

Table A.1.1. List of the taxa identified in this study. List of the taxa identified within the assemblages dominated by the red gorgonian *Paramuricea clavata* and the red coral *Corallium rubrum* in three regions of the NW Mediterranean.

	Paramuricea clavata assemblage			*Corallium rubrum* assemblage		
Taxa	Catalonia	Provence	Corsica	Catalonia	Provence	Corsica
Chlorophyta						
Flabellia petiolata	+	-	+	+	-	-
Halimeda tuna	-	-	+	-	-	+
Palmophyllum crassum	+	+	+	+	+	-
Valonia macrophysa	+	-	+	+	-	-
Rhodophyta						
Lithophyllum stictaeforme	+	-	+	+	+	+
Mesophyllum alternans	+	+	+	+	+	+
Peyssonnelia sp.	+	+	+	+	-	-
Protozoa						
Miniacina miniacea	-	+	+	+	+	+
Porifera						
Acanthella acuta	+	+	+	+	+	+
Agelas oroides	+	+	+	+	+	+
Aplysilla sulfurea[b]	-	-	-	+	+	-
Aplysina cavernicola	-	+	+	+	+	-
Axinella damicornis	+	+	+	+	+	+
Cacospongia sp.	+	+	+	+	+	+
Chondrosia reniformis	+	+	+	+	+	-
Clathrina clathrus	+	+	+	+	-	+
Clathrina coriacea~	+	+	+	+	-	-
Cliona sp.	+	+	-	+	-	+
Corticium candelabrum	+	+	+	+	+	+
Crambe crambe	+	+	+	+	+	-
Crella (Grayella) pulvinar	+	+	+	+	+	+
Dendroxea lenis	+	+	-	+	+	+
Dictyonella sp.	+	+	+	+	+	-
Fasciospongia cavernosa	+	+	+	+	+	+
Haliclona (Halichoclona) fulva	+	-	+	+	+	+
Haliclona (Reniera) mediterranea	-	-	+	-	+	+
Haliclona (Soestella) mucosa	+	+	+	+	+	+
Haliclona sp.	-	+	+	+	-	+
Hemimycale columella	+	+	+	+	+	-
Hexadella pruvoti	+	+	-	+	-	+
Hexadella racovitzai	+	+	-	+	-	+
Ircinia oros	+	-	+	+	+	+
Oscarella sp.	+	+	-	+	+	+
Petrosia ficiformis	+	+	+	+	+	+
Phorbas tenacior	+	+	+	+	+	+

Phorbas topsenti[b]	-	-	-	-	+	-
Plakina sp.	-	-	+	+	+	+
Plakortis sp.	-	-	+	+	+	-
Prosuberites longispinus	+	+	+	+	+	+
Raspaciona aculeata	+	+	-	+	+	-
Sarcotragus foetidus	-	-	+	+	-	+
Spirastrella cunctatrix	-	+	+	+	+	+
Spongia (Spongia) officinalis	+	-	+	+	+	+
Spongia virgultosa	-	-	+	+	+	+
Terpios granulosa	+	+	+	+	+	-
Unidentified white calcareous sponge	+	+	+	-	+	-
Unidentified white Dendroceratida^^	+	+	+	+	+	+
Hydrozoa						
Unidentified Hydrozoa	+	+	+	+	+	+
Anthozoa						
Alcyonium acaule	+	-	-	-	-	+
Alcyonium coralloides	+	+	+	-	+	-
Caryophyllia inornata	+	+	+	+	+	+
Corallium rubrum	-	+	-	+	+	+
Corynactis viridis	-	-	+	-	+	-
Eunicella cavolini[a]	-	+	+	-	-	-
Hoplangia durotrix	+	+	+	+	+	+
Leptopsammia pruvoti	+	+	+	+	+	+
Paramuricea clavata[a]	+	+	+	-	-	-
Parazoanthus axinellae[a]	+	+	+	-	-	-
Polychaeta						
Filograna implexa / Salmacina dysteri	+	+	+	+	+	+
Protula sp.	+	+	+	+	+	+
Serpula vermicularis	+	+	+	+	+	+
Serpulidae	+	+	+	+	+	+
Bryozoa						
Adeonella calveti/Smittina cervicornis	+	+	+	+	-	+
Beania hirtissima	-	+	-	+	-	+
Beania magellanica	+	+	-	+	-	-
Caberea boryi~	+	+	+	-	+	+
Chartella sp.	+	-	+	-	-	+
Cellaria sp.~	+	+	+	+	-	+
Celleporina sp.*	-	+	+	+	+	+
Crisia sp.~	-	+	+	+	+	+
Disporella hispida~	+	-	+	+	+	+
*Dentiporella sardonica**	+	+	+	+	+	-
Idmidronea sp.~	+	-	+	+	+	-
Margaretta cereoides[a]	+	+	+	-	-	-
Myriapora truncata	+	+	+	+	-	-
Pentapora fascialis[a]	-	+	+	-	-	-
Reteporella grimaldii	+	+	+	+	+	+
Rhynchozoon sp.*	+	-	-	+	-	+
Schizomavella sp.*	+	+	+	+	+	+
Scrupocellaria sp.~	+	+	+	+	-	+
Smittoidea sp.*	-	-	+	+	+	+
Turbicellepora sp.	+	+	+	+	-	+
Tunicata						

Aplidium sp.	-	+	+	+	+	+
Aplidium undulatum	-	-	+	+	+	+
Clavelina dellavallei[a] ~	-	-	+	-	-	-
Clavelina lepadiformis[a] ~	+	-	+	-	-	-
Pycnoclavella nana ~	-	+	+	-	+	-
Cystodytes sp.	+	-	-	+	-	-
Unidentified Didemnidae	+	+	+	+	+	+
Didemnum coriaceum	-	+	-	+	-	+
Halocynthia papillosa	+	+	-	+	-	+

* grouped as "encrusting bryozoans" in later analysis
~ species showing clear seasonality that were excluded from subsequent analysis
^^ includes the sponges *Pleraplysilla spinifera*, *Dysidea* sp. and *Aplysilla sulfurea*
[a] species found only in the *Paramuricea clavata* assemblage
[b] species found only in the *Corallium rubrum* assemblage

A.2. Structure, biodiversity and landscape pattern indices of *Corallium rubrum* assemblages over broad spatial scales

Table A.2.1. Latitude/longitude for the sites studied.

Site	Locality	Codes	Latitude N	Longitude E
Cova del Dofí	Catalonia	CatDof	42° 2’ 51.07’’	3° 13’ 31.73’’
Cova de la Reina	Catalonia	CatRei	42° 2’ 46.14’’	3° 13’ 29.03’’
Pota del Llop	Catalonia	CatLlo	42° 2’ 58.20’’	3° 13’ 31.94’’
Maïre Grotte	Provence	ProMai	43°12’ 36.72’’	5° 19’ 57.83’’
Plane Grotte Pérès	Provence	ProPer	43°11’ 12.48’’	5° 23’ 25.04’’
Riou Grotte Sud	Provence	ProRio	43°10’ 22.44’’	5° 23’ 21.88’’
Palazzu	Corsica	CorPal	42° 22’ 48.72’’	8° 32’ 44.70’’
Palazzinu	Corsica	CorPlu	42° 22’ 47.71’’	8° 33’ 0.90’’
Passe Palazzu	Corsica	CorPas	42° 22’ 47.64’’	8° 32’ 51.29’’

Table A.2.2. List of the taxa surveyed in the three studied localities of the NW Mediterranean. *Morphological growth form*: encrusting (Enc), tree (Tree), cup (Cup), massive (Mas), turf (Turf), epibiont (Epi). *Life span*: seasonal (Sea), perennial (Per). SIMPER perennial species dataset: species that belong to similarity group of 50% of cumulative contribution in similarity percentage analysis (denoted by *) and presence by +; CatDof = 68.28%; CatRei = 63.02%; CatLlo = 62.43%; ProPer = 77.22%; ProRio = 66.13%; ProMai = 70.57%; CorPal = 68.23%; CorPas = 65.96%; CorPlu = 70.14%.

Taxa	Growth form	Life span	Catalonia			Provence			Corsica		
			CatDof	**CatRei**	**CatLlo**	**ProRio**	**ProMai**	**ProPer**	**CorPas**	**CorPlu**	**CorPal**
Clorophyta											
Palmophyllum crassum	EncPer		+						+		
Flabellia petiolata	ErePer		+								
Rhodophyta											
Lithophyllum cabioache/stictaeforme	Enc	Per		+							
Mesophyllum alternans	Enc	Per		*	+	*				*	
Peyssonnelia sp.	Enc	Per		*	+	+				*	
Red encrusting	Enc	Per		+		+		+			
Mixture complex	Turf	Sea/Per	+	+	+	+	+	+	+	+	+
Protozoa											
Miniacina miniacea	Enc	Per		+		+	+	+	+	+	+
Porifera											
Acanthella acuta	Mas	Per		+	+			+			
Agelasor oïdes	Mas	Per	+	*	+			+	+		
										+	
Aplysina cavernicola	Mas	Per					*	*			
										+	
Aplysilla sulfurea	Enc	Per		+	+	+	+	+	+		
Axinella damicornis	Mas	Per	+	+	*	+	+	+	+	+	+
Chondrosia reniformis	Mas	Per			+					+	
Clathrina sp.	Mas	Per	+	*	+	+			+	+	
Cliona sp.	Bor	Per			+			+			
Corticium candelabrum	Mas	Per	+	+	+						
Crambe crambe	Enc	Per		+	*	+	+	+	+	+	
Crell apulvinar	Enc	Per	+	*	*	*	*	*	+	+	
Dendroxea lenis	Enc	Per	*	+	+	+	+	*	+	*	*
Diplastrella bistellata	Enc	Per	*	+							
Fasciospongia cavernosa	Enc	Per		+	+	+	+	+		+	
Haliclona fulva	Enc	Per	+	*	+	+	+	+		*	*
Haliclona mediterranea	Enc	Per				+				+	+
Haliclona mucosa	Enc	Per	*	+	+		+	*	*	*	+
*Hymedesmia*_type	Enc	Per								+	
Hemimycale columella	Enc	Per			+	+		+			
Hexadella pruvoti	Enc	Per	+	+					+		
Hexadella racovitzai	Enc	Per	+		+	+			+		
Ircinia fasciculata	Mas	Per	+								
Ircinia variabilis	Mas	Per	+	*	+	*	*	+	+	*	+
Ircinia oros	Mas	Per	+	*	+	+	+	+			+
Oscarella sp.	Mas	Per	+	*	+	*	*	+			+
Petrosia fisciformis	Mas	Per	*	+	+	+	*	*	+	+	*
Phorbas tenacior	Enc	Per	+	*	+		+	+		+	
Phorbas topseti/fictitius	Enc	Per				+					
Plakina sp.	Enc	Per				+		+	+	+	
Plakortis sp.	Enc	Per					+				+
Pleraplysilla spinifera	Enc	Per	+	*	*	*	*	*	*	*	*
Prosuberites longispinus	Enc	Per		*		+	+	*	+	*	+
Raspaciona aculeata	Enc	Per		+	+			+		+	
Sarcotragus muscarum	Mas	Per	+							+	
Sarcotragus foetidus	Mas	Per			+	+	+				
Scalarispongia scalaris	Enc	Per	+	+	*	*	+	*	+	+	
Spongia officinalis	Mas	Per						+			
Spongia virgultosa	Mas	Per					+	+			
Spirastrella cunctatrix	Enc	Per		+	+		+			+	
Terpios granulosa	Enc	Per		+		+	+	+	+	+	

Other sponge	Enc/ Mas	Per	*	*	*	*	*	*	+	*	*
Anthozoa											
Alcyonium acaule	Mas	Per			+	+					
Alcyonium coralloides	Enc	Per	+		+						
Caryophyllia inornata	Cup	Per	+	+	+	+	*	*	+	+	+
Corallium rubrum	Tree	Per	*	*	*	*	*	*	*	+	*
Corynactis viridis	Mas	Per				+					
Eunicella cavolinii	Tree	Per					+				
Hoplangia durotrix	Cup	Per	*	+	+	+	*	+	+	+	*
Leptopsammia pruvoti	Cup	Per	*	*	*	+		+	*	*	*
Paramuricea clavata	Tree	Per			*	+				+	
Parazoanthus axinellae	Enc	Per		+	*						
Parerythropodium coralloïdes	Enc	Per				+					
Polychaeta											
Filograna implexa/*Salmacina dysteri*	Epi	Per	+		+	+			+	+	+
*Protula*sp./*Serpula vermicularis*	Enc	Per	+	+	+	+	+	*	+	+	+
Serpulidae	Enc	Per	*	+	+	+	+	*	*	+	
Mollusca											
Bivalvia	Enc	Per				+		+			
Bryozoa											
Adeonella calveti/*Smittina cervicornis*	Tree	Per			*			+	+	+	
Beania magellanica	Enc	Per								+	
Celleporina caminata	Epi	Per				+	+	*			
*Chartella*sp.	Epi	Per			*	+		+	+		+
Dentiporella sardonica	Enc	Per					+				
Disporella hispida	Turf	Sea			+	+	+	+			
Encrusting Bryozoans	Enc	Per	*	+	*	+	+	*	*	*	*
Gregarinidra gregaria	Enc	Per							*	+	
Myriapora truncata	Tree	Per		+	+					+	
Reteporella grimaldii	Tree	Per			+	+		+	*	*	+
Rhynchozoon sp.	Enc	Per			+	+	+	+			
Schizomavella linearis	Enc	Per			+		+	+			
Turbicellepora sp	Epi	Per								+	
Tunicata											
Aplidium sp.	Enc	Per			+		*	+		+	+
Cystodites dellechiajei	Mas	Per	*		*						
Didemnidae	Enc	Per			+		+	+	+	+	+
Didemnum coriaceum	Enc	Per							+		
Halocynthia papillosa	Mas	Per	+		+						

Table A.2.3. Cover % values (mean ± SD) for benthic categories for each site studied of the NW Mediterranean region.

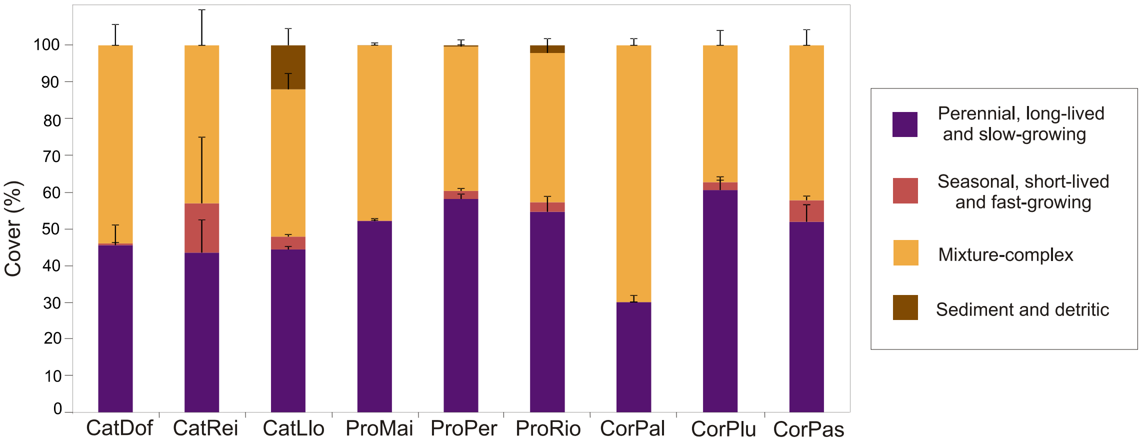


Table A.2.4. Diversity measures at different hierarchical spatial scales: alpha diversity (α-diversity ± SD) % of unshared species (β-diversity) and local diversity (γ–diversity).

Site and locality	α	β (Unshared species %)	Gamma diversity
Catalonia		34.45± 1.78	68
Dofí	24 ± 2	17.81 ± 3.66	
Reina	33 ± 0.58	20.03 ± 0.68	
PotaLlop	41 ± 5.29	20.61 ± 1.65	
Provence		31.10± 1.61	72
Maïre	30 ± 5.78	21.60 ± 0.77	
Planes Pérès	39 ± 3.2	19.43 ± 1.34	
Riou sud	34 ± 3.2	24.00 ± 1.33	
Corsica		32.21± 1.12	57
Palazzinu	40 ± 3.1	14.93 ± 1.48	
Palazzu	21 ± 4.2	17.40 ± 1.39	
PassePalazzu	26 ± 1.53	17.69 ± 1.19	

A.3. Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales

Table A.3.1. List of the taxa surveyed in the three studied localities of the NW Mediterranean. *Morphological growth form*: encrusting (Enc), tree (Tree), cup (Cup), massive (Mas), turf (Turf), erect (Erect), epibiont (Epi). *Life span*: seasonal (Sea), perennial (Per). SIMPER perennial species dataset: species that belong to similarity group of 75% of cumulative contribution in similarity percentage analysis (denoted by *) and presence by +; Average similarity (time pooled): MME = 73.92%; MPT = 75.09%; SGL = 70.24%; SPA = 66.65%; PGP = 73.83%; PCO = 65.66%.

Taxa	Growth form	Life span	Catalonia		Provence		Corsica	
			MME	**MPT**	**PGP**	**PCO**	**SGL**	**SPA**
Chorophyta								
Flabellia petiolata	Erect	Per	*	+	+	-	*	*
Halimeda tuna	Erect	Per	+	-	-	-	+	+
Dictyotales	Erect	Sea	+	-	-	-	-	+
Palmophyllum crassum	Enc	Per	*	-	*	*	+	*
Valonia macrophysa	Enc	Sea	+	-	-	-	*	-
Rhodophyta								
Lithophyllum cabioache/stictaeforme	Enc	Per	*	+	*	*	*	+
Mesophyllum alternans	Enc	Per	*	*	*	*	*	*
Peyssonnelia sp.	Enc	Per	*	*	*	*	*	*
Liagora sp.	Erect	Per	+	-	-	-	+	+
Schottera/Rhodymenia type	Turf	Sea	+	-	+	+	+	*
*Womersleyella*_type	Turf	Per	-	-	-	*	-	-
Other algae								
Mixture complex turf algae	Turf	Sea	+	+	+	+	+	+
Protozoa								
Miniacina miniacea	Enc	Per	-	-	+	+	+	*
Porifera								
Acanthella acuta	Mas	Per	*	*	*	+	+	-
Agelas oroïdes	Mas	Per	*	*	*	*	+	-
Aplysina cavernicola	Mas	Per	-	-	-	-	+	-
Axinella damicornis	Mas	Per	*	*	*	*	*	+
Chondrosia	Mas	Per	+	+	+	+	-	+

reniformis								
Clathrina sp.	Mas	Per	+	+	+	+	*	+
Cliona sp.	Bor	Per	*	-	*	*	-	-
Corticium candelabrum	Mas	Per	+	+	+	+	+	-
Crambe crambe	Enc	Per	*	*	*	*	+	+
Crella pulvinar	Enc	Per	+	*	*	-	+	*
Dendroxea lenis	Enc	Per	-	-	+	-	-	-
Dictyonella sp.	Enc	Per	+	*	+	*	-	-
Diplastrella bistellata	Enc	Per	+	-	-	-	-	-
Dysidea avara	Mas	Per	+	+	+	-	+	-
Eurypon clavatum	Enc	Per	-	-	-	-	+	-
Fasciospongia cavernosa	Enc	Per	+	-	+	+	+	+

Table A.3.2. Diversity measures at different hierarchical spatial scales: alpha diversity (α-diversity) average richness (± SD), % of unshared species (β-diversity) and gamma diversity (γ-diversity).

2006

Site and locality	α	β (Unshared species %)	γ
Catalonia		18.44± 1.20	**52**
MPT	32 ± 2	12.78 ± 0.63	
MME	33 ± 1	15.75 ± 0.85	
Provence		16.06± 1.75	**66**
PCO	36 ± 6	16.50 ± 0.95	
PGP	41 ± 2	16.07 ± 1.75	
Corsica		23.03± 1.92	**62**
SGL	32 ± 4	15.09 ± 1.24	
SPA	37 ± 6	15.98 ± 1.82	

2008

Site and locality	α	β (Unshared species %)	γ
Catalonia		19.77± 0.86	**54**
MPT	31 ± 3	12.78 ± 0.63	
MME	31 ± 2	15.75 ± 0.85	
Provence		25.34± 1.81	**61**
PCO	28 ± 6	20.57 ± 1.99	
PGP	40 ± 4	16.02 ± 0.40	
Corsica		26.50± 3.42	**54**
SGL	22 ± 6	13.76 ± 0.23	
SPA	29 ± 7	23.32 ± 3.00	

2010

Site and locality	α	β (Unshared species %)	γ
Catalonia		20.22± 0.54	**53**
MPT	33 ± 3	14.81 ± 1.2	
MME	30 ± 3	14.14 ± 0.62	
Provence		27.57± 1.15	**63**
PCO	23 ± 2	20.86 ± 2.64	
PGP	42 ± 3	16.02 ± 0.51	
Corsica		28.06± 3.22	**55**
SGL	25 ± 6	13.23 ± 0.82	
SPA	28 ± 9	21.22 ± 0.71	

A.4. Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm

Table A.4.1. List of the taxa identified in this study. Boring (BOR), Cup (CUP), Encrusting algae (ENA), Encrusting (ENC), Massive (MAS), Pedunculated (PEN), Tree (TREE).

Taxa	Growth Form	Carall Bernat	Tascó Petit	Medallot	Punta Salines
Chlorophyta					
Flabellia petiolata	PEN	+	+	+	+
Halimeda tuna	PEN	-	-	+	-
Palmophyllum crassum	ENA	+	-	+	-
Valonia macrophysa	ENC-MAS	+	-	+	-
Rhodophyta					
*Lithophyllum stictaeforme**	ENA	+	+	+	+
*Mesophyllum alternans **	ENA	+	+	+	+
Peyssonnelia sp.*	ENA	+	+	+	+
Porifera					
*Acanthella acuta**	MAS	+	+	+	+
*Agelas oroides**	MAS	+	+	+	+
*Axinella damicornis**	MAS	+	+	+	+
Cacospongia sp.	MAS	+	+	+	-
*Chondrosia reniformis**	MAS	+	+	+	+
*Clathrina clathrus**	MAS	+	+	+	-
Cliona sp.*	BOR	+	+	+	+
*Corticium candelabrum**	MAS	+	+	+	+
*Crambe crambe**	ENC	+	+	+	+
*Crella (Grayella) pulvinar**	ENC	+	+	+	+
Dendroxea lenis	ENC	+	-	-	-
Dictyonella sp.	ENC-MAS	+	+	+	+
*Dysidea avara**	ENC	+	+	+	+
Fasciospongia cavernosa	ENC	+	+	+	-
Haliclona (Halichoclona) fulva	ENC	-	+	+	-
Haliclona (Soestella) mucosa	ENC	+	+	+	-
Haliclona sp.	ENC	-	+	+	-
*Hemimycale columella**	ENC	+	+	+	+
*Hexadella racovitzai**	ENC	+	+	+	+
*Ircinia oros**	MAS	+	+	+	-
Ircinia fasciculata	MAS	-	-	+	-
Ircinia variabilis	MAS	+	+	+	+
Oscarella sp.*	MAS	+	+	+	-
*Petrosia ficiformis**	MAS	+	+	+	+
*Phorbas tenacior**	ENC	+	+	+	+
Phorbas topsenti	ENC	-	-	+	-
*Pleraplysilla spinifera**	ENC	+	+	+	+
Prosuberites longispinus	ENC	+	+	+	-
Raspaciona aculeata	ENC	+	+	-	+
*Spirastrella cunctatrix**	ENC	+	+	+	+
Spongia (Spongia) officinalis	MAS	+	+	-	-
Spongia virgultosa	MAS	+	-	+	-
Terpios granulosa	ENC	+	+	-	-
Anthozoa					
*Alcyonium acaule**	MAS	+	+	+	+
Alcyonium coralloides	ENC	+	+	+	+
*Caryophyllia inornata**	CUP	+	+	+	+
Corallium rubrum	TREE	-	+	-	-
Hoplangia durotrix	CUP	+	+	-	-
*Leptopsammia pruvoti**	CUP	+	+	+	+

*Paramuricea clavata**	TREE	+	+	+	+
*Parazoanthus axinellae**	ENC	+	+	+	+
Polychaeta					
*Filograna implexa / Salmacina dysteri**	TREE	+	+	+	+
Protula sp./*Serpula vermicularis*	ENC	+	+	+	-
Serpulidae	ENC	+	+	+	-
Bryozoa					
*Adeonella calveti/Smittina cervicornis**	TREE	+	+	+	+
Beania magellanica	ENC	-	+	+	-
Chartella tenella	TREE	-	+	+	+
Margaretta cereoides	TREE	-	-	+	-
*Myriapora truncata**	TREE	+	+	+	+
Pentapora fascialis	TREE	-	-	+	-
*Reteporella grimaldii**	TREE	-	+	+	+
Schizomavella sp.	ENC	+	+	+	+
Turbicellepora sp.	TREE	+	+	+	-
Tunicata					
*Cystodytes dellechiajei**	ENC	+	+	+	+
Didemnum sp.1	ENC	+	-	+	-
Didemnum sp. 2	ENC	+	-	+	-
*Halocynthia papillosa**	MASS	+	+	+	+

* taxa accounted for 90% of the SIMPER analysis

Table A.4.2. Cover area (%) of the representative macrobenthic taxa before and after the physical disturbance generated by the storm. Boring (BOR), Cup (CUP), Encrusting algae (ENA), Encrusting (ENC), Massive (MAS), Tree (TREE). nc: no change

Taxa	Group	Carall Bernat		Tascó Petit		Medallot		Punta Salines	
Rhodophyta		Before	After	Before	After	Before	After	Before	After
Lithophyllum stictaeforme	ENA	2.3	0.3	4.5	3.3	10.0	9.0	5.5	nc
Mesophyllum alternans	ENA	13.8	7.6	14.5	nc	19.8	9.6	18.6	nc
Palmophyllum crassum	ENA	1.5	0.3	-	-	1.3	nc	-	-
Peyssonnelia sp.	ENA	5.5	0	9.5	nc	40.0	36.0	22.0	nc
Porifera									
Acanthella acuta	MAS	1.16	0.16	3.5	2.3	1.3	nc	4.5	nc
Agelas oroides	MAS	3.3	3.1	7.5	7	6.1	nc	1.3	nc
Axinella damicornis	MAS	2	0.5	2.8	nc	3.6	nc	3.0	nc
Chondrosia reniformis	MAS	3.2	3	3	nc	3	nc	-	-
Clathrina clathrus	MAS	1.2	0	5.2	1.6	0.8	nc	-	-
Cliona sp.	BOR	0.8	1.2	0.5	nc	3.7	4.5	9.5	nc
Corticium candelabrum	MAS	1.8	1.5	-	-	-	-	-	-
Crella pulvinar	ENC	-	-	3.3	nc	1	nc	-	-
Crambe crambe	ENC	12.1	9.6	8	8	10	nc	16.3	nc
Disydea avara	ENC	7.8	2.8	1.6	nc	2	1	6.0	nc
Hemimycale columella	ENC	0.16	0	3.2	nc	0.5	nc	-	-
Hexadella racovitzai		-	-	-	-	4	3.3	-	-
Ircinia oros	MAS	-	-	4.8	nc	0.8	nc	-	-
Ircninia variabilis	MAS	-	-	3	2.8	0.3	nc	-	-
Oscarella sp.	MAS	-	-	24.5	21.8	-	-	-	-
Petrosia fisciformis	MAS	-	-	1.3	nc			-	-
Phorbas tenacior	ENC	8.2	4.2	2.5	1.8	2	nc	5.5	nc
Pleraplysilla spinifera	ENC			1.5	nc	2	nc	-	-
Spirastrella cunctatrix	ENC	-	-	3.3	nc	8.6	nc	-	-
Anthozoa									
Alcyonium acaule	MAS	1.6	0.9	3.6	nc	6	nc	2.2	nc
Caryophyllia inornata	CUP	0.3	0.16	2.6	nc	1.6	nc	-	-
Leptopsammia pruvoti	CUP	5.5	2.6	21.5	nc	5	nc	5	nc
Paramuricea clavata	TREE	8.8	2.6	12	11	9.6	7.8	5	nc
Parazoanthus axinellae	ENC	10.6	3.1	17.5	16.5	17.2	15.6	9	nc
Polychaeta									
Filograna implexa / Salmacina dysteri	TREE	-	-	0.3	0.1	1.8	0	-	-
Bryozoa									
Adeonella calveti/Smittina cervicornis	TREE	1.7	0	13	10.5	6.8	5	2.5	nc
Myriapora truncata	TREE	1.3	0	0.6	nc	1	0	1	nc
Reteporella sp.	TREE	-	-	0.8	nc	0.3	0.16	0.5	nc
Tunicata									
Cystodytes	ENC	4.5	2.1	10.5	nc	9.1	nc	10.6	nc
Halocynthia papillosa	MAS	-	-	2	0	0.3	nc	0.3	nc

Table A.4.3. Results of 2-way PERMANOVA analyses based on Bray-Curtis dissimilarity for macrobenthic taxa. Pair-wise comparisons using permutations of the *t*-statistic for the factor Site and the interaction term Site*Before/After are also indicated.

Source	**df**	**SS**	**MS**	**Pseudo_F**	**P**	**Pair-wise comparisons**
Site	3	9625	3208.3	20.25	0.0001	Carall Bernat ≠ Tascó Petit t=5.04 (p<0.0001) Carall Bernat ≠ Medallot t=3.66 (p<0.0001) Carall Bernat ≠ Punta Salines t=4.964 (p<0.0001) Tascó Petit ≠ Medallot t=3.70 (p<0.0001) Tascó Petit ≠ Punta Salines t=6.29 (p<0.0001) Medallot ≠ Punta Salines t=3.94 (p<0.0001)
Before/After	1	543.2	543.2	1.24	0.3894	
Site*BA	3	1410.5	470.17	2.96	0.0001	Carall Before ≠ Carall After t= 2.98 (p<0.0002) Tascó Petit Before = Tascó Petit After t= 1.48 (p>0.05) Medallot Before = Medallot After t= 1.36 (p>0.05)

						Pta Salines Before = Pta Salines After t= 1.40 (p>0.05)
Residual	43	6810.6	158.39			
Total	50	18539				

Table A.4.4. Results of 2-way PERMANOVA analyses based on Euclidian distances for the number of species. Pair-wise comparisons using permutations of the t-statistic for the factor Site and Site*BA (Before/After) effects are also indicated.

Permanova	df	SS	MS	Pseudo_F	P	Pair-wise
Site	3	998.05	332.68	34.416	0.0001	Carall Bernat ≠ Tascó Petit t=4.3292, p<0.0001 Carall Bernat ≠ Medallot t=4.7922, p<0.001 Carall Bernat ≠ Pta Salines t=6.5, p<0.001 Tascó Petit = Medallot t=0.108 ; p=>0.05 Tascó Petit ≠ Pta Salines t=8.2, p<0.0001 Medallot ≠ Pta Salines t=9.9, p<0.0001
Before/After	1	34.77	34.77	0.445	0.581	
Site*BA	3	258.41	86.13	8.910	0.0001	Carall (B) ≠ Carall (A) t=5.995; p<0.0009 Tascó Petit (B) = Tascó Petit (A) t=1.145; p>0.05 Medallot (B) = Medallot (A) t=0.553; p>0.05 Punta Salines (B) = Punta Salines (A) t=1.25; p>0.05
Residual	43	415.67	9.66			

Table A.4.5. Results of 2-way PERMANOVA analyses based on Euclidian distances for the cover area of the principal groups of sessile organisms and bare substrate. Pair-wise comparisons using permutations of the *t*-statistic for the factor Site and Site*BA (Before/After) effects are also indicated.

Permanova	**df**	**SS**	**MS**	**Pseudo_F**	**P**	**Pair-wise**
Site	2	8030.4	4015.2	12.08	0.0001	Carall Bernat ≠ Tascó Petit t=5.3038, p<0.0001 Carall Bernat ≠ Medallot t=2.39, p<0.005 Tascó Petit ≠ Medallot t=5.30 ; p<0.0001
Before/After	1	8017.6	8017.6	2.25	0.266	
Site*B/A	2	7102.4	3551.2	10.69	0.0001	Carall (B) ≠ Carall (A) t=4.085; p<0.001 Tascó Petit (B) = Tascó Petit (A) t=2.63; p>0.05 Medallot (B) ≠ Medallot (A) t=3.107; p<0.001
Residual	39	12953	333.14			

Table A.4.6. Results of 2-way PERMANOVA analyses based on Euclidian distances for the cover of growth forms of sessile species. Pair-wise comparisons using permutations of the *t*-statistic for the factor Site and Site*BA (Before/After) effects are also indicated.

Permanova	df	SS	MS	Pseudo_F	P	Pair-wise
Site	2	10006	5003	113.26	0.0001	Carall Bernat ≠ Tascó Petit t=12.7, p<0.0001 Carall Bernat ≠ Medallot t=9.1, p<0.001 Tascó Petit ≠ Medallot t=10.60 ; p<0.0001
Before/After	1	945.1	945.1	5.76	0.104	
Site*BA	2	327.8	163.9	3.71	0.0063	Carall (B) ≠ Carall (A) t=4.76; p<0.01 Tascó Petit (B) = Tascó Petit (A) t=2.13; p>0.05 Medallot (B) = Medallot (A) t=2.45; p>0.05
Residual	9	397.5	44.1			

B Publication Chapter 3

OPEN ACCESS Freely available online

PLoS one

Rapid Biodiversity Assessment and Monitoring Method for Highly Diverse Benthic Communities: A Case Study of Mediterranean Coralligenous Outcrops

Silvija Kipson[1,3]*, Maïa Fourt[2], Núria Teixidó[1,6], Emma Cebrian[4], Edgar Casas[1], Enric Ballesteros[5], Mikel Zabala[6], Joaquim Garrabou[1,2]

1 Institut de Ciències del Mar (ICM-CSIC), Barcelona, Catalonia, Spain, **2** UMR 6540 - DIMAR CNRS - Université de la Méditerranée, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Marseille, France, **3** Division of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia, **4** Departament de Ciències Ambientals, Facultat de Ciències, Universitat de Girona, Girona, Catalonia, Spain, **5** Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Blanes, Catalonia, Spain, **6** Departament d'Ecologia, Universitat de Barcelona, Barcelona, Catalonia, Spain

Abstract

Increasing anthropogenic pressures urge enhanced knowledge and understanding of the current state of marine biodiversity. This baseline information is pivotal to explore present trends, detect future modifications and propose adequate management actions for marine ecosystems. Coralligenous outcrops are a highly diverse and structurally complex deep-water habitat faced with major threats in the Mediterranean Sea. Despite its ecological, aesthetic and economic value, coralligenous biodiversity patterns are still poorly understood. There is currently no single sampling method that has been demonstrated to be sufficiently representative to ensure adequate community assessment and monitoring in this habitat. Therefore, we propose a rapid non-destructive protocol for biodiversity assessment and monitoring of coralligenous outcrops providing good estimates of its structure and species composition, based on photographic sampling and the determination of presence/absence of macrobenthic species. We used an extensive photographic survey, covering several spatial scales (100s of m to 100s of km) within the NW Mediterranean and including 2 different coralligenous assemblages: *Paramuricea clavata* (*PCA*) and *Corallium rubrum* assemblage (*CRA*). This approach allowed us to determine the minimal sampling area for each assemblage (5000 cm^2 for PCA and 2500 cm^2 for CRA). In addition, we conclude that 3 replicates provide an optimal sampling effort in order to maximize the species number and to assess the main biodiversity patterns of studied assemblages in variability studies requiring replicates. We contend that the proposed sampling approach provides a valuable tool for management and conservation planning, monitoring and research programs focused on coralligenous outcrops, potentially also applicable in other benthic ecosystems.

Citation: Kipson S, Fourt M, Teixidó N, Cebrian E, Casas E, et al. (2011) Rapid Biodiversity Assessment and Monitoring Method for Highly Diverse Benthic Communities: A Case Study of Mediterranean Coralligenous Outcrops. PLoS ONE 6(11): e27103. doi:10.1371/journal.pone.0027103

Editor: Simon Thrush, National Institute of Water & Atmospheric Research, New Zealand

Received May 27, 2011; **Accepted** October 10, 2011; **Published** November 2, 2011

Funding: The research was funded by the Spanish International Cooperation Agency for Development (AECID) (S. Kipson, Research Fellowship 2009-11), the French Government (Service de coopération et d'action culturelle) (S. Kipson, Fellowship 2007), the Spanish Ministry of Science and Innovation (E. Casas, Doctoral Fellowship), Parc National de Port-Cros, French Agence Nationale pour la Recherche (ANR) (MEDCHANGE Project), the Total Foundation (MedDiversa Project) and the Spanish Ministry of Science and Innovation (Biorock project ref. CTM2009-08045). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: silvija.kipson@zg.htnet.hr

Introduction

Coastal ecosystems are among the most diverse, highly productive and complex biological systems [1]. At the same time, they are highly threatened by a combination of anthropogenic impacts, such as overfishing, habitat loss, eutrophication, introductions of exotic species and climate change [2,3], leading to profound structural and functional changes [4,5]. However, future shifts in the species composition of assemblages cannot be evaluated without knowledge and understanding of the present state of marine biodiversity. Obtaining this baseline information represents a key step in exploring future modifications of coastal ecosystems.

The Mediterranean Sea is considered a marine biodiversity hotspot, harboring approximately 10% of world's marine species while occupying only 0.82% of the ocean surface [6,7]. Unfortunately, the impacts of human activities are proportionally stronger in the Mediterranean than in the other seas, raising concerns regarding threats to the conservation of the rich Mediterranean biodiversity [6]. Coralligenous outcrops, which are hard bottoms of biogenic origin that thrive under dim light conditions, are among the habitats faced with major threats in the Mediterranean Sea. These outcrops are highly diverse (harboring approximately 20% of Mediterranean species) and exhibit great structural complexity [8 10]. The species that dominate coralligenous seascapes are encrusting calcareous algae, sponges, cnidarians, bryozoans and tunicates. Some of the engineering species in these environments are long-lived, and their low dynamics make coralligenous outcrops exceptionally vulnerable when faced with sources of strong disturbances, such as destructive fishing practices, pollution, invasive species or mass mortality outbreaks [8,11 13].

The immediate consequences and long-lasting effects of these disturbances have mostly been addressed at the population level, focusing on certain structurally important species (e.g., [12,14 18]). Despite the ecological, aesthetic and economic value of coralligenous outcrops, coralligenous biodiversity patterns at the community level over regional scales remain poorly understood ([8,19] and references therein). This lack of information is partially due to the complexity involved in studying these highly diverse systems with slow dynamics, coupled with general logistical constraints related to sampling at deep rocky habitats.

Most of the previous studies at the assemblage level have been largely descriptive [20 23]. There are a few quantitative studies available, restricted to small or medium spatial scales, but their results are not comparable due to the differences in sampling methodology (e.g., scraped samples *vs.* photographic sampling) [10,24 28]. Therefore, an accurate overview of the general biodiversity patterns associated with coralligenous outcrops is lacking.

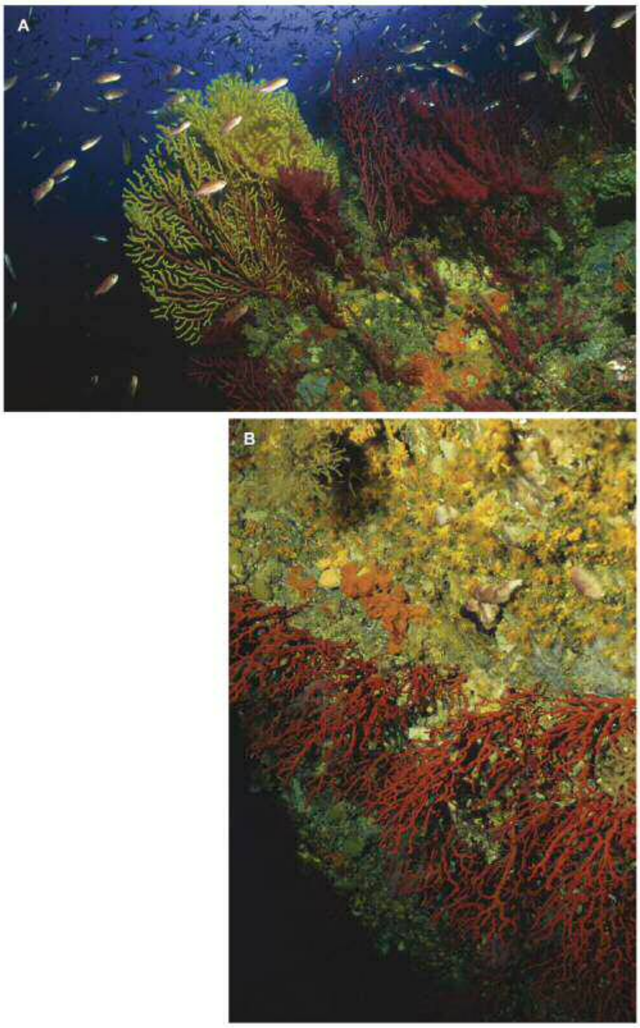


Figure 1. General aspect of 2 facies of the coralligenous outcrops considered in this study. (A) *Paramuricea clavata* assemblage (*PCA*) and (B) *Corallium rubrum* assemblage (*CRA*). Photos by E. Ballesteros.
doi:10.1371/journal.pone.0027103.g001

Ecologists, conservation practitioners, managers and policy makers highlight the need to develop cost-effective sampling methods to provide comparative measures of biodiversity and to create a platform of "biodiversity baselines". There is currently no single sampling method that has been demonstrated to be sufficiently representative to provide adequate community assessment and monitoring in coralligenous outcrops [29].

To ensure the representativeness and time- and cost-efficiency of any benthic community survey, aiming to capture the original community structure and to account for its natural variability, an adequate sampling unit size and sampling effort (i.e. the number of replicates) should be determined [30,31]. Therefore, when the goal is to assess the complexity of the system, a good representation of the species pool should be achieved and therefore the minimal sampling area for the assemblage should be defined, i.e. the sampling unit size over which an increase of area does not yield a significant increase in the number of species [32 34]. Both the sampling unit size and sampling effort will influence the representativeness of a sample data set in terms of accuracy (the ability to determine the true value) and precision (the ability to detect differences) of the estimates [29]. While accuracy and precision generally increase with sampling effort [29], the high small-scale heterogeneity of coralligenous habitats additionally implies that large sampling areas are required to achieve representative results [8]. However, optimization of the sampling strategy is indispensable given the considerable depths where coralligenous outcrops usually develop and the limited information that can be obtained in the restricted diving time.

Taking into account the priorities and activities defined by the Action Plan for the Conservation of the Coralligenous [13], we aimed to provide guidelines for the application of a rapid, non-destructive protocol for biodiversity assessment and monitoring in coralligenous habitat. The sampling procedure used in this study was designed to assess the natural spatio-temporal variability of coralligenous outcrops, which is crucial information for *a posteriori* assessment of the impact of anthropogenic activities.

The aims of this study were three-fold: (1) to determine the minimal sampling area required to assess the sessile macrobenthic species composition in the studied assemblages, (2) to estimate the minimal sampling effort needed to obtain a good representation of the number of species and the complexity of the overall community and (3) to explore the capacity of the proposed

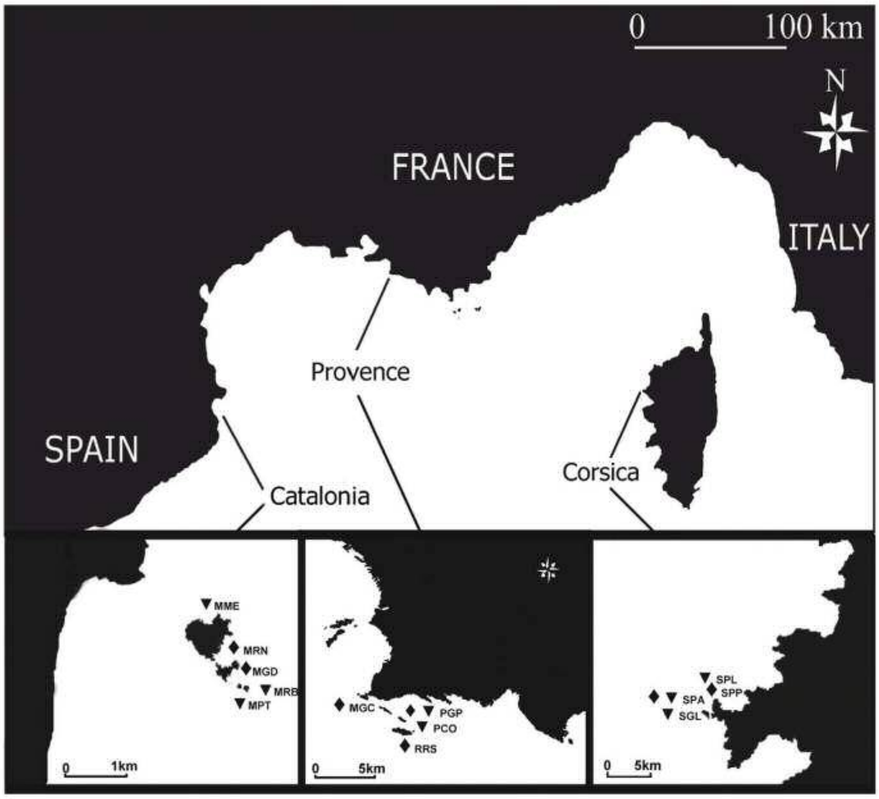


Figure 2. Map of the study area in the NW Mediterranean Sea. Three studied regions in the NW Mediterranean and sites within them (triangles = sites with *Paramuricea clavata* assemblage and diamonds = sites with *Corallium rubrum* assemblage). See Table 1 for site abbreviations.
doi:10.1371/journal.pone.0027103.g002

approach to account for assemblage composition variability on different spatial scales and among different assemblages. The application of this approach to characterizing coralligenous outcrops and detecting future changes was also assessed.

Materials and Methods

Ethics Statement

Institut de Ciències del Mar (ICM-CSIC), Centre d'Océanologie de Marseille, University of Zagreb (Faculty of Science), Universitat de Girona (Facultat de Ciències), Centre d'Estudis Avançats de Blanes-CSIC and Universitat de Barcelona approved this study.

Communities studied and study areas

Coralligenous outcrops comprise a complex of assemblages ranging from algal dominated ones to others completely dominated by macroinvertebrates with almost no algal growth [8]. Here we selected two assemblages that are dominated by the long-lived gorgonians *Paramuricea clavata* (Risso 1826) and *Corallium rubrum* (L. 1758) (Fig. 1) and that displayed the same aspect at all studied sites, always thriving under dim light conditions. The *P. clavata* assemblage (hereafter *PCA*) was sampled on rocky walls at depths ranging from 17 to 24 m, whereas the *C. rubrum* assemblage (hereafter *CRA*) was sampled on overhangs and cave entrances at depths between 14 and 20 m. Further, we consider these assemblages among the most complex ones within the coralligenous outcrops, enabling us to develop a representative sampling method that would perform well in less complex coralligenous assemblages.

We studied a total of 15 sites (8 sites for *PCA* and 7 sites for *CRA*) located in three regions: northern Catalonia, Provence and Corsica, covering more than 400 km of the coastline (Fig. 2). Two to three sites per region and assemblage were sampled (sites within regions were separated by hundreds of meters to a few kilometers). The selected regions encompass a high temperature-productivity gradient in the NW Mediterranean. Provence is characterized by cold, relatively eutrophic waters maintained by local upwellings. Northern Catalonia is characterized by waters largely influenced by river discharges [35,36], whereas Corsica is characterized by warmer and more oligotrophic waters [36]. Therefore, each region presents particular environmental conditions, thus providing a good dataset for testing the potential of the biodiversity assessment method for detecting natural inter-regional variability. In fact, along this gradient, shifts in the zonation patterns have been reported with coralligenous assemblages developing at shallower depths in the cold-eutrophic areas than in the warm-oligotrophic ones [37]. The observed depth of the coralligenous outcrops ranges from 10 to 50 55 m in Provence (Marseille area) and Catalonia (Medes Islands) [38 40] while in Corsica it ranges from 20 to 80 m [38].

Photographic sampling

The proposed method for biodiversity assessment was based on analysis of the presence/absence of macro-species dwelling in the understory of the selected assemblages that were identified from photographs (see below). To facilitate identification of these species, we sampled the assemblages using quadrats of 25×25 cm for *PCA* and 20×20 cm for *CRA*. The photographs were taken with a Nikon D70S digital SLR camera fitted with a Nikkor 20 mm DX lens and housed in Subal D70S housing. Lighting was provided by two electronic strobes fitted with diffusers. Sampling was conducted during spring and summer of 2006 and 2007. A total of 475 and 486 photographs were analyzed for *PCA* and *CRA*, respectively.

Species identification

Using these photographs, species were identified to the lowest possible taxonomic level. When further clarification was needed, working with marked plots (see below) allowed us to precisely track down an organism in the field and collect a voucher specimen. Thus, a total of 208 specimens were collected for further identification in the laboratory. Visually similar taxa that could not be consistently identified from photographs were grouped as indicated in Table S1. Furthermore, because the time of sampling differed for different sites, the species showing clear seasonality were excluded from the subsequent analysis (see Table S1).

Determination of a sampling method for biodiversity assessment in coralligenous outcrops

To determine the sampling method to be used for biodiversity assessment in coralligenous outcrops, we established the minimal sampling area (hereafter MSA) and minimal sampling effort required to provide good estimates of the species number and composition for each studied assemblage.

Table 1. Logarithmic functions fitted to spatially explicit species-area curves based on the original order of contiguous samples.

Region	Site		Function	r^2	k	A_{min}/cm^2
a) *Paramuricea clavata* assemblage						
Catalonia	El Medallot	(MME)	$y = 9.26\ln(x) - 45.09$	0.99	131	4999
	El Tascó Petit	(MPT)	$y = 6.84\ln(x) - 27.16$	0.973	53	2029
	Carall Bernat	(MRB)	$y = 8.57\ln(x) - 40.83$	0.988	117	4481
Provence	Petit Conglué	(PCO)	$y = 9.29\ln(x) - 49.27$	0.998	202	7718
	Plane-Grotte Pérès	(PGP)	$y = 10.66\ln(x) - 55.2$	0.992	177	6787
Corsica	Gargallu	(SGL)	$y = 8.68\ln(x) - 41.59$	0.996	121	4622
	Palazzino	(SPL)	$y = 6.85\ln(x) - 29.97$	0.999	80	3050
	Palazzu	(SPA)	$y = 9.04\ln(x) - 43.57$	0.995	124	4755
b) *Corallium rubrum* assemblage						
Catalonia	Cova de la Reina	(MRN)	$y = 9.19\ln(x) - 43.47$	0.984	113	4336
	Cova de Dofi	(MGD)	$y = 5.46\ln(x) - 21.33$	0.997	50	1899
Provence	Riou-Grotte Riou Sud	(RRS)	$y = 5.49\ln(x) - 20.39$	0.987	41	1573
	Plane Grotte Pérès	(PGP)	$y = 5.89\ln(x) - 19.67$	0.969	28	1079
	Maïre Grotte à Corail	(MGC)	$y = 5.83\ln(x) - 22.92$	0.999	51	1950
Corsica	Palazzu	(SPA)	$y = 7.61\ln(x) - 36.51$	0.922	121	4645
	Passe Palazzu	(SPP)	$y = 4.48\ln(x) - 18.79$	0.978	66	2530

Logarithmic functions, goodness of fit measure (r^2), k parameter and minimal sampling areas (A_{min}) calculated for each study site of the *Paramuricea clavata* and *Corallium rubrum* assemblages in the 3 regions of the NW Mediterranean. Site names are provided with abbreviations.

doi:10.1371/journal.pone.0027103.t001

a) Estimation of minimal sampling areas. To estimate MSA, we analyzed the species-area relationship [32,33,41,42], taking into account the spatial arrangement of species, to obtain a good representation of the species pool, as well as the structure of the community [34,43].

Therefore, we applied a spatially explicit design based on contiguous sampling of quadrats arrayed to cover rectangular plots. At each site, we employed plots ranging from 3.2 to 4 m^2 for *PCA* and from 1.76 to 3.72 m^2 for *CRA*. The plots were marked with screws fixed to the rock by putty, and quadrats inside the plots were sequentially positioned and photographed. Overall, 51 to 64 quadrats were photographed per site for *PCA*, whereas 44 to 93 quadrats were photographed per site for *CRA*.

For further determination of MSA, we followed the method described by Ballesteros [44]. A species-area curve for each plot was produced from the subset of all possible combinations of increasing numbers of the originally ordered contiguous quadrats. Thus, mean values of species numbers for successively larger areas were obtained and plotted *vs.* their respective areas. The curve was fitted to a logarithmic function [45]:

$$S = z \ln A + c$$

where S is the number of species, and A is the sampling area in cm^2. To evaluate the model's performance, r^2 was used as a standard goodness-of-fit measure. Based on this equation, the parameter k was calculated, which describes the shape of the curve and provides information on the qualitative distribution of species within the community [44,46]:

$$k = e^{-c/z}$$

The higher the value of k, the larger the sampling area needed to obtain a representative number of species in the community due to their more dispersed distribution [44]. In this study, the qualitative minimal sampling area was determined as the point at which an increase of the sampling area by 20% yields a 5% increment in species number (Molinier point M 20/5) using the following equation:

$$A = k * e^{[\ln(1 + dA)/dS]}$$

where dA and d'S are the relative increments of the surface area and species number (expressed as percentages), respectively. Hence, the Molinier point chosen in this study can be expressed as M 20/5 = Amin = 38.3 * k [44].

b) Estimation of sampling effort needed to maximize species number. In communities with a patchy distribution of species, such as coralligenous assemblages [8], combining small separate areas will usually result in a higher species count than will be obtained for a contiguous area of the same size [47]. Therefore, we also determined the minimal number of separate quadrats required to assess the maximum number of species present at each site (hereafter random quadrats). Consequently, we produced a second set of species-area curves based on 999 permutations, ignoring the spatial arrangement of these quadrats.

Finally, we also explored the increase in the number of species associated with increasing surface area when the MSAs determined for each assemblage were considered as sampling units (replicates).

Tests for pattern assessment within the coralligenous outcrops

We applied multivariate analytical procedures to explore the suitability of the proposed methods for the detection of the variability of biodiversity within coralligenous outcrops on different spatial scales and among the two studied assemblages. More specifically, we explored whether the methods were able to cope with the intraregional variability (hundreds of meters to a few

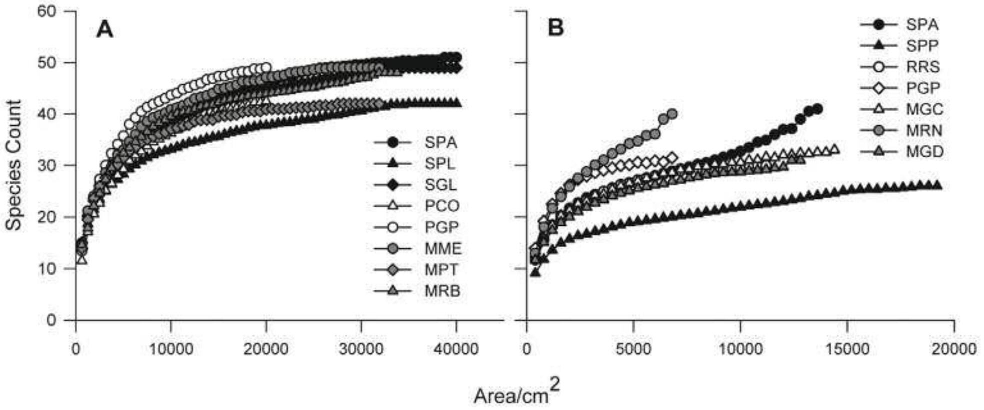


Figure 3. Spatially explicit species-area curves for each site within the 3 regions of the NW Mediterranean. (A) *Paramuricea clavata* assemblage and (B) *Corallium rubrum* assemblage (black = Corsica, white = Provence and gray = Catalonia). In a given area, each point represents multiple measures obtained from a subset of all possible combinations of increasing numbers of the originally ordered contiguous samples, with the curve based on the mean of those measures (SD not shown). See Table 1 for site abbreviations.
doi:10.1371/journal.pone.0027103.g003

Table 2. The local species number per unit area estimated through spatially non-explicit species-area curves.

			Species				% Species			
Region	**Sites**	**Total N**	**16**	**24**	**32**	**3×8**	**16**	**24**	**32**	**3×8**
a) *Paramuricea clavata* assemblage										
Catalonia	El Medallot	52	44	47	49	44	84	90	94	85
	El Tascó Petit	44	40	42	43	40	91	95	97	91
	Carall Bernat	50	43	46	48	44	86	92	95	88
Provence	Petit Conglué	52	41	45	47	41	79	87	91	79
	Plane-Grotte Pérès	58	49	53	54	48	85	91	94	83
Corsica	Gargallu	52	41	45	48	40	80	87	92	77
	Palazzino	45	36	38	40	36	80	84	90	80
	Palazzu	56	45	49	51	45	81	88	91	80
b) *Corallium rubrum* assemblage										
Catalonia	Cova de la Reina	57	40	44	47	43	71	77	82	75
	Cova de Dofí	37	28	30	31	31	75	81	85	84
Provence	Riou-Grotte Riou Sud	42	33	37	39	36	80	88	92	86
	Plane-Grotte Pérès	35	32	33	34	32	90	94	97	91
	Maïre Grotte à Corail	37	32	34	35	34	85	92	95	92
Corsica	Palazzu	49	32	36	38	34	66	73	77	69
	Passe Palazzu	26	21	23	24	21	81	88	92	81

The local species number per unit area estimated through spatially non-explicit species-area curves (Fig. 4) for each site within each region. Total N: total number of species recorded at each site; Species: number of species observed by analyzing a different number of random quadrats (16, 24, 32) or a combination of contiguous quadrats (3×8 = 3 replicates of 8 contiguous quadrats); % Species: percentage of species observed in comparison to the total species number recorded. For random quadrats, calculations were based on 999 permutations of replicate samples, whereas for replicates of 8 contiguous quadrats, calculations were based on a subset of all potential replicate combinations (SD not shown).

doi:10.1371/journal.pone.0027103.t002

kilometers) and interregional variability (hundreds of kilometers) in the species composition of the two selected assemblages. Finally, we also explored the existence of differences between these assemblages.

Because many statistical analyses (e.g., analysis of variance) use replicate measurements to account for the amount of variation, we decided to use the MSA values obtained in this study (8 contiguous quadrats, see Results and Table 1) as replicates. Therefore, prior to analysis, presence/absence data were expressed for combinations of 8 contiguous quadrats (= replicates, measuring 50×100 cm for *PCA* and 40×80 cm for *CRA*). The total number of replicates per site ranged from 5 to 10.

To determine the minimum number of replicates needed to assess biodiversity patterns, we compared the outcomes of the analysis using the overall dataset (all replicates available per site) and those using 3, 4, 5 and 6 replicates.

Similarly, we explored the potential effects on biodiversity patterns when smaller sampling unit sizes were used. For this purpose, we compared the results of a multivariate analysis based on a dataset using MSA values as replicates with those based on a dataset using single quadrats as replicates (25×25 cm for *PCA* and 20×20 cm for *CRA*).

Data treatment

A Bray-Curtis similarity [48] matrix was constructed on the basis of presence/absence data. Non-metric multidimensional scaling (MDS) ordination [49] was performed to visualize patterns of community similarities.

Non-parametric analysis of variance PERMANOVA [50] was used to test for spatial variability. We applied a hierarchical design with 2 factors: Region (3 levels), as a random factor, and Site (8 and 7 levels for *PCA* and *CRA*, respectively), as a random factor nested in Region. Tests of significance were based on 9999 permutations of residuals under a reduced model [51,52]. One-way PERMANOVA was applied to test for differences in species composition between the two assemblages (fixed factor). The test of significance was based on 9999 unrestricted permutations of raw data. All computations were performed using the PRIMER v6 software program with the PERMANOVA+ add-on package [53,54].

Results

Categories identified

A total of 93 macrobenthic taxa were identified: 7 macroalgae, 1 protozoan, 39 sponges, 10 anthozoans, 1 hydrozoan, 5 polychaetes, 21 bryozoans and 9 tunicates (Table S1). Following appropriate grouping and elimination of seasonal taxa (see Methods), a total of 77 taxa were retained for further analysis. Of these, 75 taxa were recorded in *PCA* and 72 taxa in *CRA*. A total of 23 taxa were present in all regions within both communities, while 5 taxa were recorded exclusively within *PCA* and 2 taxa within *CRA* (Table S1). Of all identified categories (including taxa and groups), approximately 70 could be identified solely from photographs (without samples taken), upon a certain training. However, in general, the identification ability depended on the quality of photographs examined as well as whether the organisms were present in a typical morphological form or not (e.g., for the bryozoan *Turbicellepora* sp.).

Determination of sampling method

Minimal sampling area (MSA). Spatially explicit species-area curves exhibited a fairly similar shape in the case of *PCA*, whereas they were more variable both in their shape and relative completeness in the case of *CRA* (Fig. 3). A good fit of the function to the data was indicated by r^2 values higher than 0.90 in all cases (Table 1).

The mean value for the qualitative minimal sampling areas was approximately 5000 cm^2 for *PCA* and half the size, 2500 cm^2, for *CRA* (Table 1). Bearing in mind the size of the quadrats used in this study (see methods), approximately 8 contiguous quadrats (corresponding to surfaces of 50×100 cm for *PCA* and 40×80 cm for *CRA*) should be used to reach the MSAs for both assemblages as a replicate for biodiversity assessment studies.

Similar inter-site differences in MSAs were observed within each assemblage (Table 1). For *PCA*, the estimated area varied between 2000 and 8000 cm^2, with the sites from the Provence region showing the largest MSA (around 7000 cm^2). In the case of *CRA*, the values obtained were slightly lower, varying between 1000 and 5000 cm^2 (Table 1).

Estimation of minimum sampling effort to maximize species number. Through analysis of all quadrats considered in this study, we determined the total number of species found at each site. For *PCA*, the species number ranged between 44 and 58, whereas for *CRA*, the number ranged between 26 and 57 (Table 2). Analysis of the species-area curves performed with random quadrats indicated that sampling efforts covering total areas of approximately 10,000 cm^2 for *PCA* and 5000 cm^2 for *CRA* would detect approximately 80% of all macrobenthic species recorded at the study sites (Fig. 4 and Table 2), whereas doubling the analyzed surface yielded more than 90% of the recorded species (Table 2). Therefore, to obtain good estimates of species number, approximately 16 to 32 random quadrats should be analyzed.

When MSAs were used as sampling units, analysis of only 3 replicates of 8 contiguous quadrats provided approximately 80% of the total species found at each site (Table 2).

Test for pattern assessment

a) Characterizing the regional variability of biodiversity patterns. Disregarding the number of replicates used per site (3, 4, 5 or 6), the patterns revealed by MDS and PERMANOVA were similar to those obtained using datasets based on the maximum possible number of replicates per site (5–10). Here, only the results of the analyses based on datasets with 3 and the maximum possible number of replicates per site (5–10) are shown (Fig. 5A–5D). For both assemblages, MDS ordination revealed 3 distinct clusters, corresponding to different regions (Fig. 5A and 5B; Fig. 5C and 5D), whereas PERMANOVA indicated significant variability at spatial levels for both region and site (Table 3). In the case of *PCA*, the greatest variation occurred at the regional scale, followed by sites and, finally, individual quadrats, whereas in the case of *CRA*, the greatest variation was observed at the site level, followed by regions and individual quadrats (Table 3). Similar levels of significance and explained variability were found, independent of the number of replicates used (Table 3).

Likewise, the use of a different number of replicates did not change the outcome of comparisons of selected assemblages. In all cases, the MDS ordinations performed revealed two distinct clusters, clearly separating one assemblage from the other (Fig. 5E and 5F), while PERMANOVA indicated significant differences between them (Table 4).

b) Analyzing the effect of different sampling unit sizes on biodiversity pattern assessment. The comparison of patterns using datasets based on individual quadrats (N = 475 for *PCA* and N = 486 for *CRA*) and 3 (or more) replicates of 8 contiguous quadrats revealed differences in the patterns and hierarchy of the spatial scales considered.

In the case of *PCA*, MDS ordination performed on the dataset based on individual quadrats revealed one distinct cluster corresponding to Corsica, whereas Catalonia and Provence overlapped (Fig. 6A). In the case of *CRA*, all clusters corresponding to different regions overlapped to a certain extent (Fig. 6B). In contrast, the MDS ordination performed on the dataset based on replicates of 8 contiguous quadrats clearly distinguished the regional clusters in both assemblages (Fig. 5A and 5C). While variability remained significant at both the region and site spatial levels, regardless of the dataset used, PERMANOVA revealed a different hierarchy of spatial scales depending on the sampling unit used. For both assemblages, in the case of datasets based on individual quadrats, the greatest component of variation was associated with the smallest spatial scale, i.e., individual quadrats (Table 3), whereas

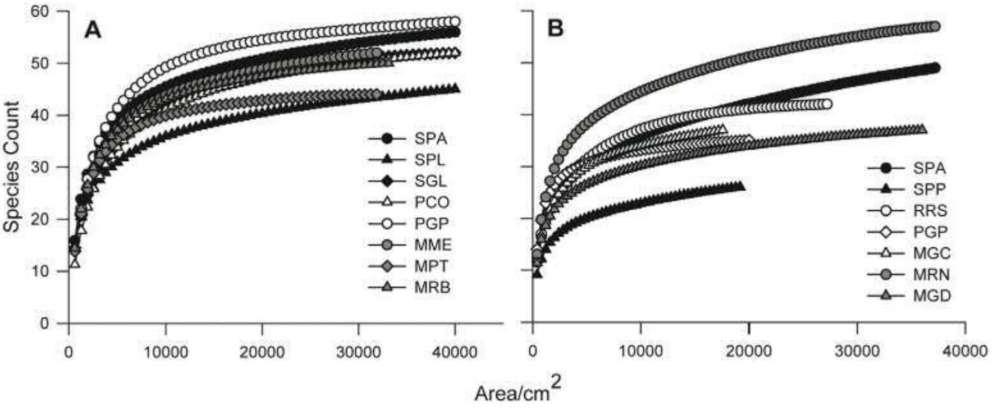


Figure 4. Spatially non-explicit species-area curves for each site within the 3 regions of the NW Mediterranean. (A) *Paramuricea clavata* assemblage and (B) *Corallium rubrum* assemblage (black = Corsica, white = Provence and gray = Catalonia). Data were based on 999 permutations of replicate samples (SD not shown). See Table 1 for site abbreviations.
doi:10.1371/journal.pone.0027103.g004

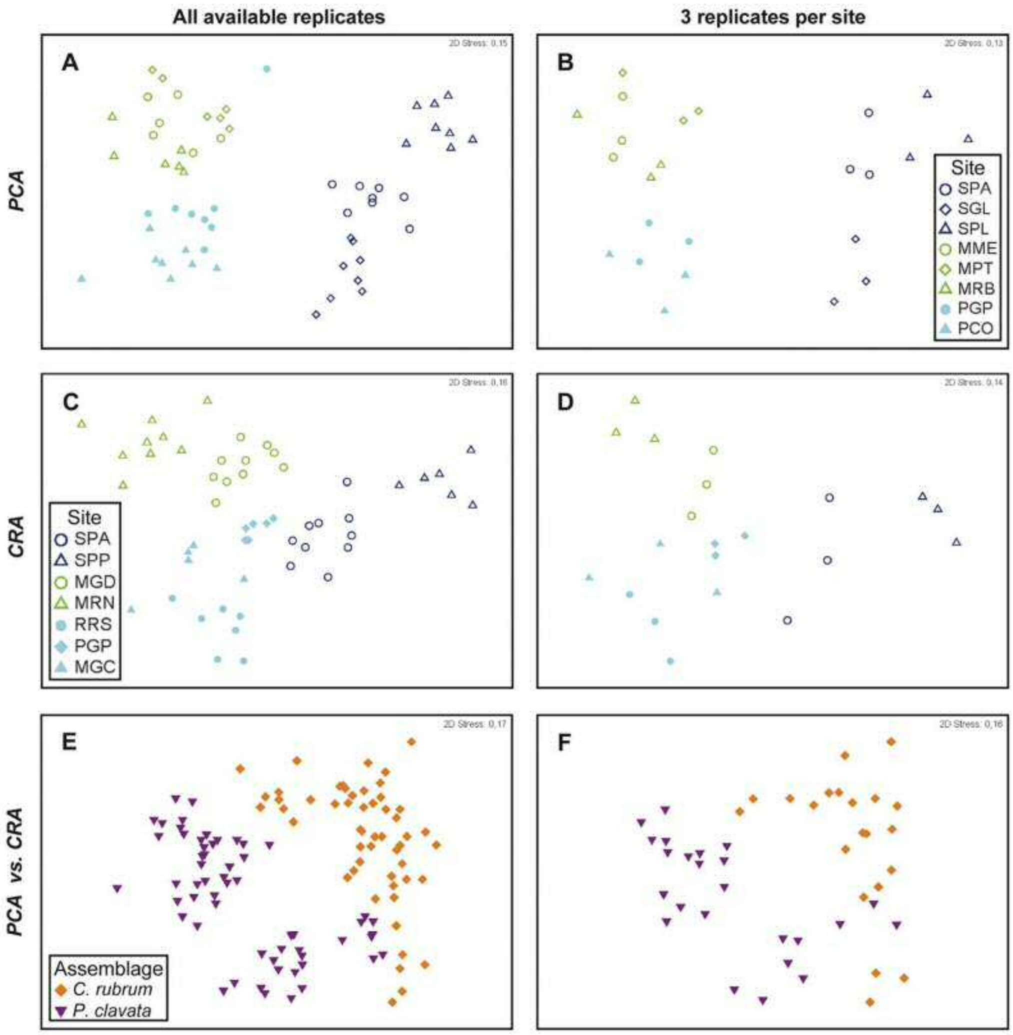


Figure 5. Non-metric multidimensional scaling (MDS) for all possible replicates and 3 replicates per site within the *Paramuricea clavata* (*PCA*) and *Corallium rubrum* (*CRA*) assemblages. Each replicate corresponds to 8 contiguous quadrats, creating a sampling unit of 50×100 cm for *PCA* and 40×80 cm for *CRA*. Three studied regions of the NW Mediterranean are depicted by colors (dark blue = Corsica, green = Catalonia and light blue = Provence). See Table 1 for site abbreviations.
doi:10.1371/journal.pone.0027103.g005

in the case of datasets based on replicates of 8 contiguous quadrats, the greatest component of variation was observed at larger spatial scales (regional level for *PCA* and site level for *CRA*). Finally, the use of smaller sampling units (individual quadrats) for comparison of selected assemblages revealed similar patterns to when larger sampling units (replicates of 8 contiguous quadrats) were used (Fig. 6C *vs*. Fig. 5E and 5F; Table 4), although the former method did not account for the particular structure of the assemblages because sampling unit size employed did not comply with the MSA.

Discussion

Here, we propose, for the first time, a standardized biodiversity assessment method for coralligenous assemblages that provides good estimates of assemblage structure and species composition based on photographic sampling and determination of the presence/absence of macrobenthic species. We used an extensive photographic survey (almost 1000 photographs) covering several spatial scales (hundreds of meters to hundreds of kilometers) and

Table 3. Summary of PERMANOVA analyses based on Bray-Curtis dissimilarity for macrobenthic taxa within the studied assemblages.

		A) *Paramuricea clavata* assemblage				**B) *Corallium rubrum* assemblage**			
Sampling unit and effort	**Source**	**df**	**Pseudo-F**	**VC**	**BC diss (%)**	**df**	**Pseudo-F**	**VC**	**BC diss (%)**
		AI) sampling unit size 50 cm×100 cm				BI) sampling unit size 40 cm×80 cm			
3 replicates	Region	2	56.19**	669.28	25.87	2	28.74*	408.70	20.22
	Site (Region)	5	40.83***	287.16	16.95	4	62.53***	418.79	20.46
	Residual	16		279.45	16.72	14		239.15	15.47
	Total	23				20			
4 replicates	Region	2	5.66**	625.67	25.01	2	2.52*	332	18.22
	Site (Region)	5	4.91***	280.34	16.74	4	9.54***	447.64	21.16
	Residual	24		287.08	16.94	21		209.57	14.48
	Total	31				27			
5 replicates	Region	2	6.17**	658.8	25.67	2	2.75**	363.79	19.07
	Site (Region)	5	6.17***	280.35	16.74	4	9.36***	424.56	20.61
	Residual	32		271.23	16.47	28		253.97	15.94
	Total	39				34			
6 replicates	Region	2	5.74**	632.5	25.15	2	2.64**	342.44	18.51
	Site (Region)	5	8.32***	308.11	17.55	4	11.14***	434.3	20.84
	Residual	40		252.65	15.90	34		249.31	15.79
	Total	47				40			
All replicates	Region	2	5.29**	607.82	25.00	2	2.33*	287.72	17.00
	Site (Region)	5	9.94***	331.93	18.00	4	13.76***	440.91	21.00
	Residual	50		267.3	16.00	46		249.28	16.00
	Total	57				52			
		AII) sampling unit size 25 cm×25 cm				BII) sampling unit size 20 cm×20 cm			
All quadrats	Region	2	2.68**	529.53	23.00	2	2.52*	396.64	20.00
	Site (Region)	5	37.32***	791.64	28.00	4	39.37***	548.78	23.00
	Residual	499		1367.3	37.00	479		932.88	31.00
	Total	506				485			

The results were obtained from datasets based on different number of replicates of 8 contiguous quadrats and individual quadrats. VC = Variance Components; BC diss = Bray Curtis dissimilarity.

P (perm) values.

*<0.05.

**<0.01.

***<0.001.

doi:10.1371/journal.pone.0027103.t003

including 2 different coralligenous assemblages (*PCA* and *CRA*), which allowed us to determine the MSA for each assemblage and optimize the sampling effort to assess biodiversity patterns and provide estimates of species number. Furthermore, we propose MSAs as unitary sampling units for variability studies requiring replicates. Three replicates measuring 5000 cm^2 for *PCA* and 2500 cm^2 for *CRA* were found to be sufficient to maximize the species number and to assess the main biodiversity patterns present (Tables 2 and 3). To ensure species identification and to facilitate the sampling procedures, we propose that photographs of smaller quadrats than the MSA arrayed to cover MSA surfaces should be obtained (e.g., 8 quadrats of 25×25 cm for *PCA* and 8 quadrats of 20×20 cm for *CRA*).

By combining a photographic survey and data acquired at the presence-absence level, the proposed method allows a large number of samples to be obtained during the limited diving time periods that are possible in deep water habitats (down to 50 m) [55,56] and thus, to cope with the high spatial heterogeneity of coralligenous assemblages, while greatly reducing image time processing, which is one of the main constrains of photosampling. Recent studies comparing commonly used sampling methods in hard bottom communities also advocate the use of photo-quadrats attaining adequate sampling areas in change/impact studies or whenever a large number of replicates is needed [56,57]. Additionally, the proposed protocol enables obtaining permanent objective records of both qualitative and quantitative data that can be further analyzed. For instance, analysis of species presence/absence datasets allows identifying the determinant species for such assemblages (SIMPER analysis, Primer, [58]), which can be further used to focus the quantitative (cover area) studies on these determinant species and thus optimize the image processing involved, alongside other methods that improve time efficiency in quantitative studies, such as recording frequencies instead of estimating cover [59] and/or applying an automated software [60]. Likewise, analysis of species presence/absence datasets allows establishment of species area relationships (SARs), which

Table 4. Summary of PERMANOVA analyses for the comparison of *Paramuricea clavata* (*PCA*) and *Corallium rubrum* (*CRA*) assemblages.

Sampling unit and effort	Source	df	Pseudo-F	VC	BC diss (%)
3 replicates	Assemblage	1	14.03***	558.22	23.63
	Residual	43		959.82	30.98
	Total	44			
All replicates	Assemblage	1	35.58***	561.93	23.71
	Res	109		899.97	30.00
	Total	110			
All quadrats	Assemblage	1	256.48***	1072.4	32.75
	Residual	959		2016.6	44.91
	Total	960			

The analyses were based on Bray-Curtis dissimilarity for macrobenthic taxa within the studied assemblages. The results were obtained from datasets based on different number of replicates of 8 contiguous quadrats and individual quadrats (25×25 cm for *PCA* and 20×20 cm for *CRA*). VC = Variance Components; BC diss = Bray Curtis dissimilarity.

P (perm) values:

*<0.05.

**<0.01.

***<0.001.

doi:10.1371/journal.pone.0027103.t004

have been recently proposed as indicators of community-level changes in biodiversity and may be useful in quantifying human impact [61].

One of the key aspects of the proposed method is the determination of MSAs as sampling units for the characterization of the coralligenous assemblages. To our knowledge, MSAs had only previously been estimated for studying cnidarian species dwelling in coralligenous assemblages [62,63]. Interestingly, both studies determined comparable values for areas required to reach at least 80% of species: approximately 5000 cm^2 for *PCA* and 4000 cm^2 for *CRA*. In the present study, use of the MSA as a sampling unit was crucial for the assessment of biodiversity patterns. Comparison of the patterns obtained using MSA and smaller individual quadrats (used in the photo sampling) as replicates clearly showed a shift in the hierarchy of the estimates of variance components from large to small spatial scales. In general, the variation in the observed similarities among samples increases as the size of the sampling unit decreases [64]. Thus, using sampling units smaller than the MSA may have resulted in increased stochastic variability in the species composition at the smallest spatial scale. Similar effects have been reported previously in different habitats (e.g., [56,65,66]). However, previous studies on coralligenous outcrops adopted sampling units ranging between 240 and 600 cm^2 (e.g., [21,24 28,67 69]), which were therefore much lower than MSA values, and found the highest variability at the replicate scale (e.g., [24,25]). Hence, we emphasize the necessity to determine MSAs and use them as sampling units in the assessment of biodiversity patterns within coralligenous (and other) assemblages.

Although coralligenous assemblages harbor a significant proportion of the biodiversity that exists in the Mediterranean Sea [8], little is known about the biodiversity patterns within them. Bearing in mind the current pressures on coralligenous habitats [8], methods are urgently needed to assess prevailing patterns, evaluate impacts to which they are subjected and provide baseline data to explore future trajectories of these high diversity assemblages. We contend that the adoption of the method proposed in this study could furnish the required data to address these timely issues. In our opinion, three main research domains could be easily addressed using this method in a reasonable time framework to facilitate the development of meaningful management and conservation plans for coralligenous assemblages.

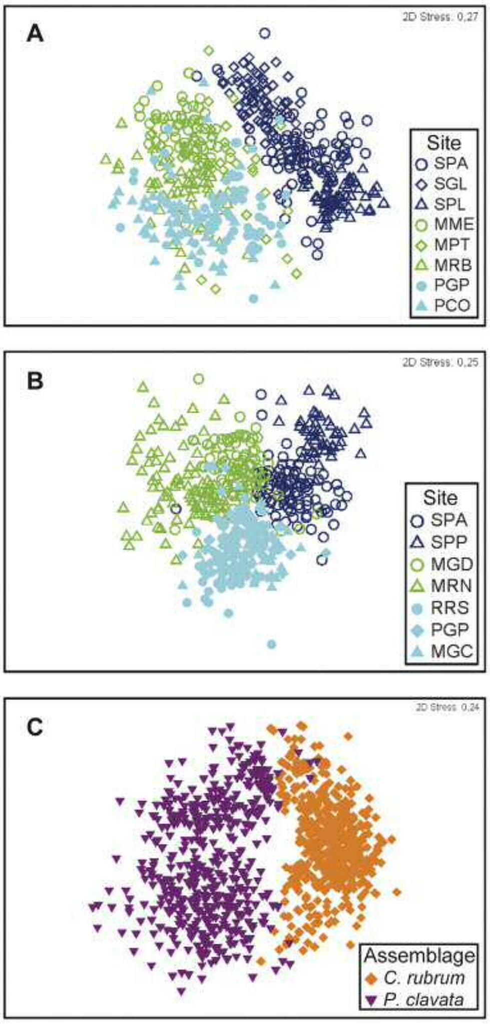


Figure 6. Non-metric multidimensional scaling (MDS) for the studied assemblages and their comparison. (A) *Paramuricea clavata* assemblage (sampling unit of 25×25 cm), (B) *Corallium rubrum* assemblage (sampling unit of 20×20 cm) and (C) comparison of *P. clavata* and *C. rubrum* assemblages in the 3 regions of the NW Mediterranean (dark blue = Corsica, green = Catalonia and light blue = Provence). See Table 1 for site abbreviations.

doi:10.1371/journal.pone.0027103.g006

First, the method displayed potential for the characterization of biodiversity patterns. Its application to the analysis of spatial patterns at different scales (1 to 10^3 km), including areas with differential environmental conditions and anthropogenic pressures, could help to establish conservation status baselines for coralligenous assemblages and, consequently, identify potential management actions needed for the recovery of areas with a low conservation status. Additionally, the method developed in this study could be used to address rarely surveyed deep coralligenous banks (extending from 60 down to 120 m, depending on the geographical position and local light conditions [8]), as ROVs (remotely operated vehicles) or research submersibles have the operational capability to collect high-resolution digital photographs that we contend are compatible with the proposed method. However, it has to be emphasized that the application of the proposed method for the assessment of deep coralligenous banks would be comparatively more difficult, since in our study scuba divers could manage to obtain the images even in coralligenous assemblages displaying high structural complexity (e.g. high density of vertical stratum) and/or developing on complex substrates such as overhangs or vaults. Obtaining the required sets of images with remote devices can be more challenging in deep coralligenous banks due to operational difficulties. Despite of this, we emphasize that the applicability of our approach is already suitable here by adapting the process of image acquisition. For instance, to ensure acquisition of spatially contiguous photographs of a standard size in these conditions of reduced operability at depth, individual still photographs could be obtained from a high resolution video transect. Besides, we strongly recommend to verify the actual number and size of replicates during the preliminary assessment, as the knowledge on the structure of deep coralligenous banks is very scarce. Finally, we believe that future technical advancements and improved operating abilities of ROVs/submersibles ensure the interest for developing biodiversity assessment methods based on the acquisition of images.

Second, the method could be applied to the evaluation of temporal changes in coralligenous assemblages, which would allow identification of impacts on the monitored assemblages. In this sense, it is crucial to establish temporal baselines to properly evaluate the significance of observed changes. Our results detected significant differences at the intra-regional scale, indicating that a reliable assessment of temporal trends should be carried out at the site level.

Finally, the proposed method proved to be sufficiently sensitive to detect significant differences between the studied coralligenous assemblages at both the community and geographic levels. Considering that coralligenous outcrops are regarded as a complex of assemblages [8], this approach may help to provide an objective basis to identify assemblages within coralligenous outcrops.

Application of unified sampling approaches over different regions, depths and times will allow tremendous progress to be made in our understanding of the biodiversity patterns of coralligenous outcrops. In this study, we developed a robust method for biodiversity assessment with the intention of providing a useful tool for management and conservation planning, monitoring and research programs focused on one of the most highly valued and emblematic Mediterranean habitats. We further contend that this method is potentially applicable in other benthic ecosystems.

Supporting Information

Table S1 List of the taxa identified in this study. List of the taxa identified within the assemblages dominated by the red gorgonian *Paramuricea clavata* and the red coral *Corallium rubrum* in three regions of the NW Mediterranean.
(DOC)

Acknowledgments

The authors are very grateful for the helpful assistance during the photographic surveys and/or biological data collection of J.M. Dominici (Réserve Naturelle de Scandola, Parc Naturel Régional de Corse), B. de Ligondes, R. Graille and F. Zuberer (Diving staff, Centre d'Océanologie de Marseille), C. Marschal, O. Torrents, J.B. Ledoux, O. Bianchimani (UMR 6540 DIMAR), C. Linares (Universitat de Barcelona) and the Medes Marine Reserve Staff. We further thank J.G. Harmelin, H. Zibrowius, J. Vacelet and N. Boury- Esnault for taxonomic assistance.

Author Contributions

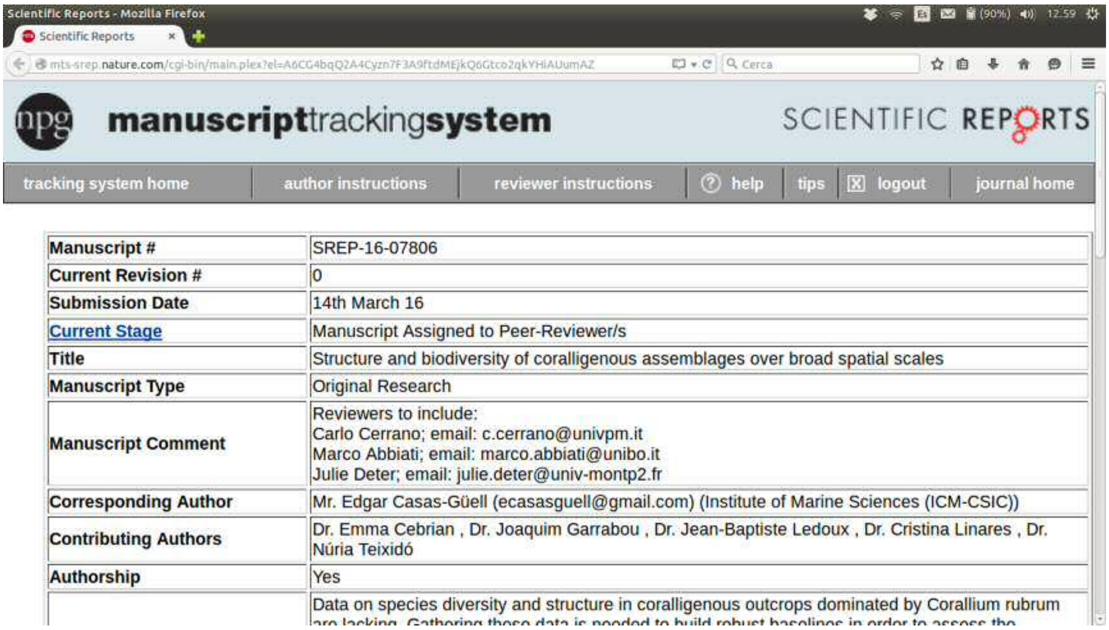
Analyzed the data: SK MF NT E. Cebrian E. Casas EB MZ JG. Wrote the paper: SK NT E. Cebrian E. Casas EB JG. Designed the study: MZ JG. Collected the data: SK MF NT E. Cebrian E. Casas EB MZ JG.

References

1. Agardy T, Alder J, Dayton P, Curran S, Kitchingman A, et al. (2005) Coastal systems. In: Reid W, ed. Millennium Ecosystem Assessment: Ecosystems & Human Well-Being. Washington: Island Press. pp 513 549.
2. Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, et al. (2008) A Global Map of Human Impact on Marine Ecosystems. Science 319: 948 952.
3. Rockstrom J, Steffen W, Noone K, Persson A, Chapin FS, et al. (2009) A safe operating space for humanity. Nature 461: 472 475.
4. Jackson JBC (2001) Historical Overfishing and the Recent Collapse of Coastal Ecosystems. Science 293: 629 637.
5. Walther G-R, Post E, Convey P, Menzel A, Parmesan C, et al. (2002) Ecological responses to recent climate change. Nature 416: 389 395.
6. Coll M, Piroddi C, Steenbeek J, Kaschner K, Ben Rais Lasram F, et al. (2010) The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. PLoS ONE 5(8): e11842. doi:101371/journalpone0011842.
7. Bianchi CN, Morri C (2000) Marine Biodiversity of the Mediterranean Sea: Situation, Problems and Prospects for Future Research. Marine Pollution Bulletin 40: 367 376.
8. Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Ocean Mar Biol Annu Rev 44: 123 195.
9. Gili JM, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol Evol 13: 316 321.
10. Garrabou J, Ballesteros E, Zabala M (2002) Structure and Dynamics of Northwestern Mediterranean Rocky Benthic Communities along a Depth Gradient. Estuar Coast Shelf Sci 55: 493 508.
11. Coma R, Polà E, Ribes M, Zabala M (2004) Long-term assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecol Appl 14: 1466 1478.
12. Garrabou J, Coma R, Bensoussan N, Bally M, ChevaldonnÉ P, et al. (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global Change Biology 15: 1090 1103.
13. UNEP-MAP-RAC/SPA (2008) Action plan for the conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean Sea. RAC/SPA, ed. Tunis: RAC/SPA. pp 21.
14. Bavestrello G, Cerrano C, Zanzi D, Cattaneo-Vietti R (1997) Damage by fishing activities to the Gorgonian coral *Paramuricea clavata* in the Ligurian Sea. Aquat Conserv: Mar Freshw Ecosyst 7: 253 262.
15. Garrabou J, Perez T, Sartoretto S, Harmelin JG (2001) Mass mortality event in red coral *Corallium rubrum* populations in the Provence region (France, NW Mediterranean). Mar Ecol Prog Ser 217: 263 272.
16. Giuliani S, Virnolamberti C, Sonni C, Pellegrini D (2005) Mucilage impact on gorgonians in the Tyrrhenian sea. Sci Tot Environ 353: 340 349.
17. Linares C, Coma R, Diaz D, Zabala M, Hereu B, et al. (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Mar Ecol Progr Ser 305: 127 137.
18. Cupido R, Cocito S, Barsanti M, Sgorbini S, Peirano A, et al. (2009) Unexpected long-term population dynamics in a canopy-forming gorgonian coral following mass mortality. Mar Ecol Prog Ser 394: 195 200.

19. Casellato S, Stefanon A (2008) Coralligenous habitat in the northern Adriatic Sea: an overview. Mar Ecol 29: 321 341.
20. Laubier L (1966) Le coralligène des Albères: monographie biocénotique. Ann Inst Océanogr Monaco 43: 139 316.
21. True MA (1970) Étude quantitative de quatre peuplements sciaphiles sur substrat rocheurs dans la région marseillaise. Bull Inst Océanogr Monaco 60: 1 41.
22. Hong JS (1982) Contribution à l'étude des peuplements d'un fond coralligène dans la région marseillaise en Méditerranée Nord-Occidentale. Bull Korea Ocean Research Develop Inst 4: 27 51.
23. Gili JM, Ros J (1985) Study and cartography of the benthic communities of Medes islands (NE Spain). Pubbl Staz Zool Napoli I: Marine Ecology 6: 219 238.
24. Acunto S, Balata D, Cinelli F (2001) Variabilita spaziale nel coralligeno e considerazioni sul metodo di campionamento. Biol Mar Medit 8: 191 200.
25. Ferdeghini F, Acunto S, Cocito S, Cinelli F (2000) Variability at different spatial scales of a coralligenous assemblage at Giannutri Island (Tuscan Archipelago, northwest Mediterranean). Hydrobiologia 440: 27 36.
26. Piazzi L, Balata D, Pertusati M, Cinelli F (2004) Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substratum inclination. Bot Mar 47: 105 115.
27. Balata D, Piazzi L, Cecchi E, Cinelli F (2005) Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposition. Mar Environ Res 60: 403 421.
28. Virgilio M, Airoldi L, Abbiati M (2006) Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. Coral Reefs 25: 265 272.
29. Bianchi CN, Pronzato R, Cattaneo-Vietti R, Benedetti-Cecchi L, Morri C, et al. (2004) Mediterranean marine benthos: a manual of methods for its sampling and study. Hard bottoms. Biol Mar Medit 11: 185 215.
30. Muxika I, Ibaibarriaga L, Saiz J, Borja A (2007) Minimal sampling requirements for a precise assessment of soft-bottom macrobenthic communities, using AMBI. Journal of Experimental Marine Biology and Ecology 349: 323 333.
31. Kronberg I (1987) Accuracy of species and abundance minimal areas determined by similarity area curves. Mar Biol 96: 555 561.
32. Cain SA (1938) The species-area curve. Am Midl Nat 19: 573 581.
33. Braun-Blanquet J (1932) Plant sociology: the study of plant communities. New York: McGraw-Hill. 439 p.
34. Niell FX (1977) Método de recolección y área mínima de muestreo en estudios estructurales del macrofitobentos rocoso intermareal de la Ría de Vigo. Inv Pesq 41: 509 521.
35. Flos J (1985) The driving machine. In: Margalef R, ed. Western Mediterranean. Oxford: Pergamon. pp 60 99.
36. Bensoussan N, Romano J-C, Harmelin J-G, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: To better understand responses of benthic communities to climate change. Estuar Coast Shelf Sci 87: 431 441.
37. Zabala M, Ballesteros E (1989) Surface-dependent strategies and energy flux in benthic marine communities or, why corals do not exist in the Mediterranean. Sci Mar 53: 3 17.
38. Laborel J (1961) Le concretionnement algal "coralligène" et son importance géomorphologique en Méditerranée Recueil des Travaux de la Station Marine d'Endoume 23(37): 37 60.
39. Hong JS (1980) Étude faunistique d'un fond de concrétionnement de type coralligène soumis à un gradient de pollution en Méditerranée nord-occidentale (Golfe de Fos) . PhD Thesis. Université d'Aix-Marseille II.
40. Gili JM, Ros J (1984) L'estatge circalitoral de les illes Medes: el coralligen. In: Ros J, *et al.*, editors. Els Sistemes Naturals de les Illes Medes. Arxius Secció Ciències 73: 677 705.
41. Arrhenius O (1921) Species and area. J Ecol 9: 95 99.
42. Connor EF, McCoy ED (1979) The statistics and biology of the species/area relationship. Am Nat 113: 791 833.
43. Boudouresque CF (1971) Méthodes d'étude qualitative et quantitative du benthos (en particuler du phytobenthos). Tethys 3: 79 104.
44. Ballesteros E (1986) Métodos de análisis estructural en comunidades naturales, en particular del fitobentos. Oecologia Aquatica 8: 117 131.
45. Gleason HA (1922) On the relation between species and area. Ecology 3: 158 162.
46. Martin D, Ballesteros E, Gili JM, Palacín C (1993) Small-scale structure of infaunal polychaete communities in an estuarine environment: methodological approach. Estuar Coast Shelf Sci 36: 47 58.
47. Hawkins SJ, Hartnoll RG (1980) A Study of the Small-scale Relationship Between Species Number and Area on a Rocky Shore. Estuar and Coast Mar Sci 10: 201 214.
48. Bray JR, Curtis JT (1957) An ordination of the upland forest communities of Southern Wisconsin. Ecol Monogr 27: 325 349.
49. Kruskal JB, Wish M (1978) Multidimensional Scaling. Beverly Hills: Sage Publications. pp 93.
50. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32 46.
51. Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58: 626 639.
52. Anderson MJ, ter Braak CJF (2003) Permutation tests for multi-factorial analysis of variance. J Stat Comput Simul 73: 85 113.
53. Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial. Plymouth: PRIMER-E. 192 p.
54. Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. Plymouth: PRIMER-E. 213 p.
55. Bohnsack JA (1979) Photographic quantitative sampling of hard-bottom benthic communities. Bull Mar Sci 29: 242 252.
56. Parravicini V, Morri C, Ciribilli G, Montefalcone M, Albertelli G, et al. (2009) Size matters more than method: Visual quadrats vs photography in measuring human impact on Mediterranean rocky reef communities. Estuar Coast Shelf Sci 81: 359 367.
57. Leujak W, Ormond R (2007) Comparative accuracy and efficiency of six coral community survey methods. J Exp Mar Biol Ecol 351: 168 187.
58. Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117 143.
59. Parravicini V, Micheli F, Montefalcone M, Villa E, Morri C, et al. (2010) Rapid assessment of epibenthic communities: A comparison between two visual sampling techniques. Journal of Experimental Marine Biology and Ecology 395: 21 29.
60. Teixido N, Albajes-Eizagirre A, Bolbo D, Le Hir E, Demestre M, et al. (2011) Hierarchical segmentation-based software for cover classification analyses of seabed images (Seascape). Mar Ecol Progr Ser 431: 45 53.
61. Tittensor DP, Micheli F, Nyström M, Worm B (2007) Human impacts on the species-area relationship in reef fish assemblages. Ecol Lett 10: 760 772.
62. Weinberg S (1978) The minimal area problem in invertebrate communities of Mediterranean rocky substrata. Mar Biol 49: 33 40.
63. Gili JM, Ballesteros E (1991) Structure of cnidarian populations in Mediterranean sublittoral benthic communities as a result of adaptation to different environmental conditions. Oecologia Aquatica 10: 243 254.
64. Nekola JC, White PS (1999) The distance decay of similarity in biogeography and ecology. J Biogeogr 26: 867 878.
65. Steinitz O, Heller J, Tsoar A, Rotem D, Kadmon R (2006) Environment, dispersal and patterns of species similarity. J Biogeogr 33: 1044 1054.
66. Rocchini D, He K, Oldeland J, Wesuls D, Neteler M (2010) Spectral variation versus species beta-diversity at different spatial scales: a test in African highland savannas. J Environ Monitor 12: 825 831.
67. Hong JS (1983) Impact of the pollution on the benthic community: environmental impact of the pollution on the benthic coralligenous community in the Gulf of Fos, northwestern Mediterranean. Bulletin of the Korean Fisheries Society 16: 273 290.
68. Cocito S, Bedulli D, Sgorbini S (2002) Distribution patterns of the sublittoral epibenthic assemblages on a rocky shoal in the Ligurian Sea (NW Mediterranean). Sci Mar 66: 175 181.
69. Piazzi L, Balata D, Cecchi E, Cinelli F, Sartoni G (2010) Species composition and patterns of diversity of macroalgal coralligenous assemblages in the northwestern Mediterranean Sea. J Nat Hist 44: 1 22.

C Publication Chapter 4



Manuscript #	SREP-16-07806
Current Revision #	0
Submission Date	14th March 16
Current Stage	Manuscript Assigned to Peer-Reviewer/s
Title	Structure and biodiversity of coralligenous assemblages over broad spatial scales
Manuscript Type	Original Research
Manuscript Comment	Reviewers to include: Carlo Cerrano; email: c.cerrano@univpm.it Marco Abbiati; email: marco.abbiati@unibo.it Julie Deter; email: julie.deter@univ-montp2.fr
Corresponding Author	Mr. Edgar Casas-Güell (ecasasguell@gmail.com) (Institute of Marine Sciences (ICM-CSIC))
Contributing Authors	Dr. Emma Cebrian , Dr. Joaquim Garrabou , Dr. Jean-Baptiste Ledoux , Dr. Cristina Linares , Dr. Núria Teixidó
Authorship	Yes
	Data on species diversity and structure in coralligenous outcrops dominated by Corallium rubrum are lacking. Gathering these data is needed to build robust baselines in order to assess the

D Publication Chapter 5

Mar Biol
DOI 10.1007/s00227-015-2635-7

ORIGINAL PAPER

Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales

Edgar Casas-Güell · Núria Teixidó ·
Joaquim Garrabou · Emma Cebrian

Received: 26 September 2014 / Accepted: 12 February 2015

Abstract Coralligenous outcrops rank among the most important ecosystems in the Mediterranean Sea, primarily because of their biodiversity. Information on the spatial and temporal variability of the composition and structure of coralligenous assemblages is essentially lacking for most regions. We explored the variability of the biodiversity patterns of coralligenous outcrops dominated by the red gorgonian *Paramuricea clavata* in the NW Mediterranean region using a hierarchical sampling design. The study addressed two overlooked spatial and temporal scales: from 1 to >100 km and a 5-year period. Overall, no temporal changes were detected in either species composition or the 12 morphofunctional groups considered. Significant differences in species composition were found at the various spatial scales. However, variation in composition at the locality level (>100 km apart) showed the lowest values in comparison with the differences found at the site level (<1 km apart). Despite the differences, the sites displayed high similarity (average similarity 55.7 %) and shared approximately 50 % of the species. Similarly, the patterns of diversity at different scales, the alpha (site) and gamma (locality) were consistent with the specific composition trends, whereas the beta diversity showed the greatest differences among sites. Our results demonstrate the moderate spatial variability in biodiversity in the NW Mediterranean region and an extremely low temporal variability. This study provides baselines for detecting potential effects due to global change, and it furnishes a basis for the implementation of monitoring schemes of coralligenous assemblages. The development of similar sampling schemes in other Mediterranean regions will provide a global view of the biodiversity of coralligenous outcrops.

Introduction

Subtidal reefs foster a high biodiversity of animal and plant species (Witman and Dayton 2001; Knowlton and Jackson 2001), representing one of the most productive habitats in marine ecosystems and offering substantial, commercial, recreational and cultural value to society. During recent decades, however, an increasing proportion of the world's reefs have been reported to be severely impacted (Halpern et al. 2008; Crain et al. 2009; Jackson 2010). From a conservation perspective, the collection of data on species composition and assemblage structure over a variety of spatial scales is indispensable for understanding the variation of biodiversity at local and regional scales (Lourie and Vincent 2004). Additionally, these baseline datasets are valuable for assessing changes associated with several anthropogenic threats and the outcome of management actions.

Communicated by F. Bulleri.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-015-2635-7) contains supplementary material, which is available to authorized users.

E. Casas-Güell (✉) · N. Teixidó · J. Garrabou
Institut de Ciències del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain
e-mail: ecasas@cbiolegs.cat; ecasas@icm.csic.es

J. Garrabou
UM110, CNRS/INSU, IRD, Université du Sud Toulon Var, Mediterranean Institute of Oceanography (MIO), Aix-Marseille Université, Marseille, France

E. Cebrian
Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Accés Cala Sant Francesc 14, 17300 Blanes, Girona, Spain

E. Cebrian
Departament de Ciències Ambientals, Facultat de Ciències, Universitat de Girona, Girona, Spain

Knowing and understanding patterns of variability of benthic assemblages at a range of spatial and temporal scales are crucial for effective management and conservation of coastal marine habitats (Ferdeghini et al. 2000; Piazzi et al. 2004; Virgilio et al. 2006). This is even more relevant in key habitats such as the coralligenous outcrops, which are considered one of the most important ecosystems in the Mediterranean Sea (Ballesteros 2006; Piazzi et al. 2012). Coralligenous outcrops have been identified as Mediterranean priority habitats by the EU Habitats Directive (92/43/CE) and foster some of the richest assemblages found in Mediterranean, harboring approximately 10 % of marine Mediterranean species (Pérès and Picard 1964; Laubier 1965; Ros et al. 1985; Bouduresque 2004; Ballesteros 2006). The species that characterize such assemblages belong to various taxonomic groups, primarily encrusting calcareous algae, sponges, cnidarians, polychaetes, bryozoans and tunicates (Garrabou et al. 2002; Ballesteros 2006). Most of these species are long-lived and exhibit low dynamics, making them vulnerable to various types of threats (Garrabou et al. 1998; Teixidó et al. 2011). In fact, many coralligenous outcrops have already been affected by various stressors such as nutrient enrichment, overexploitation, invasive species, increase in sedimentation, mechanical impacts as well as climate change, resulting in dramatic consequences for many species and even for the structure of the whole assemblages (Balata et al. 2007; Garrabou et al. 2009; Piazzi et al. 2011; Cebrián et al. 2012; Teixidó et al. 2013; Cecchi et al. 2014). However, much of our understanding of the composition and structure of coralligenous outcrops is still limited and based mainly on descriptive studies restricted to smaller or medium spatial scales—from a few meters to a few km (10 s of km) (reviews by Ballesteros 2006; Giaccone 2007; Casellato and Stefanon 2008 and references therein) and some experimental studies (e.g., Balata et al. 2007; Gennaro and Piazzi 2011). To our knowledge, few studies addressing coralligenous assemblages have extended to larger spatial scales (up to 200 km of coastline), and most of them focused on the phytobenthic component (Piazzi et al. 2004, 2010). Thus, the structure of coralligenous outcrops has been poorly understood because there are no spatiotemporal studies or baseline data at the assemblage level over large scales. This situation has prevented a proper assessment of the current state of biodiversity and future changes in this valuable assemblage. Difficulties related to the study of these highly diverse and structurally complex systems, coupled with general logistic constraints related to the sampling of intermediate–deep rocky habitats, have hindered progress in our knowledge of this topic (Parravicini et al. 2010).

The aim of this study was to quantify the variability of the structure and dynamics of coralligenous assemblages dominated by the red gorgonian *Paramuricea clavata* across different localities in the NW Mediterranean separated by hundreds of km and over a 5-year period. The long-lived gorgonian *P. clavata* is considered a key species in Mediterranean coralligenous assemblages (Gili and Coma 1998) and provides biomass and biogenic substrata that significantly shapes the assemblage (Harmelin and Marinopoulos 1994; Gili and Coma 1998). It contributes greatly to the aesthetic value of the Mediterranean sublittoral seascape (Bianchi et al. 1995) and attracts high numbers of recreational divers (Coma et al. 2004). We assessed structural complexity in terms of the specific and morpho-functional composition of the sessile perennial species associated to *P. clavata*. Additionally, we assessed various components of species diversity (alpha, beta and gamma diversity) over time. The ultimate goal of this study is to provide assemblage structure baseline data to furnish a solid basis for comparisons in terms of potential changes in species composition changes in response to multiple threats.

Materials and methods

Study sites and sampling design

A hierarchical sampling design was used to assess spatial and temporal patterns of coralligenous assemblages dominated by the red gorgonian *Paramuricea clavata*. Three localities were selected in the NW Mediterranean region (Catalonia, Provence and Corsica), covering more than 400 km (5°E–W) and more than 200 km (1°N–S) of the coastline (Fig. 1). All localities were located in marine protected areas. At each locality, two sites (separated by approximately 1 km) were sampled: in Catalonia, Tascó Petit (MPT) and Medallot (MME) located in the Natural Park of Montgrí, Medes Islands and Baix Ter; in Provence, Petit Conglué (PCO) and Plane-Grotte Pérès (PGP) located in Riou Archipelago in the National Park of Calanques; and in Corsica, Palazzu (SPA) and Gargallu (SGL) located in the Scandola Nature Reserve (Fig. 1).

At each site between depths of 17–25 m, one or two permanent plots were set up to cover a total area of approximately 4 m^2. The plots were photographically sampled during 5 years (2006–2010) using 25 × 25 cm (625 cm^2) to facilitate species identification. For this study, only 3 years were considered within a 5-year period. From each site and year considered, three different groups of eight contiguous quadrats of 25 × 25 cm (625 cm^2) that corresponded to the minimum sampling area (hereafter MSA; 8 quadrats = 5000 cm^2, for more information see Kipson et al. 2011) were randomly chosen within the permanent plots, resulting in a total of 432 photographs (24 photos × 6 sites × 3 years). The photographs

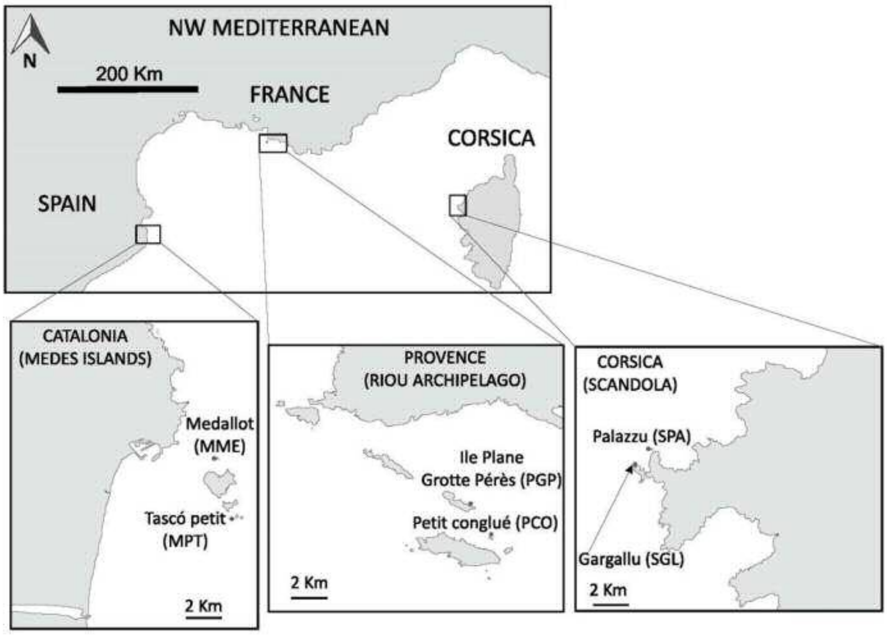


Fig. 1 Map showing the Mediterranean geographic area where the photographic surveys were conducted: region, localities and sites. Geographic information (latitude/longitude): MPT (42°2′31.82″N/3°13′32.14″E), MME (42°3′6.04″N/3°13′18.88″E), PCO (43°10′43.89″N/5°23′45.79″E), PGP (43°11′12.48″N/5°23′25.04″E), SPA (42°22′48.72″N/8°32′44.70″E), SGL (42°22′20.61″N/8°32′29.17″E)

were taken with a Nikon D70S digital SLR camera fitted with a Nikkor 20 mm DX lens (3000 × 2000 pixel resolutions) and housed in a Subal D70S housing. Lighting was achieved by two electronic strobes fitted with diffusers. Specimens were also collected in order to facilitate species identification by photographic samples. Photographic sampling was chosen as it provides a good compromise between optimal resolution (Bohnsack 1979; Foster et al. 1991; Deter et al. 2012) and conservation of the habitat, although could probably underestimate small and cryptic species. This study did not involve sampling or damage of any endangered or protected species. Furthermore, the non-destructive methodological approach obtaining biodiversity data through the analysis of images is suitable to study marine benthic communities in marine protected areas.

Analysis of photographs

From each photograph, sessile macrobenthic species were identified at the lowest taxonomic level (whenever possible). The species lists were cross-checked with the World Register of Marine Species database (worms, available at: http://www.marinespecies.org). Sessile macrobenthic species were classified into 12 morphofunctional groups based on their taxonomy and growth form (three seasonal and nine perennial groups) (see Table 1) (adapted from Garrabou et al. 2002 and Teixidó et al. 2011).

We calculated (1) the percentage of species belonging to the various morphofunctional groups found in each site and year and (2) a semiquantitative abundance for each morphofunctional group estimated from the species presence/absence data in each of the MSA (group of eight contiguous 25 × 25 cm photographs) sampled at each site. For instance, a presence in only one photograph corresponded to 12.5 % of cover [(one presence/eight photographs) × 100]. Finally, the values for each MSA were averaged to obtain site estimates. We focused on perennial species for specific composition and diversity indices analyses (see below).

Statistical analyses

Presence/absence specific data were analyzed by a non-metric multi-dimensional scaling (nMDS) ordination analysis to visualize spatial and temporal patterns based on the

Table 1 Biological categories used in this study combining taxonomy, life span and morphological growth forms (adapted from Garrabou et al. 2002 and Teixidó et al. 2011)

Biological categories	Description
Seasonal algal turf	Annual erect or semierect fleshy algal species, with one or multiple zones of attachment to the substratum; generally is constituting algal cushions or thin sheets with mixtures of algal species
Seasonal animal turf	Small seasonal animal species, mainly bryozoans and hydrozoans; usually forming animal cushions or thin sheets with mixtures species
Seasonal mixture complex turf	Small seasonal algae and animal species (mainly bryozoans and hydrozoans), sediment, detritus and fragments; normally forming cushions or thin sheets with mixtures of species
Perennial algal encrusting	Species growing mainly as two-dimensional sheets; more or less completely attached to the substratum
Perennial algal erect	Species attached to the substratum usually with a unique zone (visible even in winter) of basal attachment to the substratum
Perennial algal turf—invasive	Perennial dense thick filamentous turf algae with the ability to maintain permanent carpets (e.g., the invasive species *Womersleyella setacea*)
Perennial animal encrusting	Species of sponges, cnidarians, bryozoans and tunicates growing as two-dimensional sheets; more or less completely attached to the substratum
Perennial animal massive	Mound species of sponges and cnidarians with vertical and lateral growth; normally attached to the substratum all along their basal area
Perennial animal tree	Erect species of cnidarians and bryozoans, more or less branched; usually with a single point of attachment to the substratum
Perennial animal cup	Solitary corals attached to the substratum all along their basal area
Perennial animal boring	Excavating organisms living into the rock (e.g., *Cliona viridis*)
Perennial animal epibiont	Species growing over other invertebrates or calcareous algae (mainly polychaetes, e.g., *Salmacina dysteri* or *Filograna implexa* and bryozoans, e.g., *Chartella tenella*)

Bray–Curtis similarity measure. Furthermore, a similarity percentage procedure analysis (SIMPER, Clarke and Warwick 1994) was performed to identify the percentage contribution of taxa and morphological groups to the significant dissimilarities among sites and localities.

Species composition, morphofunctional groups and diversity indices were analyzed by a nonparametric analysis of variance, PERMANOVA (Anderson 2001a, b; Anderson et al. 2008), applied using Bray–Curtis and Euclidean distances for multivariate and univariate analyses, respectively. The model included three factors: location "Lo" (random factor, three levels), site "Si" (random factor, two levels, nested in location) and time (random factor, three levels, crossed to location and site).

Spatial and temporal patterns of diversity measures were assessed by quantifying alpha diversity (average number of species per sampling unit), gamma diversity (the total number of species within a locality) and beta diversity (multivariate distance between group centroids determined with the PERMDISP procedure). PERMDISP is an approach used to compare the degree of sample dispersion of different groups based on a distance matrix. When PERMDISP is used on a Jaccard distance presence/absence matrix, it is directly interpretable as a test for similarity in beta diversity among groups (Anderson et al. 2006, 2011).

The PERMANOVA and PERMDISP analyses were computed using the program PRIMER v6 with the PERMANOVA + add-on package.

Results

Species composition

A total of 106 macrobenthic taxa were identified across the region studied: 13 macroalgae, 1 protozoan, 42 sponges, 3 hydrozoans, 10 anthozoans, 1 mollusk, 4 polychaetes, 22 bryozoans and 10 tunicates (see Appendix 1 of ESM for the species list and the assignment to the various morphofunctional groups). Of these taxa, 88 were perennial and 18 seasonal.

Structural complexity—morphofunctional groups

Overall, the structure of morphofunctional groups showed a consistent pattern at spatial and temporal scales in terms of either percentages of species per group or their abundance (Figs. 2, 3). The principal group that characterized the outcrops in terms of species was the *Animal encrusting*, with values of approximately 30–40 % of the species found in each site, followed by *Animal massive* and *Animal tree*, which encompassed 10–20 % of the species in most of the sites. The remaining categories, *Algae encrusting*, *Algal erect*, *Animal cup* and others represented approximately 0–10 % of species (Fig. 2).

The morphofunctional groups displaying the highest constant abundances (>50 % of cover) at all sites and over time were *Algal encrusting*, *Animal encrusting*, *Animal*

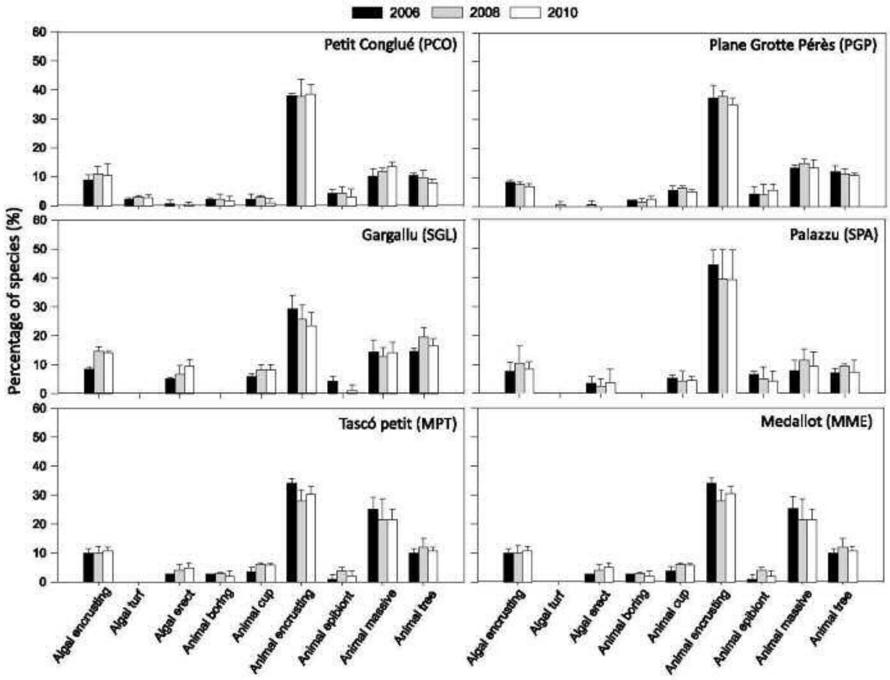


Fig. 2 Percentage of perennial species relative to morphological growth forms for each site and year studied

massive and *Animal tree* (Fig. 3). *Animal cup* and *Animal boring* showed moderate to high abundances (ranging between 20 and 100 % cover), with a degree of temporal variability depending on the site (Fig. 3). Finally, the categories *Algal turf*, *Algae erect* and *Animal epibionts* exhibited a higher degree of variability (ranging from 0 to 100 %).

The abundance of the different morphofunctional groups was similar among localities ($F_{2,53} = 1.19$, $p > 0.05$, Table 2) and time ($F_{3,53} = 1.94$, $p > 0.05$, Table 2) but showed differences among sites ($F_{3,53} = 17.49$, $p < 0.05$, Table 2). Variability among sites correspond to a comprehensive variability in the contribution of *Algal*, *Animal encrusting*, *Animal Massive, Animal cup* and *Animal Tree* within sites except for, PCO, in Provence, where exceptional high abundances (up to 100 %) of *Algal turf* were present (Fig. 3).

Structural complexity—specific composition

The specific composition of the perennial macrobenthic species was constant over time for all localities and sites (Fig. 4; Table 3) but differed significantly among localities and sites (Table 3). The *Locality* and *Site* nested in *Locality* factors explained a high percentage of the variation with 18.49 and 20.56 %, respectively (Table 3). Variability among replicate units explained the 20.65 % indicating a high spatial variability at the smallest (replicate) scale.

Parallel to these results, the SIMPER analysis showed an overall average similarity in specific composition, up to 55.74 % across localities and time. The number of species contributing to 50 % of the similarity for each site ranged between 10 and 16 species (Table 4). Overall, 33 different species contributed to the similarity for each site. Most of the species belonged to the morphofunctional groups displaying the highest number of species and abundance, i.e., *Algae encrusting, Animal encrusting, Animal massive* and *Animal tree* (Table 4). For these groups, certain species played determining roles at most sites, such as *Mesophyllum alternans* and *Peysonnelia* sp. for *Encrusting algae*; *Parazoanthus axinellae*, *Crambe crambe* and Serpulidae for *Animal encrusting*, *Paramuricea clavata* for *Animal tree* and *Leptopsammia pruvoti* for *Animal cup*.

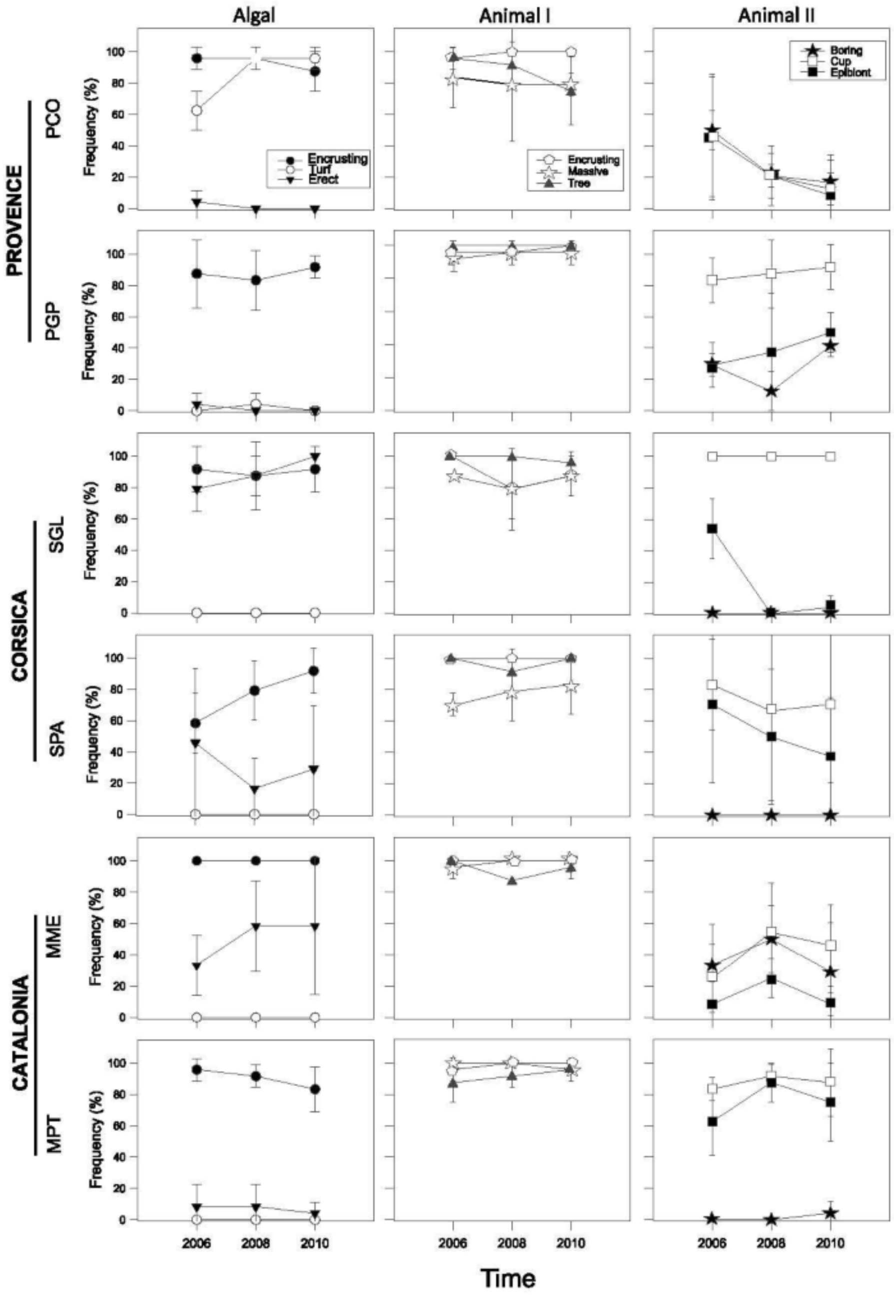


Fig. 3 Abundance (expressed as %) of each morphofunctional group for each site and year studied

Diversity indices

Alpha diversity (mean ± SD) remained constant over time at almost all the studied sites (Fig. 5) and similar for all localities (Table 5), but it showed significant differences among sites (Table 5). The values of alpha diversity ranged from 23 ± 2 species in PCO to 42 ± 3 species in PGP. A constant pattern of beta diversity was found over time (Fig. 5; Table 5b). The sites located in Catalonia showed lower mean values (ranged from 12.7 % ± 0.6 to 15.7 % ± 0.8) compared with the sites in Corsica (ranged from 13.2 % ± 0.8 to 23.3 % ± 3.0) and in Provence (ranged from 16.0 % ± 0.5 to 20.8 % ± 2.6) (Table 5). Gamma diversity showed similar and constant values among the three localities studied (Fig. 5; Appendix 2 of ESM).

Table 2 PERMANOVA analysis testing the significance level of the different factors for the abundance of the different perennial morpho-functional groups

Source of variation	*df*	SS	MS	Pseudo-*F*	*P* (perm)
Locality	2	3420.6	1710.3	1.2566	0.3514
Site (locality)	3	4077.8	1359.3	13.51	0.0001
Time	2	103.26	51.631	0.63095	0.6842
Locality × time	4	327.32	81.831	0.81334	0.6058
Site (locality) × time	6	603.67	100.61	1.129	0.3498
Residual	36	3208.3	89.119		
Total	53	117.1			

Discussion

Studies addressing spatial and temporal patterns of biodiversity are urgently needed to identify the most effective monitoring schemes for in situ conservation and sustainable use (Gaston 2000). In assemblages dominated by long-lived assemblages (such as the coralligenous ones), the challenge is even greater since declining or recovery trajectories are

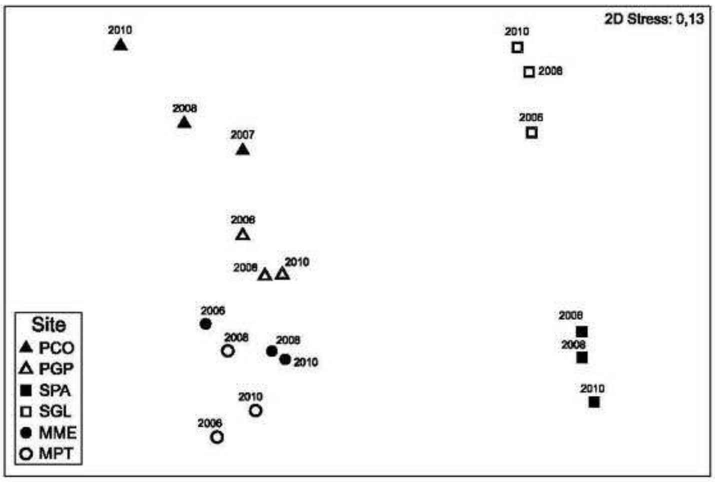


Fig. 4 Non-metric multidimensional scaling (nMDS) ordination plot of macrobenthic species in the three regions of the NW Mediterranean Sea over time (2006, 2008 and 2010). Analysis performed on Bray–Curtis dissimilarities of presence/absence data. For each locality, the two sites are shown by open or closed dots. See "Materials and methods" for site abbreviations

Table 3 PERMANOVA analysis testing the significance level of the different factors in the specific composition on the basis of Bray–Curtis dissimilarity measure

Source	*df*	SS	MS	Pseudo-*F*	*P* (perm)	Estimates components of variation (Sq. root)
Locality	2	20,802	10,401	2.3016	0.0088	18.491
Site (locality)	3	12,855	4284.9	8.8866	0.0001	20.555
Time	2	1410.3	705.14	1.5897	0.1937	3.8121
Locality × time	4	1774.3	443.56	0.9199	0.5714	−2.5368
Site (locality) × time	6	2893.1	482.18	1.1304	0.2797	−4.3066
Res	36	15,355	426.54			20.653
Total	53	55,089				

Table 4 Species by morphofunctional groups contributing more than 50 % to the similarity of studied sites

	Provence		Corsica		Catalonia	
	PCO	PGP	SPA	SGL	MME	MPT
Algae encrusting	*Lithophyllum* spp. *Palmophyllum crassum* *Mesophyllum alternans* *Peyssonnelia* sp.	*Mesophyllum alternans* *Peyssonnelia* sp.	*Mesophyllum alternans* *Peyssonnelia* sp.	*Lithophyllum* spp. *Mesophyllum alternans* *Peyssonnelia* sp.	*Mesophyllum alternans* *Peyssonnelia* sp.	*Mesophyllum alternans* *Peyssonnelia* sp.
Algae turf		–	–	*Flabellia petiolata*	–	–
Algae filamentous	*Womersleyella_type*		–	–	–	–
Animal encrusting	*Parazoanthus axinellae* *Crambe crambe*	*Crella pulvinar* *Rhynchozoon sp.* *Haliclona mucosa* *Oscarella sp.* *Pleraplysilla sp.inifera* *Crambe crambe* Serpulidae	*Cacospongia* *Crella pulvinar* *Miniacina miniacea* Serpulidae	Serpulidae	*Pleraplysilla sp.inifera* *Parazoanthus axinellae* *Crambe crambe* Serpulidae	*Oscarella sp.* *Dictyonella sp.* *Parazoanthus axinellae* *Crambe crambe*
Animal massive	*Axinella damicornis*	*Acanthella acuta* *Agelas oroides* *Axinella damicornis*	*Ircinia variabilis* *Spongia officinalis*	–	*Agelas oroïdes* *Axinella damicornis Cystodites dellechiajei*	*Acanthella acuta* *Axinella damicornis* *Cystodites dellechiajei*
Animal tree	*Eunicella cavolinii Paramuricea clavata*	*Adeonella/Smittina* *Paramuricea clavata*	*Reteporella grimaldii* *Paramuricea clavata*	*Myriapora truncata Adeonella/Smittina* *Paramuricea clavata*	*Margaretta cereoides* *Adeonella/Smittina* *Paramuricea clavata*	*Adeonella/Smittina* *Paramuricea clavata*
Animal cup	–	*Caryophyllia inornata* *Leptopsammia pruvoti*	*Leptopsammia pruvoti*	*Caryophyllia inornata* *Leptopsammia pruvoti*	*Leptopsammia pruvoti*	*Leptopsammia pruvoti*
Animal boring	–	–	–	–	–	–
Animal epibiont	–	–	–	–	–	*Chartella sp.*

The underlined species are those contributing to the similarity of most of the sites

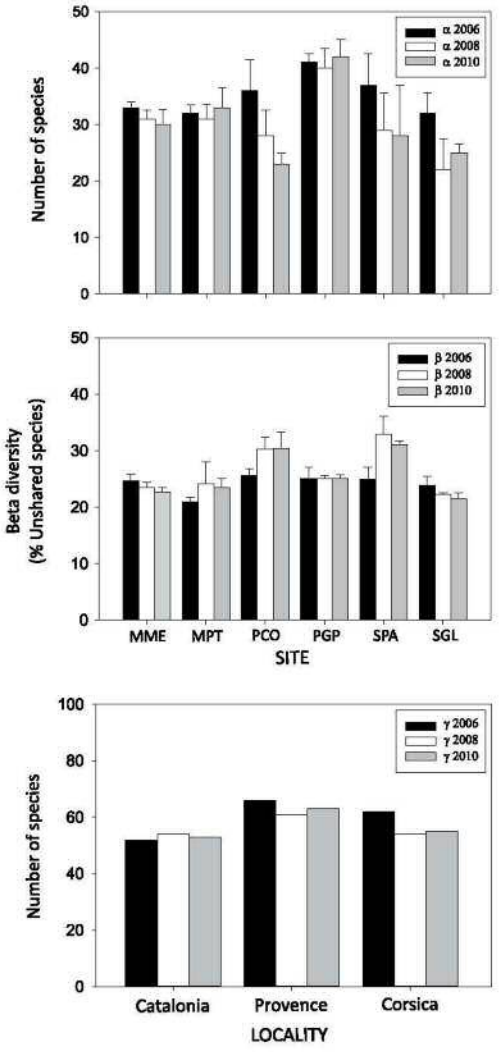


Fig. 5 Diversity metrics (α-diversity, β-diversity and γ-diversity) over time

more difficult to detect due to the slow pace of their population dynamics (Hughes et al. 2013). In this context, large-scale, long-term biodiversity datasets are a basic resource that furnishes the essential tools to provide information to promote sound conservation actions (Magurran et al. 2010). The present study provides the first baseline information on temporal and spatial patterns of coralligenous assemblages, highlighting no temporal changes in the patterns in the different components of biodiversity over the study period and most of the spatial variation in species composition among sites distant appreciatively 1 km.

Interestingly, the morphofunctional groups tended toward common patterns at spatial and temporal scales. This result supports the hypothesis that coralligenous outcrops dominated by the red gorgonian *P. clavata*, despite differences in species composition, displayed similar structural complexity, at least within the NW Mediterranean. This common composition of the assemblages was complemented by the abundance of other morphofunctional groups, which displayed a greater abundance and/or a higher degree of temporal variability such as *Algal turf*, *Algae erect* and *Animal epibionts*. In fact, these groups could be related to dysfunctions in the assemblages since most are characterized by faster growth rates (especially, in comparison with other perennial species included in the other groups). For instance, *algal turf* abundance resulted from a recent introduction, such as the *Womersleyella* type, which only showed high abundance at one site (Petit Conglué), highlights the suitability and interest of using the considered morphofunctional groups.

The present study found differences in specific composition among the three localities studied (see below). However, it is important to note the relatively high similarity found among them (overall average similarity 55.7 %). In addition, 51 % of the perennial species were always found at the three localities (see Appendix 1 of ESM). Interestingly, the greatest variability was found at the site and replicate level. Other studies in coralligenous assemblages have also found the highest variability at the smallest scales investigated (e.g., among replicates) (Ferdeghini et al. 2000; Virgilio et al. 2006; Terlizzi et al. 2007; Abbiati et al. 2009; Ponti et al. 2011). Similarly, patterns of diversity at different scales, the alpha (site) and gamma (locality) values were congruent with the specific composition trends.

In short, significant differences in species composition were found at the different spatial scales analyzed. However, variation in composition at the large scale considered here (at the locality level) showed the lowest values compared with the differences found at the small scale. This general finding was also consistent with the beta diversity values, where the highest differences were found among sites. The processes behind these multi-scale patterns in assemblages are difficult to discern. In any case, species composition across localities does not appear to be determined primarily by the differences in physicochemical conditions (Bensoussan et al. 2010) and/or the differential impact of major disturbances (e.g., mass mortality) (Garrabou et al. 2009). Instead, biological factors (growth rates, recruitment, competition and successional patterns) should be the major driver of patterns found at the site level (or, alternatively, should explain most of the variability found at the site level). As mentioned previously, coralligenous

Table 5 Univariate tests based on Euclidean distances for alpha and gamma diversities and PERMDISP test for beta diversity

Source	*df*	SS	MS	Pseudo-*F*	*P* (perm)
(a) Alpha diversity (PERMANOVA)					
Locality	2	337.04	168.52	0.7275	0.6453
Site (locality)	3	725.50	241.83	8.0018	0.0180
Time	2	306.81	153.41	4.8931	0.0922
Locality × time	4	125.41	31.352	1.0374	0.4588
Site (locality) × time	6	181.33	30.222	1.753	0.1409
Residual	36	620.67	17.241		
Total	53	2296.8			
Source	*df*1	*df*2		*F*	*P*
(b) Beta diversity (PERMDISP)					
Locality	2	51		11.4792	0.0005
Site	5	48		6.7646	0.0001
Time	2	51		4.8931	0.4123

assemblages are typically dominated by long-lived species characterized by slow population dynamics (Garrabou 1999; Linares et al. 2007; Teixidó et al. 2009, 2011). Additionally, these species generally display a limited dispersal capacity (Uriz 1998; Duran 2003; Abbiati et al. 2009; Ledoux et al. 2010b). These life history traits may shape the high heterogeneity observed at a small scale. For instance, the recruitment rates for most species are low (Garrabou 1999; Mariani et al. 2006; Teixidó et al. 2011) but can also show low-frequency pulses (e.g., Garrabou and Harmelin 2002). The arrival of pulses of new offspring can shape the assemblage for long periods because of the longevity of the coralligenous species. Besides, the limited dispersal ability, based either on sexual or asexual reproduction, implies that local persistence is enhanced once the populations are established (Costantini et al. 2007; Ledoux et al. 2010a). Finally, the slow growth rates hinder competition displacement between species, which could result in the dominance of competitive (Huston 1979; Garrabou et al. 2002). Overall, we argue that the interaction of these different biological processes appears to shape the species composition and abundance at each site (or even patches within sites). Thus, the observed patterns would result from the contribution of each factor over contemporary (several decades) or historical scales (hundreds of years) to the species pool and to the abundance of the species at each site. In fact, in agreement with other studies on coralligenous assemblages, that variability observed at the smallest scale (replicates or patches) is considerably high showing a patch distribution of organisms linked both to the heterogeneity of substrate and to the interactions among sessile organisms (Acunto et al. 2001; Ferdeghini et al. 2000; Balata et al. 2005). The observation that characteristic facies occur within the coralligenous assemblages (i.e., patches within the assemblages with a high abundance of certain species—for example, *Parazoanthus axinellae* or *Leptopsamia pruvoti*) appears to corroborate this hypothesis (Pérès and Picard 1964).

Coralligenous outcrops are reported to occur primarily in the northern part of the Mediterranean Basin, with few records from the eastern or southern basin (Martin et al. 2014). However, the datasets reviewed contained information for 30 % of the total coastline of the Mediterranean Basin, indicating an extremely limited basic knowledge of these bioconstructions (Martin et al. 2014). Thus, the identification of similar structure in the coralligenous outcrops is important for the effective management and conservation of this sensitive habitat (Mokhtar-Jamaï et al. 2011; Giakoumi et al. 2013). Fine-scale knowledge on the distribution of species, such as that produced by this study, is crucial to furnish data for studies on marine conservation planning and the implementation of conservation plans (Giakoumi et al. 2013; Martin et al. 2014). In this context, a large-scale study on spatial variation on coral reefs composition and abundance revealed substantial changes at regional scales along the Great Barrier Reef, with profound implications for future projections of regional-scale impacts of climate change (Hughes et al. 2012). We believe that the development of this type of fine-scale study in various regions of the Mediterranean Basin will provide a global perspective on and characterization of the distribution of the biodiversity of the area and will support critical data to protect the full range of biodiversity (e.g., genes, species and life history stages), with implications for predictions of climate change impacts on coralligenous outcrops. Finally, the results obtained in this study could already help in the definition of monitoring schemes (periodicity and spatial coverage) required for the assessment of conservation status of Mediterranean marine habitats. This kind of information is especially timely to fulfill the requirements of the European Marine Strategy Framework Directive (Piazzi et al.

2014). Overall, thus, pursuing the characterization through the adoption of standardized methods could provide to the coastal management authorities (from local to international level) enormous benefits for the design of effective management and conservation plans.

Acknowledgments The authors thank C. Linares, J. B. Ledoux and S. Kipson for field assistance. The study has been funded by the Spanish Ministry of Science and Innovation to E. Casas-Güell, a doctoral fellowship linked to the Biorock Project (CTM2009–08045, http://biorock.medrecover.org/) and the Total Foundation (medDiversa project, http://meddiversa.medrecover.org/). ECG thanks helpful and valuable comments on earlier drafts of the manuscript to Dr. M. J. Anderson during a research visit fellow (EEBB-I-13-06897) at NZIAS-INMS (Massey University), and to Dr. M. Pawley and A. N. H. Smith for made my stay especially comfortable. The authors are members of the Marine Conservation Research Group (http://medrecover.org) of the Generalitat de Catalunya.

Conflict of interest The authors declare that they have no conflicts of interest concerning this article.

References

Abbiati M, Airoldi L, Costantini F, et al (2009) Spatial and temporal variation of assemblages in Mediterranean coralligenous reefs. UNEP-RAC/SPA

Acunto S, Balata D, Cinelli F (2001) Variabilità spaziale del coralligeno e considerazioni sul metodo di campionamento. Biologia Marina Mediterranea 8:191–200

Anderson MJ (2001a) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Anderson MJ (2001b) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58:626–639

Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. Ecol Lett 9:683–693. doi:10.1111/j.1461-0248.2006.00926

Anderson MJ, Gorley RN, Clarke RK (2008) Permanova + for primer: guide to software and statistical methods PRIMER-E, Plymouth

Anderson MJ, Crist TO, Chase JM et al (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol Lett 14:19–28. doi:10.1111/j.1461-0248.2010.01552.x

Balata D, Piazzi L, Cecchi E, Cinelli F (2005) Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposition. Mar Environ Res 60:403–421

Balata D, Piazzi L, Benedetti-Cecchi L (2007) Sediment disturbance and loss of beta diversity on subtidal rocky reefs. Ecology 88:2455–2461

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanogr Mar Biol Annu Rev 44:123–195

Bensoussan N, Romano J-C, Harmelin J-G, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: to better understand responses of benthic communities to climate change. Estuar Coast Shelf Sci 87:431–441. doi:10.1016/j.ecss.2010.01.008

Bianchi CN, Dore G, Morri C (1995) Guida del subacqueo naturalista: Mediterraneo e tropici. Editrice Archivio Fotografico Sardo, Nuoro, pp 1–278

Bohnsack JA (1979) Photographic quantitative sampling of hard-bottom benthic communities. Bull Mar Sci 29:242–252

Bouduresque CF (2004) Marine biodiversity in the Mediterranean: status of spicies, populations and communities. Sci Rep Port-Cros Natl Park Fr 20:97–146

Casellato S, Stefanon A (2008) Coralligenous habitat in the northern Adriatic Sea: an overview. Mar Ecol 29:321–341. doi:10.1111/j.1439-0485.2008.00236

Cebrián E, Linares C, Marschal C, Garrabou J (2012) Exploring the effects of invasive algae on the persistence of gorgonian populations. Biol Invasions 14:2647–2656. doi:10.1007/s10530-012-0261-6

Cecchi E, Gennaro P, Piazzi L, Ricevuto E, Serena F (2014) Development of a new biotic index for ecological status assessment of Italian coastal waters based on coralligenous macroalgal assemblages. Eur J Phycol 49(3):298–312. doi:10.1080/09670262.2014.918657

Clarke KR, Warwick RM (1994) Change in Marine Communities: an approach to statistical analysis and interpretation. Bourne Press Limited, Bournemouth

Coma R, Pola E, Ribes M, Zabala M (2004) Long-term assessment of temperate octocoral mortality patterns, protected vs unprotected areas. Ecol Appl 14:1466–1478. doi:10.1890/03-5176

Costantini F, Fauvelot C, Abbiati M (2007) Genetic structuring of the temperate gorgonian coral (Corallium rubrum) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. Mol Ecol 16:5168–5182. doi:10.1111/j.1365-294X.2007.03579

Crain CM, Halpern BS, Beck MW, Kappel CV (2009) Understanding and managing human threats to the coastal marine environment. Ann NY Acad Sci 1162:39–62. doi:10.1111/j.1749-6632.2009.04496

Deter J, Descamp P, Boissery P et al (2012) A rapid photographic method detects depth gradient in coralligenous assemblages. J Exp Mar Bio Ecol 418–419:75–82. doi:10.1016/j.jembe.2012.03.006

Duran S (2003) Phylogeography, gene flow and population structure of (Porifera : Poecilosclerida) Disseration, University of Barcelona

Ferdeghini F, Acunto S, Cocito S, Cinelli F (2000) Variability at different spatial scales of a coralligenous assemblage at Giannutri Island (Tuscan Archipelago, northwest Mediterranean). Hydrobiologia 440:27–36

Foster MS, Harrold C, Hardin DD (1991) Point vs. photo quadrat estimates of the cover of sessile marine organisms. J Exp Mar Bio Ecol 146:193–203. doi:10.1016/0022-0981(91)90025-R

Garrabou J (1999) Life-history traits of Alcyonium acaule and Parazoanth us axinellae (Cnidaria, Anthozoa), with emphasis on growth. Mar Ecol Prog Ser 178:193–204

Garrabou J, Harmelin J-G (2002) A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean: and management needs insights into conservation and management needs. J Anim Ecol 71:966–978

Garrabou J, Sala E, Arcas A, Zabala M (1998) The impact of diving on Rocky Sublittoral Communities: a case study of a bryozoan population. Conserv Biol 12:302–312

Garrabou J, Ballesteros E, Zabala M (2002) Structure and dynamics of north-western Mediterranean rocky benthic communities along a depth gradient. Estuar Coast Shelf Sci 55:493–508. doi:10.1006/ecss.2001.0920

Garrabou J, Coma R, Bensoussan N et al (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol 15:1090–1103. doi:10.1111/j.1365-2486.2008.01823

Gaston KJ (2000) Global patterns in biodiversity. Nature 405:220–227. doi:10.1038/35012228

Gennaro P, Piazzi L (2011) Synergism between two anthropic impacts: *Caulerpa racemosa* var. *cylindracea* invasion and seawater nutrient enrichment. Mar Ecol Prog Ser 427:59–70. doi:10.3354/meps09053

Giaccone G (2007) Coralligenous assemblage as underwater seascape: distribution off Italian coasts. Biol Mar Mediterr 14:124–141

Giakoumi S, Sini M, Gerovasileiou V et al (2013) Ecoregion-based conservation planning in the Mediterranean: dealing with large-scale heterogeneity. PLoS ONE 8:e76449. doi:10.1371/journal.pone.0076449

Gili JM, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–321. doi:10.1016/S0169-5347(98)01365-2

Halpern BS, Walbridge S, Selkoe KA et al (2008) A global map of human impact on marine ecosystems. Science 319:948–952. doi:10.1126/science.1149345

Harmelin J-G, Marinopoulos J (1994) Population structure and partial mortality of the gorgonian *Paramuricea clavata* (Risso) in the north-western Mediterranean (France, Port-Cros Island). Mar Life 4:5–13

Hughes TP, Baird AH, Dinsdale EA et al (2012) Assembly rules of reef corals are flexible along a steep climatic gradient. Curr Biol 22:736–741. doi:10.1016/j.cub.2012.02.068

Hughes TP, Linares C, Dakos V, Van de Leemput I, Van Nes E (2013) Living dangerously on borrowed time during slow, unrecognized regime shifts. Trends Ecol Evol 28:149–155

Huston M (1979) A general hypothesis of species diversity. Am Nat 113:81–101

Jackson JBC (2010) The future of the oceans past. Philos Trans R Soc Lond B Biol Sci 365:3765–3778. doi:10.1098/rstb.2010.0278

Kipson S, Fourt M, Teixidó N, Cebrián E, Casas E et al (2011) Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops. PLoS ONE 6(11):e27103. doi:10.1371/journal.pone.0027103

Knowlton and Jackson (2001) Rocky subtidal communities. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Associates, Inc., Sunderland

Laubier L (1965) Le "coralligène" des Albères: monographie biocénotique. Ann Instit Océanogr Monaco 43:139–316

Ledoux J-B, Garrabou J, Bianchimani O et al (2010a) Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, Corallium rubrum. Mol Ecol. doi:10.1111/j.1365-294X.2010.04814

Ledoux J-B, Mokhtar-Jamaï K, Roby C et al (2010b) Genetic survey of shallow populations of the Mediterranean red coral [Corallium rubrum (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. Mol Ecol 19:675–690. doi:10.1111/j.1365-294X.2009.04516

Linares C, Doak DF, Coma R et al (2007) Life history and viability of a long-lived marine invertebrate: the octocoral *Paramuricea clavata*. Ecology 88:918–928

Lourie SA, Vincent ACJ (2004) Using biogeography to help set priorities in marine conservation 18:1004–1020

Magurran AE, Baillie SR, Buckland ST et al (2010) Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. Trends Ecol Evol 25:574–582. doi:10.1016/j.tree.2010.06.016

Mariani S, Uriz M-J, Turon X, Alcoverro T (2006) Dispersal strategies in sponge larvae: integrating the life history of larvae and the hydrologic component. Oecologia 149:174–184. doi:10.1007/s00442-006-0429-9

Martin CS, Giannoulaki M, De Leo F et al (2014) Coralligenous and maërl habitats: predictive modelling to identify their spatial distributions across the Mediterranean Sea. Sci Rep. doi:10.1038/srep05073

Mokhtar-Jamaï K, Pascual M, Ledoux J-B et al (2011) From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. Mol Ecol 20:3291–3305. doi:10.1111/j.1365-294X.2011.05176

Parravicini V, Micheli F, Montefalcone M et al (2010) Rapid assessment of epibenthic communities: a comparison between two visual sampling techniques. J Exp Mar Bio Ecol 395:21–29. doi:10.1016/j.jembe.2010.08.005

Pérès JM, Picard J (1964) Nouveau Manuel de Bionomie Benthique de la Méditerranée. Rec Trav St Mar Endoume 47:5–137

Piazzi L, Balata D, Pertusati M, Cinelli F (2004) Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substratum inclination. Bot Mar 47:105–115. doi:10.1515/BOT.2004.010

Piazzi L, Balata D, Cecchi E et al (2010) Species composition and patterns of diversity of macroalgal coralligenous assemblages in the north-western Mediterranean Sea. J Nat Hist 44:1–22. doi:10.1080/00222930903377547

Piazzi L, Gennaro P, Balata D (2011) Effects of nutrient enrichment on macroalgal coralligenous assemblages. Mar Poll Bul 62:1830–1835. doi:10.1016/j.marpolbul.2011.05.004

Piazzi L, Gennaro P, Balata D (2012) Threats to macroalgal coralligenous assemblages in the Mediterranean Sea. Mar Poll Bul 64:2623–2629. doi:10.1016/j.marpolbul.2012.07.027

Piazzi L, Balata D, Cecchi E, Gennaro P, Serena F (2014) Effectiveness of different investigation procedures in detecting anthropogenic impacts on coralligenous assemblages. Sci Mar 78(3):319–328. doi:10.3989/scimar.03989.28A

Ponti M, Fava F, Abbiati M (2011) Spatial–temporal variability of epibenthic assemblages on subtidal biogenic reefs in the northern Adriatic Sea. Mar Biol 158:1447–1459. doi:10.1007/s00227-011-1661-3

Ros JD, Romero J, Ballesteros E, Gill JM (1985) Diving in blue water. The benthos. In: Margalef R (ed) Western Mediterranean. Pergamon Press, Oxford, pp 233–295

Teixidó N, Pineda M-C, Garrabou J (2009) Decadal demographic trends of a long-lived temperate encrusting sponge. Mar Ecol Prog Ser 375:113–124. doi:10.3354/meps07757

Teixidó N, Garrabou J, Harmelin J-G (2011) Low dynamics, high longevity and persistence of sessile structural species dwelling on Mediterranean coralligenous outcrops. PLoS ONE 6:e23744. doi:10.1371/journal.pone.0023744

Teixidó N, Casas E, Cebrián E, Linares C, Garrabou J (2013) Impacts on coralligenous outcrop biodiversity of a dramatic coastal storm. PLoS ONE 8(1):e53742. doi:10.1371/journal.pone.0053742

Terlizzi A, Anderson MJ, Fraschetti S, Benedetti-cecchi L (2007) Scales of spatial variation in Mediterranean subtidal sessile assemblages at different depths. Mar Ecol Prog Ser 332:25–39

Uriz M-J (1998) How do reproductive output, larval behaviour, and recruitment contribute to adult spatial patterns in Mediterranean encrusting sponges ? Mar Ecol Prog Ser 167:137–148

Virgilio M, Airoldi L, Abbiati M (2006) Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. Coral Reefs 25:265–272. doi:10.1007/s00338-006-0100-2

Witman JD, Dayton PK (2001) Rocky subtidal communities. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Associates, Inc., Sunderland

E Publication Chapter 6

Impacts on Coralligenous Outcrop Biodiversity of a Dramatic Coastal Storm

Núria Teixidó[1,2]*, **Edgar Casas**[2], **Emma Cebrián**[3], **Cristina Linares**[1], **Joaquim Garrabou**[2]

1 Ecology Department, Biology Faculty, University of Barcelona, Barcelona, Spain, **2** Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain, **3** Centre for Advanced Studies of Blanes (CEAB-CSIC), Blanes, Spain

Abstract

Extreme events are rare, stochastic perturbations that can cause abrupt and dramatic ecological change within a short period of time relative to the lifespan of organisms. Studies over time provide exceptional opportunities to detect the effects of extreme climatic events and to measure their impacts by quantifying rates of change at population and community levels. In this study, we show how an extreme storm event affected the dynamics of benthic coralligenous outcrops in the NW Mediterranean Sea using data acquired before (2006–2008) and after the impact (2009–2010) at four different sites. Storms of comparable severity have been documented to occur occasionally within periods of 50 years in the Mediterranean Sea. We assessed the effects derived from the storm comparing changes in benthic community composition at sites exposed to and sheltered from this extreme event. The sites analyzed showed different damage from severe to negligible. The most exposed and impacted site experienced a major shift immediately after the storm, represented by changes in the species richness and beta diversity of benthic species. This site also showed higher compositional variability immediately after the storm and over the following year. The loss of cover of benthic species resulted between 22% and 58%. The damage across these species (*e.g.* calcareous algae, sponges, anthozoans, bryozoans, tunicates) was uneven, and those with fragile forms were the most impacted, showing cover losses up to 50 to 100%. Interestingly, small patches survived after the storm and began to grow slightly during the following year. In contrast, sheltered sites showed no significant changes in all the studied parameters, indicating no variations due to the storm. This study provides new insights into the responses to large and rare extreme events of Mediterranean communities with low dynamics and long-lived species, which are among the most threatened by the effects of global change.

Citation: Teixidó N, Casas E, Cebrián E, Linares C, Garrabou J (2013) Impacts on Coralligenous Outcrop Biodiversity of a Dramatic Coastal Storm. PLoS ONE 8(1): e53742. doi:10.1371/journal.pone.0053742

Editor: Simon Thrush, National Institute of Water & Atmospheric Research, New Zealand

Received September 21, 2012; **Accepted** December 3, 2012; **Published** January 10, 2013

Funding: This study has been funded by the Agence National de la Recherche (Medchange project) and Total Foundation (medDIVERSA project, http://meddiversa.medrecover.org), as well as the CSIC project (PIEC 200430E599). Support of this work was also provided by the Agency for Management of University and Research Grants (to NT, research contract Beatriu de Pinós 2009-BP-263), the Spanish Ministry of Science and Innovation (to E. Casas, doctoral fellowship linked to Biorock project CTM2009-08045; to CL, research contract Ramón y Cajal), and the Spanish National Research Council (to E. Cebrián, research contract JAE doc). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: nteixido@icm.csic.es

Introduction

Extreme events are rare, stochastic perturbations that can cause abrupt and dramatic ecological change within a short period of time relative to the lifespan of organisms [1], [2], [3]. Extreme events are also considered rapid drivers with the potential to alter the state and trajectory of community structure and dynamics at wide spatial scales [4 5], [6], quickly forcing the system away from its equilibrium state and shaping its dynamics far into the future [7], [8], [9]. When ecosystems are forced beyond a threshold, regime shifts occur and the system enters into alternate stable states with a structure and function that are fundamentally different from the previous regime [10], [11]. Thus, understanding the community dynamics affected by extreme events is crucial for ecology and conservation research in a climatically changing world. As a consequence, interest in large phase-shifts and ecosystem resilience related to extreme events has increased considerably during recent decades due to the high level of disturbances that both terrestrial and marine ecosystems are suffering [10], [11], [12].

Studies characterizing marine ecosystem responses to anthropogenic climate change have revealed decreases in ocean productivity, alterations in food web dynamics, changes in physiology, increases in disease incidence, shiftis in species distributions, and reduced abundance of habitat-forming species [13], [14], [15]. In contrast, little is known about how extreme events affect marine communities. Under the conditions of ongoing climate change, observations and global change models predict increases in the frequency and intensity of extreme weather and climatic events, including heat waves, droughts, and intense tropical and mid-latitude storms [2], [16]. Extreme storms, such as hurricanes and severe storms in the tropics and mid-latitude storms in temperate areas abruptly alter ecological processes and structure and severely affect marine littoral communities [17], [18], [19], [6]. In comparison with our understanding regarding the effects of hurricanes and tropical storms affecting coral reefs (*e.g.* [20], [21], [22], [23], [24]), there is little knowledge about how extreme storms affect rocky benthic communities in temperate regions such as the Mediterranean Sea. This lack of knowledge may partially be explained by the rarity and stochastic nature of extreme storms in the Mediterranean Sea, combined with the scarcity of baseline data and long-term studies, making it difficult to study the effects of these events. Nevertheless, analyzing the impacts of these events may provide new insights into processes that shape the structure of benthic communities in this region.

The Mediterranean Sea is considered a hotspot of marine biodiversity, harboring approximately 10% of the world's marine species while covering less than 1% of the world ocean surface [25], [26]. This region has a long history of modification of natural ecosystems by human activities [26]. In the Mediterranean Sea, coralligenous outcrops are of special concern, as they represent one of the most important hotspots for biological diversity (harboring approximately 20% of Mediterranean species), exhibit great structural complexity, and are among the habitats facing major threats [27], [28]. The species that characterize coralligenous seascapes are encrusting calcareous algae, sponges, cnidarians, bryozoans, and tunicates. Some of the engineering species in these environments are long-lived; hence, their low dynamics make coralligenous outcrops exceptionally vulnerable to anthropogenic disturbances, such as destructive fishing practices, pollution, invasive species or mass mortality outbreaks linked to climate change [29], [30], [31], [32], [33]. Moreover, the Mediterranean basin is also considered to represent a climate change hotspot and will undergo one of the largest changes in climate worldwide, with an increase in the frequency of hot wave extremes of 200 to 500% predicted at the end of the twenty-first century [34], [35], [36], [16].

Studies over time provide exceptional opportunities to reveal the effects of extreme climatic events and to measure their impacts by quantifying rates of change at population and community levels. These studies are even more valuable when addressing slow-growing, long-lived species, which do not often undergo marked declines and in which adult mortality is rarely observed [37], [38], [32]. Since 2006, we have annually surveyed coralligenous outcrops in the Medes Islands Marine Reserve in the western Mediterranean, and we were able to detect the impact of a dramatic coastal storm in December 2008 [39], [40], [41] that shifted the community composition and structure of the most common long-lived benthic species in the area. Storms of comparable severity have been documented to occur irregularly within 50 year periods in the Mediterranean Sea [41], [42]. Here, we provide evidence of the immediate impact of this severe coastal storm on the coralligenous outcrops and their responses over the following year. We assessed changes in the dynamics of the benthic community structure using data from before (2006 2008) and after the impact (2009 2010) and by analyzing: i) the community composition, species richness and beta diversity of sessile benthic perennial species with low dynamics, ii) community cover dynamics, and iii) the sensitivity of representative benthic species to the effects of the storm by quantifying cover changes. The final aim of the study is to identify the responses of communities with low dynamics and long-lived species to large and rare extreme events, providing new insights to understand and predict how present and future impacts affect these communities.

Extreme Storm Event on December 26th 2008

The December 26th 2008 storm was an extreme event considered to be one of the strongest impacting the Catalan coast in the last 50 years [43], [41],[42]. Storms of an equivalent intensity were reported for the same area in the early (31/01/1911) (Meteorological Service of Catalonia, http://www20.gencat.cat/docs/meteocat/Continguts/Noticies/2011/Gener/pdf/31degenerde1911.pdf), [44] and mid-twentieth century (22/02/1948) (La Vanguardia newspaper archives, http://hemeroteca.lavanguardia.com/preview/1948/02/22/pagina-4/34354259/pdf.html); but there are no instrumental wave records of these storms. On December 25th 2008, a strong high pressure system developed over northern Europe (1047 hPa) blocking the western atmospheric circulation and forcing northern cold air and a deep cyclone to flow towards the NW Mediterranean Basin [41], [39]. This convergence caused maritime eastern winds and stormy seas to reach the Catalan coast. The storm reached category 5 [45] as it moved from the Gulf of Genoa to the Catalan coast, where it hit the shore on December 26th, with wind gusts up to 20 m s^{-1}, wave heights of 8 m with peaks of 14.4 m, and wave periods of 14 s [39], [41]. The damage caused by the intense waves was accentuated by the dislodged material that they carried, scouring sand and the displacement of large rocks [41], [N. Teixidó *pers. observ*]. Shallow sublittoral communities (5 10 m) in the Natural Park of Montgrí, Medes Islands and Baix Ter and adjacent areas showed high abrasion, with density reductions of 50 80% of sea urchin populations and loss of algal cover up to 90% within a depth range of 5 10 m [46],[47]. Although these shallow habitats were the most impacted, damage was also registered in deeper habitats (20 m depth), with 80% of mortality of the brown alga *Cystoseira zosteroides* [40]. The most damaged communities were dwelling on surfaces facing the East. An exploratory dive immediately after the storm at a depth of approximately 16 20 m (one week afterward, 03/01/2009) revealed detached living colonies and fragments of gorgonians (*Paramuricea clavata* and *Eunicella singularis*) and massive sponges (*Ircinia oros*) on the sea floor, torn skeleton bases with living tissue of *P. clavata*, rhizomes of *P. oceanica*, and displacement of large rocks with compressed sessile organisms (N. Teixidó *pers. observ.*). Additionally, the storm caused a significant decline of the sea bass population (*Dicentrarchus labrax*), the burial of 20% of *Posidonia oceanica* meadows and affected the deep-sea environment (300 1500 m) through increases of current speed, sediment transport, and the grain size of particles [43],[41].

Materials and Methods

Study Area

We assessed the impact of this dramatic storm on coralligenous outcrops in the Natural Park of Montgrí, Medes Islands and Baix Ter of the NW Mediterranean Sea (42° 3′N 3° 13′E, NE Spain). This area harbors well-developed coralligenous outcrops with a depth distribution of 15 70 m [48]. Due to their beauty and aesthetic value, these outcrops are among the most attractive areas for recreational scuba diving and are subjected to diving impacts [49], [30].

Field Activity and Data Collecting

We quantified the immediate impact of the storm on the benthic community and the following year using before-and-after data (Fig. 1). Sampling site locations had different exposure orientation, where the most exposed face the East and the most sheltered the North-West: Carall Bernat faces the NE, Medallot the SW, Tascó Petit the NW, and Punta Salines the N. The sites are separated by few hundreds of meters to 3 kilometers. Carall Bernat was the site most exposed, whereas Tascó Petit and Punta Salines were the most sheltered; thus used as controls. We present data from surveys that were performed annually before the storm event (July August 2006, 2007, and 2008), shortly after the storm (February 2009) and one year later (August 2010). Data available from Punta Salines cover only 2008 and February 2009. However, the Punta Salines data set has a meaningful ecologic value because it covers the most relevant time span of the analyzed temporal variation (before and immediately after); thus, we considered as a valid


A1
A2
B1
B2
C1
C2
D1
D2

Figure 1. Photographs showing the impact of the coastal storm on coralligenous outcrops. These photographs show the same quadrat (25*25 cm) before (2008) and after the storm event (2009). A: Carall Bernat, dates A1:23/06/2008, A2:10/02/2009; B: Tascó Petit dates B1:24/06/2008, B2 16/02/2009; C: Medallot, dates C1:22/06/2008, C2:09/02/09, D: Punta Salines dates: D1:25/06/2008, D2:09/02/2009.
doi:10.1371/journal.pone.0053742.g001

control site. This severe storm was a natural experiment affecting sublittoral communities with differences in exposure among sites and offered the possibility to reveal the effects produced after this severe meteorological event.

We used data from 4 permanent plots (4 m long * 0.8 m wide, total area $= 3.2$ m^2) located haphazardly at a depth of ~ 20 m at four different sites. The corners of each plot were marked with PVC screws fixed in holes in the rocky substratum with two-component putty [37]. During each survey, elastic bands were placed around the corners to facilitate the recognition of plot borders. Then, each plot was monitored photographically using quadrats of 25*25 cm to facilitate species identification [50]. The photographs were taken with a Nikon D70S digital SLR camera fitted with a Nikkor 20 mm DX lens (3000 * 2000 pixel resolution) and housed in Subal D70S housing. Lighting was achieved using two electronic strobes fitted with diffusers. Approximately 64 quadrats covered the entire surface of the permanent plot. In each permanent plot, we analyzed 3 replicates of 8 photographic quadrats (5000 cm^2) as a minimal sampling area as the optimal sampling effort [50]. These 3 replicates per site ($n = 51$ in total: 3 sites* 3 replicates* 5 years $= 45$; 1 site * 3 replicates* 2 years $= 6$) allowed replication and further statistical comparisons. A total of 404 photographs were analyzed.

All necessary permits for the described field studies were obtained from the authority responsible for this Protected Area. The locations are not privately-owned. This study did not involve endangered or protected species. Moreover, we did not perform any disturbance to species during our fieldwork. Our data were based on the analysis of images, a non-destructive technique to study marine benthic communities.

Benthic Communities

Natural variability of coralligenous outcrops shows little changes over time [51], [32], [52]. Changes in the benthic coralligenous outcrops due to the severe storm were evaluated in three ways. i) Changes in community composition, species richness, and beta diversity were measured based on the presence-absence of perennial sessile macro-species. Overall, these perennial species are characterized by slow growth and low dynamics (hereafter referred to as SG, see Table S1 for the species list) [53], [54], [55], [32]. They mainly consisted of macroalgae and encrusting red algae, sponges, anthozoans, bryozoans and tunicates. A total of 64 SG species were identified at the lowest taxonomic level from photographs. Additional dives were performed for species identification (see [50] for further details). This approach, based on presence-absence analysis is an optimal method for coralligenous biodiversity assessment and monitoring, providing good estimates of the composition and structure of these communities [50]. ii) The percent cover of functional groups of sessile organisms and substrates was measured. These groups were classified as slow growing species (hereafter SG, i.e., the 64 species mentioned above with low dynamics); fast growing species (hereafter FG, including small, filamentous and seasonal hydrozoans and bryozoans with high dynamics); turf of algae (hereafter TA, corresponding to a multispecific assemblage of small and filamentous algae); detritic matrix (hereafter DM, consisting of conglomerates of detritus and microalgae); and bare substrate (hereafter BS). iii) The percent cover of the representative slow growing species was determined by the similarity percentage procedure (SIMPER analysis) (see below). Then, the 37 representative species were grouped into 6 different morphological forms: Boring (BOR), Cup (CUP), Encrusting (ENC), Encrusting algae (ENA), Massive (MAS), and Tree (TREE). Furthermore, we measured the sensitivity of these 37 representative species by comparing the change in the percentage of cover before and after the storm (see Table S2 for cover values). The sensitivity values ranged from -100% (total disappearance of cover after the storm) to 0% (no cover change) to positive values (increased cover). To perform the cover analyses, each photograph was projected onto a grid of 25 squares (5 cm×5 cm), and abundances were quantified by counting the number of squares filled in the grid by either each functional group or representative species and expressing the final values as percentages [56], [57]. For the red gorgonian *Paramuricea clavata*, which exhibits an arborescent form, cover was calculated as the area occupied by its base. Percent cover of functional (ii) and morphological (iii) groups over 5 years were calculated for sites where all temporal range was available.

Statistical Analysis

Changes in community composition were investigated using non-metric multidimensional scaling (MDS) on the basis of the Bray-Curtis dissimilarities of the presence-absence of 64 perennial macro-species as well as the presence-absence of the functional groups described above (FG, TA, DM, BS). The null hypothesis of no structure in the data was tested using the similarity profile test (SIMPROF) (with 9999 permutations and a 0.1% significance level [58] on the Bray-Curtis matrix). This technique is a permutation-based ranking procedure aimed at testing genuine clusters in samples with no *a priori* assumptions about group membership. Differences in beta-diversity (% of unshared perennial macro-species) among sites and before and after the impact were analyzed using the **PERMDISP** routine. This is a routine for comparing the degree of dispersion of different groups of samples based on a distance matrix. We tested for similarity in the beta-diversity among groups on a Jaccard distance matrix [59]. The representative taxa for each site before the storm were determined using the similarity percentage procedure (SIMPER) [60]. Then, we measured the sensitivity of these taxa by comparing the percentage of cover change before and after the storm.

Non-parametric analysis of variance PERMANOVA [61] was used to examine the changes generated by the storm. The sampling design included 2 factors: *Site*, which was random with 4 (changes in community composition) or 3 (cover of functional and morphologic groups) levels; and *Before/After*, which was fixed with 2 levels. Differences between samples were quantified using i) Bray-Curtis dissimilarities for the multivariate perennial macro-species data matrix and ii) Euclidean distances for univariate analyses. Analyses were performed with 9999 unrestricted random permutations of the raw data. Pair-wise comparisons for all combinations of *Site x Before/After* were also carried out using t-tests and 9999 permutations of the raw data. Chi-squared tests were carried out to test for differences in the frequency of sensitivity among the sites and taxonomic as well as morphological form groups. The analyses were computed using the program Primer v6 with the PERMANOVA+add-on package [62] and Statistica (version 8.0 StatSoft).

Results

The community composition of sessile macro-species showed a major shift after the immediate impact of the storm (Fig. 2). The most exposed site Carall Bernat was the most impacted and underwent a change in benthic structure, resulting in a distinct cluster (SIMPROF test $p<0.01$, see Figure S1 for all the SIMPROF groups) containing the immediate post-storm (2009) data and those of the following year (2010). This post-storm group showed a higher dissimilarity and larger multivariate dispersion than the pre-storm data (2006 2008) and those from the other three sampling sites; no significant changes were observed (Fig. 2). There was a significant interaction between sites and before-after the storm impact ($F_{3,43} = 2.96$, $p<0.0001$) (see Table S3 for 2-way PERMANOVA and pair-wise tests). Considering the pair-wise comparisons, only Carall Bernat showed a significant difference before (85% similarity) and after the storm (74% similarity) ($t=2.98$, $p<0.0001$, Table S3). This shifting pattern was corroborated by a significant decrease in the mean species number ($F_{3,43} = 8.91$, $p<0.001$), from the mean values of 35.5±0.57 before to 27±0.62 sessile species after the storm, representing a decline of 24% (pair-wise comparisons $t = 5.99$, $p<0.0001$) (Fig. 3) (see Table S4 for 2-way PERMANOVA and pair-wise tests). The clear shift in the community composition was also evident based on the beta-diversity analysis ($F_{7,43} = 5.8$, $p<0.001$) (Fig. 4). Carall Bernat showed a significant increase of beta-diversity after the storm (18.2% ±0.7 before vs. 22.33% ±1.4 after) ($t = 1.78$, $p<0.01$), indicating higher variation in the benthic composition, whereas Medallot exhibited a decrease, which was not significant (24.4% ±1.2 before vs 18.7% ±1.4 after) ($t=2.97$, $p>0.05$). Regarding the non- impacted sites, no changes were observed in the community structure (Fig. 2, Table S3), mean species number (before: Tascó Petit 34.3±0.43, Punta Salines 31.1±1.1; after: Tascó Petit 35.3±1.0, Punta Salines 30.5±0.8) (Fig. 3, Table S4) and beta diversity (Fig. 4) ($t=0.9$ $p>0.05$ for Tascó Petit, $t=0.9$, $p>0.05$ for Punta Salines).

Before the storm event (2006 2008), the coralligenous outcrops were characterized by a high cover of perennial and slow growing species (SG) (mean values of 87% ±0.9 Carall Bernat, 88% ±0.8 Tascó Petit, and 84% ±0.7 Medallot) (Figs. 5 and 6), such as encrusting and fragile calcareous algae, encrusting sponges, tree bryozoans and gorgonians, massive sponges and tunicates, and an overall high structural complexity. These patterns were constant over the three years and reflected the low natural variability of coralligenous outcrops (Fig. 5). There was a significant change in the percentage of cover of the principal functional groups among the three sites and before-after the storm ($F_{2,39} = 10.7$, $p<0.0001$) (Fig. 5), and these differences were significant for the pair-wise comparisons of the interaction term at Carall Bernat and Medallot ($p<0.001$, see Table S5). The cover of SG at Carall Bernat was by far the most severely damaged, showing a decrease to 37% ±3.9 of the total area immediately after the impact (2009), but increased to 46% ±4.7 in the following year (2010) (Fig. 5). The distribution of damage also depended on small-scale position effects at Carall Bernat, where 16% and 25% of the area analyzed in 2009 showed values as low as 10% and 50% of SG cover, respectively. The scouring effect of the storm was evident in the peak of bare substrate (BS) (mean value 63% ±4) observed immediately after the storm (2009) at Carall Bernat, which was replaced by turf algae (TA) in 2010 (mean value 44% ±3). Interestingly, surveys at Medallot showed a moderate decrease of SG, with a reduction from 84% ±0.7 (before) to 74% ±2.3 (2009), followed by a further decrease down to 56% ±3.8 (2010), suggesting a delayed loss of SG cover (Fig. 5). Furthermore, there was an increase of detritic matrix (DM) in 2009 (mean value 12% ±0.2) and a 3-fold increment from the pre-storm cover value of TA (mean value 33.3% ±3.6 in 2010). In contrast, Tascó Petit was almost not affected after the storm, exhibiting a discrete peak of 5% BA and a reduction of 66% in fast growing species (FG) (12% ±1.8 before vs. 4% ±1 after) (Fig. 5). No significant change in cover percentage of the principal functional groups was observed before and after the storm (pair-wise comparisons $t=2.63$; $p>0.05$, see Table S5).

The damage to the cover of different growth forms differed significantly among localities and before-after the storm (Fig. 6) ($F_{2,9}=3.7$, $p<0.01$) The cover loss of massive (MAS) and encrusting (ENC) sponges and tunicates, scleractinian corals (CUP), encrusting algae (ENA), and arborescent gorgonians and bryozoans (TREE) ranged from 45% to 66% in relation to the pre-storm cover at Carall Bernat (Fig. 6). The damage to ENC and ENA was particularly striking due to the high pre-storm cover (approximately 46.5% and 23% before and 21.8% and 9.5% after the storm, respectively), as well as for the TREE category, which despite its low cover before the storm (approximately 12%) declined considerably to 4% (Fig. 6). Medallot and Tascó Petit did not show significant differences before and after the storm (pair-wise comparisons $t = 2.13$ and $t=2.45$, $p>0.05$, see Table S6). The sensitivity of the representative species most affected by the storm was significantly different across sites, with 95% of species being affected at Carall Bernat ($n=24$), 38% at Tascó Petit ($n=31$), 34% at Medallot ($n=32$), and less than 1% at Punta Salines ($n = 19$) ($X^2=54.2$, $df=3$, $p<0.0001$) (Fig. 7). The alga *Peyssonnelia sp.*, the encrusting and delicate sponges *Hemimycale columella* and *Pleraplysilla spinifera*, the massive-ropy fragile sponge *Clathrina clathrus* and the bryozoans *Adeonella calveti* and *Myriapora truncata* were reduced by up to 100% at Carall Bernat (Fig. 7, Table S2). In addition, among the species that exhibited high coverage before the storm (each species showing a cover value of approximately 10%), *Lithophyllum stictaeforme* (ENC) was reduced to 85%, *Parazoanthus axinellae* (ENC) to 72%, *Paramuricea clavata* (TREE) to 70%, *Disydea avara* (ENC) to 64%, *Phorbas tenacior* (ENC) to 49%, and *Crambe crambe* (ENC) to 20%. Similar patterns of damage were not found at the other sites. For example, at Medallot, the reduction was 100% only for *F. implexa* and for *M. truncata* (TREE*),* and other species showed values lower than 50% such as *D. avara* (ENC), *Reteporella spp.* (TREE), and *P. clavata* (TREE). Only 3 species out of 31 showed high to moderate values of cover loss at Tascó Petit: 100% for *Halocynthia papillosa* (MAS), 67% for *C. clathrus* (MAS), and 50% for *Filograna implexa* (TREE). No evident changes of cover loss were detected in Punta Salines (Fig. 7). Overall, there was no significant difference regarding taxonomic groups ($X^2=6.9$, $df=5$, $p>0.05$) or morphological forms ($X^2=9.8$, $df=5$, $p>0.05$). The massive and robust sponges *Chondrosia reniformis* and *Agelas oroides* appeared to be less affected, showing approximately 5% cover loss at Carall Bernat and Tascó Petit and no change at Medallot. After the storm, The removal of sessile organisms on the boring sponge *Cliona sp. during the storm* increased the exposed area by 45% at Carall Bernat and +20% at Medallot, respectively (Fig. 7).

Discussion

The storm of December 26th 2008 was considered to be the strongest recorded in the last 50 years in the northern part of the Catalan coast (41°N - 42°30′N), with the greatest wave power, the highest wave heights, and the longest duration [41], [39], [45]. Its impact, including abrasion of sediment particles, severely affected the littoral communities in the region, causing mortality of sessile

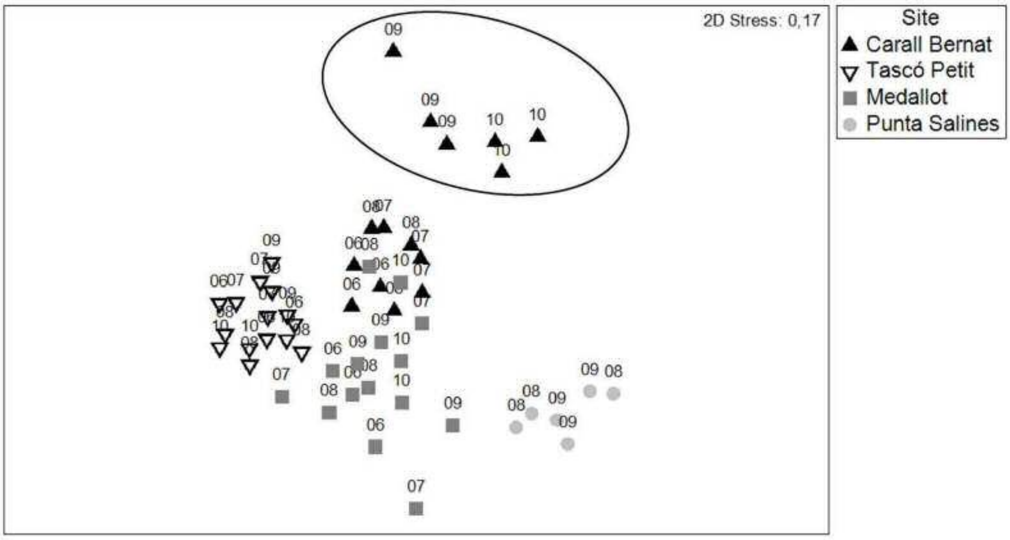


Figure 2. Shift in the structure of the perennial benthic species in coralligenous outcrops in the Medes Islands in response to the 2008 dramatic storm episode. Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis resemblance measure for species presence/absence data from 2006 to 2010. The circle indicates a SIMPROF group containing the immediate post-storm (2009) and following year (2010) data for Carall Bernat. Each symbol represents 8 analyzed photographs.
doi:10.1371/journal.pone.0053742.g002

benthic organisms, including some long-lived species (mainly sponges and anthozoans) estimated to be more than 50 years old [53],[32]. Coralligenous outcrops exhibit low dynamics and few changes over time at population and community level in absence of large disturbances [51], [63], [28], [32], which makes the impact of extreme events very important for community dynamics.

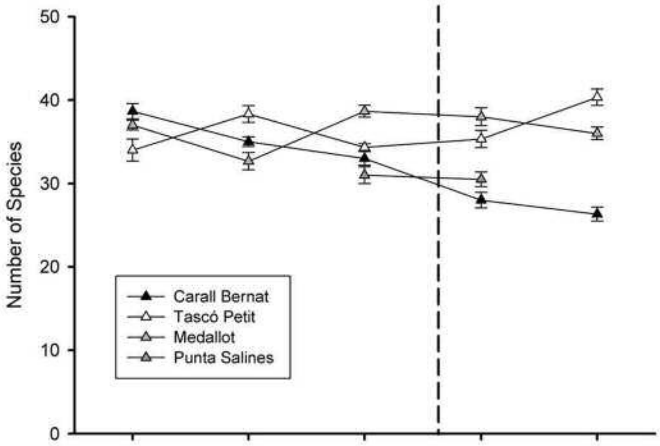


Figure 3. Mean number of sessile species (± SE) over time in the Medes Islands. The dotted line represents the impact of the unusual storm in December 2008.
doi:10.1371/journal.pone.0053742.g003

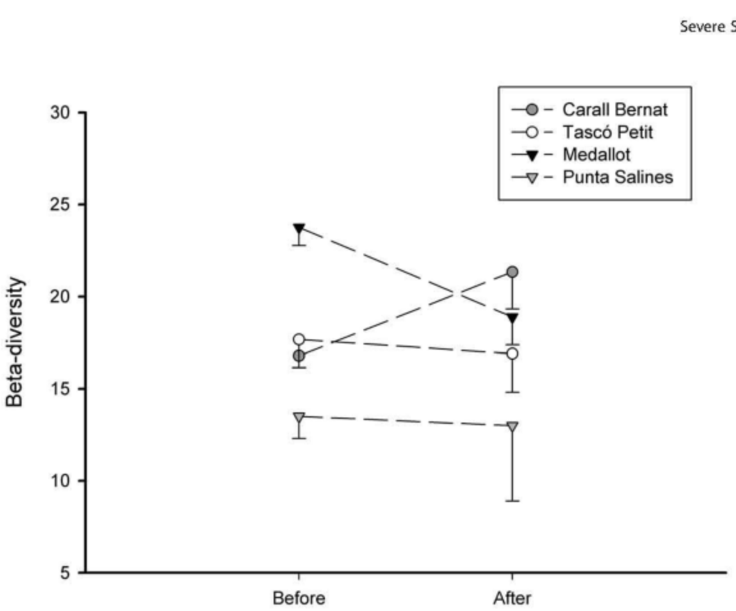


Figure 4. Mean (±1 SE) beta-diversity (as the percentage of unshared species) before and after the storm for each site. The results of PERMDISP analyses are shown.
doi:10.1371/journal.pone.0053742.g004

This is even more important in the Mediterranean Sea, which is considered a hotspot of climate change, where exceptional events such as this storm or heat-waves in summers are predicted to increase [34], [35], [36], [16]. Overall, our results quantified the different effects of this rare, extreme event on the community structure dynamics of long-standing coralligenous outcrops. This study is unique in using high-resolution sampling over time to reveal how extreme events can shift coralligenous outcrops characterized by long-lived species and may be of general interest regarding ecological responses to extreme and unusual climatic events.

Spatial Patterns of the Storm Impacts

The loss of cover of slow growing benthic species was between 58% and 22% immediately after the storm at two of the studied sites, Carall Bernat and Medallot, respectively (Fig. 5). The damage caused by the storm (with an eastern wind direction) was influenced by aspects of orientation, local habitat profiles, depth, and the presence of boulders. The most impacted site, Carall Bernat (wall facing NE), is located within a narrow channel surrounded by large stone blocks, whereas Medallot (wall facing SW) is located in the most westerly part of an archipelago and is better protected from easterly wave swells. Our data showed that approximately 18 out of 24 species at Carall Bernat were severely affected, showing cover loss values higher than 50% (Fig. 7), accompanied by a significant decrease of perennial species richness (24%) (Fig. 3). Although the impact was local and restricted to the northernmost part of the Catalan coast, the damage was among the highest registered for coralligenous outcrops of the NW Mediterranean Sea. High mortality rates of gorgonian populations have been reported to be up to 10–60% after major episodes of mass mortality, such as those related to positive temperature anomalies in the summers of 1999 and 2003 in the NW Mediterranean Sea [29], [64], [31]. However, these studies did not explore overall community shifts (including changes in calcareous algae, sponges, anthozoans, bryozoans, and tunicates). Other studies quantifying the impacts of severe hurricanes and cyclones on coral reefs have also focused on primary framework corals e.g., [65], [20] and have reported reef losses, with values ranging from 17% to 60% [66], [19], [67], [23], [68], thereby highlighting the importance in terms of the broader community changes.

In this study, the effects of the storm were not found to be uniform and synchronous. Although Carall Bernat was the most impacted site, it showed a low recovery of perennial species after one year (see below), whereas Medallot exhibited little cover loss immediately after the storm (cover loss of approximately 8%) but showed a further decline in the following year (23% cover loss of slow growing species), accompanied by a considerable increase in turf algae (~ 33%) (Fig. 5). We acknowledge the different responses of benthic community dynamics, which integrate different complex history processes and disturbances, and highlight the complexity of identifying unique, combined and/or synergetic effects of disturbance when most coastal habitats are exposed to multiple stressors [67], [69], [70]. Based on this complexity, our results showed that benthic communities dwelling in rather small areas (less than ~ 10 km^2) can exhibit significantly different responses to sudden disturbances.

An abrupt shift in the multivariate structure of coralligenous outcrops after the storm was only observed at the most exposed and impacted site, Carall Bernat, which showed the highest compositional variability in response to the disturbance (Fig. 2). Tascó Petit and Punta Salines did not show any significant change on community structure before and after the storm, indicating no major effects of this severe storm at the sheltered sites. This pattern of greater variability was corroborated by an increase of beta diversity in the perennial species composition after the disturbance (immediately after and in the following year) (Fig. 4). After extreme

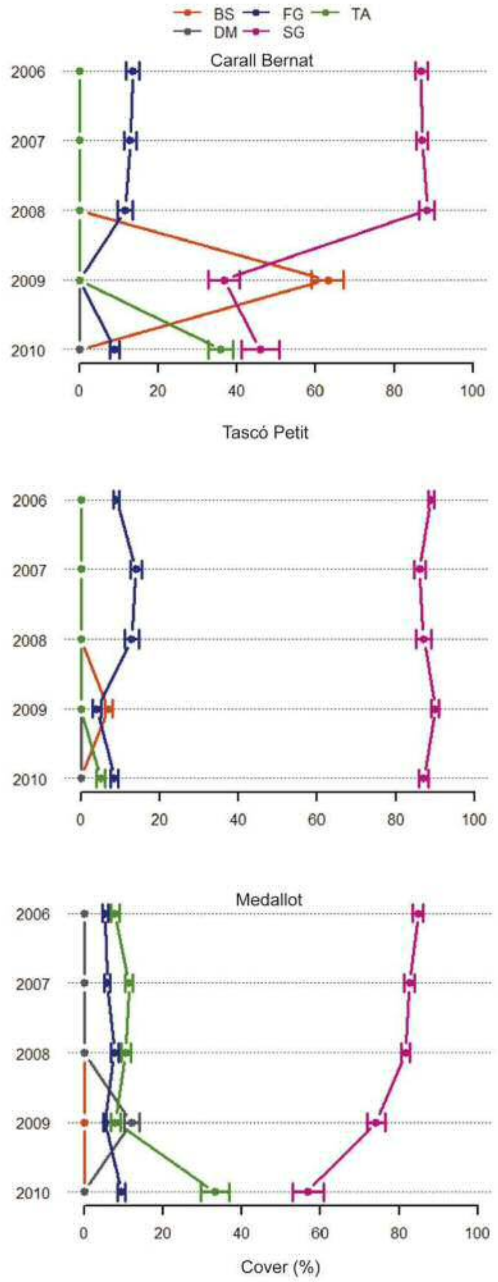


Figure 5. Response to the dramatic 2008 storm episode regarding the cover area of the principal groups of sessile organisms and bare substrate. The principal categories are Bare substrate (BS), Detritic Matrix (DM), Turf algae (TA), Fast growing species (FG, i.e., small animal species mainly bryozoans and hydrozoans), and Slow growing species (SG, i.e., perennial algae and animal species).
doi:10.1371/journal.pone.0053742.g005

events such as this storm, post-disturbance variability is expected to be elevated and to persist for a longer period of time relative to pre-disturbance conditions, and this variability will be stabilized more gradually, only after the disturbed state has returned to the baseline condition [71], [72]. Our results demonstrate that this unusual storm produced a mosaic of small remaining survivor patches in the most impacted site and reduced the structural complexity of perennialslow-growing benthic species, creating a seascape habitat exhibiting higher fluctuations in the presence/absence of component species, accompanied by a reduction in the number of species, thus reducing the species pool (a decrease of 8 perennial species) (Figs. 2 and 3). Interestingly, this severe storm appeared to have opposite effects on beta diversity (Fig. 4), such that it increased significantly in Carall Bernat due to a high variability in composition, whereas it declined significantly at Medallot. This increased similarity at Medallot between pre-and post-storm conditions may be explained as a consequence of the change in the relative cover of perennial-slow benthic species (decreasing) and turf algae (increasing). Overall, this change in beta diversity (increase or decrease) was accompanied by a loss of functional groups in Carall Bernat and Medallot (Figs. 6 and 7), with a shift in dominance from encrusting algae and perennial animal species to turf-forming algae. Encrusting calcareous algae are the major contributors to coralligenous outcrops and, together with sponges, cnidarians, bryozoans, and tunicates, are the species that characterize this habitat [28]. Thus, their replacement by turf-forming algae may increase the sensibility to invasion, as some of these algae belong to the most invasive species in the Mediterranean Sea, triggering substantial changes in the structure and dynamics of rocky communities and rendering surfaces inhospitable to the recruitment of native invertebrates [73], [74], [75].

Species Sensitivity

Species sensitivity showed a gradient regarding the site exposure: from high through intermediate to low values of cover loss at Carall Bernat, Medallot, Tascó Petit, and Punta Salines (Fig. 7). Our findings indicated that the damage across perennial species was uneven and those with fragile forms, irrespective of their morphology, were the most impacted, showing cover losses between 50 and 100% (Fig. 7). These results agree with the general observation that fragile branching and foliose corals are the most susceptible to hurricane damage to coral reefs [19], [67], as well as in the NW Mediterranean Sea, a severe winter storm caused high mortality of the fragile bryozoan *Pentapora facialis* [76]. In the present study, the species ranged from short-lived perennial species with estimated ages of 2 5 years (e.g., the crustose coralline alga *Peyssonnelia sp.*, the massive-ropy fragile sponge *Clathrina clathrus*, and the delicate tree-like bryozoans *Adeonella calveti* and *Myriapora truncata* to persistent and long-lived perennial species with estimated longevities of 50 100 years (e.g., the gorgonian *Paramuricea clavata*, the scleractinians *Leptopsammia pruvoti* and *Caryophyllia inornata*, and the alcyonacean *Alcyonium acaule)* and encrusting calcareous algae (e.g., *Lithophyllum stictaeforme and Mesophyllum alternans)* with low natural adult mortality [53], [28], [37], [32]. Thus, this unusual event produced high episodic mortality of adults in a community in which this rarely occurs under natural conditions. These observations are in agreement with the fact that large and infrequent disturbances such as this storm are considered to drive species interactions and community

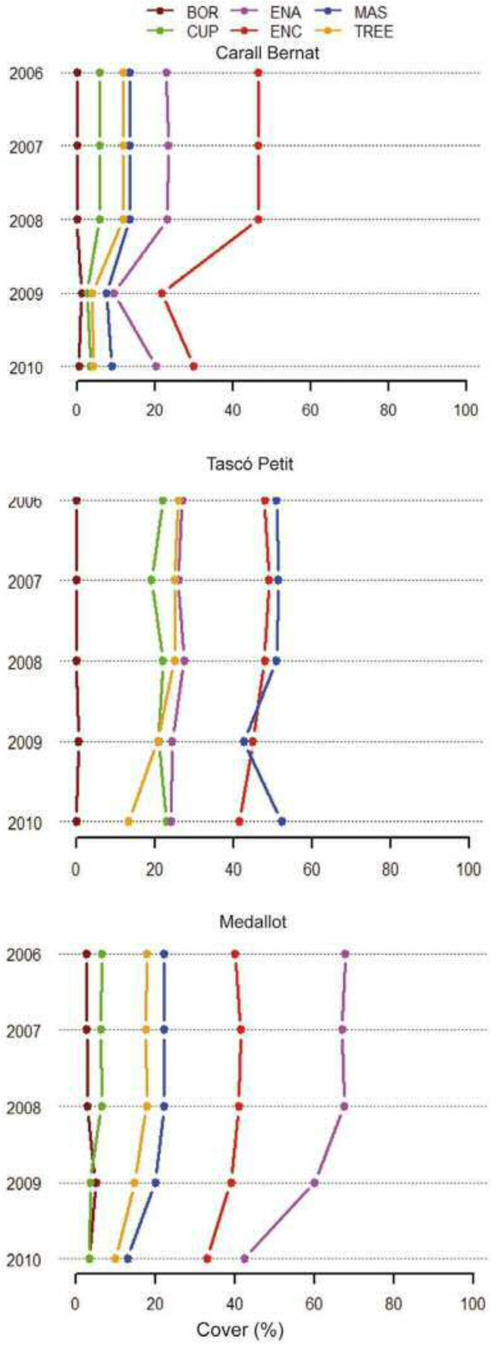


Figure 6. Response of the cover area of the representative macrobenthic taxa to the dramatic 2008 storm episode. These taxa account for 90% of the average similarity within each site (SIMPER analysis). The taxa are represented as 5 morphological forms: Boring (BOR), Cup (CUP), Encrusting algae (ENA), Encrusting (ENC), Massive (MAS), and Tree (TREE).
doi:10.1371/journal.pone.0053742.g006

dynamics, which cause long-term effects on both marine and terrestrial communities [6], [77], [5], [67].

Patterns of Surviving Patches

The strong abrasive effect of the storm did not completely homogenize the available space by creating a seascape of bare substrate at the most impacted site; rather, it produced a mosaic of small remaining surviving patches of perennial benthic species (with values of perennial-slow growing species cover ranging from 10% to 50%), associated with a decrease of habitat complexity and heterogeneity. Spatial heterogeneity following large disturbances has been widely documented in both marine and terrestrial ecosystems [77], [78], [79], and it has been recognized that biotic residuals (e.g., surviving roots and rhizomes of plants, as well as fragments of corals and sponges) are regularly available, even following a large disturbance [80], [81], [82]. In the present study, the existence of small patches after the storm (mainly encrusting algae and clonal animals such as encrusting sponges, anthozoans, and tunicates) at the most impacted site was fundamental for slight recovery, with a minor increase of cover being observed during the following year. This increase of perennial-slow growing species represented an increase of 10% (37% ±3.9) immediately after the impact (2009) to 46% ±4.7 (2010) (Fig. 5). We hypothesize that these surviving colonies and fragments favored faster recovery via vegetative regrowth and this partial recovery occurred more rapidly than could take place through the growth of new recruits via larvae. Our results showed that one year is not enough to re-establish the community to its prior state before the storm (Figs. 2, 3, 4, 5, and 6). Only two encrusting calcified algae (*Lithophyllum stictaeforme and Mesophyllum alternans)* and six clonal species (the sponges *Crambe crambe*, *Dysidea avara*, *Corticium candelabrum*, *Phorbas tenacior*, the anthozoans *Parazoanthus axinellae*, and the tunicate *Cystodytes dellechiajei*) contributed to regrowth from the remnant tissue. The finding of small remaining surviving patches is of special interest to understand community responses due to the overall low dynamics of coralligenous species combined with the infrequent or unsuccessful recruitment events recorded for sexually produced larvae of clonal organisms [83], [37], [32].

Conclusion

With the increasing threat to coastal habitats due to global warming and other interacting factors, there is growing concern about the capacity of ecosystems to absorb multiple disturbances occurring over short time periods [8], [10], [11]. Global warming is predicted to increase the frequency and magnitude of extreme climate and weather events [2], [16]. For the western Mediterranean Sea, a decrease in the total number of cyclones has been predicted [84], but an increase of wind and wave intensity [85], [86]. Consequently, the observed damage makes it evident that recurrent severe storms will seriously affect coralligenous outcrops, posing threats to their resilience. Based on the complex responses to disturbance, efforts to acquire and analyze data over time are fundamental to quantify these changes and evaluate the ecological mechanisms behind them, which will ultimately allow us to develop our capacity to predict long-term and larger scale community shifts. The effects of this storm were difficult to predict, but now that they have been registered, they provide new

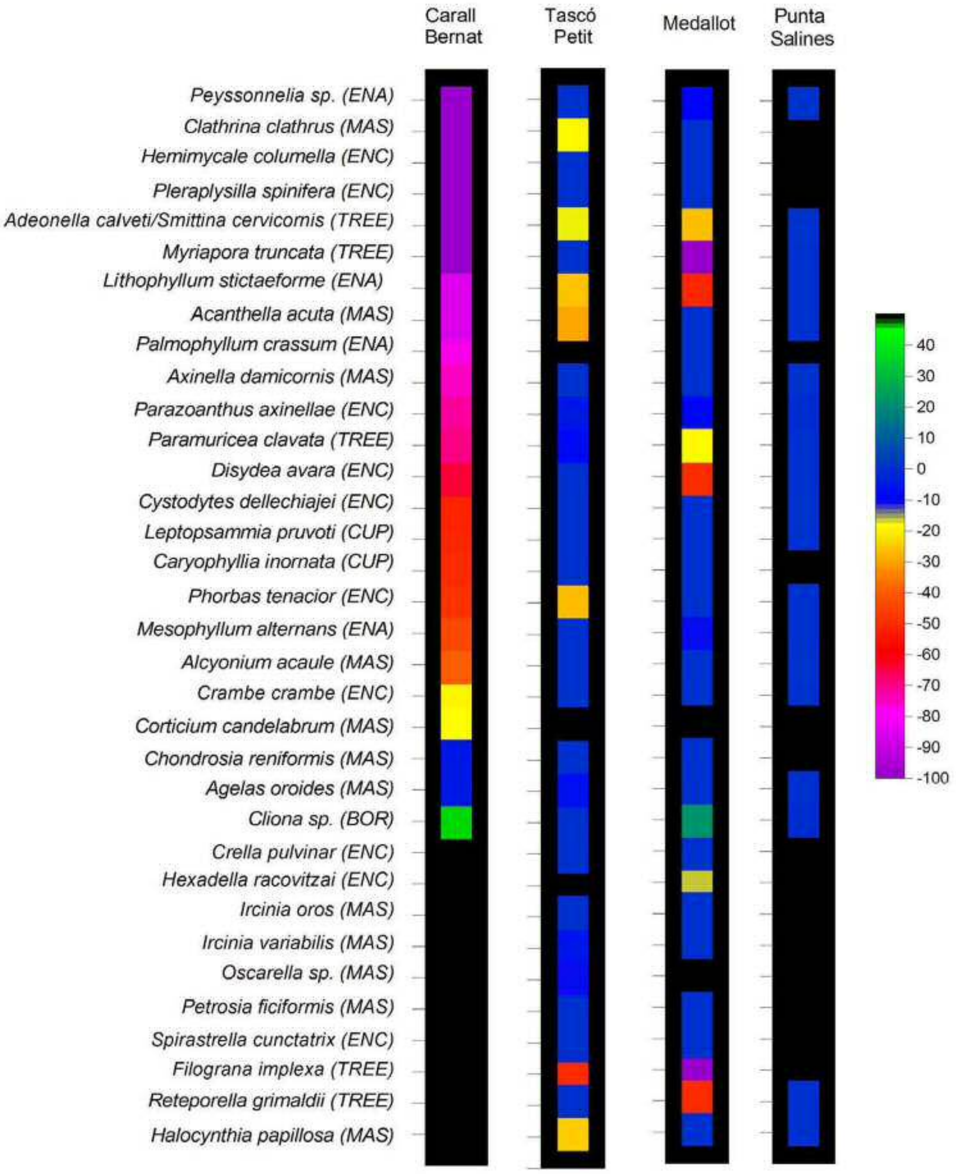


Figure 7. Sensitivity (as a percentage of cover change) of the representative macrobenthic taxa to the physical disturbance generated by the storm. Cover change (from highest, −100%, to lowest, 0%) at Carall Bernat defines the order of the taxa at the other sites. Representative macrobenthic taxa were chosen from SIMPER analysis. Black areas indicate that taxa were not representative for the specific site.
doi:10.1371/journal.pone.0053742.g007

insights into population and community dynamics. Consequently, under the present warming scenario and due to the high diversity that the Mediterranean Sea harbors [87], [26], we emphasize the need for long-time empirical and modeling studies on sublittoral benthic communities. This information is crucial not only for understanding the mechanisms underlying the dynamics of these communities and the ecological consequences of global climate change but also for determining effective management and conservation approaches to maintain the biodiversity of the Mediterranean.

Supporting Information

Figure S1 Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis resemblance measure for species presence/absence data from 2006 to 2010. A 4- group model was obtained by SIMPROF analysis: Tascó Petit (2006 2010); Punta Salines (2008 2009), Medallot (2006 2010) and the pre-storm years of Carall Bernat (2006 2008); the immediate post-storm years (2009 2010) of Carall Bernat and an independent group of Medallot (2007). Each symbol represents 8 photographs analyzed.
(TIF)

Table S1 List of the taxa identified in this study. Boring (BOR), Cup (CUP), Encrusting algae (ENA), Encrusting (ENC), Massive (MAS), Pedunculated (PEN), Tree (TREE).
(DOCX)

Table S2 Cover area (%) of the representative macrobenthic taxa before and after the physical disturbance generated by the storm. Boring (BOR), Cup (CUP), Encrusting algae (ENA), Encrusting (ENC), Massive (MAS), and Tree (TREE).
(DOCX)

Table S3 Results of 2-way PERMANOVA based on Bray-Curtis dissimilarity for macrobenthic taxa. Pair-wise comparisons using permutations of the *t*-statistic for the factor Site and the interaction term Site*Before/After are also indicated.
(DOCX)

Table S4 Results of 2-way PERMANOVA based on Euclidian distances for the number of species. Pair-wise comparisons using permutations of the *t*-statistic for the factor Site and Site*BA (Before/After) effects are also indicated.
(DOCX)

Table S5 Results of 2-way PERMANOVA analyses based on Euclidian distances for the cover area of the principal groups of sessile organisms and bare substrate. Pair-wise comparisons using permutations of the *t*-statistic for the factor Site and Site*BA (Before/After) effects are also indicated
(DOCX)

Table S6 Results of 2-way PERMANOVA analyses based on Euclidian distances for the cover of growth forms of sessile species. Pair-wise comparisons using permutations of the *t*-statistic for the factor Site and Site*BA (Before/After) effects are also indicated
(DOCX)

Acknowledgments

Special recognition is given to R. Coma, R. Graille, E. Isla, S. Kipson, M. Zabala, and F. Zuberer for field assistance. The authors are very grateful to E. Ballesteros and MJ. Uriz for taxonomic advices. N. Teixidó thanks JLL Riera and J. Santiago for help with graphic and image editing and W. Arntz for critical reading. An anonymous reviewer and a former Academic Editor, Tasman Crowe, provided valuable comments on an earlier draft. The authors are members of the Marine Conservation Research Group (http://medrecover.org) of the Generalitat de Catalunya.

Author Contributions

Conceived and designed the experiments: NT E.Cebrian CL JG. Performed the experiments: NT E.Casas E.Cebrian CL JG. Analyzed the data: NT E. Casas. Wrote the paper: NT E. Cebrian CL JG.

References

1. Turner MG, Baker WL, Peterson CJ, Peet RK (1998) Factors influencing succession: lessons from large, infrequent natural disturbances. Ecosystems 1: 511 523.
2. Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, et al. (2000) Climate extremes: observations, modeling, and impacts. Science 289: 2068 2074.
3. Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate-change experiments: events, not trends. Frontiers in Ecology and the Environment 5: 365 374.
4. White PS (1979) Pattern, process, and natural disturbance in vegetation. The Botanical Review 45: 229 299.
5. Petraitis PS, Latham RE, Niesenbaum RA (1989) The maintenance of species diversity by disturbance. Quarterly Review of Biology: 393 418.
6. Sousa WP (2001) Natural disturbance and the dynamics of marine benthic communities. In: Bertness MD, Gaines SD, Hay ME, editors. Marine community ecology. Massachusetts: Sinauer Associates. 85 130.
7. Holling CS (1973) Resilience and stability of ecological systems. Annual Review of Ecology and Systematics 4: 1 23.
8. Gunderson LH (2000) Ecological resilience in theory and application. Annual Review of Ecology and Systematics: 425 439.
9. Hughes TP, Graham NAJ, Jackson JBC, Mumby PJ, Steneck RS (2010) Rising to the challenge of sustaining coral reef resilience. Trends in Ecology & Evolution 25: 633 642.
10. Scheffer M, Carpenter S, Foley JA, Folke C, Walker B, et al. (2001) Catastrophic shifts in ecosystems. Nature 413: 591 596.
11. Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, et al. (2004) Regime shifts, resilience, and biodiversity in ecosystem management. Annual Review of Ecology, Evolution, and Systematics: 557 581.
12. Carpenter SR, Brock WA (2006) Rising variance: a leading indicator of ecological transition. Ecology Letters 9: 311 318.
13. Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, et al. (2006) The impacts of climate change in coastal marine systems. Ecology Letters 9: 228 241.
14. Rosenzweig C, Casassa G, Karoly DJ, Imeson A, Liu C, et al. (2007) Assessment of observed changes and responses in natural and managed systems. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, editors. Climate change 2007: Impacts, Adaptation, and Vulnerability Contribution of Working Group II to the Fouth Assessment Report of the Intergovernmental Panel on Climate Change: Cambridge University Press. 79 131.
15. Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328: 1523 1528.
16. IPCC (2007) The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA 996: 2007 2007.
17. Dayton PK (1971) Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs 41: 351 389.
18. Underwood AJ (1999) Physical disturbances and their direct effect on an indirect effect: responses of an intertidal assemblage to a severe storm. Journal of Experimental Marine Biology and Ecology 232: 125 140.
19. Woodley JD, Chornesky EA, Clifford PA, Jackson JBC, Kaufman LS, et al. (1981) Hurricane Allen's impact on Jamaican coral reefs. Science 214: 749 755.
20. Knowlton N, Lang JC, Rooney MC, Clifford P (1981) Evidence for delayed mortality in hurricane-damaged Jamaican staghorn corals. Nature 294: 251 252.
21. Harmelin-Vivien ML (1994) The effects of storms and cyclones on coral reefs: a review. Journal of Coastal Research: 211 231.
22. Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265: 1547 1551.
23. Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR (2005) Hurricanes and Caribbean coral reefs: impacts, recovery patterns, and role in long-term decline. Ecology 86: 174 184.
24. Walker SJ, Degnan BM, Hooper JNA, Skilleter GA (2008) Will increased storm disturbance affect the biodiversity of intertidal, nonscleractinian sessile fauna on coral reefs? Global Change Biology 14: 2755 2770.
25. Bianchi C, Morri C (2000) Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. Marine Pollution Bulletin 40: 367 376.
26. Coll M, Piroddi C, Steenbeek J, Kaschner K, Lasram FBR, et al. (2010) The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. PLoS One 5: e11842 e11842.
27. Gili JM, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology & Evolution 13: 316 321.

28. Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanography and Marine Biology: An Annual Review 44: 123 195.
29. Cerrano C, Bavestrello G, Bianchi CN, Cattaneo-Vietti R, Bava S, et al. (2000) A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. Ecology Letters 3: 284 293.
30. Coma R, Pola E, Ribes M, Zabala M (2004) Long-term assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecological Applications 14: 1466 1478.
31. Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonné P, et al. (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global Change Biology 15: 1090 1103.
32. Teixidó N, Garrabou J, Harmelin JG (2011) Low Dynamics, High Longevity and Persistence of Sessile Structural Species Dwelling on Mediterranean Coralligenous Outcrops. PLoS One 6: e23744 e23744.
33. Cebrian E, Linares C, Marschal C, Garrabou J (2012) Exploring the effects of invasive algae on the persistence of gorgonian populations. Biological Invasions 14: 2647 2656.
34. Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Global and Planetary Change 63: 90 104.
35. Déqué M (2007) Frequency of precipitation and temperature extremes over France in an anthropogenic scenario: model results and statistical correction according to observed values. Global and Planetary Change 57: 16 26.
36. Diffenbaugh NS, Pal JS, Giorgi F, Gao X (2007) Heat stress intensification in the Mediterranean climate change hotspot. Geophysical Research Letters 34: L11706 L11706.
37. Linares C, Doak DF, Coma R, Díaz D, Zabala M (2007) Life history and viability of a long-lived marine invertebrate: the octocoral Paramuricea clavata. Ecology 88: 918 928.
38. Miriti MN, Rodríguez-Buriticá S, Wright SJ, Howe HF (2007) Episodic death across species of desert shrubs. Ecology 88: 32 36.
39. Jimenez J (2012) Characterizing Sant Esteve's storm (26th December 2008) along the Catalan coast (NW Mediterranean). In: Mateo M, Garcia-Rubies A, editors. Assessment of the ecological impact of the extreme storm of Sant Esteve's Day (26 December 2008) on the littoral ecosystems of the north Mediterranean Spanish coasts Final Report (PIEC 200430E599) Centro de Estudios Avanzados de Blanes, Consejo Superior de Investigaciones Científicas, Blanes, Spain. 31 44.
40. Navarro L, Ballesteros E, Linares C, Hereu B (2011) Spatial and temporal variability of deep-water algal assemblages in the Northwestern Mediterranean: The effects of an exceptional storm. Estuarine, Coastal and Shelf Science 95: 52 58.
41. Sanchez-Vidal A, Canals M, Calafat AM, Lastras G, Pedrosa-Pàmies R, et al. (2012) Impacts on the Deep-Sea Ecosystem by a Severe Coastal Storm. PLoS One 7: e30395 e30395.
42. Bolanos R, Jorda G, Cateura J, Lopez J, Puigdefabregas J, et al. (2009) The XIOM: 20 years of a regional coastal observatory in the Spanish Catalan coast. Journal of Marine Systems 77: 237 260.
43. Mateo MA, Garcia-Rubies T (2012) Assessment of the ecological impact of the extreme storm of Sant Esteve's Day (26 December 2008) on the littoral ecosystems of the north Mediterranean Spanish coasts. Final Report (PIEC 200430E599): Centro Estudios Avanzados de Blanes, Consejo Superior Investigaciones Cientificas, Blanes, Spain.
44. Ansell TJ, Jones PD, Allan RJ, Lister D, Parker DE, et al. (2006) Daily mean sea level pressure reconstructions for the European-North Atlantic region for the period 1850 2003. Journal of Climate 19: 2717 2742.
45. Mendoza Ponce ET, Jiménez Quintana JA (2009) Regional vulnerability analysis of Catalan beaches to storms.Institution Civil Engineers 162: 127 135.
46. Hereu B, Garcia-Rubies A, Linares C, Navarro L, Bonaviri C, et al. (2012) Impact of the Sant Esteve's storm (2008) on the algal cover in infralittoral rocky photophilic communities. In: Mateo MA, Garcia-Rubies A, editors. Assessment of the ecological impact of the extreme storm of Sant Esteve's Day (26 December 2008) on the littoral ecosystems of the north Mediterranean Spanish coasts Final Report (PIEC 200430E599): Centro Estudios Avanzados de Blanes, Consejo Superior Investigaciones Cientificas, Blanes, Spain. 123 143.
47. Hereu B, Linares C, Sala E, Garrabou J, Garcia-Rubies A, et al. (2012) Multiple Processes Regulate Long-Term Population Dynamics of Sea Urchins on Mediterranean Rocky Reefs. PLoS One 7: e36901 e36901.
48. Gili JM, Ros J (1985) Study and cartography of the benthic communities of Medes Islands (NE Spain). Marine Ecology 6: 219 238.
49. Garrabou J, Sala E, Arcas A, Zabala M (1998) The impact of diving on rocky sublittoral communities: a case study of a bryozoan population. Conservation Biology 12: 302 312.
50. Kipson S, Fourt M, Teixido N, Cebrian E, Casas E, et al. (2011) Rapid Biodiversity Assessment and Monitoring Method for Highly Diverse Benthic Communities: A Case Study of Mediterranean Coralligenous Outcrops. PLoS One 6: e27103 e27103.
51. Garrabou J, Ballesteros E, Zabala M (2002) Structure and dynamics of north-western Mediterranean rocky benthic communities along a depth gradient. Estuarine, Coastal and Shelf Science 55: 493 508.
52. Teixidó N, Casas-Güell E, Cebrián E, Garrabou J, Harmelin J, et al. (2011) Integrating life-history traits and biodiversity patterns of coralligenous communities in the NW Mediterranean Sea. World Conference on Marine Biodiversity. Aberdeen, Scotland (UK).
53. Coma R, Ribes M, Zabala M, Gili JM, Zabala M (1998) Growth in a modular colonial marine invertebrate. Estuarine, Coastal and Shelf Science 47: 459 470.
54. Garrabou J, Ballesteros E (2000) Growth of Mesophyllum alternans and Lithophyllum frondosum (Corallinales, Rhodophyta) in the northwestern Mediterranean. European Journal of Phycology 35: 1 10.
55. Teixidó N, Pineda MC, Garrabou J (2009) Decadal demographic trends of a long-lived temperate encrusting sponge. Marine Ecology Progress Series 375: 113 124.
56. Sala E, Ballesteros E (1997) Partitioning of space and food resources by three fish of the genus Diplodus (Sparidae) in a Mediterranean rocky infralittoral ecosystem. Marine Ecology Progress Series 152: 273 283.
57. Balata D, Piazzi L, Cecchi E, Cinelli F (2005) Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposition. Marine Environmental Research 60: 403 421.
58. Clarke KR, Somerfield PJ, Gorley RN (2008) Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. Journal of Experimental Marine Biology and Ecology 366: 56 69.
59. Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. Ecology Letters 9: 683 693.
60. Clarke KR, Warwick RM (1994) Change in marine communities: an approach to statistical analysis and interpretation. Nat Env Res Counc UK.
61. Anderson MJ (2001) A new method for non parametric multivariate analysis of variance. Austral Ecology 26: 32 46.
62. Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. Primer-e, Plymouth, UK 214.
63. Garrabou J, Harmelin JG (2002) A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean: insights into conservation and management needs. Journal of Animal Ecology 71: 966 978.
64. Linares C, Coma R, Diaz D, Zabala M, Hereu B, et al. (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Marine Ecology Progress Series 305: 127 137.
65. Edmunds PJ, Witman JD (1991) Effect of Hurricane Hugo on the primary framework of a reef along the south shore of St. John, US Virgin Islands. Marine Ecology Progress Series 78: 201 204.
66. Stoddart DR (1974) Post-hurricane changes on the British Honduras reefs: re-survey of 1972. Proc Second Int Coral Reef Symp 2, Great Barrier Reef Committee, Brisbane: 473 483.
67. Hughes TP, Connell JH (1999) Multiple stressors on coral reefs: a long-term perspective. Limnology and Oceanography: 932 940.
68. Alvarez-Filip L, Millet-Encalada M, Reyes-Bonilla H (2009) Impact of hurricanes Emily and Wilma on the coral community of Cozumel island, Mexico. Bulletin of Marine Science 84: 295 306.
69. Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, et al. (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293: 629 637.
70. Wilson SK, Graham NAJ, Pratchett MS, Jones GP, Polunin NVC (2006) Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? Global Change Biology 12: 2220 2234.
71. Collins SL (2000) Disturbance frequency and community stability in native tallgrass prairie. American Naturalist 155: 311 325.
72. Fraterrigo JM, Rusak JA (2008) Disturbance-driven changes in the variability of ecological patterns and processes. Ecology Letters 11: 756 770.
73. Airoldi L (1998) Roles of disturbance, sediment stress, and substratum retention on spatial dominance in algal turf. Ecology 79: 2759 2770.
74. Piazzi L, Ceccherelli G, Cinelli F (2001) Threat to macroalgal diversity: effects of the introduced green alga Caulerpa racemosa in the Mediterranean. Marine Ecology Progress Series 210: 149 159.
75. Linares C, Cebrian E, Coma R (2012) Effects of turf algae on recruitment and juvenile survival of gorgonian corals. Marine Ecology Progress Series 452: 81 81.
76. Cocito S, Sgorbini S, Bianchi CN (1998) Aspects of the biology of the bryozoan Pentapora fascialis in the northwestern Mediterranean. Marine Biology 131: 73 82.
77. Pickett STA, White PS, editors. (1985) The ecology of natural disturbance and patch dynamics: Academic Press. New York.
78. Foster DR, Knight DH, Franklin JF (1998) Landscape patterns and legacies resulting from large, infrequent forest disturbances. Ecosystems 1: 497 510.
79. Turner MG (2010) Disturbance and landscape dynamics in a changing world. Ecology 91: 2833 2849.
80. Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. American Naturalist: 1119 1144.
81. Highsmith RC (1982) Reproduction by fragmentation in corals. Marine Ecology Progress Series 7: 207 226.
82. Teixidó N, Garrabou J, Gutt J, Arntz WE (2007) Iceberg disturbance and successional spatial patterns: the case of the shelf Antarctic benthic communities. Ecosystems 10: 143 158.
83. Hughes TP, Baird AH, Dinsdale EA, Moltschaniwskyj NA, Pratchett MS, et al. (2000) Supply-side ecology works both ways: the link between benthic adults, fecundity, and larval recruits. Ecology 81: 2241 2249.

84. Ulbrich U, Leckebusch GC, Pinto JG (2009) Extra-tropical cyclones in the present and future climate: a review. Theoretical and Applied Climatology 96: 117 131.
85. Young IR, Zieger S, Babanin AV (2011) Global trends in wind speed and wave height. Science 332: 451 451.
86. Marcos M, Jordà G, Gomis D, Pérez B (2011) Changes in storm surges in southern Europe from a regional model under climate change scenarios. Global and Planetary Change 77: 116 128.
87. Somot S, Sevault F, Déqué M, Crépon M (2008) 21st century climate change scenario for the Mediterranean using a coupled atmosphere ocean regional climate model. Global and Planetary Change 63: 112 126.