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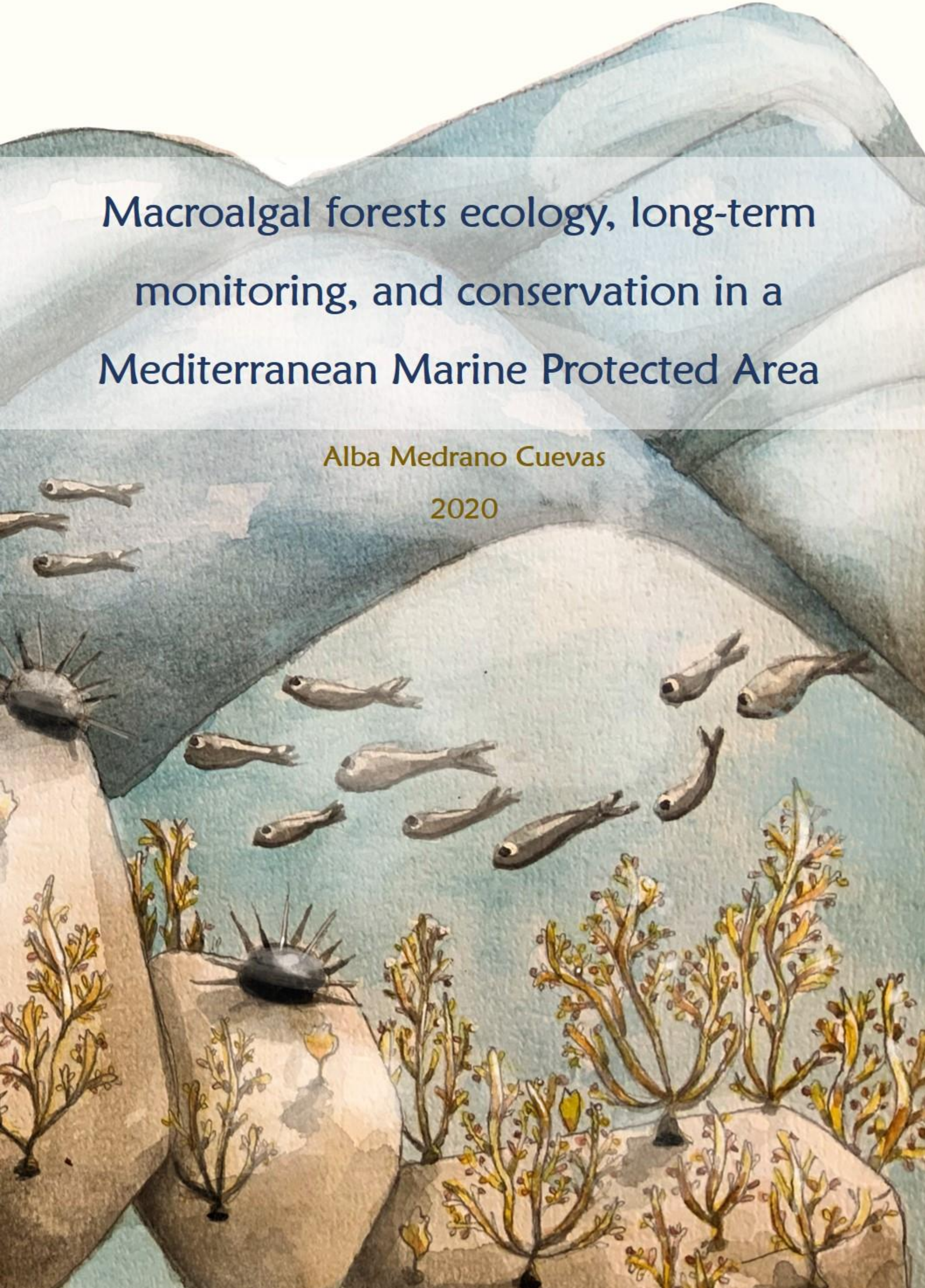
Alba Medrano Cuevas



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2020

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**TESI DOCTORAL**

Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
Doctorat en Ecologia, Ciències Ambientals i Fisiologia Vegetal

# Macroalgal forests ecology, long-term monitoring, and conservation in a Mediterranean Marine Protected Area

## Ecologia dels boscos de macroalgues, estudis a llarg termini i conservació en una Àrea Marina Protegida del Mar Mediterrani

*Memòria presentada per* ***Alba Medrano Cuevas*** *per optar*
*al Grau de Doctora per la Universitat de Barcelona*

**Alba Medrano Cuevas**
Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
Universitat de Barcelona
Desembre de 2019

La directora i tutora de la tesi:
**Dra. Cristina Linares Prats**
Universitat de Barcelona

El director de la tesi:
**Dr. Bernat Hereu Fina**
Universitat de Barcelona

*A l’Elliott,*

*Als meus pares i germà,*

*I al De Mata.*

*-Ama usted el mar, capitán.*
*-¡Sí! ¡Lo amo! ¡El mar es todo! Cubre las siete décimas partes del globo terrestre. Su aliento es puro y sano. Es el inmenso desierto en el que el hombre no está nunca solo, pues siente estremecerse la vida en torno suyo. El mar es el vehículo de una sobrenatural y prodigiosa existencia; es movimiento y amor; es el infinito viviente, como ha dicho uno de sus poetas. [...] El mar no pertenece a los déspotas. En su superficie pueden todavía ejercer sus derechos inicuos, batirse, entredevorarse, transportar a ella todos los horrores terrestres. Pero a treinta pies de profundidad, su poder cesa, su influencia se apaga, su potencia desaparece. ¡Ah! ¡Viva usted, señor, en el seno de los mares, viva en ellos! Solamente ahí está la independencia. ¡Ahí no reconozco dueño ni señor! ¡Ahí yo soy libre!*

Julio Verne, Veinte mil leguas de viaje submarino

# Agraïments

**-95% de les persones que m'envolten** (darrers 10 anys):

*"Alba, i tu, algún dia acabaras d'estudiar i tindràs una feina normal?"*

**-Alba** (Desembre 2019, 33 anys):

*"SI!! Ara!! (a la primera part de la pregunta només)."*

I no haguès pogut arribar aquest final sense el suport, confiança, ajuda, ensenyament, paciència i amor de moltes persones que m'han acompanyat en aquest camí.

Primer de tot, he d'agraïr als meus dos directors de tesi. A la Cristina ja que, sense ella, ni hagués pogut començar el doctorat, ni l'hagués acabat. Gràcies per fer tot més fàcil i aixecar-nos sempre. I al Bernat, perquè amb ell he aprés molt, tant d'ecologia com de la vida. També vull agraïr la constructiva involucració dels tres membres de la comissió de seguiment d'aquesta tesi, en Kike Ballesteros, en Javier Romero i, especialment, al Simone Mariani, qui ha sigut un exemple de feina ben feta i un bon amic.

L'etapa final d'una tesi no és fàcil però he tingut la sort de compartir aquest moment amb els millors. Marta, Yaiza y Dani, la única cosa que volvería a repetir de terminar una tesis, sería volver a hacerlo a la vez que vosotros. Vivir esta etapa juntos ha sido lo major de este duro final, ¡gracias por tanto! Marteta, amb tu no només hem compartit el final sinò tot el camí i estic segura i feliç de que continuarem caminant plegades!

MiniMeds! Graciel·la, Dani, Ignasi, Eneko, Pol, Júlia (i Marta again), què haguéssin sigut sense vosaltres les més de 300 immersions d'aquesta tesi!!! Feliç d'haver-les compartit amb vosaltres però, sobretot, d'haver gaudit junts de moltes més hores fora de l'aigua. No podria haver tingut més sort amb uns companys com vosaltres. I això no només va pels MiniMeds propers, també pels blanencs-gironins, i en especial, la Jana.
Vull agraïr també l'ajuda i suport dels membres del grup de recerca MedRecover, especialment la Paula, en Quim i en Mikel pel seu caliu i confiança. És un plaer treballar amb vosaltres!

I am also very grateful to the members of Ester Serrão's Lab, who warmly hosted and guided me during my research stay in Faro. Special thanks to João and Cristina for their friendly support inside and outside the lab and to Aschwin for his joyfulness. Y a Rosa, porque la mayor pena de que no se pudiera hacer un modelo de nicho realista con 7 tristes presencias de la especie, ha sido no continuar colaborando contigo.

Y recordando los lejanos inicios, agradezco a Evie y Jenna por ponerme las alas y enseñarme a volar en ciencia. Y a mis queridos y añorados Michel y Henri García Baral por descubrirme el amor más verdadero por el mar.

Però si he arribat fins aquí també ha sigut gràcies a molta gent important per a mi amb qui l'ecologia marina no ha sigut un punt de partida. D'entrada els meus amics, la meva estimada Arca de tota una vida que, setmana rere setmana, em regalen alegria i amor. A tu De Mata que, tot i que el tema de la tesi no sigui del teu interés, estic segura que arribaras a llegir aquestes línies perquè tinc la sort de que sempre ens hem acompanyat en tot (i així seguirà sent!). A la Beta i la Itzi, regalassos de la vida que sempre sento aprop encara que estiguem lluny. Beta, saps que sóc molt feliç de tenir un trocet teu a la tesi, mil gràcies per la portada, que és tant preciosa com ho ets tu! I a les estupendes 'vahines', amb qui les setmanes s'omplen d'alegria i desconnexió.

Per últim, però més important, estaré eternament agraïda a la meva familia, als meus pares, al meu germà i als meus avis, que han sigut, són i seran un pilar clau en tots els pasos de la meva vida. A les estimadíssimes Anoka i Cumbia, per no deixar que un mal dia continuï sent-ho quan entro per la porta de casa. Y a ti Elliott, que has sido mi cable a tierra durante toda la tesis (y unos cuantos años más). Gracias por cuidarme y quererme, la vida es mejor juntos!

**Gràcies! Gracias! Thanks! Obrigada!**

# Advisors' report

Dra. Cristina Linares and Dr. Bernat Hereu, professors at Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals (Universitat de Barcelona), advisors of the PhD thesis entitled "**Macroalgal forests ecology, long-term monitoring, and conservation in a Mediterranean Marine Protected Area**",

INFORM, that the research studies developed by Alba Medrano Cuevas for her Doctoral Thesis have been organized in four chapters, which correspond to four scientific papers listed below: two of them are already published, one is in review, and the last one will be submitted in the next few weeks;

and CERTIFY, that the work has been carried out by Alba Medrano Cuevas, participating actively in all the tasks: conceiving and setting the objectives, conceiving and performing the analyses, participating actively in the field work, carrying out the experiments and writing the manuscripts.

Finally, we certify that the co-authors of the publications listed below and that conform this doctoral thesis, will not use these manuscripts in another PhD thesis.

Barcelona, 13th December 2019

| | |
|---|---|
| Advisor: | Advisor: |
| **Dra. Cristina Linares Prats** | **Dr. Bernat Hereu Fina** |
| Universitat de Barcelona | Universitat de Barcelona |

## List and publication status of the chapters of this thesis

**CHAPTER I:** Medrano, A.[1], Linares, C.[1], Aspillaga, E. [1,2], Capdevila, P.[1,3], Montero-Serra, I.[1], Pagès-Escolà, M.[1], Zabala, M.[1], Hereu, B.[1] (2019). Long-term monitoring of temperate macroalgal assemblages inside and outside a No take marine reserve. Marine environmental research, 104826. 5-year impact factor (2018): 3.815.

**CHAPTER II:** Medrano, A.[1], Linares, C.[1], Aspillaga, E. [1,2], Capdevila, P.[1,3], Montero-Serra, I.[1], Pagès-Escolà, M.[1], Hereu, B.[1] (2019). No-take marine reserves control the recovery of sea urchin populations after mass mortality events. Marine environmental research, 145, 147-154. 5-year impact factor (2018): 3.815.

**CHAPTER III:** Medrano A.[1], Hereu, B.[1], Mariani, S.,[1,4], Neiva, J.[5], Pagès-Escolà, M.[1], Paulino, C.[5], Rovira, G.[1], Serrão, E. A.[5], Linares, C.[1] Expansion in times of decline: *Treptacantha elegans* ecology, genetic diversity, and distribution in the Catalan Coast. To be submitted at Frontiers in Marine Science Journal. Impact factor (2018): 3.086. This journal was launched in 2014.

**CHAPTER IV:** Medrano A.[1], Hereu, B.[1], Cleminson, M.[1], Pagès-Escolà, M.[1], Rovira, G.[1], Solà, J.[1,6], Linares, C.[1] From marine deserts to algal forests: *Cystoseira* forestation to reverse stable degraded ecosystems inside and outside a No-take marine reserve. Under review at Restoration Ecology. 5-years impact factor (2018): 2.888

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# Contents

**Chapter IV. From marine deserts to algal forests: *Cystoseira* forestation to reverse stable degraded ecosystems inside and outside a No-take marine reserve**

**General discussion and conclusions**

# Summary

All marine ecosystems in the Mediterranean Sea are highly threatened by anthropogenic stressors that can alter their structure and function, especially in rocky shores. Moreover, extreme climate events are becoming more frequent and intense in our times. To detect the potential impacts and the vulnerability of any ecosystem, the combination of experimental and observational studies in the field is vital. In addition, long-term monitoring programs carried out simultaneously on human-protected and human-impacted environments may be crucial to discern the nature of the impacts.

Macroalgal beds dominate the shallow benthic Mediterranean habitats where they play a pivotal role. Among them, the canopy-forming *Cystoseira sensu lato* species represent the highest structural complexity level and provide unique habitats with ecological services comparable to terrestrial forests. Canopy-forming algae are in decline in many coastal areas where, among other impacts, overgrazing by herbivorous can lead to the loss of these diverse habitats shifting towards degraded sea urchin barren grounds. Conservation tools such as marine reserves or No-take zones (NTZs) have the potential to reduce some of the anthropogenic threats and to restore benthic habitats through trophic cascade effects caused by the major abundance of predator. Besides, active ecosystem restoration strategies may speed up the recovery of impacted ecosystems. Nevertheless, there is an important lack of continuous and long-term studies providing robust ecological data of the natural dynamic and vulnerability of macroalgal assemblages while integrating the role of marine conservation.

In this dissertation, different methodological approaches were combined to explore the long-term dynamics of macroalgal communities and the role of different conservation strategies (NTZs and active restoration) in the Montgrí, Medes Islands, and Baix Ter (MIMBT) Natural Park (NW Mediterranean Sea). In the first two chapters, the analysis of long-term monitoring datasets provided essential information to understand how macroalgal assemblages and sea urchin populations respond to natural fluctuations and anthropogenic disturbances, mainly overfishing. In the third chapter, field monitoring and sampling were combined with genetic analyses to increase the ecological knowledge of the canopy-forming alga *Treptacantha elegans* as well as to describe their recent expansion. In the fourth and last chapter, active restoration actions as seeding experiments were conducted in aquaria and in the field to optimize restoration techniques to recover degraded shallow ecosystems. In addition, different restoration strategies were combined in the field inside and outside the Medes Islands NTZ to evaluate the role of marine protection on restoration activities.

The results of this thesis showed that the abundance and structure of the main macroalgal assemblages in the MIMBT Natural Park were stable at large over the last fifteen years. Overall, any effect of marine protection was observed on the most representative species of this habitat but we found a higher abundance of canopy-forming algae inside the NTZ than in unprotected areas. Contrarily, sea urchin populations were deeply affected by a severe storm in 2008 which caused the almost depletion of its populations in all the studied areas. Although similar trajectories of sea urchin abundance have been observed over the years between both protection regimes due to the large stability of the sea urchin high-density state, clear differences in the recovery of sea urchin populations were found after the storm linked to marine protection. The sea urchin populations inside the NTZ recovered slowly than the populations outside the NTZ inside due to the higher predatory fish abundance inside the NTZ.

In contrast to the global widespread decline of canopy-forming macroalgal assemblages across many regions during the last decades, *Treptacantha elegans* has increased their distributional range and has shown an extraordinary expansion along the Catalan northern coast over the last two decades. The results of this thesis contributed to explaining this geographical and depth range extension, which could be linked to some ecological attributes such as their relative fast-growing dynamics, early fertile maturity, and high turnover rate. Besides, the molecular analyses have shown that all the populations of *T. elegans* in the Catalan coast constitute a single genetic group that could be originated in the MIMBT Natural Park under the marine protection benefit. Given the fast and stable population dynamics of *T. elegans*, this species was selected as a potential species to actively restore degraded shallow rocky ecosystems (e.g., sea urchin barren grounds) turning them into productive marine forests. In this way, the effectiveness of active restoration actions combined with passive strategies such as marine protection (e.g., NTZs) was experimentally demonstrated.

This thesis addressed marine vegetation changes in the shallow rocky shores of the MIMBT Natural Park integrating the macroalgal and sea urchin dynamics in front of natural and human-related impacts, and the role and effectiveness of Marine Protected Areas and restoration actions as conservation tools at lower trophic levels.

Besides, since most of this thesis is based on long-term monitoring data, a valuable baseline of the algal community's structure and functioning was provided here which could be vital to predict and detect ecological changes that could jeopardize the preservation of marine forests.

# Resum

Els ecosistemes bentònics mediterranis es troben fortament amenaçats per pressions, tant a nivell local com global, que poden alterar la seva estructura i funcionament, especialment en els fons rocosos litorals. A més, els episodis climàtics extrems esdevenen cada vegada més freqüents i intensos. Per a detectar els possibles impactes sobre qualsevol ecosistema així com la seva vulnerabilitat, la combinació d'estudis observacionals i experimentals al camp és primordial. En aquest sentit, els estudis a llarg termini realitzats simultàniament en zones impactades i en zones protegides de l'acció humana, poden ser crucials per a identificar la naturalesa dels impactes.

Al mar Mediterrani, les comunitats de macroalgues dominen els hàbitats bentònics soms, on tenen un paper primordial. D'entre elles, les algues de tipus arborescent del gènere *Cystoseira* representen el nivell més elevat de complexitat estructural i proporcionen hàbitats amb serveis comparables als boscos terrestres. Aquestes algues es troben en declivi en diverses zones costaneres. És aquí on, entre altres impactes, la sobrepastura dels herbívors pot portar a la pèrdua d'aquests hàbitats rics i diversos i la seva transició cap a fons degradats dominats pels eriçons. Les eines de conservació, així com ara les reserves marines o les àrees on està prohibida completament l'extracció (NTZs), tenen el potencial de reduir algunes de les amenaces derivades de l'acció humana i de restaurar els hàbitats bentònics gràcies als efectes dels depredadors a través de les cascades tròfiques. A més de la restauració passiva a través de la creació d'aquestes àrees protegides, la restauració activa dels ecosistemes impactats pot accelerar la seva recuperació. No obstant això, hi ha una manca important d'estudis continuats i a llarg termini que proporcionin dades ecològiques robustes sobre la dinàmica natural i la vulnerabilitat dels hàbitats de macroalgues i que alhora integrin el paper de les diferents eines de conservació marina.

En aquesta tesi, s'han combinat diferents metodologies per explorar la dinàmica de les comunitats de macroalgues juntament amb el paper de diferents estratègies de conservació (NTZs i restauració activa) al Parc Natural del Montgrí, les Illes Medes i el Baix Ter (MIMBT), situat al Nord-oest del mar Mediterrani. En els primers dos capítols, les anàlisis de dades d'estudis a llarg termini han proporcionat informació essencial per millorar la nostra comprensió sobre com les comunitats de macroalgues i les poblacions d'eriçons responen a les fluctuacions naturals i a les pertorbacions d'origen antròpic, principalment, la sobrepesca. En el tercer capítol, mostrejos en el camp s'han combinat amb anàlisis genètics per augmentar el coneixement ecològic de l'alga arborescent

*Treptacantha elegans* i per descriure la seva recent expansió. Al quart i últim capítol, s'han realitzat accions de restauració activa, com ara experiments de sembra en aquaris i al camp per optimitzar les tècniques de restauració dels ecosistemes poc profunds degradats. A més, s'han combinat diferents estratègies de restauració a camp, dins i fora de la NTZ de les Illes Medes, per avaluar el paper de la protecció en les activitats de restauració.

Els resultats d'aquesta tesi mostren que la abundància i la composició de les principals comunitats algals del Parc Natural del MIMBT s'han mantingut estables al llarg dels darrers quinze anys. En general, no es va observar cap efecte de la protecció en les espècies d'algues més representatives, tot i que si que es van trobar abundàncies més elevades de l'alga *T. elegans* a dins de la NTZ que a les zones no protegides de l'acció humana. Al contrari, les poblacions d'eriçons es van veure profundament afectades per una forta tempesta l'any 2008, el que va provocar el declivi gairebé total d'aquestes poblacions a les zones estudiades. Tot i que s'havien observat trajectòries similars en l'abundància d'eriçons al llarg del temps, tant a dins com a fora de la NTZ, degut a la gran estabilitat de les poblacions d'eriçons quan les poblacions presenten elevades densitats, en aquesta tesi es van trobar clares diferències en la recuperació d'aquestes poblacions després de la tempesta que estarien vinculades a l'efecte de la protecció marina. Les poblacions d'eriçons a dins de la NTZ es van recuperar més lentament que les de les zones no protegides degut a la major abundància de peixos depredadors a dins de la NTZ.

Davant de la davallada generalitzada de les macroalgues arborescents que s'ha pogut observar a moltes regions al llarg de les darreres dècades, l'alga *Treptacantha elegans* ha demostrat una extraordinària expansió a la costa nord catalana al llarg de les dues darreres dècades, augmentat considerablement la seva distribució. Els resultats d'aquesta tesi contribueixen a explicar aquesta expansió, tant en fondària com a nivell geogràfic, que podria estar relacionada amb alguns trets ecològics, com ara la seva dinàmica de creixement relativament ràpid, la maduresa reproductiva precoç i la seva elevada taxa de renovació poblacional. A més, les anàlisis moleculars mostren que totes les poblacions de *T. elegans* a la costa catalana constitueixen un únic grup genètic que podria tenir el seu origen al Parc Natural del MIMBT com a conseqüència de la protecció marina. Atesa la dinàmica ràpida i estable de les poblacions de *T. elegans*, es va seleccionar aquesta com a potencial espècie per restaurar activament els ecosistemes rocosos poc profunds degradats (els blancalls originats per la sobrepastura d'eriçons) convertint-los en productius boscos marins. Gràcies a això, també s'ha demostrat

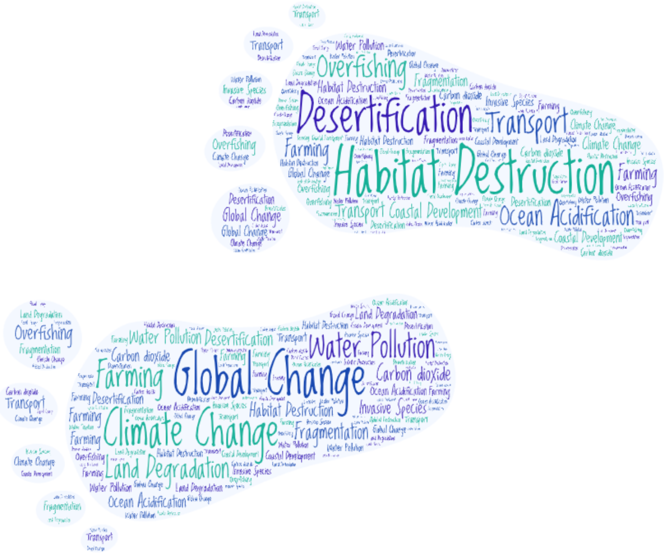
experimentalment l'èxit de combinar accions de restauració activa amb estratègies passives com la protecció marina (p.e. NTZs).

Aquesta tesi aborda els canvis de vegetació marina als fons soms i rocosos del Parc Natural del MIMBT integrant la dinàmica de les macroalgues i els eriçons enfront dels impactes naturals i humans, i, el paper i l'eficàcia de les zones marines protegides i de les accions de restauració com a eines de conservació per als nivells tròfics més basals. A més, atès que la major part d'aquesta tesi es basa en dades d'estudis a llarg termini, aquesta tesi proporciona una valuosa referència de la dinàmica i de l'estat actual de les comunitats infralitorals dominades per algues, el que podria ser vital per predir i detectar canvis ecològics que puguin posar en perill la preservació dels boscos marins.

# General introduction and objectives

# Introduction

## Long-term studies to understand changes in marine environments



There is no doubt that humans have deeply altered all major ecosystems on Earth but understand how the structure and functioning of these ecosystems have changed is challenging.

The scale, frequency, and nature of the threats determine their potential impacts on ecosystems. Some threats act at global, regional, or local scales (e.g., climate change vs. land degradation). Ecosystems resistance and recovery times can be modulated by the frequency and intensity of the threats, which can occur naturally (e.g., volcano eruption) or as a result of the human activities (e.g., water pollution).

Long-term ecological studies provide core ecological data fundamental to establish baselines from which we could assess the signature of ecosystem changes (Magurran et al., 2010; Navarrete et al., 2010) and design effective ecosystem management (Lindenmayer et al., 2012). Most of long-term datasets were initiated to answer ecological questions, which places long-term studies as old as ecology itself (May and McLean, 2007). To achieve a robust understanding of the ecosystems, communities or populations dynamics, the studies should comprise and continue beyond the life spans of dominant organisms (Strayer, 1996).

There is not a single marine ecosystem unthreatened by anthropogenic stressors (Halpern et al., 2007) with the hard-bottom coastal areas and rocky reefs being the highest impacted (Halpern et al., 2008; 2019). Nevertheless, ecological field studies conducted systematically over many years to detect the vulnerability of the ecosystems represent an outstanding challenge in marine environments (Vitousek et al., 1997). Hence, long-term monitoring programs have commonly focused on single species or populations, such as corals, seagrasses, sea urchins or kelp species among others, while the vulnerability of the ecosystem functioning have been usually assessed by short-term ‘snapshot’ studies or ‘before-after’ studies (e.g., Kirkman and Kirkman, 2000; Steneck et al., 2002; Babcock et al., 2010; Gross and Edmuns, 2015).

In aims to give some real numbers on the priority topics of ecological long-term studies, I did a systematic review on Web of Science using the keywords (“Temporal patterns” or “Temporal distribution” or “Multi-year monitoring” or “Long-term monitoring” or “Temporal variation” and “Community ecology” and “Population ecology”) and a deep search on GoogleScholar selecting the 198 references published during the last decade. I found 64 relevant studies (S1) of long-term ecological studies (> 5 years) and classified them according to its studied target (plants, animals, or both animals and plants together) and to their environment (freshwater, marine or terrestrial) (Fig. 1).

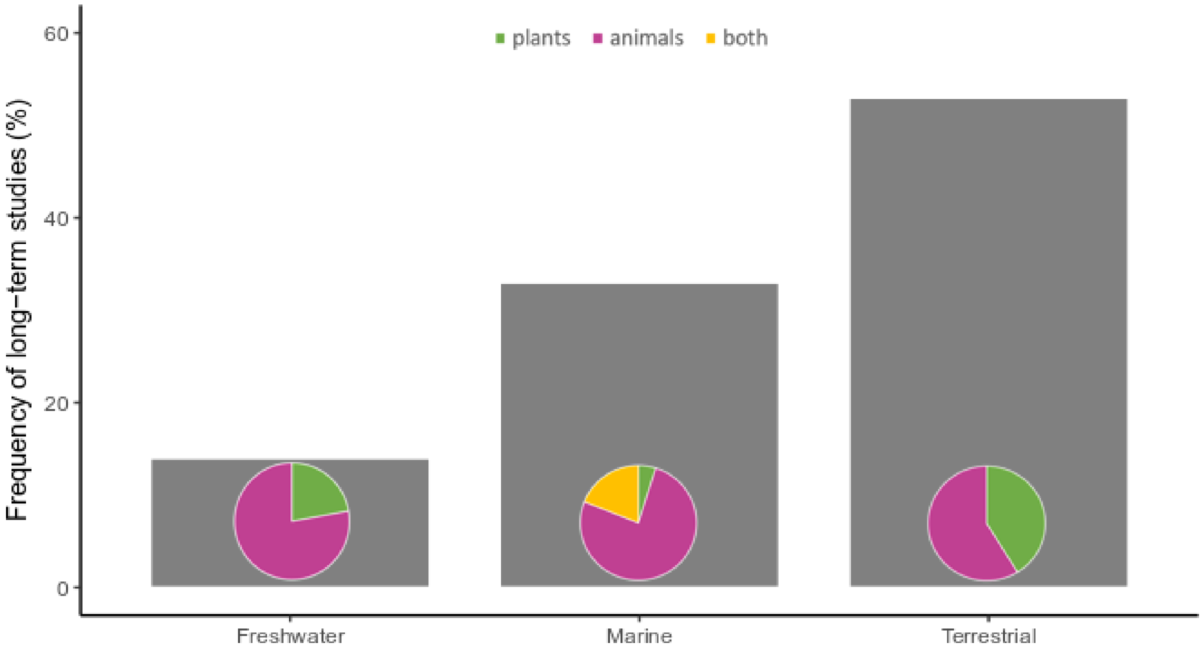


**Figure 1**. Grey bars represent the percentage of long-term studies (> 5 years) classified by their environmental realm (freshwater, marine, or terrestrial). Pie charts inside the bars represent the frequency of the main ecological targets in these studies (purple color: animals, green color: plants, yellow color: both). Total number of studies: 65.

Although simultaneous long-term monitoring on human-protected and human-impacted environments may be fundamental to distinguish between natural and anthropogenic changes (Navarrete et al., 2010), less than one-third of the reviewed studies took place in environments where some kind of protection exists (e.g., natural parks, marine reserves, etc.). It was also remarkable that, in marine ecosystems, less than one-fourth of the reviewed long-term studies focused their attention on plants (Fig. 1) and they usually do when considering ecological interactions between them and other emblematic species such as charismatic corals or commercial fishes. In other words, there is an important lack of continuous and long-term studies that integrate marine vegetation changes together with their long-term dynamics and with the conservation role, which is one of the purposes of this thesis.

## Macroalgal ecosystems

Macroalgae (or seaweeds) are ecologically key species in temperate coastal ecosystems worldwide, where they are one of the dominant primary producers with a key role on the ecosystem functioning (Jones et al., 1994; Duarte and Cebrian, 1996; Teagle et al., 2017). Seaweeds represent an important source of carbon sequestration (Krause-Jensen and Duarte, 2016) and provide invaluable ecosystem services in many shallow coastal systems (Blamey and Bolton, 2018). Consequently, changes in macroalgal composition may sway in the whole coastal ecosystem (Lotze et al., 2006; Mineur et al., 2015).

In temperate coastal ecosystems, kelps (Laminariales) and fucoids (Fucales) are the large canopy-forming algae in the intertidal and subtidal zones. These brown algae create structurally complex habitats and play a key role in facilitating biodiversity and driving food webs (Dayton, 1985; Steneck et al., 2002; Schiel and Foster, 2006). Under their canopy many associated species find food and shelter contributing to the ecosystems functioning and increasing the biodiversity of wide trophic levels (Boudouresque et al., 2017). In addition, their three-dimensional structure has a nursery value for early life stages of many species (Cheminée et al., 2013). Thus, canopy-forming algae provide unique habitats with services comparable to terrestrial forests (Fig. 2) (Dayton, 1985; Graham, 2004; Ballesteros et al., 2009; Wernberg et al., 2011a).

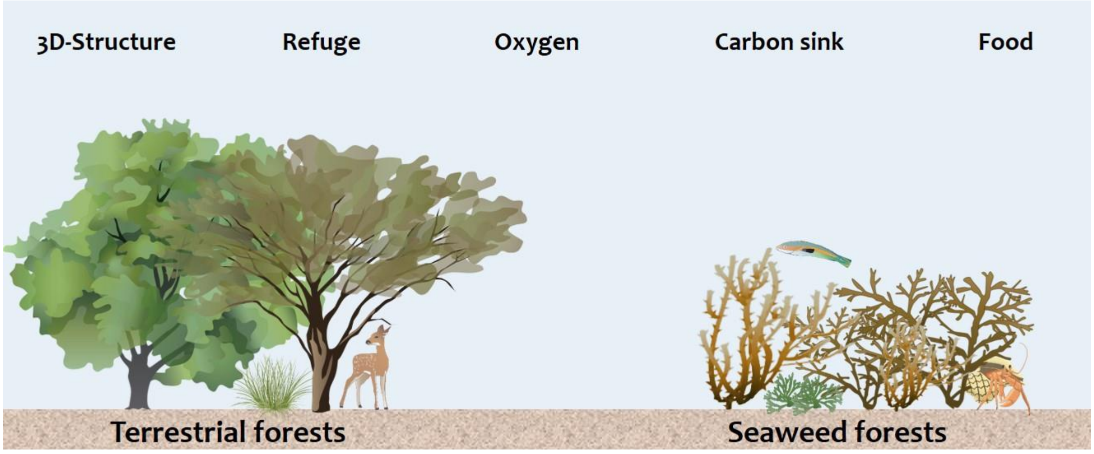


**Figure 2**. Schematic representation of the main ecosystem services provided by terrestrial and seaweed forests. Images source: IAN Symbols Library, University of Maryland

Kelps and fucoids are closely related taxa inhabit mainly on rocky bottoms from the intertidal zone down to the photic limit worldwide. Despite these and other shared characteristics, they can display different biological and ecological traits, outstanding here the potential scale of dispersion (Schiel and Foster, 2006). Fucoids have large and easily sinking propagules (zygots) while kelps have smaller ones (spores). Because of this, kelps have the potential to disperse at hundreds of meters while the dispersal ability of fucoids generally not exceeds scarce meters (Gianni et al., 2013; Capdevila et al., 2018). This may be an explanatory factor behind the limited recovery of fucoid populations when impacted (Jenkin et al., 2004; Mangialajo et al., 2012).

Marine forests are influenced by several environmental factors such as light, turbulence, nutrients, and temperature (Schiel and Foster, 2006). Notwithstanding the natural threats, coastal pollution, overfishing, ocean warming, and other human disturbances are causing the widespread decline of canopy-forming algae (Steneck et al., 2002; Wernberg et al., 2011a; Campbell et al., 2014), with the consequent loss of ecosystem functioning (Lorentsen et al., 2010; Mineur et al., 2015).

## Macroalgal beds and sea urchin barrens as alternative states

All ecosystems are subjected to natural or anthropogenic changes. The state of the ecosystems may responds in a different way when changes are gradual or abrupt (Scheffer et al., 2001). Some states may recover to their original state when restoring the disturbance to levels before the change but it could be insufficient when the ecosystem has two alternative stable states for certain level of disturbance (Fig. 3). Transitions between alternative stable states are also known as ‘regime shifts’ or ‘phase shifts’. The magnitude of disturbance that a system can suffer before it shifts into a different state is known as resilience (Folke et al., 2004; Filbee-Dexter and Scheibling, 2014). The presence of different states can be characterized by hysteresis, which occurs when various feedback mechanisms maintain the alternative state and inhibit the return to the pre-disturbed state even if the critical parameters changed (Filbee-Dexter and Scheibling, 2014; Melis et al., 2019). The presence of alternative stable states has profound implications in the response of the ecosystems to environmental changes.

The most important herbivores feeding on macroalgal forests are sea urchins. Not all urchins have this ability, but most temperate areas have at least one species in this category (Tegner and Dayton, 2000). Sea urchin outbreaks can boost overgrazing and cause one of the most widespread and abrupt catastrophic shifts in nature, the transition from productive marine forests toward low productive habitats such as turf-forming algae or sea urchin barren grounds (Fig. 3) (Pinnegar et al., 2000, Airoldi and Beck, 2007; Ling et al., 2015; Maggi et al., 2018; Filbee-Dexter and Wernberg, 2018).

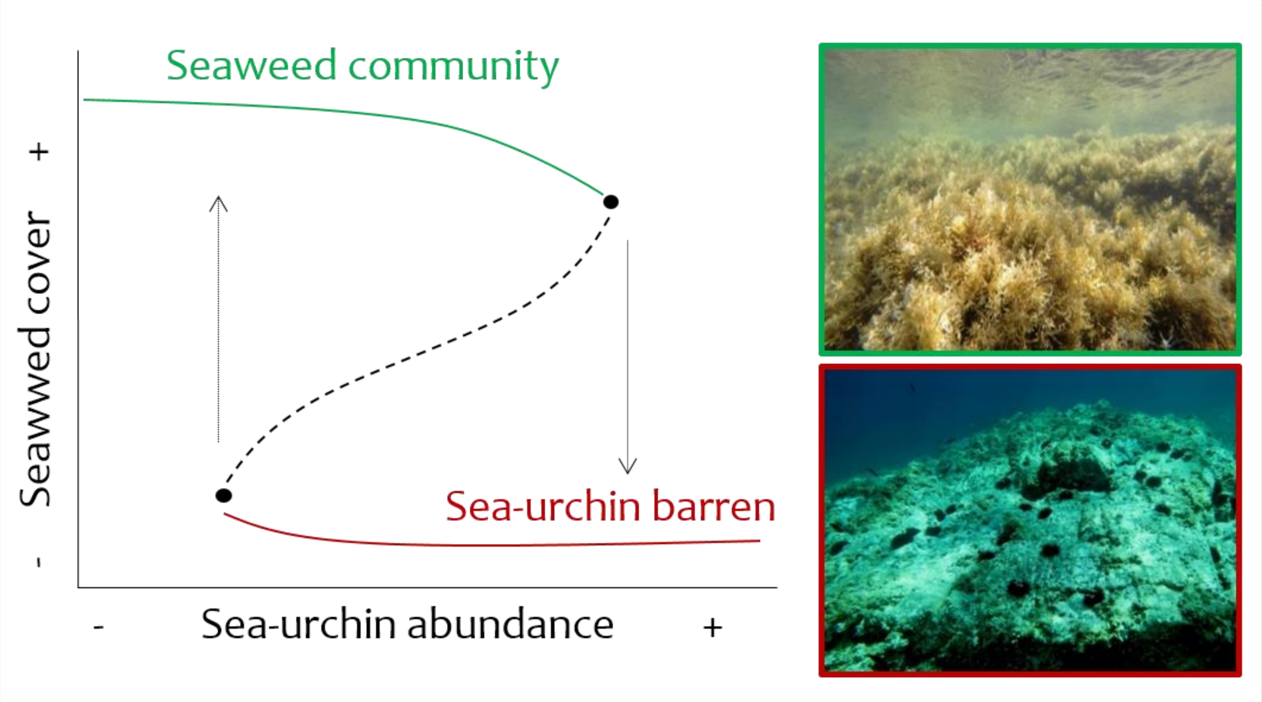


**Figure 3**. Macroalgal forests and sea urchin barrens as alternative stable states. Adapted from Filbee-Dexter and Scheibling, 2014.

Probably, although consequences of sea urchins overgrazing were already known (e.g., Paine and Vadas, 1969), the most famous observation of this catastrophic shift was described by Estes and Palmisano (1974). In this classic study, Ester and Palmisano identified that increased sea urchin overgrazing resulted from the reduction of their predator populations. In other words, top-down control of sea urchins is key in the maintenance of canopy-forming forests. Sea urchin populations follow natural fluctuations that can lead to gradual increases in abundance. However, abrupt increases (i.e., sea urchin outbreaks) are usually related to trophic changes (i.e., decrease of their main predators due to direct effects of overfishing) and environmental changes which can facilitate large recruitment events (Steneck, 1998; Guidetti and Dulcic, 2007; Ling et al., 2009; 2019).

Overgrazing by sea urchin outbreaks can then result in sea urchin barren grounds (Fig. 3). Once established, they represent an alternative and stable state maintained by several feedback mechanisms that increase the hysteresis and prevent the recovery of macroalgal forests (Fig. 4). Recruitment variability, spatial refuge availability, escaping behavior, and adult-juvenile associations can maintain dense sea urchin populations even when predatory fishes are abundant (Nishizaki and Ackerman, 2004; Zhang et al., 2011; Bonaviri et al., 2012; Oliva et al., 2016). On the other side, the physical structure provided by the macroalgae, the wave-induced sweeping, and the local supply of macroalgal propagules are some of the feedbacks than maintain the macroalgal dominant state (Fig. 4) (Konar and Estes, 2003; Hoey and Bellwood, 2011; Mangialajo et al., 2012).

Understanding macroagal beds/sea urchin barrens regime shift is important not only for ecology but also for considering appropriate marine management strategies to preserve macroalgal states. Given the high ecological hysteresis of this regime shift between alternative stable states, management strategies to reduce the sea urchin abundance or to restore macroalgal forests following sea urchin overgrazing are challenging (Filbee-Dexter and Scheibling, 2014; Ling et al., 2015).

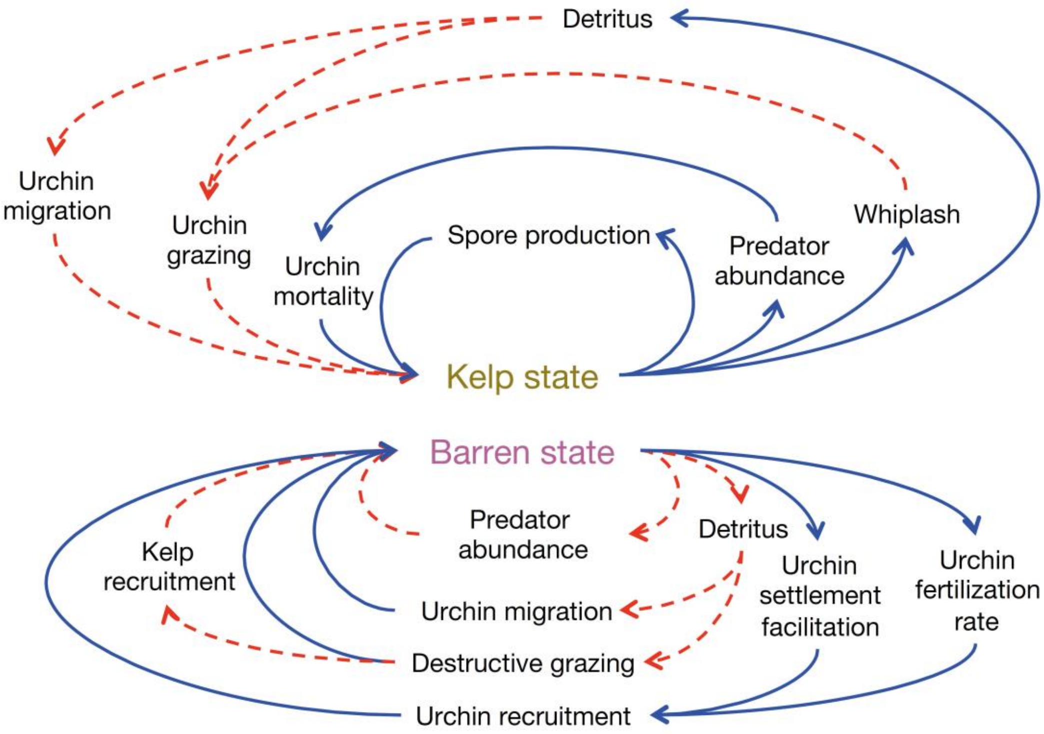


**Figure 4**. Stabilizing feedback mechanisms of sea urchin barren grounds and macroalgal beds extracted from Filbee-Dexter and Scheibling, 2014. Blue lines represent positive feedbacks and red dashed lines represent negative ones.

## Conservation tools

Classical conservation management actions such as Marine Protected Areas have been recognized as a tool to protect and restore marine ecosystems around the world. The term 'marine protected area' (MPA) refers to areas in which human activities that cause reductions in populations either directly through exploitation or indirectly through habitat alteration are eliminated or greatly reduced (Carr, 2000). The Convention on Biological Diversity (CBD) calls for 10% of coastal and marine areas to be conserved by 2020 through effectively managed, ecologically representative, and well-connected systems of MPAs (Strategic Plan for Biodiversity 2011-2020, CBD, 2010). To date (ending the year 2019), only 4.8% of the world's oceans are protected in implemented and properly managed MPAs and approximately half of them (2.2%), are highly protected in No-Take marine reserves (Fig. 5).

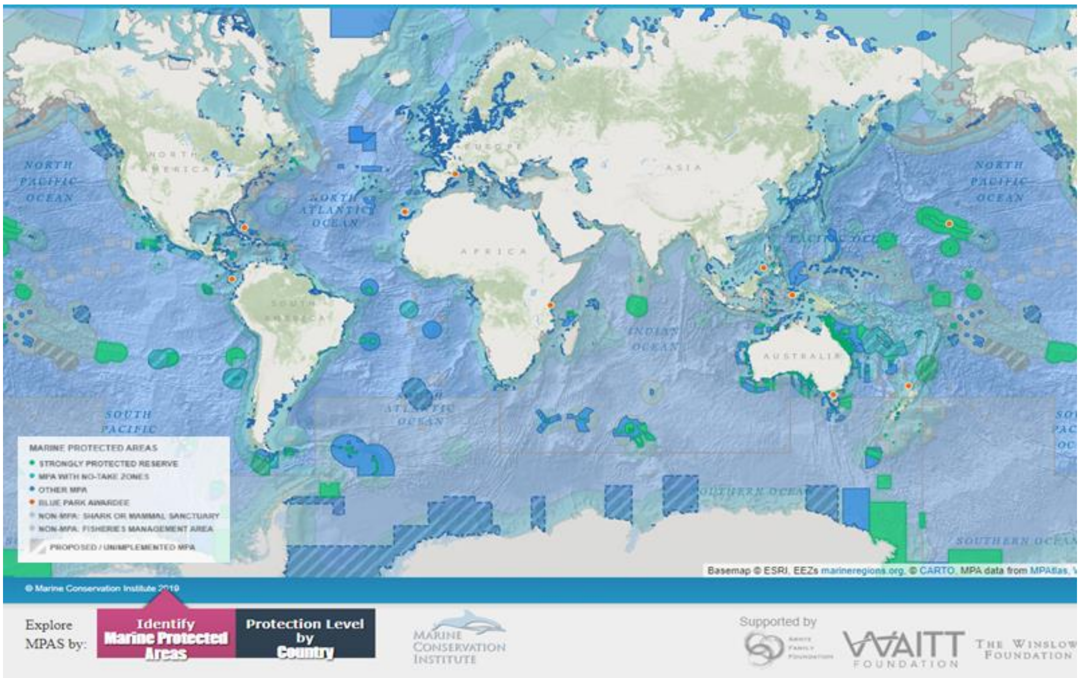


**Figure 5**. Map of updated global MPAs extracted from MPAtlas as of October 2019 (http://www.mpatlas.org/map/mpas/).

No-Take marine reserves or No-Take Zones (NTZs) are the MPAs with the stronger protection, where no extractive activity is allowed (Sala and Giakoumi, 2017). NTZs prevent the overexploitation of key structural species, allowing for the reduction or removal of associated impacts, maintaining and restoring ecosystem functioning, with the subsequent improvement of trophic regulation on populations of consumers (i.e., trophic cascades). However, not much evidence supports NTZs as effective enough to restore degraded ecosystems (Huntington et al., 2011; Sangil et al., 2012; Toth et al., 2014; Cox et al., 2017). The natural variability on species dynamics and ecological interactions limit recovery benefits only after long term periods (i.e., decades Babcock et al., 2010; Strain et al., 2019).

A more suitable tool for recovery in the shorter term is ecosystem restoration, which involves the active assistance in the recovery of a degraded, damaged, or destroyed habitat (SER 2004). Active restoration allows for accelerated ecosystem recovery by implementing management techniques (e.g., transplanting) (Perrow and Davy, 2002; Holl and Aide, 2011; Bayraktarov et al., 2016). Combining well-designed active and passive restoration practices have become indispensable to reverse widespread ecosystem degradation (Lotze et al., 2006; Mitsch, 2014; Possingham et al., 2015).
Given the existence of sea urchin barrens, several control measures to reduce sea urchin abundance and restore kelp and fucoid forests have been described such as sea urchin

eradication (Leighton et al., 1966; Watanuki et al., 2010; Sangil et al., 2012; Piazzi and Ceccherelli, 2019) and algal transplantation (Carney et al., 2005; Whitaker et al., 2010). Despite these efforts, attempts to recover macroalgal forests from sea urchin barren grounds remain challenging due to the high hysteresis of stable barren state and the difficulty of reestablishing populations of natural predators and consequent trophic cascades (i.e., in old and well-enforced NTZs) that help reduce pervasive sea urchin overgrazing (Ling et al., 2015; 2019). Therefore, the combination of active (i.e. algal transplantation and sea urchin removal) and passive restoration (i.e., establishment of marine protected areas) in priority sites for restoration (i.e., NTZs, Gianni et al., 2013), provide a unique yet largely unexplored opportunity for successful forestation from sea urchin barren grounds.

Restoration actions of kelp and fucoid forests have mostly considered transplanting individuals (Vasquez and Tala, 1995; Falace et al., 2006; Campbell et al., 2014). Notwithstanding that, the different algal reproductive strategies and the feedbacks preventing the recovery of healthy ecosystems have been taken also into account to design and implement local restoration actions. Recently, non-destructive restoration techniques such as the enhancement of recruitment potential (Gianni et al., 2013; Falace et al., 2018; Verdura et al., 2018) or the ex situ outplanting (De La Fuente et al., 2019) has been also proposed to restore marine forests.

## Macroalgal communities in the Mediterranean Sea

The Mediterranean Basin is the largest quasi-enclosed sea, considered a hotspot of marine biodiversity (Bosc et al., 2004; Coll et al., 2010). Mediterranean waters are considered oligotrophic in general although there are important regional and seasonal variations (Bosc et al., 2004). Mediterranean coasts support high levels of anthropogenic influence, not only by the dense population inhabiting but also due to overexploitation and habitat destruction (Lotze et al., 2011; Sala et al., 2012).

Macroalgal communities dominate the shallow benthic habitats in the Mediterranean Sea (Zabala and Ballesteros, 1989; Sala et al., 2012). There, the highest level of structural complexity is represented by canopies of fucoid algae, mostly species of *Cystoseira sensu lato* genus, which form extensive beds on exposed or sheltered rocky shores from the upper infralittoral zone down to the photic limit (Sales and Ballesteros, 2009; Mariani et al., 2019). The *Cystoseira* genus is considered polyphyletic with high morphological similarity between species (Draisma et al., 2010; de Sousa et al., 2019). A recent study resolves that species of the *Cystoseira* genus were included in three genera: Carpodesmia, Cystoseira, and Treptacantha (Orellana et al., 2019). *Cystoseira sensu lato* is currently constituted by 46 species, most of them endemic from the Mediterranean Sea (Giaconne, 1991; Orellana et al., 2019). *Cystoseira* (*sensu lato*) fucoids are characterized by a single or several main axes attached to the substratum which can conform arborescent and dense canopies (Gómez-Garreta et al., 2001; Cormaci et al., 2012). They are considered late successional species and tend to dominate the low impacted rocky coastal habitats (Ballesteros et al., 2007). All *Cystoseira* (*sensu lato*) fucoids except *C. compressa* have been listed under Annex II of the Barcelona convention (2010) among those species that are considered threatened or endangered and need protection measures (Gianni et al., 2013). Mediterranean fucoid forests suffered a global decline in the last century due to a multiple direct and indirect human impacts such as water pollution, herbivores overgrazing or coastal development (Thibaut et al., 2005; Mangialajo et al., 2008; Perkol-Finkel and Airoldi, 2010; Thibaut et al., 2015; Mineur et al., 2015; Blanfuné et al., 2016).

In the NW Mediterranean Sea, the sea urchin *Paracentrotus lividus* (Lamark) is considered the most abundant and important local herbivore feeding on fleshy algae of the shallow reefs. The sea urchin *Arbacia lixula* (Linnaeus) (Fig. 6) may also have large consequences for macroalgal communities. *A. lixula* preferentially graze on encrusting coralline algae (Privitera et al., 2008), having an important role in the maintenance of sea urchin barrens once they are established (Agnetta et al., 2015). Although the role of the herbivorous fish *Sarpa salpa* (Fig. 6) (Sala and Zabala, 1996; Sala and Boudouresque, 1997; Hereu, 2005) has been often overlooked (Gianni et al., 2017), their effects on macroalgal beds are weaker than the sea urchins ones due to their feeding preference and behavior (Hereu, 2006; Hereu et al., 2008; Ahmed et al., 2014).

**Figure 6**. Images of the herbivores in the NW Mediterranean Sea. A) The sea urchin *Paracentrotus lividus,* B) The sea urchin *Arbacia lixula*, and C) The herbivore fish *Sarpa salpa.*

# Objectives

The objectives of this thesis are twofold. The first goal is to contribute to the understanding of the dynamic of macroalgal communities and their main herbivores. The second goal is to enlarge the knowledge of the ecology and conservation of human-impacted macroalgal forests in the Northwestern Mediterranean enhancing the effectiveness of management and restoration actions.

Different methodological approaches were combined here to explore the ecological dynamic of algal communities in The Montgrí, Illes Medes and Baix Ter Natural Park (Box 1). A large and valuable number of monitoring and research programs have been performed in this Natural Park before and after the establishment of the marine reserve in 1983, which constitutes an excellent framework to study how human impacts could shape the benthic communities. Human impacts (mainly fishing consequences) over the long-term monitoring were evaluated here. Apart from anthropogenic impacts, benthic communities suffered severe damages owing to an extraordinary storm observed over the course of this long-term monitoring, in 2009. Therefore, impacts on benthic communities by human-related and natural threats were included in this thesis.

Although recent studies have reported an important decline of several canopy-forming *Cystoseira sensu lato* populations, *Treptacantha elegans* (Box 2 and Box 3) has shown an exceptional expansion of its distribution in the studied area. Because of this, we considered *T. elegans* as the model species in this thesis.

## **BOX 1** Study site: the Montgrí, Illes Medes, and Baix Ter Natural Park

The Montgrí, Illes Medes and Baix Ter Natural Park covers a marine area of 2.037 ha and has its core in the no-take zone (NTZ) of the Medes Islands (Fig. 7), which comprises the Archipelago of the Medes Islands (93 ha), where all fishing and harvesting activities have been prohibited since 1983 (García-Rubies and Zabala, 1990). Outside the NTZ, the nearby Montgrí coast is divided into a peripheral zone (Fig. 7), a contiguous beltway zone to the Medes Islands that was established in 1990 where artisanal fishing and recreational angling are allowed under restriction, and a no reserve zone (Fig. 7), where artisanal, recreational and spearfishing are allowed.

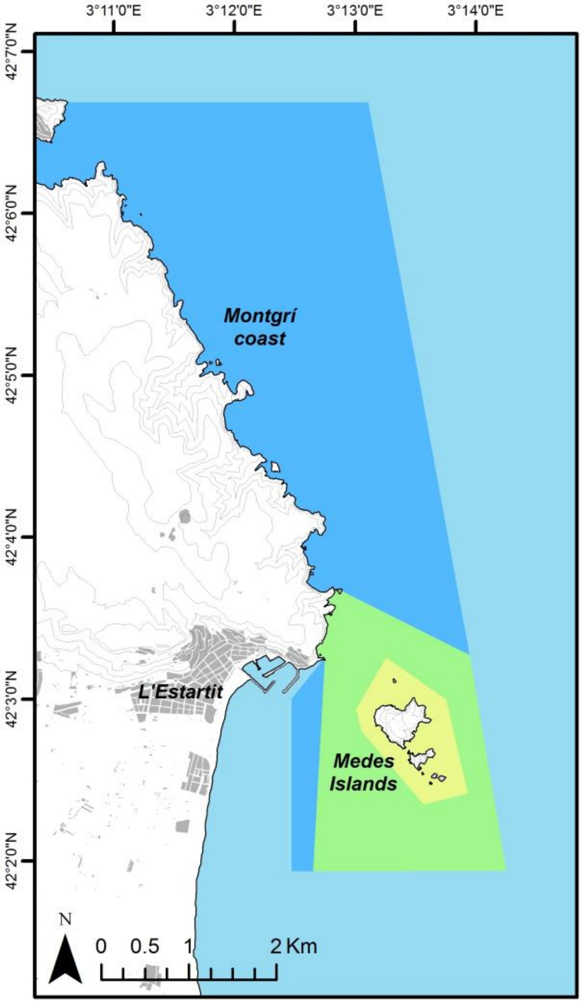


**Figure 7.** Map of the Montgrí, Illes Medes and Baix Ter Natural Park in the NW Mediterranean Sea. The perimeter of the Medes Islands NTZ is delimited by the yellow polygon; the peripheral zone is delimited by the green polygon; and the no reserve zone, by the dark blue polygon.

**BOX 2** The model species: *Treptacantha elegans*

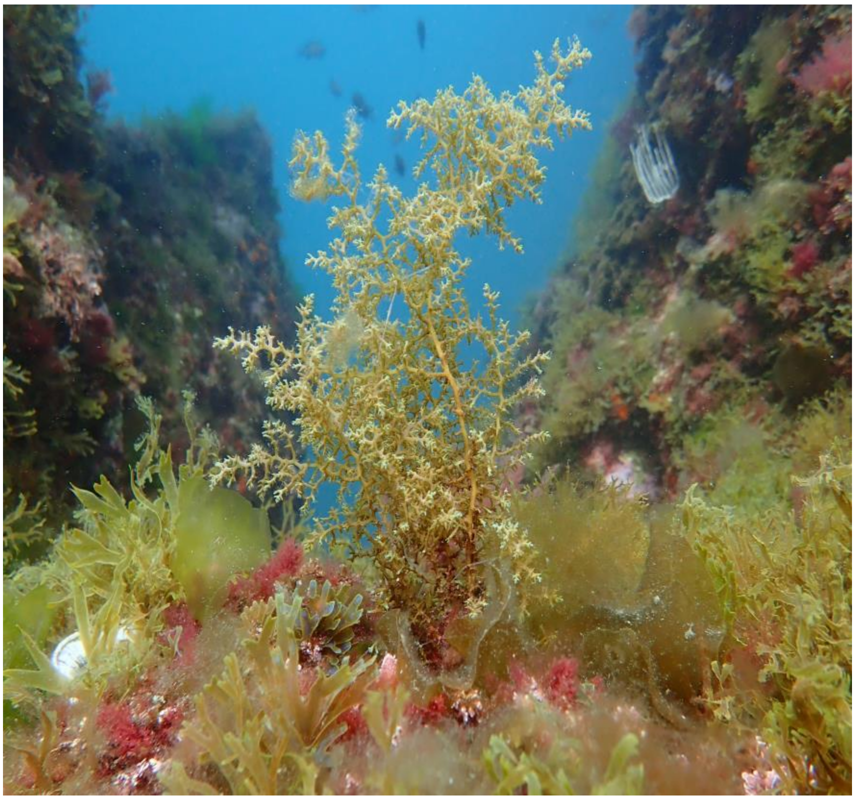
Among Cystoseira sensu lato algae, *Treptacantha elegans* is a Mediterranean endemic alga recently assigned to *Treptacantha* genera by Orella et al. (2019). Its original description (as *Cystoseira elegans* by Sauvageau 1912, pp. 292) was located in Banyuls-sur-Mer (South-Eastern France), where they made extensive meadows at the innermost areas of the coves and other sites with medium to low water movement, down to 2–3 m depth (Feldmann, 1937).

The canopy-forming branches of *Treptacantha elegans* grow in spring, reaching the maximum development and the fertile maturity in early summer when larger specimens could exceed fifty centimeters height (Barceló et al., 2001), although main axis is relatively short (8 cm maximum length). Specimens are attached to the substrate perennially by a robust basal disc. Their deciduous branches are always cylindrical, without aerocysts, and show spiny appendages. Tophules could be spiny or verrucous (Cormaci et al., 2012; Mariani et al., 2019).

## **BOX 3** *Treptacantha elegans* in the study area

Although *Treptacantha elegans* typically inhabits shallow and shelter littoral environments, the occurrence for the species on infralittoral habitats (down to 5 m depth) was reported in the Medes Islands NTZ for first time by Sala (1996). The specimens found by these authors were originally assigned to *Cystoseira spinosa* (today accepted as *Treptacantha ballesterosii*) because of similar morphological characters and their depth range distribution (E. Sala, personal communication). These *T. elegans* specimens thriving in more exposed and deeper environments presented thicker branches and bear more abundant and rigid spiny appendages than those described for this species (Mariani et al., 2019; Box 2).

An exceptional expansion of deeper *T. elegans* specimens has been observed in the Medes Island Marine Reserve and nearby areas (Catalonia coast) in the last decades.



The present thesis has been structured in 4 chapters detailed below. In the first two chapters, the analysis of long-term monitoring datasets provided essential information to understand how macroalgal assemblages and sea urchin populations respond to natural fluctuations and anthropogenic disturbances, mainly overfishing. In the third chapter, field monitoring and sampling were combined with molecular analyses to increase the ecological knowledge on the canopy-forming algae *Treptacantha elegans* as well as to describe their recent expansion. In the fourth and last chapter, seeding experiments were parallelly conducted in aquaria and in the field to optimize restoration techniques for degraded shallow ecosystems. Besides, different restoration techniques were tested in the field which involved a large experimental effort.

**Specific objectives:**

**Chapter I**: Benthic shallow-rocky habitats in the Northwestern Mediterranean Sea are mostly represented by photophilic algae communities of small and erect algae and calcareous algae formations. The aim of this chapter is to describe the temporal dynamics of the macroalgal communities considering their main drivers and environmental stressors and to evaluate the effect of protection in macroalgal beds over time in the Montgrí, Illes Medes and Baix Ter Natural Park. To do this, I analyzed a long-term monitoring dataset of the infralittoral macroalgal communities, annually monitored inside and outside the NTZ of Medes Islands during the last 15 years.

**Chapter II**: In the Montgrí, Illes Medes and Baix Ter Natural Park, sea urchins and, in particular, the purple sea urchin *Paracentrotus lividus*, are considered the most important herbivores controlling the macroalgal dynamics on the shallow reefs. Due to their key role in structuring the infralittoral seaweed communities, understanding how fully protected areas can shape their population structure and dynamics may be crucial for enhancing the effectiveness of conservation actions for temperate benthic communities. Here, I analyzed a long-term monitoring dataset (nearly three decades) of sea urchin populations inside and outside the NTZ of Medes Islands to describe the role of marine protected areas on the sea urchins populations facing human-related and natural threats.

**Chapter III**: Mediterranean *Cystoseira (sensu lato*) forests are experiencing a global decline, driving to a reduction of the habitat complexity, with important consequences for biodiversity and ecosystem functioning. Against all odds, the fast-growing *Treptacantha elegans* (Box 2 and 3) has increased their distributional range and has shown an extraordinary expansion in the North Catalan coast over the last two decades. Here, I recreated their temporal and spatial spread and untangled their ecological attributes such as population dynamics and population connectivity to provide a baseline of their actual status.

**Chapter IV**: In light of the global decline of canopy-forming macroalgal forests, forestation of sea urchin barren grounds is encouraged in this chapter as a tool to shift from low productive and structurally poor barren grounds to high productive and diverse marine forests. Following the results of the chapter 3, the expanding and fast-growing alga *Treptacantha elegans* (Box 2 and 3) is proposed here as model species to promote the forestation of barren grounds. A three-step forestation protocol was assessed combining the active control of sea urchin populations and the *T. elegans* forestation. In addition, forestation success was assessed inside and outside the Medes Islands NTZ to evaluate the potential of active (restoration), passive (NTZs), and combined active with passive (NTZ + restoration) conservation strategies.

# Chapter I

# Long-term monitoring of temperate macroalgal assemblages inside and outside a No-Take marine reserve

# Abstract

Macroalgal communities have an essential role in the shallow benthic habitats of temperate seas, where changes in their composition can resonate through entire coastal ecosystems. As all major ecosystems on Earth, algal beds have already been affected by multiple disturbances. Passive conservation tools, such as marine protected areas or No-take zones, have the potential to reduce some of the anthropogenic impacts by limiting human activity. However, without a good knowledge of the natural community dynamics, it is not easy to discern between changes fruit of the intrinsic variability of biological communities and the ones caused by human-related stressors. In this study, we evaluated the natural variability of macroalgal communities' composition inside and outside a Mediterranean No-Take marine reserve during 15 years. We described their temporal dynamics considering their main drivers and we tested the effect of protection in seaweed beds. We did not find differences either in the composition of the macroalgal assemblages or the total algal cover between protected and non-protected locations over the fifteen years of study. Nevertheless, we observed a positive effect of the protection increasing the cover of some specific species, such as the canopy-forming *Treptacantha elegans*. Our results highlight the importance of obtaining long-term data in ecological studies to better understand the natural variability of marine communities. Accordingly, a robust understanding of the community dynamics would help us to avoid misinterpretations between 'impacted' or 'in-recovery' communities when recovery times are longer than the study periods.

# 1. Introduction

Global and local human disturbances have affected all major ecosystems on Earth, including coastal algal communities (Halpern et al., 2007; Gianni et al., 2017), the dominant primary producers in the coastal zone (Krause-Jensen and Duarte, 2016). Overfishing, global warming, and exceptional storms further contribute to transforming rocky infralittoral algal habitats (Ling et al., 2009; Smale and Vance, 2016; Wernberg et al., 2016; Maggi et al., 2018). Conservation tools such as Marine Reserves or No-Take Zones (NTZ) have the potential to reduce some of these anthropogenic disturbances (i.e, banning fishery activities) and to restore benthic habitats through trophic cascade effects (Shears and Babcock, 2002; Guidetti, 2006; Babcock et al., 2010).

Macroalgae play a key role in the structure of temperate benthic ecosystems (Jones et al., 1994; Duarte and Cebrian, 1996; Teagle et al., 2017), representing an important source of carbon sequestration (Krause-Jensen and Duarte, 2016) and providing invaluable ecosystem services in many shallow coastal systems (Blamey and Bolton, 2018). Consequently, changes in macroalgal communities may sway in the whole coastal ecosystem (Mineur et al., 2015). In the Mediterranean Sea, algal communities dominate the shallow benthic habitats (Zabala and Ballesteros, 1989). There, the highest level of structural complexity is represented by canopies of fucoid algae, mostly *Cystoseira sensu lato* populations, providing habitat, shelter, and food to many associated organisms and harboring a high diversity and productivity (Ballesteros et al., 1998; Mangialajo et al., 2008; Sala et al., 2012; Cheminée et al., 2013). Water pollution, modifications of natural rocky coastline, and sea urchin overgrazing as a consequence of overfishing are the major drivers of the loss of fucoids in the Mediterranean Sea (Airoldi and Beck, 2007; Mineur et al., 2015). Consequently, severe declines of macroalgal forests (i.e., *Cystoseira spp.*) have been documented, with the consequent changes in the associated species, and effects cascading up to higher trophic levels (Ling et al., 2015; Cheminée et al., 2013).

Long-term ecological studies observing natural communities dynamics under the human influence are essential to understand the operation of natural systems, and most especially, to know the communities' baseline before describing pressures or impacts. Changes in marine benthic communities and conservation effects are usually reported in ‘before-after’ studies or with ‘snapshots’ of the protected area vs. the unprotected one, while long-term monitoring programs are focused on specific species or populations, such as corals, seagrasses, sea urchins or kelp species among others (e.g. Kirkman and Kirkman, 2000; Steneck et al., 2002; Babcock et al., 2010; Gross and Edmuns, 2015). Despite the essential role of macroalgal beds in benthic communities, there is an important lack of continuous and long-term studies that integrate community changes together with their long-term dynamics and with the conservation role, without which we can misinterpret communities alterations.

In this study, we annually monitored infralittoral macroalgal communities inside and outside a NTZ located in the North-Western Mediterranean Sea during 15 years. Benthic shallow-rocky habitats in the study area are mostly represented by photophilic algal communities of small and erect algae (e.g., *Dyctiota spp.*) and calcareous algal formations (Ballesteros, 1991). The sea urchin *Paracentrotus lividus* is the main herbivorous species that control algal biomass (Hereu et al., 2012a; Medrano et al., 2019a). Abundance and temporal trends on sea urchin populations were similar inside and outside the studied NTZ (Hereu et al., 2012a) until an exceptionally violent storm occurred in 2008 that drastically reduced sea urchin densities. After that, the populations of the sea urchin *P. lividus* outside the NTZ recovered faster than the population inside the NTZ (Medrano et al., 2019a). The present study aims to describe the temporal dynamics of the macroalgal communities considering their main drivers and environmental stressors and to evaluate the effect of protection in macroalgal beds over time by analyzing long-term monitoring data.

## 2. Materials and Methods

### 2.1 Monitoring and study area

Shallow rocky infralittoral macroalgal communities were monitored at eight localities in the Montgrí, the Illes Medes and the Baix Ter Natural Park, located in the North-Western Mediterranean Sea (Fig. 1). Within the Natural Park, four out of the eight sampled localities were placed inside the Medes Islands No-Take Zone (NTZ) and, the other four, outside the boundaries of the NTZ (Fig. 1).

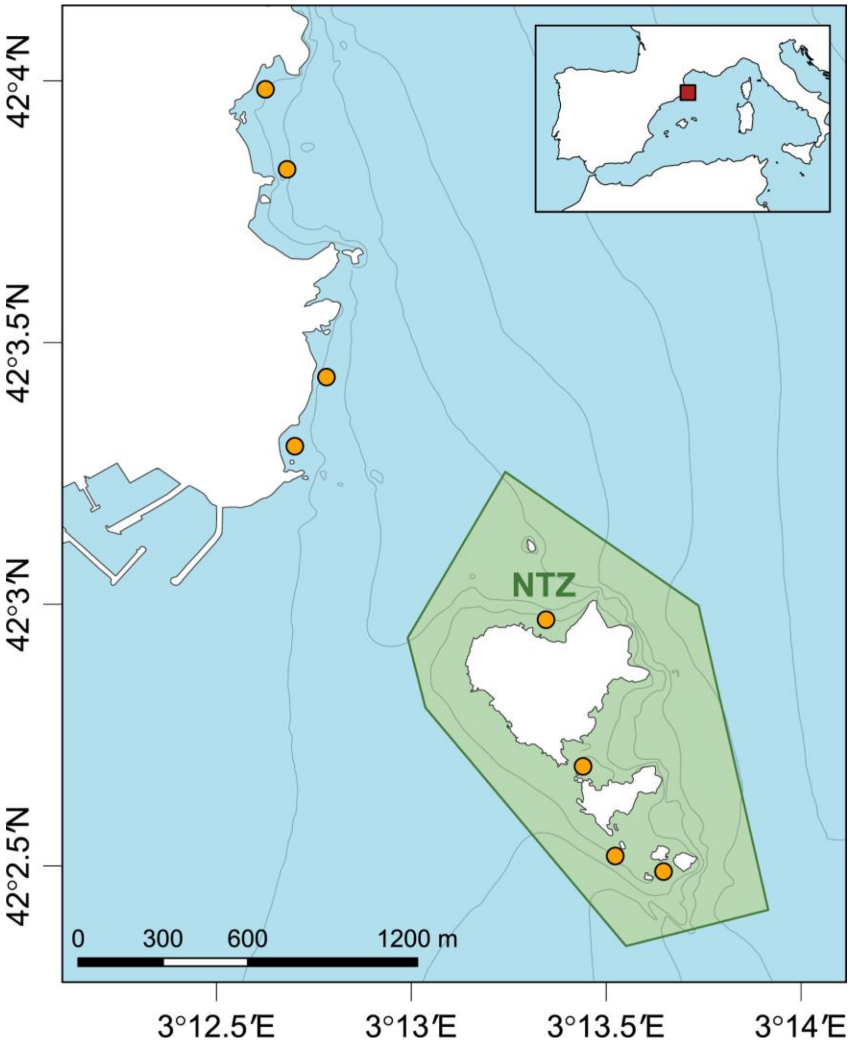


**Figure 1**. Map of the study sites in the Montgrí, Illes Medes, and Baix Ter Natural Park (NW Mediterranean Sea). Orange dots represent the long-term monitoring sites: four are located outside the NTZ, and four more are inside the Medes Islands NTZ. The perimeter of the Medes Islands NTZ is delimited by the green polygon. Unprotected zone is outside the green polygon.

To monitor macroalgal communities, we annually sampled the algal assemblage structure based on the main seaweed species (Table 1) from 2001 to 2016, excluding the years 2006, 2007, 2011 and 2015. The algal percent cover was visually quantified using 50x50 cm quadrats divided into 25 subquadrats of 10x10 cm (Hereu et al., 2008). Twenty replicate quadrats were randomly counted at each location at depths between 5 and 10 m by SCUBA divers. At each quadrat, each species percentage cover was estimated as the percentage of the numbers of subquadrats where the species was present relative to the total number of subquadrats sampled (as described in Sala and Ballesteros, 1997 and Hereu et al., 2008). To avoid the effect of seasonality, sampling was always performed at the end of May, when the most representative macroalgae (perennial and seasonal species) reach the maximum biomass in the study area (Sala and Boudouresque, 1997).

**Table 1.** List of the main macroalgal species present in the study area and included in the anual monitoring: full names, abbreviations, and authorities. The species in bold represent about 90% of total algal cover and were considered here as the fourteen major species.

| | | |
|---|---|---|
| *Rhodophyta* | | |
| *Amphiroa rigida* | Amphiroa | Lamouroux |
| ***Asparagopsis armata*** | **Asparagopsis** | **Harvey** |
| *Bonnemaisonia asparagoides* | Bonnemaisonia | (Woodward) C. Agardh |
| *Ceramium ciliatum* | Ceramium | Ducluzeau |
| ***Corallina elongata*** | **Corallina** | **Ellis and Solander (also accepted as *Ellisolandia elongata*)** |
| *Gelidium spinosum* | Gelidium | (Gmelin) Silva |
| ***Jania rubens*** | **Jania** | **(Linnaeus) Lamouroux** |
| ***Laurencia obtusa*** | **Laurencia** | **(Hudson) Lamouroux** |
| *Liagora viscida* | Liagora | (Forsskal) C. Argardh |
| ***Lithophyllum incrustans*** | **L.incrustans** | **Philippi** |
| ***Mesophyllum alternans*** | **M.alternans** | **(Foslie) Cabioch and Mendoza** |
| *Peyssonnelia bornetii* | Peysonnelia | Bourderesque and Denizot |
| ***Sphaerococcus coronopifolius*** | **Sphaerococcus** | **(Goodenough and Woodward) Stackhouse** |
| ***Wrangelia penicillata*** | **Wrangelia** | **C. Agardh** |
| *Phaeophyta* | | |
| *Cladostephus spongiosus* | Cladostephus | (Hudson) C. Agardh |
| *Colpomenia sinuosa* | Colpomenia | (Mertens ex Roth) Derbès and Solier |
| *Cystoseira compressa* | C.compressa | (Esper) Gerloff and Nizamuddin |
| ***Treptacantha elegans*** | **T.elegans** | **Sauvageau** |
| ***Dictyota spp*** | **Dictyota** | |
| ***Halopteris scoparia*** | **Halopteris** | **(Linneaus) Sauvageau** |
| ***Padina pavonica*** | **Padina** | **(Linneaus) Thivy** |
| *Zanardinia typus* | Zanardinia | (Nardo) Furnari |
| *Chlorophyta* | | |
| *Acetabularia acetabulum* | Acetabularia | (Linnaeus) Silva |
| ***Codium bursa*** | **C.bursa** | **(Linnaeus) C. Agardh** |
| *Codium effusum* | C.effusum | (Rafinesque) Delle Chiaje |
| ***Codium vermilara*** | **C.vermilara** | **(Olivi) Delle Chiaje** |
| *Flabellia petiolata* | Flabellia | (Turra) Nizamuddin |
| *Halimeda tuna* | Halimeda | (Ellis and Solander) Lamouroux |
| *Ulva rigida* | Ulva | C. Agardh |

## 2.2 Environmental drivers and stressors determining algal assemblage structure

To investigate the relation between the composition of the macroalgal assemblages and the effect of the environmental drivers inside and outside the NTZ, we used Redundancy Analysis (RDA) (Van Den Wollenberg, 1977) on fourth-root transformed percentage cover data of all the monitored macroalgal species (Table 1). The RDA is a multivariate analysis technique which allows to introduce explanatory (environmental) variables considering multiple regressions to determine linear combinations of these variables with the dependent variables (seaweeds). Water nutrients, sea-surface temperature, sea urchin abundance, and level of protection (as categorical variable) were included as environmental variables (adapted from Hereu et al., 2008).

Specifically, Chlorophyll-a (Chl-a) levels are a good proxy for nutrient levels in coastal waters (Brodie et al., 2007). Therefore, Chl-a data from the MODIS-Aqua sensor were extracted for the monitored area with a 4 km resolution from NASA Giovanni (MODISA_L3m_CHL_v2018; https://giovanni.gsfc.nasa.gov/giovanni/) from 2002 (starting date of this time-series) to 2016. In order to relate the nutrient concentration with the sampled algae cover, the average concentration of Chl-a (mg/m$^3$) was calculated for the spring season of each sampled year (March to May).

Sea-surface temperature (SST) data were obtained from the L'Estartit Meteorological Station (http://meteolestartit.cat/mar/temperatura/), where temperature has been measured 1.7 km offshore of the Medes Islands NTZ (the northwestern Mediterranean, 42°03'N 3°15'15''E) since 1974. Like Chl-a, we have considered here the mean SST of the spring season (March to May) for each year from 2001 to 2016. We used the same physical environmental variables (Chla- and SST) for locations inside and outside the NTZ, given that the average distance between the locations is lower than the spatial resolution of the described physical environmental variables (2 km).

The effect of protection on fish communities and the sea urchin abundance inside and outside the Medes Islands NTZ over the studied period were obtained from the long-term monitoring program of the Montgrí, Illes Medes and Baix Ter Natural Park (Medrano et al., 2019a). Sea urchins missing data of the years 2006, 2007, 2014 and 2015 were linearly interpolated from the known values in the time-series. It is important to note that higher fish abundance and biomass of the main sea urchin predator species have remained significantly larger inside the NTZ than outside (García-Rubies and Zabala 1990; Sala 1997; Hereu et al., 2005; Sala et al., 2012), with higher predation rates of sea urchins observed inside the NTZ over the last years (Sala, 1997; Hereu et al., 2005; 2012). Contrarily, no clear effect of protection on the unique herbivorous fish in the area, *Sarpa salpa*, on rocky bottoms was observed (García-Rubies, 1990; Macpherson et al, 2004; Hereu et al. non pub. data).

The collinearity of the four variables was checked. Although the maximum correlation was found between the variables Sea urchin abundance and Protection level (Spearman correlation: -0.7), we considered and included all four variables as reasonably explanatory in this study.

### 2.3 Long-term trends of the main macroalgal species inside and outside the NTZ

The fourteen most representative algal species (representing a total cover of 89 ± 0.06 % (Mean ± SD) in the studied monitoring period, Table 1 bolded species) were selected to describe the temporal patterns of the macroalgal dynamics across protection levels and time. To show a clearer global view rare species were excluded. First, kite diagrams of the percentage of the main algal cover were plotted to visualize the changes of these algal assemblages over the studied period inside and outside the NTZ. Second, to assess the abundance variability throughout the studied period inside and outside the NTZ, we calculated the coefficient of variation (CV) of the algal relative abundance in each location. CVs of the fourteen most representative macroalgae were estimated as the mean CV of each species between the monitoring sites and CVs of the assemblages between protection levels were estimated with the fourteen CVs mean of each site. To test pairwise comparisons among CV, we used Tukey's Honestly Significant Differences (HSD) test in each pair of means (inside/outside NTZ). Finally, to test for differences in overall macroalgal assemblage structure among protection and time, we used multivariate generalized linear models implemented in the manyglm function of the mvabund R package (Wang et al., 2017). The macroalgal abundance in each monitored site was fitted as the response variable with a negative binomial distribution after visually checked the most likely distribution of the data. The variables protection (inside/outside NTZ) and time (years) were included as main fixed effects in the model. We then ran a univariate analysis of variance to test the significance of the effects in every algal species (Wang et al. 2017).

All statistical analysis and plots were run with the software R 3.3.3 (R Core Team, 2017). Multivariate analyses were performed with the R package 'vegan' (Oksanen et al., 2017) and R Package 'mvabund' (Wang et al., 2017).

# 3. Results

## 3.1 Environmental drivers and stressors determining algal assemblage strucure

The first two axes of the RDA explained the 68 % of the species-environment relationship (Fig. 2A RDA, Table 2). The four biotic and abiotic variables were significantly correlated with the RDA axes. Protection and Sea urchins were strongly correlated with the first axis, while environmental variables related to seasonality (SST and Chl-a) were significant correlated with the second axis (Table 3).

**Table 2**. Results of redundancy analysis (RDA) on fourth-root transformed data.

| Axes | RDA 1 | RDA 2 | RDA 3 | RDA 4 |
|---|---|---|---|---|
| Eigenvalues | 2.8765 | 1.4672 | 1.2062 | 0.8398 |
| Cumulative percentage variance | | | | |
| of species data | 9.92 | 14.98 | 19.14 | 22.03 |
| of species-environment relation | 45.02 | 67.98 | 86.86 | 100 |

Along the first axis, species positioned at the far right end were more abundant in protected sites with low abundance of sea urchins (e.g., *Asparagopsis armata* and *Treptacantha elegans*). These species were separated from those most commonly observed in non-protected sites, which were correlated with higher abundances of sea urchins (e.g., *Wrangelia penicillata, Lithophyllum incrustans, Peyssonnelia bornetii).* In line with that, the species identified as canopy-forming (*Cystoseira sensu lato* species) were positioned in the right side of the axis, while the encrusting coralline algae were on the left side (e.g, *Lithophyllum incrustans* and *M. alternans)*. Along the second axis, the macroalgal seasonal species (spring-growing) related to the spring nutrient peak and cold waters were positioned in the top of the RDA biplot (e.g., *Dictyota spp., Bonnemaisonia asparagoides,* and *Ulva rigida)*. Despite being a seasonal growing alga, since *Laurencia obtusa* biomass peak takes place during the late spring-early summer, it was mostly related to warm waters as well as the perennial *Codium* species (Fig. 2A).

Macroalgal assemblages inside and outside the NTZ shifted towards the same ordination space over time, highlighting inter-annual variability of the assemblage, and homogeneous patterns of change in both protection levels (Fig. 2B). Despite this observed variability, the last dot (representing 2016 data) was located close to the origin dot (representing 2003 data), indicating that no major shifts in the algal assemblages occurred over this long-term study (Fig. 2B).

**Table 3**. Biplot scores for environmental variables, the coefficient of determination (R2), and their significance using 999 permutations on the RDA results.

| Variable | RDA 1 | RDA 2 | $R^2$ | p(>r) |
|---|---:|---:|---:|---:|
| Protection | 0.97570 | -0.21911 | 0.7393 | 0.001 |
| Sea-urchins | -0.93935 | -0.34297 | 0.4598 | 0.001 |
| SST | -0.62272 | -0.78244 | 0.2954 | 0.001 |
| Chl-a | 0.58762 | 0.80913 | 0.0859 | 0.043 |

SST:Surface Seawater Temperature. Chl-a: Chlorophyll-a as proxy of nutrients

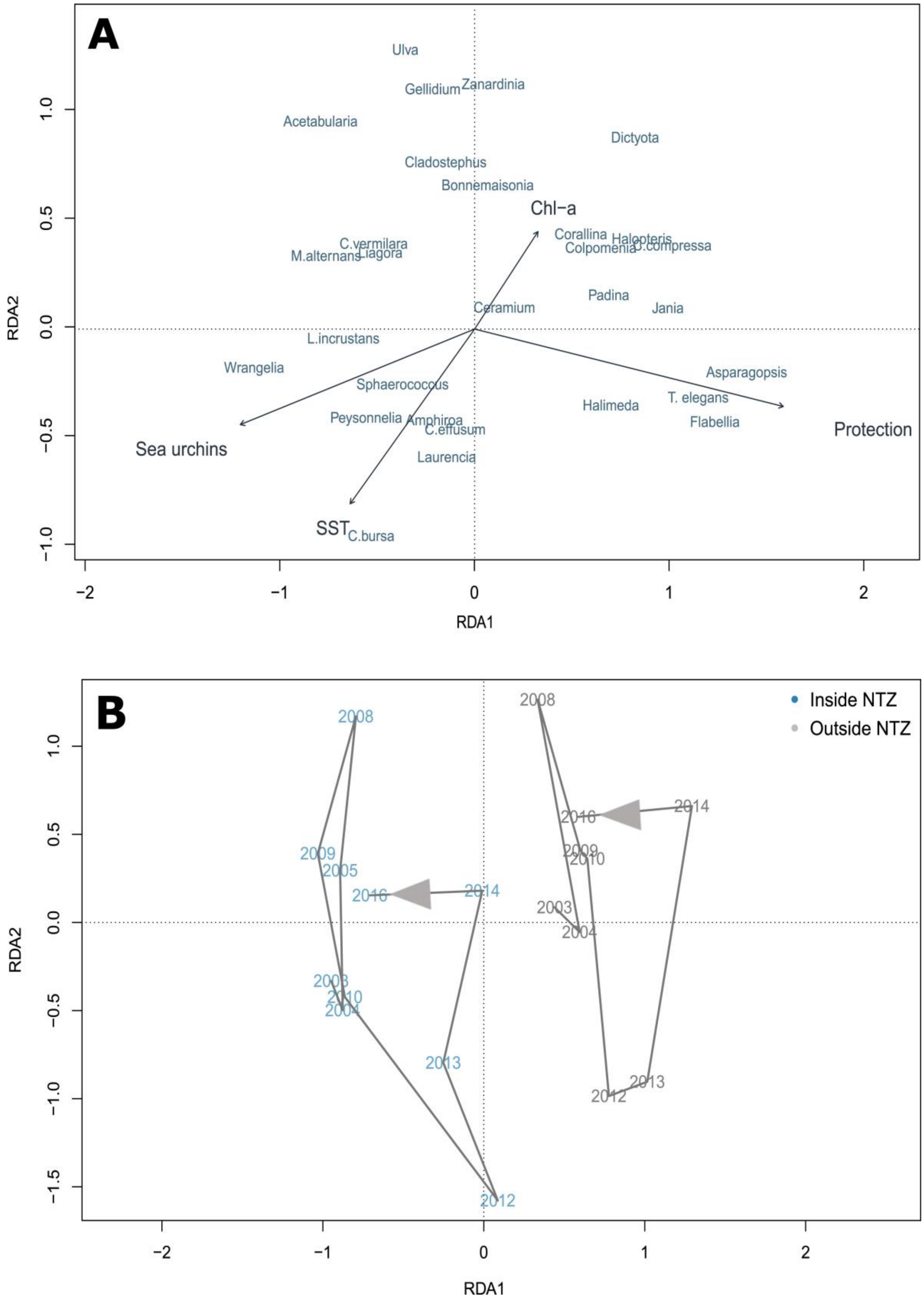


**Figure 2**. (A) Redundancy Analysis ordination for macroalgal cover data over time. Blue dots are sampling sites inside the NTZ and grey dots, outside the NTZ. Species code in Table 1. (B) Biplot of the Redundancy Analysis connecting the monitored years with grey lines within the protection levels. Font colors indicate the protection level (Blue=inside the NTZ, Grey=outside the NTZ).

## 3.2 Long-term trends of the main macroalgal species inside and outside the NTZ

The cover of the main macroalgal communities was homogeneous between the protected and the non-protected locations (inside/outside NTZ) (Fig. 3). The seasonal species of the genus *Dictyota* and the perennial *Corallina elongata* (today also accepted as *Ellisolandia elongata*) were the dominant seaweed in the study area during Spring, with a mean cover of 60 ± 15 % and 52 ± 17.3 % (Mean ± SD), respectively, across the eight monitored sites over time (Fig. 3). High stability of seaweeds cover over the fifteen years was found in most of the species in both protection levels. Annual changes in the species cover were detected simultaneously throughout the fifteen years in the protected NTZ and unprotected zones, suggesting the same variability on algal community drivers and the similar impact of stressors in both levels of protection (Fig. 3).

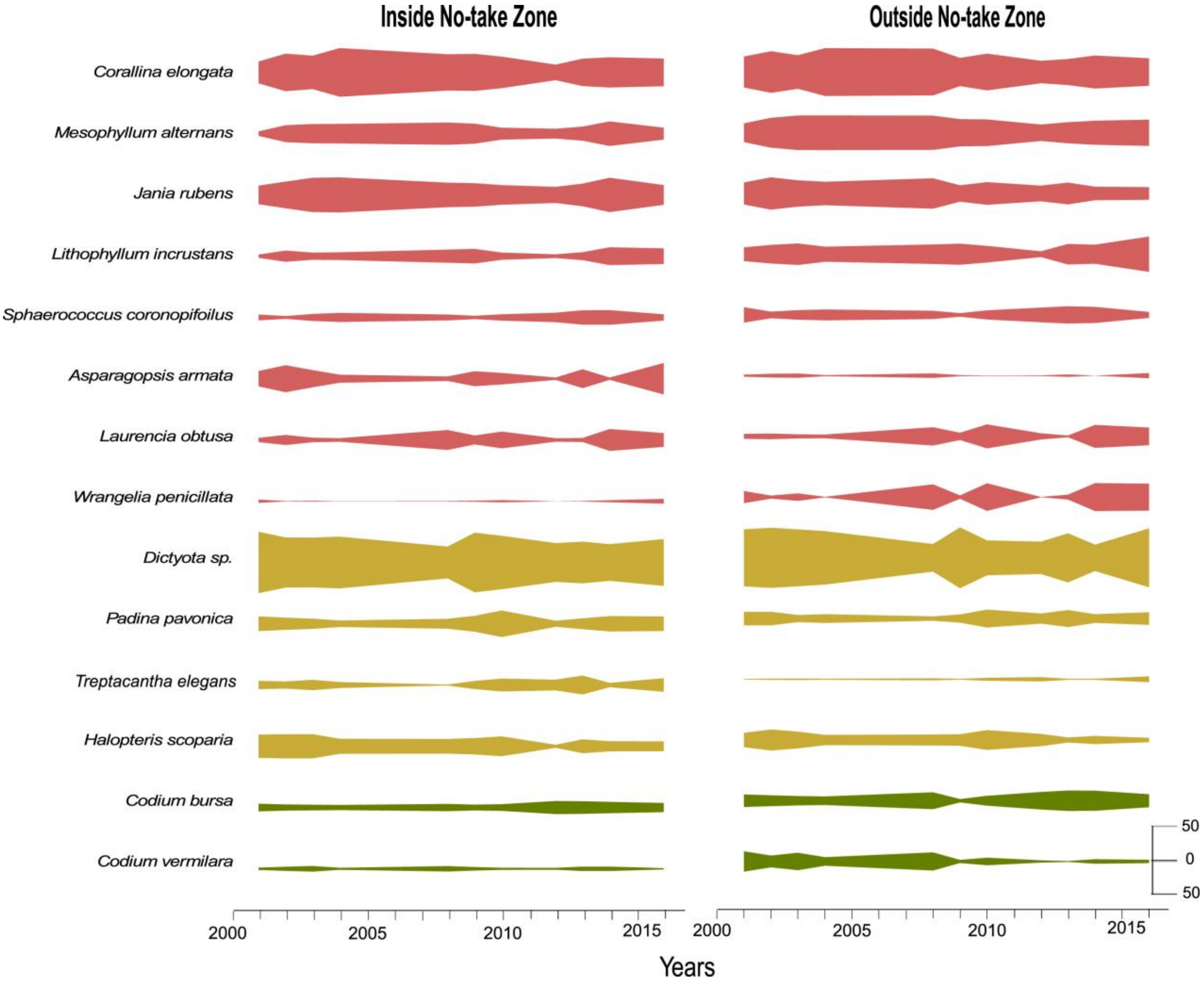


**Figure 3.** Kite diagram representing the cover of the fourteen most representative macroalgal species in the study area over the fifteen years. Left panel corresponds to the percentage cover within NTZ localities and right panel to the percentage cover of the localities outside the NTZ. Percentage cover was scaled to the maximum value of 50 and the colored diagrams show spectral values from 0 to 50 for each species. Color code indicates the corresponding macroalgae phyllum (Rhodophyta: red, Phaeophyta: yellow, and Chlorophyta: green).

The coefficient of variation showed a consistency of the total macroalgal cover estimates over time in both protection levels (Fig. 4A). For the particular species *A. armata*, *Halopteris scoparia*, *W. penicillata*, and *M. alternans,* CV pairwise comparisons showed differences within levels of protection being *A. armata* and *H. scoparia* more variable outside the NTZ while the abundances of *W. pencillata* and *M. alternans* were more variable inside the protected area (Fig. 4B, Table 1 S2). Among all the most abundant species, the canopy-forming species *Treptacantha elegans* showed the highest variability (Fig. 4B).

Short-term changes in macroalgal abundance such as the observed after the 2008 extraordinary storm occurred in the studied period (Fig. 3), but these were not significant over all the multi-year monitoring for the majority of the species (Table 4). Only three of the fourteen dominant species showed differences in their cover across protection levels (Table 4). In spite of the very low cover, the seasonal growing naturalized alga *A. armata* and the perennial canopy-forming *T. elegans* were more abundant inside the NTZ than outside. In contrast, the seasonal growing species *W. penicillata* was the most characteristic species outside the NTZ (Fig. 3, Table 4). While *L. obtusa* increased its relative cover over the study period (Fig. 3, Table 4) the calcareous algae *C. elongata* and *Jania rubens* decreased over the study period but only outside the NTZ (Fig. 3, Table 4).

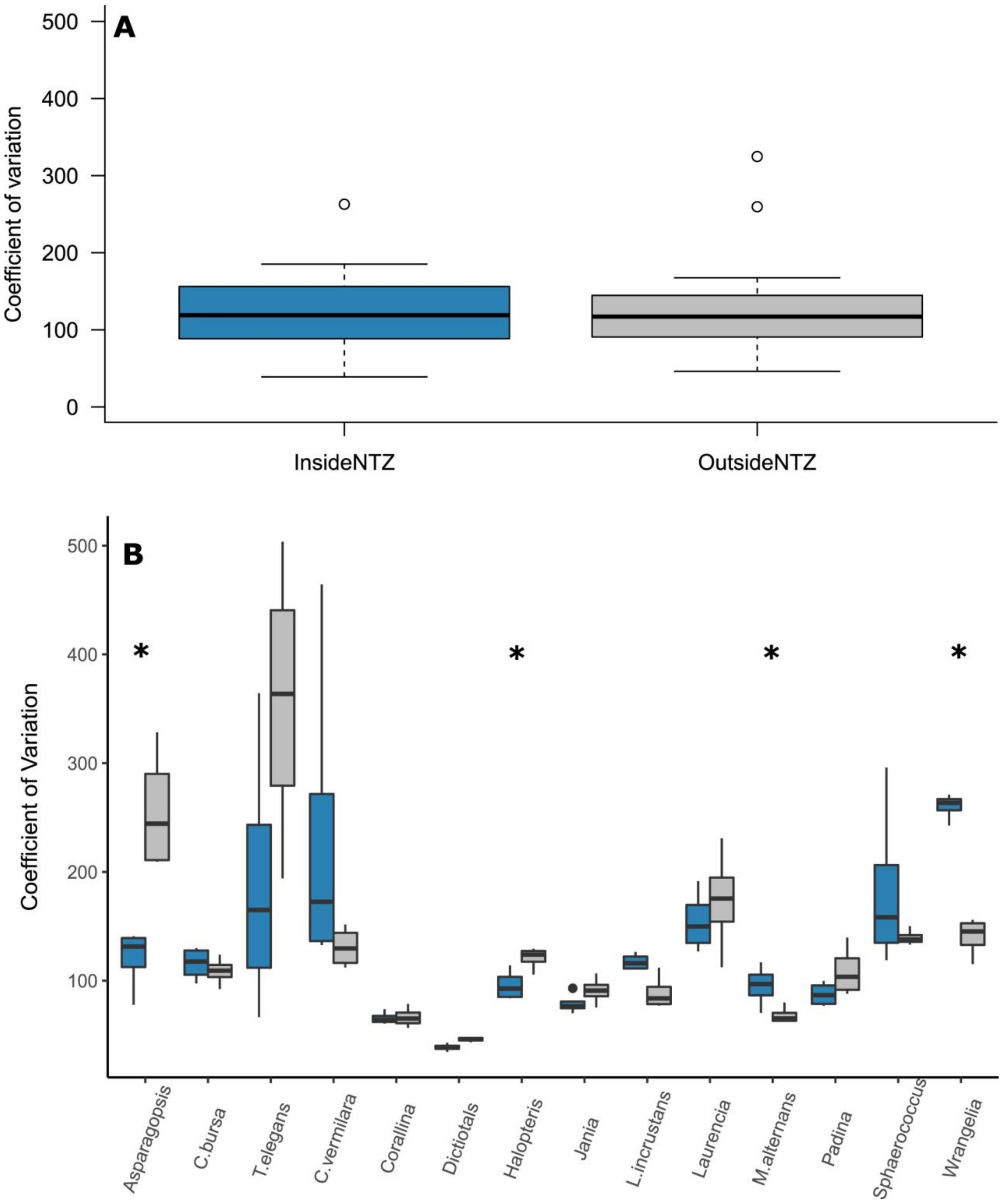


**Figure 4**. Coefficients of Variation (CVs) over time. Blue color represents the values inside the NTZ and grey color the values outside the NTZ. (A) Boxplot showing within-protection CVs in total macroalgae cover (inside/outside the NTZ). (B) Boxplot showing CVs in the cover of the fourteen main macroalgae species within protection levels. '*' indicates significance differences ($p<0.05$) in pair means using Tukey pairwise comparison. Species code in Table 1.

**Table 4.** Generalized Linear Model manyglm results (Deviation test statistic, p-value) of macroalgal cover (Percentage/0.25m$^2$) Df: degrees of freedom. Bold indicates $p < 0.05$. ':' indicates interaction. Species code in Table 1.

| Source | Df | *Asparagopsis* | *Dictyota* | *T. elegans* | *C. vermilara* | *C. bursa* |
|---|---|---|---|---|---|---|
| Protection | 1 | **25.093, 0.001** | 2.45, 0.596 | **23.324, 0.001** | 5.545, 0.209 | 2.26, 0.607 |
| Time | 10 | 31.461, 0.343 | 30.58, 0.343 | 25.542, 0.547 | 26.429, 0.531 | 39.415, 0.112 |
| Protection:Time | 9 | 17.314, 0.088 | 16.605, 0.088 | 16.276, 0.088 | **28.503, 0.020** | 6.463, 0.088 |

| Source | Df | *Halopteris* | *Laurencia* | *Padina* | *Sphaerococcus* | *Wrangelia* |
|---|---|---|---|---|---|---|
| Protection | 1 | 0.001, 0.993 | 0.14, 0.993 | 0.108, 0.993 | 0.089, 0.993 | **14.214, 0.006** |
| Time | 10 | 29.86, 0.354 | **47.096,0.010** | 27.475, 0.489 | 26.928, 0.510 | 40.56, 0.112 |
| Protection:Time | 9 | 18.854, 0.061 | 13.157, 0.088 | 19.008, 0.061 | 13.115, 0.088 | 11.207, 0.088 |

| Source | Df | *Coralina* | *Jania* | *L. Incrustans* | *M. Alternans* |
|---|---|---|---|---|---|
| Protection | 1 | 0.933, 0.889 | 1.62, 0.744 | 8.749, 0.052 | 3.736, 0.391 |
| Time | 10 | 34.044, 0.228 | 21.578, 0.547 | 33.851, 0.228 | 35.31, 0.169 |
| Protection:Time | 9 | **26.231, 0.025** | **24.497, 0.025** | 18.032, 0.077 | 15.289, 0.088 |

## 4. Discussion

Our results revealed that the coastal macroalgal assemblages of the Montgrí, the Illes Medes and the Baix Ter Natural Park were stable and similar in total algal cover inside and outside the protected NTZ over the fifteen years of study with no species replacement, although some variability were observed between years. The communities were dominated by the perennial species *C. elongata* and the seasonal *Dictyota s*pecies. *Corallina elongata* has been previously described as predominant species subjected to moderate pollution (Díez et al., 1999; Soltan et al., 2001). Species related to high-quality environmental requirements, such as *Cystoseira sensu lato* species. (Thibaut et al., 2005; Mangialajo et al., 2008) were less abundant in the study area. In addition to environmental quality, bottom-up (herbivorous control) and top-down (resources control) processes interactively influence the structure of macroalgal benthic communities (Korpinen et al., 2007; Smith et al., 2010). As we expected, bottom-up forces such as spring nutrient concentration and the ordination of the majority of seasonal algal species were associated to water temperature, in which herbivory showed the weakest influence (except for *A. armata* and *W. penicillata*). Contrarily, top-down forces may play a role in determining the abundance of fucoids such as the canopy-forming *Cystoseira sensu lato* species and the encrusting coralline algae species. It has been extensively documented that increased abundance of grazers feeding on algal beds can significantly change the structure of shallow benthic communities (Airoldi and Beck, 2007; Filbee-Dexter et al., 2014; Mineur et al., 2015).

The sea urchin *Paracentrotus lividus* is the most important herbivore in the study area, playing an important role in structuring algal communities (Sala and Zabala, 1996; Palacín et al., 1998; Hereu et al., 2008). As *P. lividus* preferentially graze on fleshy algae (Privitera et al., 2008), encrusting algae showed a positive relationship to the presence of sea urchins. Although many studies have been demonstrate that herbivorous fishes may also influence the structure of the benthic assemblages (Vergés et al., 2009; Gianni et al., 2017), we did not consider them because there are not evidences of the effect of protection on the abundance of the unique herbivore fish in this area, *Sarpa salpa* (Prado et al. 2007). Moreover, their effects on macroalgal beds in the studied area are weaker than the sea urchins one (Hereu, 2006; Hereu et al. 2008). This could be explained by the feeding preference and behavior of *Sarpa salpa*, the only strictly herbivorous fish in this area (Sala and Boudouresque, 1997). *S. salpa* has shown preference for high palatable plants such as seagrasses or fleshy algae (Vergés et al., 2009; Ali et al., 2017). In addition, fishes feeding behavior differ from the sea urchins as fishes bite the leaf while sea urchins graze the entire thalli which could deplet large algal extensions (Hereu, 2006; Jadot et al., 2006).

No-take marine reserves can indirectly restore the original trophic cascades recovering the abundance of herbivores' predators (Sala and Giakoumi, 2017) and controlling the herbivore populations (Medrano et al., 2019a). Results of this study go one step further in the trophic cascade of the studied area and also demostrate that No-Take marine reserves can influence the abundance of the canopy-forming *Cystoseira sensu lato* species, being more abundant in protected areas (as reported for the same studied NTZ in Sala et al., 2012). Without losing sight of the large variability observed in the cover of perennial canopy-forming *T. elegans* over time suggesting a high influence of local conditions on this species, this results reinforces the usefulness of marine reserves as conservation tools also at lower trophic levels, which is particularly relevant when considering the global decline of *Cystoseira sensu lato* species in the Mediterranean Sea (Thibaut et al., 2005; 2014a). Despite *Cystoseira sensu lato* species were not the dominant species in the macroalgal beds of the Natural Park, their loss or replacement could have major consequences for many associate organisms (e.g., lowering fish recruitment, Cheminée et al., 2013). On the other hand, *Codium vermilara* showed completely opposite ordination relative to the main drivers and stressors than *Cystoseira spp*. This result support the hypothesis of *C. vermilara* could have been replaced by

*Cystoseira sensu lato* species assemblages after their historical decline in the NW Mediterranean Sea (Ricart et al., 2018).Regarding the less abundant species, our study confirms the effect of protection for *A. armata, which* was already suggested by Sala and Boudouresque (1997). This unpalatable red alga was practically absent outside the NTZ, but showed a high variability over the fifteen years. The reverse pattern was observed for *W. penicillata* species, more abundant and less variable outside the NTZ. We did not detect the influence of any of the studied drivers in the filamentous turf forming *Ceramium ciliatum*, probably because of their short life cycle (Bologa et al., 1995).

Extreme climate events such as severe storms have been also considered as potential drivers eroding macroalgal beds (Navarro et al., 2011; Borja et al., 2018; Capdevila et al., 2019a). An exceptionally storm with drastic consequences on benthic communities occurred in the study area in 2008 (Mateo and Garcia-Rubies, 2012; Sanchez-Vidal et al., 2012), where we observed a short-term effect in the macroalgal communities exerted by the storm like those previously reported in other studies (Navarro et al., 2011; Michelli et al., 2016; Maggi et al., 2018). Benefiting from the available space that resulted from the storm, a rapid increase of highly seasonal *Dictyota* species were observed right after the storm (the year 2009), while most of the perennial species decreased. Over the following years, the abundance and structure of the main macroalgal assemblages recovered swiftly to pre-storm values and no long-term impacts were observed, indicating that impacts of a single extraordinary storm on rocky-shallow algal communities may be reversed relatively quickly. However, we should consider that an increase in the intensity and frequency of extreme climatic events, such as extraordinary storms, has been observed since 1950 and is expected to increase in the future (IPCC, 2014; Reguero et al., 2019), especially in the Mediterranean, which has been highlighted as a hotspot of ongoing climate change (IPCC, 2014, Cramer et al., 2018). Our results stress the importance of increasing spatial and temporal scales to better understand the natural variability of the marine communities and do not misunderstand the changes observed in algal assemblages (Lindenmayer et al., 2012). If the same dataset of this study would have been used to describe the consequences of this extraordinary storm right after the impact, notable differences could have been described.

Despite the relevance of monitoring algal communities, it is important to highlight some limitations of this study. In order to effectively document long-term changes of the macroalgal assemblages structure over time, we prioritize a broad view of the community by monitoring the algal cover of the main species in our study area and missing the minority species. This methodology allows to maintain long-term monitoring programs, involving different observers due to the easy identification of a reduced number of species but it is not the optimal ecological design in order to analyze other community indexes such as biodiversity or richness. Many studies pool species into functional groups, as a way to predict algal community composition (Steneck and Dethier, 1994). This reduction of species-specific information is generally accepted at the expense of a broader view of the changes in community structure. However, it is important to stress that this approach hinders to detect some important changes at the species level because different algal species have different responses to herbivores and other perturbations independently on their functional group (Hereu et al., 2008). Most of the results exposed here would have been lost or misinterpreted working with functional groups such as the high variability of *T. elegans*, which would have been pooled in the erect algae group.

A major insight of this study is the absence of major shifts in species replacement and abundance and the null effect of protection in the most important macroalgal communities in the rocky-shallow infralittoral zone of the studied area. Differences only regard the less abundant species, among them the canopy-forming *Treptacantha elegans* which took preference inside the protected NTZ. These results provide a current baseline of algal communities and contribute to the literature on the role of NTZs marine reserves in the benthic communities.

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# Chapter II

## No-take marine reserves control the recovery of sea urchin populations after mass mortality events

## Abstract

Understanding how no-take zones (NTZs) shape the population dynamics of key herbivores is crucial for the conservation and management of temperate benthic communities. Here, we examine the recovery patterns of sea urchin populations following a high-intensity storm under contrasting protection regimes in the NW Mediterranean Sea. We found significant differences in the recovery trends of *Paracentrotus lividus* abundance and biomass in the five years following the storm. The *P. lividus* populations outside the NTZ recovered faster than the populations inside the NTZ, revealing that predation was the main factor controlling the sea urchin populations inside the NTZ during the study period. *Arbacia lixula* reached the highest abundance and biomass values ever observed outside the NTZ in 2016. Our findings reveal that predation can control the establishment of new sea urchin populations and emphasize top-down control in NTZs, confirming the important role of fully protected areas in the structure of benthic communities

# 1. Introduction

Within marine protected areas, No-Take Zones (NTZs), where the exploitation of marine resources is not allowed, provide large-scale controlled areas for examining trophic interactions and cascade effects compared to natural ecosystems (Pinnegar et al., 2000; Guidetti, 2006). Previous empirical and observational studies within NTZs have demonstrated the role of herbivores, such as sea urchins, and their predators as major structuring forces in marine temperate benthic communities (Castilla and Durán, 1985; Shears and Babcock, 2002). As a result of prohibiting extractive activities, predation and top-down control increases as lower trophic levels decline, including sea urchins (Guidetti, 2006; Barrett et al., 2009; but see also Halpern, 2003).

Beyond the top-down control of predatory fishes, many processes, such as recruitment variability, spatial refuge availability and their escaping behaviour, influence sea urchin abundances (Sala and Zabala, 1996; Nishizaki and Ackerman, 2004; Hereu et al., 2012a; Oliva et al., 2016). Moreover, when sea urchin populations are established, adult individuals can also facilitate juvenile survival by reducing micro-predator abundance (Bonaviri et al., 2012) and offering refuge under their spine canopy for avoiding predation (Tegner and Dayton, 1977; Zhang et al., 2011; Hereu et al., 2012a).

A favorable combination of such processes can maintain dense sea urchin populations, even when they coexist with abundant fish predators within NTZs; this provides evidence of the existence of strong feed-back processes that provide resilience for sea urchin populations once they are established (Bonaviri et al., 2012; Ling and Johnson, 2012). In addition to biological interactions, other factors, such as hydrodynamic forces (Micheli et al., 2016), extraordinary storms (Sanchez-Vidal et al., 2012), sea urchin diseases (Girard et al., 2012; Clemente et al., 2014), or even human harvesting (Pais et al., 2011), can also determine the abundance of sea urchins at local scales.

To date, several studies have examined the long-term natural trends of established sea urchin populations (Lessios et al., 1984; Sala et al., 1998; Hereu et al., 2012a), where all of the processes affecting their dynamics occur together. Nevertheless, few studies have reported the rebuilding capacity of collapsed sea urchin stocks after extraordinary mass mortalities (Edmuns and Carpenter, 2001; Miller et al., 2003; Girard et al., 2012; Clemente et al., 2014; Lessios, 2016). Hence, little is known about the underlying mechanisms that drive their recovery and how conservation tools such as NTZs can influence and modulate these processes (Hunte and Younglao, 1988; Guidetti, 2006; Hamilton and Caselle, 2015).

To investigate the underlying mechanisms behind the recovery sea urchin populations and the role of fully protected areas in such recovery patterns, we used long-term monitoring data (nearly three decades) of sea urchin populations inside and outside the NTZ of the Montgrí, Illes Medes and Baix Ter Natural Park in the NW Mediterranean Sea.

In this area, the purple sea urchin, Paracentrotus lividus (Lamark), is the most abundant species and is considered the most important herbivore controlling the macroalgal dynamics on the shallow reefs (Sala and Zabala, 1996; McClanahan and Sala, 1997; Bulleri et al., 1999; Hereu, 2005). Although the black sea urchin, Arbacia lixula (Linnaeus), is also common, it is approximately one order magnitude less abundant than P. lividus on the Catalan coast (Hereu et al., 2012a). Despite its low density, A. lixula is considered a thermophilic species (Privitera et al., 2011; Wangensteen et al., 2013), and it has been suggested that climate change can increase its abundance and distribution (Francour et al., 1994; Wangensteen et al., 2012). Nevertheless, there is little quantitative evidence of these increasing patterns in the field to date. The abundance of both species did not show significant differences between contrasting protection levels from 1991 to 2010 (Hereu et al., 2012a). In December 2008, when an extraordinary storm occurred in this area (Mateo and Garcia-Rubies, 2012) that had drastic effects on the benthic communities (Sanchez-Vidal et al., 2012), a sea urchin population mass mortality event occurred (Hereu et al., 2012b; Pagès et al., 2013). The main goal of this study was to examine the recovery patterns of the devastated sea urchin populations under contrasting protection regimes. We hypothesize that top-down control could play a major role restricting the recovery of depleted sea urchin populations within the NTZ, while a fast recovery was expected outside the NTZ where the predation pressure is lower (García-Rubies et al., 2013). Since almost all of the adult individuals were lost after the storm, we anticipated that the population recovery would rely on recruitment and juvenile survival.

Due to the key role of sea urchins in structuring sublittoral communities in the Mediterranean Sea, understanding how fully protected areas can shape their population dynamics may be crucial for enhancing the effectiveness of conservation actions for temperate benthic communities.

# 2. Material and methods

## 2.1. Study site

The Montgrí, Illes Medes and Baix Ter Natural Park is located on the north-eastern coast of Catalonia, Spain (NW Mediterranean Sea, Fig. 1), covering a marine area of 2.037 ha. The park has its core in the No-Take Zone (NTZ) of the Medes Islands, which comprises the Archipelago of the Medes Islands (93 ha), where all fishing and harvesting activities have been prohibited since 1983 (García-Rubies and Zabala, 1990). Outside the NTZ, the nearby Montgrí coast is divided into a peripheral zone, a contiguous beltway zone to the Medes Islands that was established in 1990 where artisanal fishing and recreational angling are allowed under restriction, and a no reserve zone, where artisanal, recreational and spearfishing are allowed (Fig. 1). The total fish biomass and abundance of the apex predators and carnivores have remained significantly higher in the NTZ than in the two areas where some type of fishing is allowed, with no differences between them (Sala et al., 2012; García-Rubies et al., 2013; Hereu et al., 2017). Because there were no differences in the fish abundance and biomass between the peripheral zone and the no-reserve zone, both of the areas were considered outside the NTZ in this study. Accordingly, a higher fish biomass of the main sea urchin predatory species (Table 1) and higher sea urchin predation rates have been observed inside the NTZ in recent years (Sala, 1997; Hereu et al., 2005, 2012a).

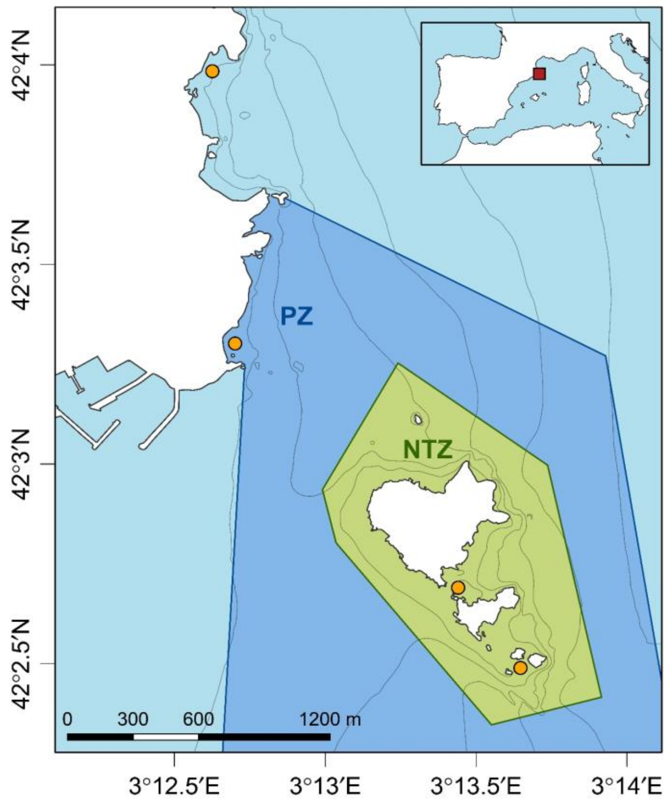

**Figure 1**. Map of the study locations in the Montgrí, Illes Medes and Baix Ter Natural Park in the NW Mediterranean Sea. Orange dots represent the long-term monitoring sites: two are located outside the NTZ, and two more are inside the NTZ. The perimeter of the Medes Islands NTZ is delimited by the green polygon, and the peripheral zone (PZ) is delimited by the blue polygon. No reserve zone is outside the green and blue polygons.

**Table 1.** Density and biomass (Mean ± SE) of the main *P. lividus* predators vulnerable to fisheries inside and outside the Medes Islands NTZ from 2009 to 2016 (Hereu et al., 2017).

| Species | Density (Ind/500m$^2$) | | Biomass (kg wet mass/500m$^2$) | |
|---|---|---|---|---|
| | NTZ | No reserve | NTZ | No reserve |
| ***Spariadae*** | | | | |
| *Diplodus sargus**<br>*main predator of juveniles and adult sea urchins (>10mm) (Sala and Zabala, 1996; Sala, 1997; Hereu et al., 2005) | 12.4 ± 1.7 | 7.9 ± 1.9 | 3.1 ± 0.5 | 1.5 ± 0.4 |
| *Diplodus vulgaris* | 29.9 ± 6.2 | 17.2 ± 3.7 | 3.6 ± 0.9 | 1.3 ± 0.4 |

## 2.2. Long-term monitoring

We monitored four sea urchin populations inhabiting extensive reef areas (150 m$^2$) with the same topography that is dominated by large limestone boulders in the natural park yearly beginning in 1991 (Fig. 1). Two of the four studied populations were located in the Medes Islands NTZ (sites coordinates: 42°2.497′N; 3°13.629′E and 42°2.658′N; 3°13.479′E), and the other two were located outside the NTZ in the nearby coast: one located inside the peripheral zone (42°3.313′N; 3°12.721′E) and one in the no-reserve zone (42°3.952′N; 3°12.591′E).

During the first monitoring years, the sea urchin population densities remained similar inside and outside the NTZ despite the higher fish abundances observed in the NTZ throughout the study period (Hereu et al., 2012a). In December 2008, an extraordinary storm struck the Catalan coast, with maximum wind speeds up to 20 m/s, significant wave heights as great as 8 m, record maximum wave heights in excess of 14 m, and wave periods up to 14 s. This storm was categorized as a highly extreme event with a return period of more than 100 years (Sanchez-Vidal et al., 2012). Substantial negative effects on the benthic populations and communities inhabiting soft and hard bottoms (e.g., macroalgae, gorgonians, seagrass meadows and sea urchins) from shallow to deep waters were documented after the storm (Mateo and García-Rubies, 2012; Sanchez-Vidal et al., 2012; Pagès et al., 2013). The impact of these forces on the sea urchin populations rendered a more than 80% loss of individuals and 90% of the biomass and levelled the sea urchin populations between the areas inside and outside the NTZ. The larger specimens of *P. lividus* were the most affected, with consequent changes in their size frequency distribution (Hereu et al., 2012a; b).

### 2.3. Sampling methodology

To describe the medium-term (5 yrs) recovery patterns of sea urchin populations inside and outside the NTZ after the 2008 mass mortality event on the *P. lividus* and *A. lixula* populations under different protection regimes, we extended the annual monitoring until 2016. Although the abundances of *Arbacia lixula* were very low in our study area for the duration of the monitoring, we also evaluated their temporal trend to detect any clear pattern in this species.

The sea urchin species, abundance and size were recorded in three 50 $m^2$ transects at 6 m depth in each study site. As described in Hereu et al. (2012a), transects were divided into five 10 $m^2$ sub-transects, and within each transect, we counted and measured the diameter of all the present sea urchins. The sea urchin biomass was calculated using the allometric length-weight relationship, $W = a(TD)^b$, where W is the wet weight (in g) and TD (in cm) is the measured sea urchin test diameter. Biomass of *P. lividus* was estimated as $W = 0.00319(TD)^{2.479}$(Ling et al., 2015) and, *A. lixula* biomass was estimated as $W = 0.8467(TD)^{2.6042}$ (Ballesteros, 1981).

### 2.4. Data analysis

To detect potential structural changes in the *P. lividus* populations due to the 2008 storm, the mean abundance per year was fitted into sequential linear regressions. Every potential change point in the data series was assessed by computing the residuals of the linear models and analysing the F statistics using the R package ‘strucchange’ for R (Zeileis et al., 2003).

We used generalized linear mixed models (GLMMs) to determine differences in the medium-term (5 yrs) recovery patterns of the sea urchin populations between the NTZ and the non-protected area. The *P. lividus* densities and biomass in the five subsequent years (2009–2013) were fitted as the dependent variables. The explanatory variables time (years) and protection level (inside/outside the NTZ) were included as fixed effects, and the sample sites nested to the protection level were a random effect. The data distribution was visually inspected by fitting the response variable to the most likely distribution (Fig. 1, S3), and the negative binomial GLMMs with a logarithm link function were conducted using the package ‘lme4’ for R (Bates et al., 2015).

Because of the low abundance and biomass of *Arbacia lixula*, which were an order of magnitude lower than those of *P. lividus*, we did not statistically examine the recovery patterns of this species.
All the statistical analyses were performed with R version 3.3.3 (R Core Team, 2017).

## 3. Results

The abundance and biomass of *P. lividus* reached the lowest values ever recorded after the storm of 2008 (Figs. 2 and 3). Despite the short-term effects of the mortality event, we found significant differences in the recovery trends of the *P. lividus* abundance and biomass over the following five years under the different protection regimes (Table 2 and Fig. 3). Sequential F tests and the critical F values identified a shift in the *P. lividus* populations inside the NTZ after the 2008 storm, resulting in a significant reduction in the population abundance from that time on (Fig. 2).

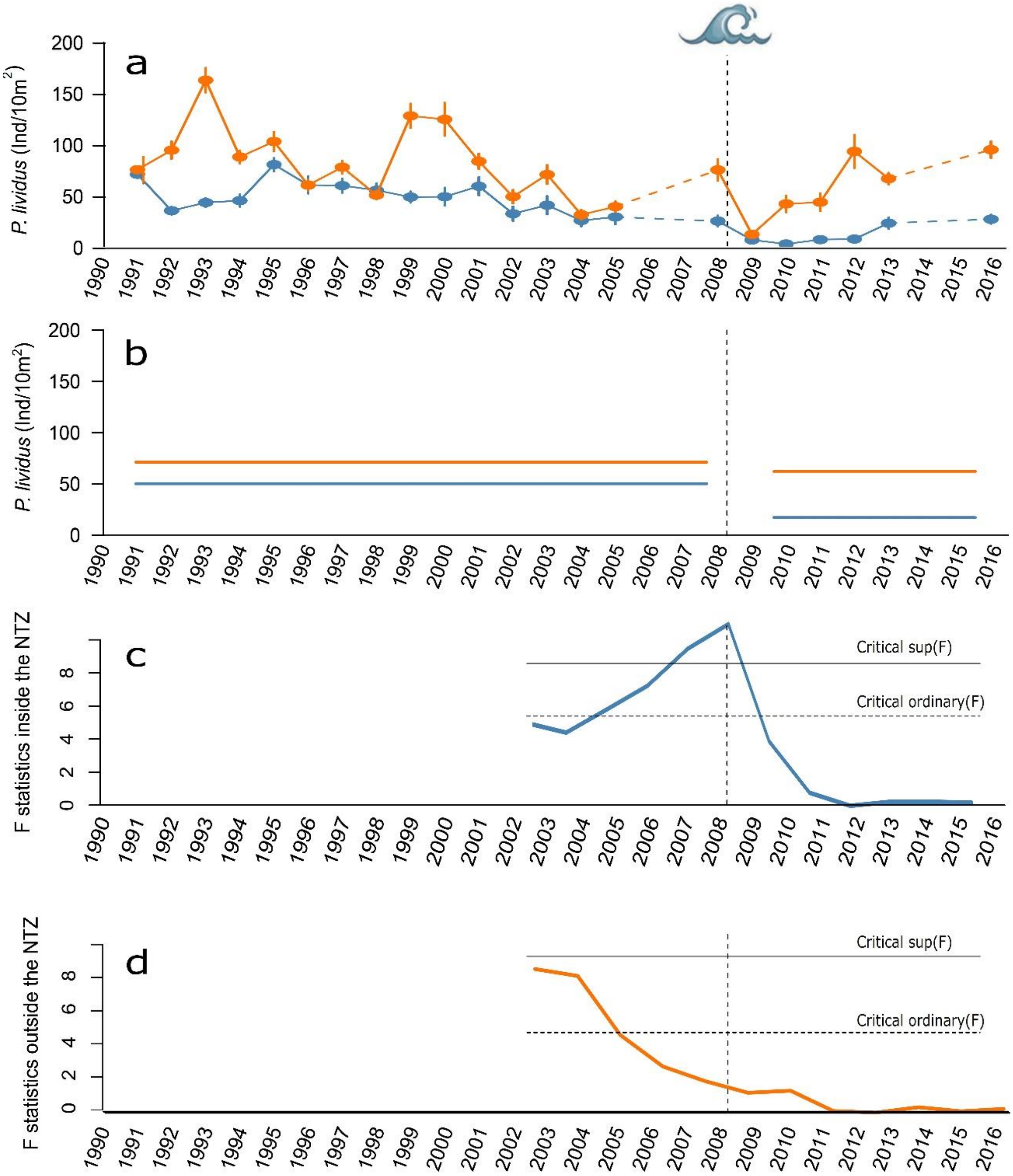

**Figure 2.** *Paracentrotus lividus* abundance over time. Blue lines represent the values inside the NTZ, and orange lines represent the values outside the NTZ. (a) The density of *P. lividus* per 10 m$^2$ (Mean ± SE) since 1990. (b) Mean values of the *P. lividus* density before (average density from 1991 to 2008) and after the extraordinary storm (average density from 2010 to 2016). (c–d) Change-point analysis by sequential F tests with the proper critical F levels. (c) Identified change point after the 2008 storm inside the NTZ, and (d) the absence of a change point after the storm outside the NTZ.

**Table 2**. Results of the generalized linear mixed models (GLMMs) testing differences in the *P. lividus* density and biomass between different protection levels (inside and outside the NTZ) and in the five subsequent years after the storm (2009–2013). To select the maximum likelihood model, we used Akaike's information criteria (AIC). According to Burnham and Anderson (2003), the model with the lowest AIC value was considered the most appropriate, which is indicated in bold.

| **Models** | **Variables** | **Coefficients** | | | | **AICs** |
|---|---|---|---|---|---|---|
| | | Estimate | Std. Error | *z value* | *p* | |
| Density ~ Protection*Years + (1\|Protection:Site) | Intercept | 3.7138 | 0.5421 | 6.851 | **<0.001** | 2104.3 |
| | Protection (MPA) | -2.0464 | 0.7673 | -2.667 | **0.0076** | |
| | Year | 0.6540 | 0.0842 | 7.768 | **<0.001** | |
| | Protection*Year | -0.3182 | 0.1132 | -2.811 | **0.0049** | |
| Density ~ (1\|Protection: Site) | Intercept | 2.8035 | 0.6739 | 4.16 | **<0.001** | 2169.3 |
| Biomass ~ Protection*Years + (1\|Protection:Site) | Intercept | 5.9833 | 0.259 | 23.101 | **<0.001** | 3447.3 |
| | Protection (MPA) | -1.6393 | 0.3645 | -4.497 | **<0.001** | |
| | Year | 0.9989 | 0.1103 | 9.057 | **<0.001** | |
| | Protection*Year | -0.7008 | 0.1454 | -4.820 | **<0.001** | |
| Biomass ~ (1\|Protection:Site) | Intercept | 5.3857 | 0.5519 | 9.759 | **<0.001** | 3520.8 |

In contrast, outside the NTZ, both the *P. lividus* abundance and biomass started to recover one year after the mortality caused by the storm, and the biomass increased fourfold in two years, from the lowest value of 119.7 ± 28.3 g/10 m$^2$ in 2009 to 445.6 ± 83.7 g/10 m$^2$ (mean ± SE) in 2011 (Fig. 3). The non-protected populations displayed a fast recovery in density in 2010 (Fig. 3), which was consistent with the high recruitment peak observed that year (61.6% of the population, Fig. 4). From these new sea urchins, the non-protected populations began to grow, reaching a similar size structure as that before the storm in 2013 (Fig. 4).

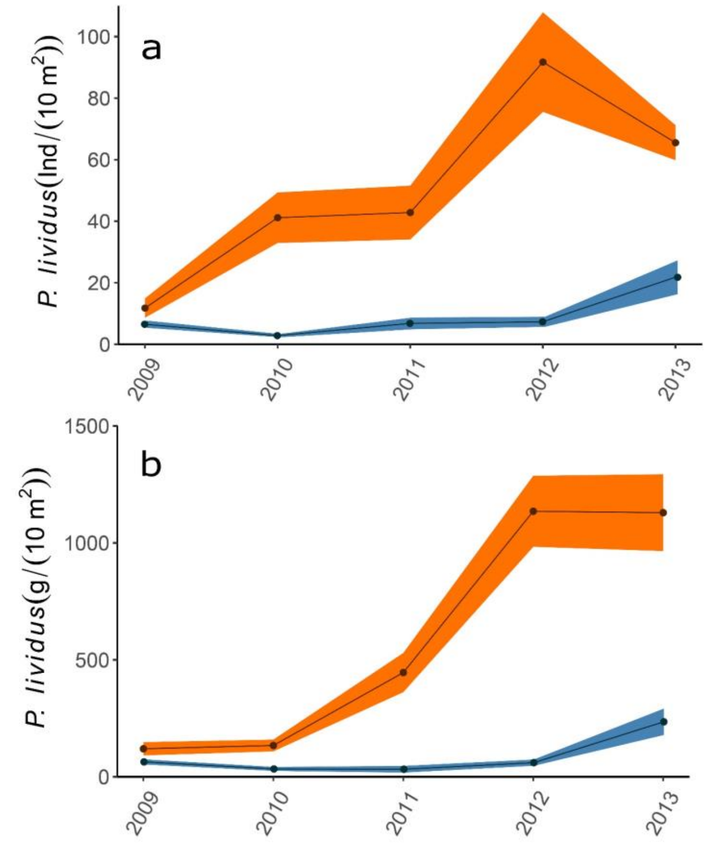

**Figure 3.** Recovery patterns of *Paracentrotus lividus* in abundance (a) and biomass (b). Black points correspond to the mean, and the colored areas correspond to the SE. The blue colour represents the values inside the NTZ, and orange represents the values outside the NTZ.

In 2016, eight years after the storm, the *P. lividus* abundance and biomass in the non-protected sites were higher than the pre-storm values in 2008 and higher than those observed inside the NTZ (Figs. 2 and 3).

In contrast, inside the NTZ, the *P. lividus* abundance and biomass continued decreasing for some of the years after the 2008 mass mortality event, reaching the lowest biomass values three years after the storm (40.8 ± 15 g/10 $m^2$ in 2011). The sea urchin biomass in the NTZ started to recover in 2013 (255.2 ± 59.6 g/10 $m^2$), and 5 years after the storm, the biomass and density values were far from the pre-storm year values (Fig. 2). No recruitment peak was observed inside the NTZ until 2011, when the populations were dominated by small individuals (> 80% of the sea urchins were < 4 cm in diameter, Fig. 4).

The abundance of the *Arbacia lixula* populations was lower than that of *P. lividus*, but it also dropped as a consequence of the 2008 storm. Nevertheless, the *A.*

*lixula* populations did not show significant contrasting short-term recovery patterns under different protection regimes, which was observed in *P. lividus*. The abundance and biomass of *A. lixula* outside the NTZ reached the highest values ever observed in this area in 2016. In contrast, inside the NTZ, the recovery was slower, and in 2016, the density values were similar to those observed before the mass mortality event (Fig. 5a–b).

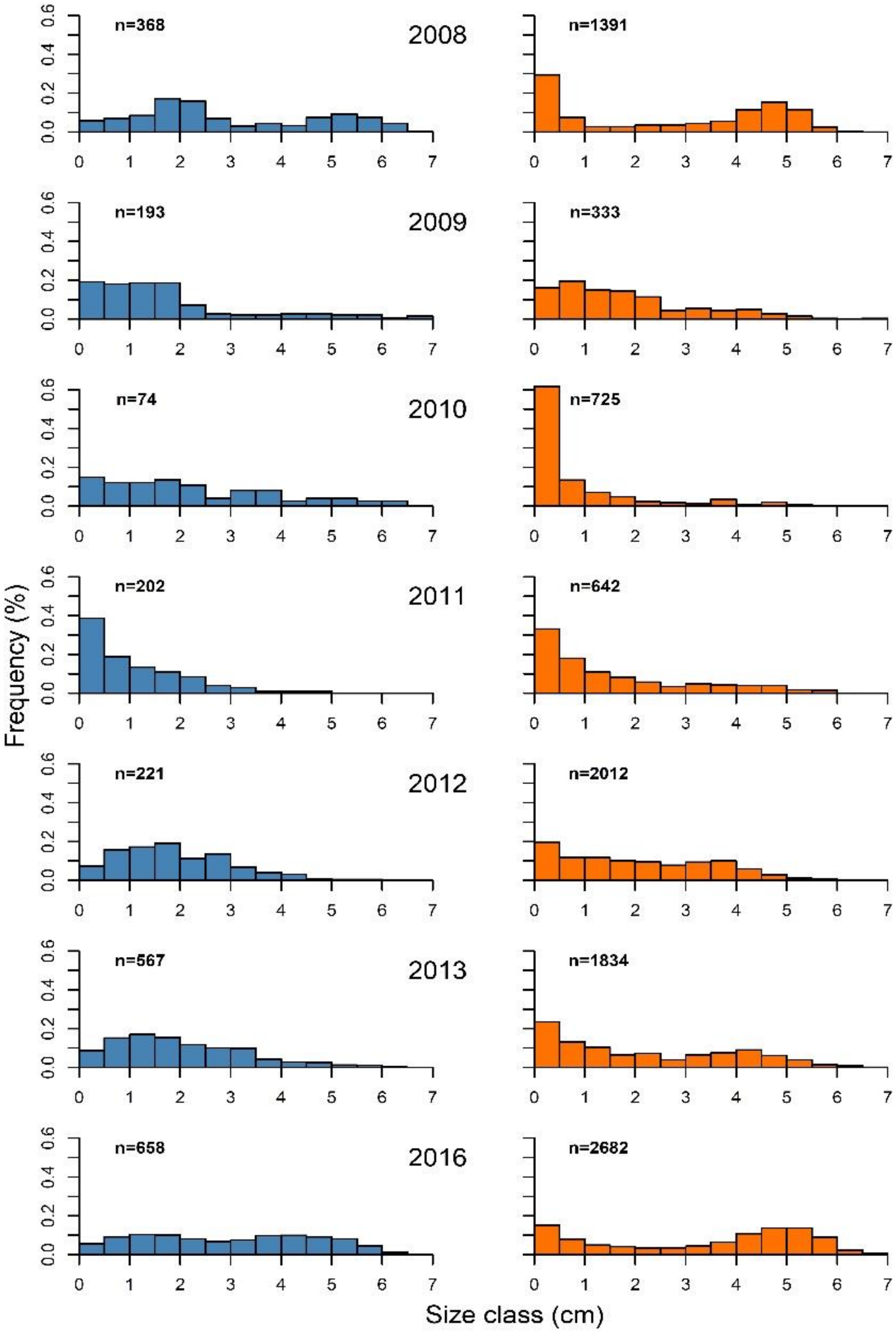

**Figure 4.** Size class distribution of *Paracentrotus lividus* before the extraordinary storm (2008) and in the years following the storm (2009–2016). The blue colour represents the frequency distribution inside the NTZ, and orange represents the frequency distribution outside the NTZ.

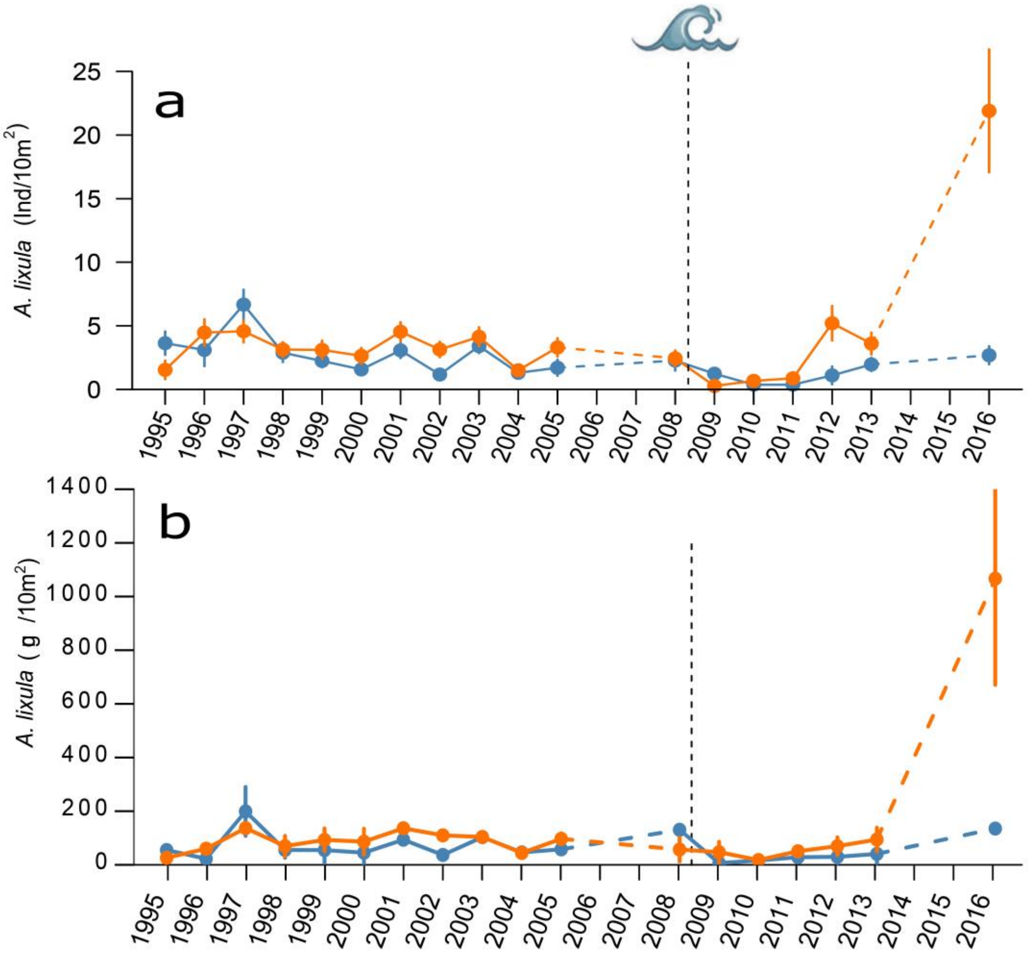

**Figure 5**. *Arbacia lixula* abundance (a) and biomass (b) since 1995. Coloured dots represent the mean per 10 m$^2$, and bars represent the SE. The blue colour represents the values inside the NTZ, and orange represents the values outside the NTZ.

## 4. Discussion

In this study, we tested the effects of a NTZ on the recovery process of a key herbivore following a high-intensity storm in a temperate benthic community. We conclude that the sea urchin *Paracentrotus lividus* populations outside the NTZ recovered faster than the populations inside the NTZ, revealing that predation is the main factor controlling the recovery of sea urchin populations in the NTZ. This is consistent with many studies that also demonstrated the role of top-down control as a major structuring force in benthic communities in other temperate systems (Shears and Babcock, 2002; Guidetti, 2006; Halpern et al., 2006; Clemente et al., 2011).

After the almost complete depletion of adult *P. lividus* populations due to the dramatic storm in 2008 and given that the foundation and recovery of benthic populations relies on the interaction between settlement and post-settlement mortality, we expected differences in one or both of the processes between the protected and non-protected areas to fully understand the contrasting recovery patterns observed.

The settlement of *P. lividus* is decoupled from local populations and can be highly variable at small scales given the long planktonic stage of sea urchin larvae (Hereu et al., 2004, 2012a). Therefore, the arrival of new individuals is not expected to explain the differences between the protected and non-protected areas, suggesting a key role of post-settlement mortality to fully understand the contrasting recovery patterns observed between the different protection levels.

In fact, the high juvenile predation rate (Hereu et al., 2005) and the absence of adult individuals that facilitate juvenile survival inside the NTZ may explain the slow sea urchin population recovery within the fully protected area. After the depletion of sea urchin populations in 2008, the size structure of the *P. lividus* populations inside and outside the NTZ changed dramatically. When the recovery began, unimodal size distributions dominated by small sizes were observed in both areas because of recruitment pulses. Although the settlement episodes were simultaneous both inside and outside the NTZ, the recruitment peak observed in 2010 outside the NTZ was not observed in the NTZ, suggesting high predation control within the NTZ. After the 2010 recruitment peak, the sea urchin populations outside the NTZ began to recover, showing immediate increases in their abundance, size and biomass according to the reported growth rates for this species (Turon et al., 1995; Ouréns et al., 2013). In addition, the annual arrival of new settlers and the low predation rate maintained the smallest size class as the most frequent class outside the NTZ over time (Sala and Zabala, 1996; Hereu et al., 2012a). Inside the NTZ, the recovery of adult individuals was slower, as it was determined by the lower survival of juveniles.

Adult *P. lividus* specimens were established in populations in both protection regimes, i.e., inside and outside the NTZ, at different times: in 2013 outside the NTZ and in 2016 inside the NTZ. In 2016, the population structures were characterized by a bimodal distribution, with one mode in the adult sea urchin size class in both areas and another in the juvenile size class, which is typical of sea urchin populations under a certain degree of predation pressure (Sala and

Zabala, 1996), but with the majority of the smallest size classes in the non-protected area due to the highest post-settlement mortality inside the NTZ. These observed patterns support that top-down control is crucial for determining the sea urchin population structure within the NTZ and reaffirm the importance of adults in maintaining sea urchin populations. When sea urchins reach the refuge size from their predators (Sala and Zabala, 1996), they facilitate post-settlement survival by conferring protection from predation (Tegner and Dayton, 1977; Ouréns et al., 2014), eliminating micropredator grazing (Bonaviri et al., 2012), and protecting them from environmental and hydrodynamic forces (Nishizaki and Ackerman, 2004).

Before the extraordinary storm, the similar abundance and biomass trajectories observed over the years between the sea urchin populations of both protection regimes reinforce that factors other than top-down control can be important over large scales, as was also revealed in Guidetti and Dulčić (2007) and in Bonaviri et al. (2012).

In 2008, the *P. lividus* densities reached the lowest values inside the NTZ throughout the long-term monitoring until the storm. This together with the time scale could be crucial in the observed responses of long-term studies (Babcock et al., 2010), and we think that predation could have become the major controlling force of sea urchin populations in- side the NTZ regardless of the storm on a timescale longer than the one studied.

The *Arbacia lixula* populations showed a similar recovery pattern to that of the *P. lividus* populations, as the density and biomass values outside the NTZ increased in 2012. In addition, *A. lixula* reached densities and biomasses never observed in this area outside the NTZ in 2016. *A. lixula* is a thermophilic species that is more abundant in the southern and eastern areas of the Mediterranean Sea. Thus, such an upward trend seems to be linked to the warming trends observed on the Catalan coast in recent decades, where the temperature has increased 0.26 ± 0.08 °C/decade from 1985 to 2015 (Vargas-Yáñez et al., 2017), and is probably enhanced by the lack of predation pressure when fishing is allowed. In addition, the preliminary results on the *A. lixula* growth rate (Barrera, 2018) have shown the faster growth of newborn *A. lixula* compared with *P. lividus*, which is suggested as a win-win strategy for *A. lixula* under expected global change conditions.

The coexisting sea urchins *P. lividus* and *A. lixula* have different diets and foraging activities (Wangensteen et al., 2011; Agnetta et al., 2013), and it has been described that *P. lividus* has a preference for fleshy algae and that *A. lixula* preferentially graze on encrusting coralline algae (Privitera et al., 2008), having an important role in the maintenance of sea urchin barrens once they are established (Agnetta et al., 2015). In addition, a wider area can be impacted by *A. lixula* grazing activity than by *P. lividus* activity due to its higher mobility on barren zones (Bonaviri et al., 2011). Therefore, the increase of this species may have large consequences for macrophyte-dominated communities.

Physical disturbances and interactions among multiple stressors acting at local and global scales can drive important changes in the structure and function of marine populations and communities. Several studies to date have demonstrated that severe storms and extreme waves can trigger mass mortalities in sea urchins populations (Scheibling and Lauzon-Guay, 2010; Scheibling et al., 2010) and have important effects on rocky shore communities in general (Denny et al., 2009; Micheli et al., 2016; Borja et al., 2018). An increase in the intensity and frequency of extreme climatic events, such as extraordinary storms (as observed in our study), has been observed since 1950 and is expected to increase in the future (IPCC, 2014; Reguero et al., 2019), especially in the Mediterranean, which has been highlighted as a hot- spot of ongoing climate change (IPCC, 2014; Cramer et al., 2018).

Our findings reveal that predation can control the establishment of new sea urchin populations and emphasize top-down control in NTZs. These results confirm the important potential role of protected zones, those areas fully protected from fishing, in the structure of benthic communities (Sangil et al., 2012a; Sala and Giakoumi, 2017). Additionally, the contrasting patterns regarding different levels of protection observed in this study highlight the relevance of well-designed long-term monitoring to better understand the natural variability of sea urchin populations and to discern the underlying mechanisms when mass mortality events occur. Long-term monitoring also provides useful insights into the management and conservation of algal-dominated benthic ecosystems.

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# Chapter III

# Expansion in times of decline: *Treptacantha elegans* ecology, genetic diversity, and distribution in the Catalan Coast

## Abstract

Widespread decline of canopy-forming macroalgal assemblages has been documented in many regions during the last decades. This pattern is often followed by the replacement of structurally complex algal canopies by more simplified habitats (e.g., sea urchin barren grounds). Against all odds, the fucoid *Treptacantha elegans*, a large Mediterranean brown macroalga, has broadened its distributional range and displayed an unexpected expansion along the northern coast of Catalonia over the last two decades. Here, we reconstructed its temporal and spatial spread and unraveled ecological traits mostly unknown such as population dynamics and genetic patterns to provide a comprehensive and integrated view of the current status and geographical expansion for this species. Fast-growing dynamics, early fertile maturity, and high turnover rate are the main competitive advantages that allow deep *T. elegans* populations to colonize available substrata and maintain dense and patchy populations. Besides, we also provided evidence that deep *T. elegans* populations across the Catalan coast constitute a single genetic group with little differentiation of populations. This may support the idea of a unique source of spread in the last decades from the Medes Islands No-Take Zone towards both southern and northern waters.

## 1. Introduction

How marine populations persist, evolve, and change their geographical distributions as a response to global change is one of the main questions in contemporary ecology (Koehn et al., 2011; Munday et al., 2013). Species may redistribute to keep their preferred environmental conditions (Perry et al., 2005; Wernberg et al., 2011b; Poloczanska et al., 2013; Pecl et al., 2017) or adapt their physiology as a response to global and local stressors (Harley et al., 2012; Poloczanska et al., 2013). Seaweeds are dominant primary producers in benthic habitats with a key role in ecosystem functioning (Wernberg et al., 2011a; Wahl et al., 2015). As a response to global change, they may show both geographical expansions, when species colonize new habitats, or contractions, when populations disappear from areas previously inside their distributional range (Wernberg et al., 2011b; Bates et al., 2014).

In the Mediterranean Sea, *Cystoseira sensu lato* fucoids (here used to designate Atlantic-Mediterranean fucoid species of *Cystoseira sensu stricto*, *Carpodesmia* and *Treptacantha*, Orellana et al., 2019) are late successional species and among the major canopy-forming seaweeds. They provide three-dimensional structure, food, and shelter to many associated species (Cheminée et al., 2013; Blanfuné et al., 2016). Decline of these species may drive a decrease in habitat complexity with important consequences for benthic biodiversity and ecosystem functioning (Thibaut et al., 2005, Mangialajo et al., 2008; Thibaut et al., 2015; Mineur et al., 2015; Blanfuné et al., 2016). All *Cystoseira* species, except *C. compressa*, appear in the Annex II of the Barcelona convention (2010) among those taxa that are considered threatened or endangered and need protection measures (Gianni et al., 2013). *Cystoseira sensu lato* show limited dispersal abilities (Gianni et al., 2013; Capdevila et al., 2018), which support the existence of monospecific stands near the parent populations (Mangialajo et al., 2012). This characteristic and the influence of other environmental factors may explain why many populations of these macroalgae are so poorly connected in many areas of the Mediteranean Sea (Buonomo et al., 2018; Mariani et al., 2019) a trait that seriously jeopardizes their conservation. Nonetheless, the few studies on population genetics suggest that water currents play a significant role in long-distance dispersal for *Cystoseira sensu lato* populations (Susini et al., 2006; Thibaut et al., 2016a; Buonomo et al., 2017). Thus, despite the well-documented general decline of local populations

(Thibaut et al., 2005; 2014a; 2015) evidences for stability and expansion have been also reported in some areas (Thibaut et al., 2014a; 2016b; Iveša et al., 2016).

The Mediterranean endemic *Cystoseira elegans* (Sauvageau) Orellana & Sansón has very recently assigned to the genus *Treptacantha* (Orellana et al. 2019). It was first described by Sauvageau (1912; pp. 292) for Banyuls-sur-Mer (South-Eastern France) where the species formed extensive stands at the innermost rocky bottoms of coves and other shallow environments characterized by low to medium water movements (Feldmann, 1937). Although recent studies have reported important regression in shallow environments (Thibaut et al., 2005; 2015; Mariani et al., 2019), an exceptional expansion of *T. elegans* (identification by M. Verlaque, personal communication) has been observed in the Medes Islands No-Take Zone (NTZ) and in nearby areas (North-Western Mediterranean Sea) across deeper habitats (between 5-20 m depth). As a result, there is strong evidence that *T. elegans* has been spreading along the coast of Catalonia in the last decades, clearly benefitting from some unknown environmental factor or a possible change in its fitness.

Here we aim at describing this recent spread throughout the North Catalan coast. Concretely, our goals are: (1) provide insights about the main traits of deep *T. elegans* specimens and the species population ecology; (2) assess the species historical and current distribution in the Catalan coast; and (3) provide an estimate of the patterns of genetic diversity and differentiation among populations including the ancient and recent expansion zones.

## 2. Materials and Methods

### 2.1 Phenology and demographic data

To untangle their population dynamics, three deep *Treptacantha elegans* populations from the Medes Islands NTZ were annually surveyed in early summer during the seaweed maximum development period. All sites were selected to show physical and biological characteristics as similar as possible. Two permanent plots of 1 and 1.5 $m^2$ were placed between 5 and 10 m depth at each site. A total of 684 specimens were mapped and individually monitored yearly from 2017 to 2019.

To accurately describe the reproductive period, 20 *T. elegans* individuals covering a wide size range (2-23 cm) were monthly sampled from Medes Islands NTZ for one year to determine the presence and maturity of receptacles of the alga in the laboratory. We categorized the maturity status within three stages: 1) immature individuals without receptacles; 2) individuals beginning their reproductive period, showing receptacles but still immature; 3) fertile and reproductive individuals.

Measurement of the main axis was reported as a good indicator in morphometric studies of other *Cystoseira* species (Ballesteros et al., 2009). However, as *T. elegans* is characterized by a tiny main axis (1-3 cm height, Cormaci et al., 2012), this trait hinders accurate measurements *in situ* unless invasive techniques are applied. To discern the best size indicator for *in situ* measurements, we collected 27 specimens and measured several morphometric parameters. Correlation analyses between the parameters were done (Table 1 S4) and the maximum height of the longest axis was selected as the best parameter. Then, all specimens inside the plots were measured using a caliper by SCUBA divers. We calculated population density and size structure pooling the individuals in 1 cm size intervals (Ballesteros et al., 2009; Capdevila et al., 2015; Verdura et al., 2018). Yearly transition measures within the studied period were used to estimate the mortality and recruitment rates. Recruits were identified as new individuals appearing in the permanent plots regardless of their size. Mortality rates for each size class were compared using a generalized linear model (GLM) fitted with a negative binomial distribution and a logarithm link function after visually checking the most likely distribution of the data and residuals (McCullagh and Nelder 1989). GLM analyses were conducted using the package "MASS" for R software (Venables and Ripley 2002) with R version 3.3.3 (R Core Team, 2017).

## 2.2 Spatial and temporal distribution

Past and present distribution of shallow and sheltered *Treptacantha elegans* in the Catalan coast has been recently reported by Mariani et al. (2019). Although descriptive studies have been carried out in the studied shorelines since the seventies (Ballesteros et al., 1984a; b), *T. elegans* was not reported for deeper and more exposed environments until the nineties. The first occurrence for the species (as *Cystoseira spinosa*; see Mariani et al. 2019) on infralittoral habitats from Catalonia was reported for the Medes Islands by Sala (1996). All available information about the spatial and temporal distribution of this species along the Catalan coast is reported in Table 1.

**Table 1**. Details of historical information on *T. elegans* compiled for the present study.

| Locality | Distance from Medes Islands (Km) | GPS coordinates | Year of first observation | Reference | Type of document | Previous studies in the area? | Current distribution |
|---|---|---|---|---|---|---|---|
| Ferranelles | 0 | 42.04195; 3.22577 | 1996 | Sala, 1996 | Article | Yes | Present |
| Tascons | 0 | 42.0416 ; 3.2268 | 1996 | Sala, 1996 | Article | Yes | Present |
| Freueto | 0 | 42.0446 ; 3.2241 | 1996 | Sala, 1996 | Article | Yes | Present |
| Falaguer | 2 | 42.0659 ; 3.2096 | 1996 | Sala, 1996 | Article | Yes | Present |
| Cova de la Reina | 0 | 42.0464 ; 3.2244 | 1998 | Hereu et al., 2008 | Article | Yes | Present |
| Sant Estiu | 0 | 42.0428 ; 3.2253 | 1998 | Hereu et al., 2008 | Article | Yes | Present |
| Raco Portitxol | 0 | 42.0434 ; 3.2241 | 1998 | Hereu et al., 2008 | Article | Yes | Present |
| Salpatxot | 0 | 42.0492; 3.2226 | 2001 | Medrano et al., 2019b | Article | Yes | Present |
| P.Deu | 0 | 42.0507 ; 3.2245 | 2001 | Medrano et al., 2019b | Article | Yes | Present |
| Molinet | 1 | 42.0555 ; 3.2120 | 2001 | Medrano et al., 2019b | Article | Yes | Present |
| Arquets | 1.2 | 42.0582 ; 3.2125 | 2001 | Medrano et al., 2019b | Article | Yes | Present |
| Dui | 1.7 | 42.0635 ; 3.2113 | 2002 | Medrano et al., 2019b | Article | Yes | Present |
| Embarcador | 0 | 42.0466 ; 3.2201 | 2003 | CEAB* | Report | Yes | Present |
| Aiguafreda, Begur | 9 | 41.9641 ; 3.2277 | 2010 | CEAB* | Report | Yes | Present |
| Messina, Cap de Creus | 29 | 42.2912 ; 3.3083 | 2011 | CEAB* | Report | Yes | Present |
| Baix de Cols | 7.5 | 42.1001 ; 3.1861 | 2011 | CEAB* | Report | Yes | Present |
| Llosa, Palamós | 25 | 41.8461 ; 3.1482 | 2012 | CEAB* | Report | Yes | Present |

(*) CEAB (Centre d'Estudis Avançats de Blanes; CSIC). Data have been collected since 1992 by the Macroalgae and invasive species monitoring group.

## 2.3 Molecular analysis

We collected twenty *Treptacantha elegans* samples in each of 3 locations along the Catalan coast, covering its range of distribution in the area (Fig. 1, locations: 1 Medes Islands NTZ, 2 Messina, and 3 Begur). The samples were dried in silica gel for DNA extraction and kept in separated bags for molecular analysis.

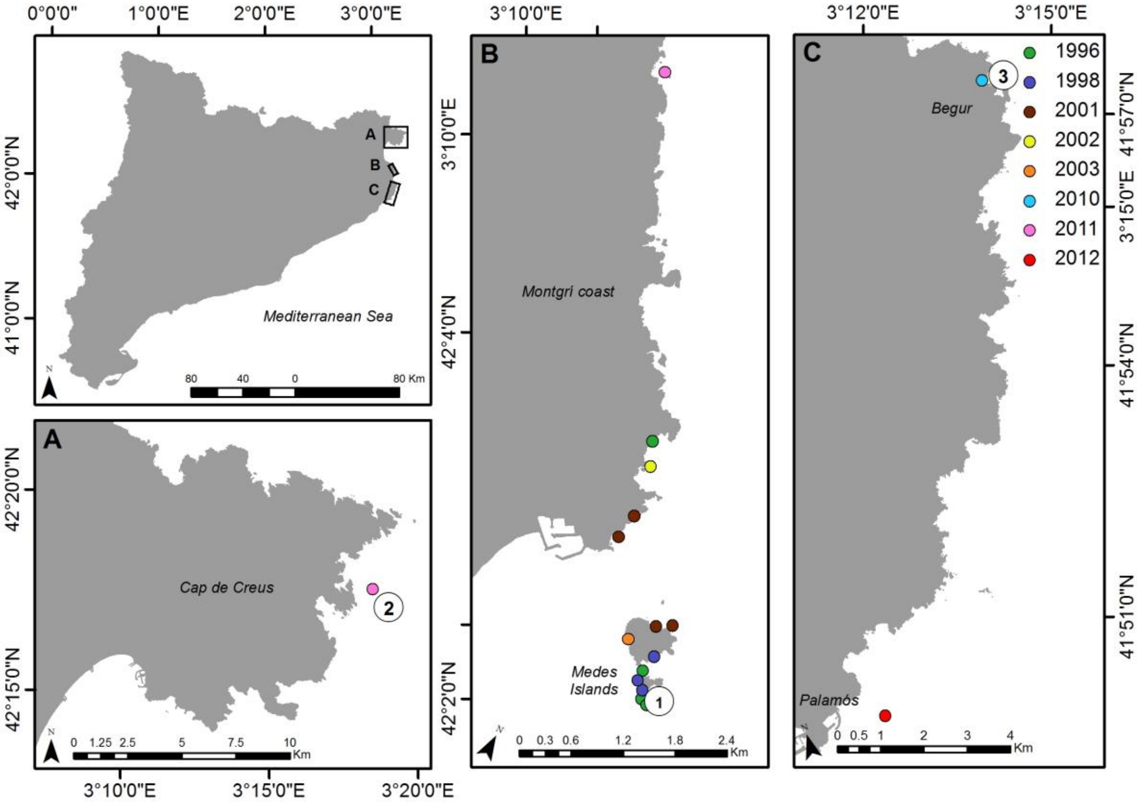

**Figure 1.** Geographical distribution of deep *Treptacantha elegans* over the studied years in the Catalan coast. Colored dots indicate the temporal sequence of their first report and circled numbers identify the populations where molecular analyses were conducted.

Genomic DNA for the three *T. elegans* populations was extracted from the dried samples using the NucleoSpin® Plant II kit (Macherey-Nagel Duren, Germany) according to the manufacturer's protocol. Eight unpublished microsatellite loci previously developed for *Cystoseira baccata* were used for genotyping. Polymerase chain reactions (PCRs) were performed in 15 μL total volume containing 1· GoTaq® Flexi buffer (Promega, Madison, WI, USA), 1.5 mm $MgCl_2$, 0.07 mm each dNTP, 0.4 μM of labelled (FAM, ATT0-550, ATT0-565 or HEX) forward primers, 0.4 μM of reverse primers, 1U GoTaq Flexi DNA Polymerase (Promega), and 5 μL of 1:100 diluted DNA template. In all PCRs, an initial denaturation step (95ºC, 2 min) was followed by 30 cycles of 95ºC for 30 s, a primer specific annealing temperature (Ta) for 30 s and 72ºC for 30 s, ending with a final elongation step at 72ºC for 5 min. Amplified fragments were separated using an ABI PRISM 3130 xl (Applied Biosystems, CCMAR, Portugal) automated capillary sequencer. Alleles were manually scored in STRand (Toonen and Hughes, 2001) using the GeneScan 500 LIZ dye size standard (Applied Biosystems).

Estimates of genetic diversity were calculated for each locus and population using GENETIX software v. 4.05 (Belkhir et al., 1996-2004). This included the mean number of alleles per locus (allelic richness), non-biased expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), number of private alleles, and inbreeding coefficient ($F_{IS}$).Pair-wise differentiation, as assessed with $F_{ST}$ (Weir and Cockerham, 1984), were calculated. Analyses of molecular variance components (AMOVA) were conducted using ARLEQUIN v3.1 (Excoffier et al., 2005).

Population structure was inferred using STRUCTURE v2.444 (Pritchard et al., 2000) considering admixture and no a priori population assignments. The correlated allele frequency model was run with a burning time of 250,000 repetitions, 1,000,000 iterations and considering a range of genetic clusters (K) from 1 to 5. The model was run 14 times for each K to check the consistency. The best K was estimated using the program Structure Harvester (Earl et al., 2012). All individuals with missing data at three or more loci were excluded (4 individuals).

## 3. Results

### 3.1 Phenological and ecological traits

Branches of *Treptacantha elegans* from deep habitats (5-15 m depth) begin to grow from the perennial basal disc (or holdfast) in early spring when sea water temperature starts to rise (Fig. 2). The branches are overall thicker and longer than those observed in specimens from shallow and sheltered environments (see Mariani et al., 2019 and Authors' personal observations). The receptacles located at the base of each spiny branch begin to mature progressively as branches approach their maximum development. Larger specimens may exceed 50 cm in height like in shallower specimens (Barceló et al., 2001). The reproductive period spans from the end of May to August, being fertile mainly when water temperature rises above 18ºC. In June and July, 90% of the sampled individuals showed receptacles (Fig. 2). The minimum size of fertile sampled individuals was 4.5 cm height. Like in the shallow *T. elegans* specimens, those from deeper habitats shed their branches after the reproductive period and remained attached to the substrate through the perennial holdfast until the following spring (Fig. 2) (Gómez-Garreta et al., 2001; Rodriguez-Prieto et al., 2013).

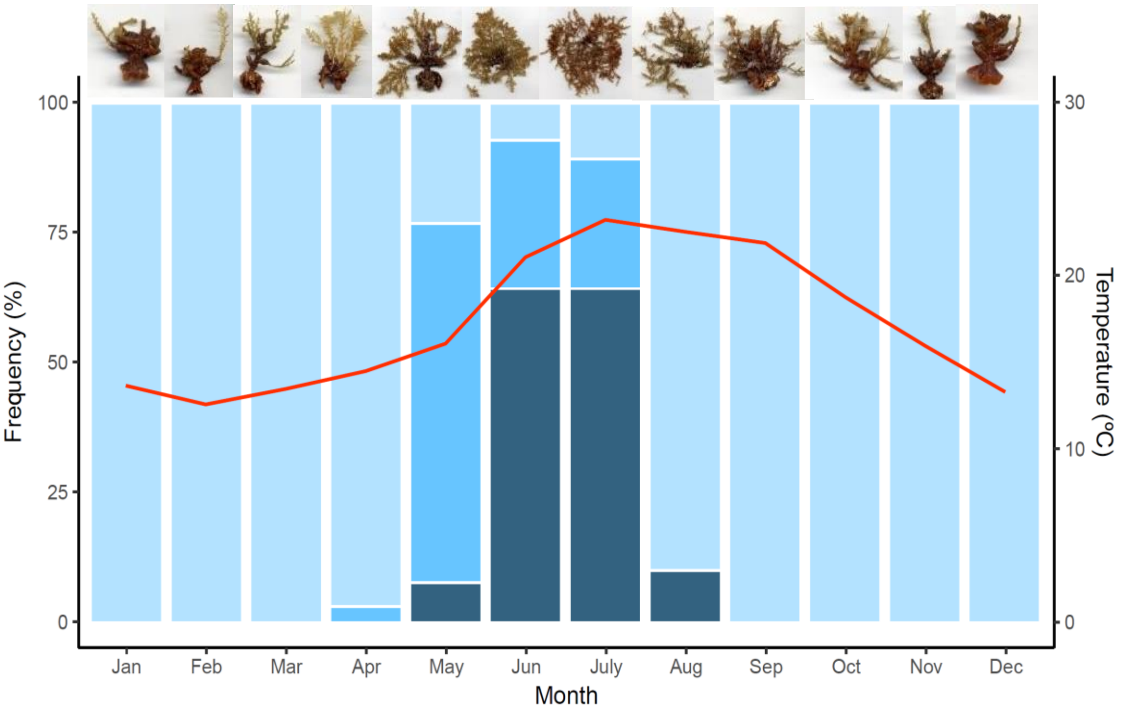

**Figure 2**. Monthly frequency of fertile *Treptacantha elegans* individuals observed during a year. Light blue bars represent the percentage of immature individuals, without the presence of reproductive structures. Medium-blue bars represent the percentage of the individuals beginning the reproductive stage, with reproductive receptacles in the maturing process. Dark blue bars represent the percentage of fertile and reproductive individuals. The red line shows the mean monthly temperature at 5 m depth. Pictures at the top illustrate the monthly canopy-forming branches development stage.

Similar high densities (51.3 ± 4.26 ind/ $m^2$, Mean ± SE) were observed over the three years in the monitored populations. These dense populations were usually dominated by large individuals of more than 15 cm length (except in 2017, Fig. 3). Some specimens of *Treptacantha elegans* can reach large sizes within their first year of life, with an observed maximum annual growth of 28 cm/year in May for an individual recruited the previous spring. New recruits that yearly appeared in the permanent plots were about one-third of the total counts (33.7 % ± 7.9, Mean ± SE), 82 % of which exceeded the fertile minimum size. The mean annual mortality rate within the 3 populations was about 36 % (35.9 % ± 9.8, Mean ± SE). Small specimens showed higher annual mortality rates than the larger ones (Fig. 4, Table 2).

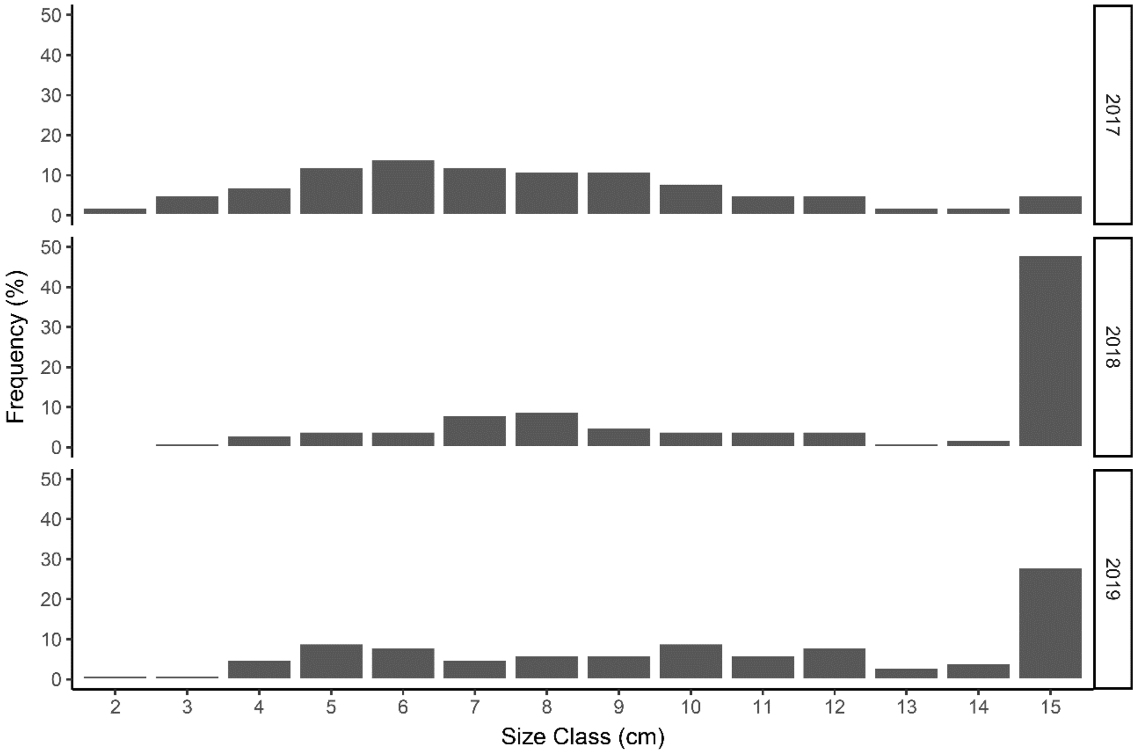

**Figure 3**. Size-class distribution of the length of the longest axis (cm) of *Treptacantha elegans* populations across the three studied years. Relative frequency of size-classes was estimated on 1cm intervals.

**Table 2.** Results of the generalized linear model (GLM) testing differences in the *T. elegans* mortality rates between the size classes.

| **Variables** | | **Coefficients** | | | | **AIC** |
|---|---|---|---|---|---|---|
| | | Estimate | Std. Error | *z value* | *p* | |
| Mortality rate ~ Size Class | Intercept | 0.7753 | 0.0843 | 9.190 | <0.001 | -9.236 |
| | Size Class (cm) | -0.0344 | 0.0089 | -3.835 | **<0.001** | |

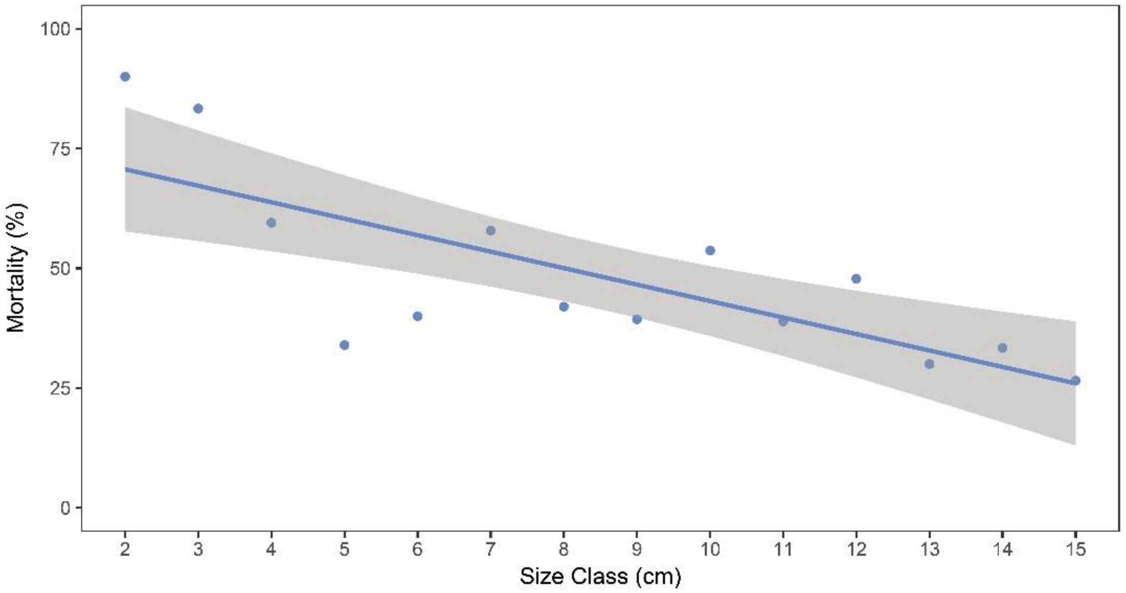

**Figure 4**. Relationship between the mortality rate and the size of the *T. elegans* stands. The blue line represented the generalized linear model fitted between the response variable and the predictor fitted to the most likely distribution (negative binomial).

## 3.2 Spatial and temporal distribution

Since their first observation in the Medes Islands NTZ by Sala and Boudouresque (1996), deep *T. elegans* populations have progressively expanded to other areas of the coast of Catalonia (Fig. 1). The farthest populations were detected 30 km away from the Medes Islands in 2011 and 2012 (Table 1). All the 17 populations detected over the last two decades were still present in 2018 on rocky, exposed bottoms between 5 and 15 m depth.

## 3.3 Population genetic structure and diversity

The 8 microsatellite loci showed moderate polymorphism, ranging from 3 to 14 total alleles per locus (64 in total). Allelic richness was higher in the Medes Islands NTZ population and lowest in the northern population (Messina) (Table 3). Although it showed the lowest number of private alleles, marginally higher genetic diversity was also observed in Medes Islands NTZ (Table 3). All populations showed positive inbreeding coefficients ($F_{IS}$) resulting from heterozygote deficiency (Table 3). Mean $F_{ST}$ across all populations and loci was 0.07.

**Table 3**. Multilocus genetic diversity estimates for *T. elegans* populations (n= 20) across Catalan coast based on 8 microsatellite loci. For A, $H_e$, and $H_o$ values are mean ± SE over all 8 loci. $H_e$ corresponds to unbiased expected heterozygosity (Nei 1978).

| **Location** | **Map code** | **Allelic richness (A)** | **Expected hetero-zygisity ($H_e$)** | **Observed hetero-zygosity ($H_o$)** | **Inbreading coefficient ($F_{IS}$)** | **Number of private alleles** |
|---|---|---|---|---|---|---|
| Mesina | 2 | 4.5 ± 0.5 | 0.7 ± 0.03 | 0.39 ± 0.09 | 0.34 | 6 |
| Medes Islands | 1 | 6 ± 1.2 | 0.74 ± 0.05 | 0.56 ± 0.1 | 0.11 | 4 |
| Begur | 3 | 5.6 ± 0.8 | 0.70 ± 0.06 | 0.46 ± 0.1 | 0.29 | 8 |

**Table 4**. Pairwise $F_{ST}$ estimates between the three populations over 8 loci. Asterisks indicate significant values of F-statistics ($P < 0.05$) for 1023 permutations

| | **$F_{ST}$** | | **Distances (km)** | |
|---|---|---|---|---|
| **Populations** | Medes Islands | Begur | Medes Islands | Begur |
| Mesina | 0.073* | 0.099* | 29 | 37 |
| Medes Islands | | 0.030 | | 9 |

The analyses of Molecular Variance (AMOVA) revealed that most genetic variation occurred within populations (93%, Table 2 S4). Pairwise $F_{ST}$ estimates revealed no differentiation between the Medes Islands and Begur (separated only by 9 km, Fig.1 locations 1 and 3). Pairwise estimates between the Medes Islands with Messina and Messina with Begur were significant (Table 4). According to Structure Harvester the best K was 3. Structure results showed negligible population differentiation within *T. elegans* populations across the Catalan coast, i.e., revealed a genetically homogeneous region (Fig. 5).

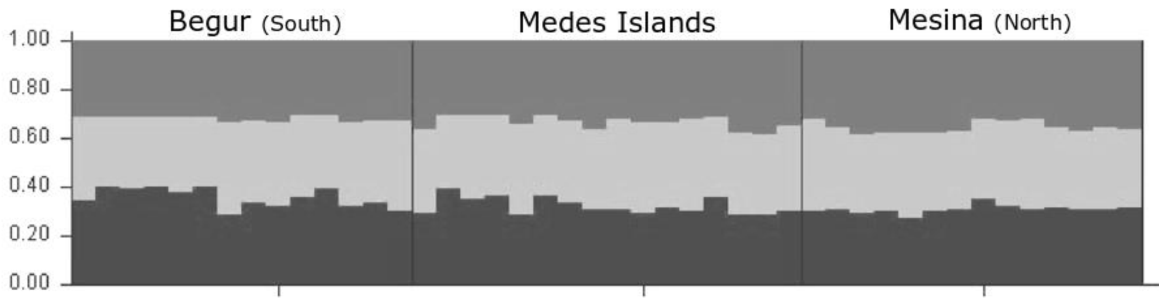

**Figure 5.** Genetic subdivision of *Treptacantha elegans* populations based on STRUCTURE, assuming 3 genetic clusters. Each vertical bar represents the proportion of genome assigned to each cluster for each individual. Vertical black lines separate different populations. Note the absence of any population differentiation.

## 4. Discussion

Most species *Cystoseira sensu lato* gather many typical traits of less competitive species (*sensu* McKinney and Lockwood, 1999): they are often considered late successional species, are especially sensitive to environmental and man-induced stressors (Ballesteros et al., 2007) and show limited dispersal abilities (Mangialajo et al., 2012; Gianni et al., 2013). This is why the results of this study are surprising. Despite its decline in shallow and sheltered environments in neighboring areas (Thibaut et al., 2005; 2015), in contrast, the brown alga *Treptacantha elegans* has remarkably increased both its depth range and spatial distribution over the last two decades along the Catalan coast.

In the Medes Islands NTZ, *T. elegans* forests showed fast-growing dynamics, and their stands were capable to reach the largest sizes and fertile maturity at 1 year old. These uncommon traits among species of the same genus clearly represent an advantage for colonizing new available substrate. In addition, no differences were observed between algal annual mortality rates and the percentage of new individuals observed within the same year, which might also represents a major advantage for *T. elegans* populations.

Increased tolerance and adaptation, and/or migration to different locations within their specific niche, are the possible responses to environmental changes in macroalgae (Harley et al., 2012). Morphological variation has been observed between *T. elegans* populations from shallow to deep waters (Mariani et al., 2019) and the presence of thicker and spinier branches is most likely a plastic response (acclimation rather than adaptation) to more exposed and deeper environments as no evidence for genetic differences has been detected in preliminary assays of these morphotypes (João Neiva pers.comm. = cox1 sequences).

Our molecular analyses of the three *T. elegans* populations across the Catalan coast showed a great homogeneity despite relatively high intra-population variation and inbreeding. The first deep *T. elegans* population was detected in the Medes Islands NTZ and its origin is believed to be related to the protection effect (Sala et al., 2012). This population also exhibited the highest allelic richness, so it may represent the focus of the geographical expansion across the Catalan coast. These results may suggest a common a common ancestral gene pool but loosely connected populations, as has been reported for other *Cystoseira sensu lato* species (Susini et al., 2007; Buonomo et al., 2017; 2018). This could be explained by the patchy distribution of *T. elegans* populations which has been related to the presence of rocky and suitable habitats and limited dispersal range of fucoid gametes (Sales and Ballesteros, 2009; Mariani et al., 2014; 2019). Unfortunately, our data are not sufficient to determine a correlation between distance and genetic differentiation within populations.

Limited range dispersal has been proposed for most fucoid species because of their large sized and fast sinking zygotes (Gianni et al., 2013). Although gametic dispersal distance of *T. elegans* is less than 10 m from source populations (Authors, manuscript under review; chapter 4), here we show the existence of two populations 30 km away from the Medes Islands NTZ. This may be explained by the fact that this species, as other fucoids, is capable to disperse through detached or drifting fertile parts of mature plants (Deysher and Norton, 1982; Mangialajo et al., 2012; Thibaut et al., 2014b) or by animals (Gianni et al., 2013). This may explain the southern spreading through the Northern Current, which flows with a permanent southwestward circulation in the Northwestern Mediterranean basin (Calvo et al., 2011). Since a spread in the opposite direction is unlikely (i.e. from the Medes Islands to the northern study area), *T. elegans* populations from the north of Catalonia may have arrived from the original population in Banyuls-sur-Mer (France) where this species was described for the first time (Sauvageau, 1912) or from shallow populations in the same area (see Mariani et al., 2019).

Several environmental drivers and the interaction of some favorable conditions may have promoted the spreading of this species. Seawater eutrophication is one of the major causes for the disappearance of *Cystoseira sensu lato* populations in the Mediterranean Sea (Sales et al., 2011; Iveša et al., 2016). Evidence of the improvement in coastal water quality in the Catalan coast, particularly during the 1990s (Pinedo et al., 2013), has been related to the recovery of *Cystoseira sensu lato* species (Ricart et al., 2018). The Medes Islands NTZ might have worked as a refuge for *T. elegans* populations until environmental conditions had improved, probably favored by the predatory fish control on their main grazers (Medrano et al., 2019a).

Our results shed new light on the capacity of *Cystoseira sensu lato* species to persist or even widen their geographical distributions under global change scenarios. Given its fast and stable population dynamics, *T. elegans* may represent an ideal organism to conduct habitat restoration actions in previously degraded systems such as sea urchin barrens or turf-dominated habitats. Due to its high genetic diversity, and the potential source for new populations, Medes Islands NTZ population may be the ideal source of *T. elegans* individuals or propagules for restoration actions.

## Acknowledgements

We thank Marc Verlaque for their help on *T. elegans* taxonomic identification and Kike Ballesteros for his helpful and constructive discussions on this study. We also thank Mikel Zabala, Ignasi Montero-Serra, and Jordi Solà for their help on the fieldwork, and to 'Macroalgae and invasive species monitoring group', especially María García, for untangling the presence of *T. elegans* populations among their data across the Catalan coast.

This work was partially funded by the long-term monitoring programme of the Natural Park of Cap de Creus and the Natural Park of Montgrí, Medes Islands and Baix Ter protected areas (public agreement PTOP- 2017-130, Departament de Territori i Sostenibilitat of the Generalitat de Catalunya) and the European Union's Horizon 2020 research and innovation programme under the grant agreement No 689518 (MERCES) and the grant agreement No 730984, ASSEMBLE Plus project. Simone Mariani was supported by the Spanish INTRAMURAL CSIC PIE201730E033.

# Chapter IV

# From marine deserts to algal forests: *Cystoseira* forestation to reverse stable degraded ecosystems inside and outside a No-Take marine reserve

## Abstract

Canopy-forming algae are in decline in many coastal areas, where overgrazing by herbivorous can lead to the loss of these highly structured and diverse habitats towards less complex sea urchin barren grounds. Once established, low productive barren grounds are considered stable states maintained by several positive feedback mechanisms that prevent the recovery of marine forests. To revert this global decline, restoration efforts and measures are being encouraged by EU regulations and local actions. Here, we tested the success of active forestation techniques as a tool to promote functional and productive *Cystoseira* forests in sea urchin barren grounds under different restoration strategies (active, and combined active with passive strategies). Active forestation was performed in 6 barren grounds, 3 located inside a Mediterranean No-Take marine reserve (active and passive strategy) and 3 outside (active strategy alone), following a three-step protocol: 1) sea urchin population eradication, 2) seeding with *Treptacantha elegans,* and 3) enhancement of *T. elegans* recruitment. Forestation success was assessed one year later in the 6 barren grounds. Successful forestation was only achieved after combining active with passive restoration strategies, which significantly improved both the associated benthic community and the cover of *T. elegans*. Our results encourage forestation of barren grounds to shift from less productive habitats to complex *Cystoseira* forests, highlight the potential of the combined passive and active restoration strategies, as well as the important role of marine reserves not only in conservation but also in ecological restoration.

## 1. Introducció

Macroalgae are ecologically pivotal species in temperate coastal ecosystems worldwide, where they are one of the dominant primary producers with a key role in the ecosystem functioning (Jones et al., 1994; Duarte and Cebrian, 1996; Teagle et al., 2017). In temperate ecosystems, kelps (Laminariales) and fucoids (Fucales) are the large canopy-forming macroalgae in the intertidal and subtidal zones, creating structurally complex habitats that provide food and shelter for many species and harbor high diversity and productivity (Dayton, 1985; Steneck et al., 2002; Schiel and Foster, 2006). Canopy-forming macroalgal species provide unique habitats with services comparable to terrestrial forests (Dayton, 1985; Graham, 2004; Ballesteros et al., 2009; Wernberg et al., 2011a). However, coastal pollution, overfishing, ocean warming, and other disturbances are causing the widespread decline of macroalgal species (Steneck et al., 2002; Wernberg et al., 2011a; Campbell et al., 2014), affecting the provision of habitat services for the associated flora and fauna, and the consequent loss of ecosystem functioning (Lorentsen et al., 2010; Cheminée et al., 2013; Mineur et al., 2015).

Increased sea urchin overgrazing resulting from the reduction of predator populations is one of the main causes for the global loss of marine macroalgal forests. Sea urchin populations follow natural fluctuations that can lead to gradual increases in abundance, but abrupt increases (i.e., outbreaks) are possible usually due to indirect effects of overfishing such as overfishing of their natural predators (Steneck, 1998; Guidetti and Dulcic, 2007; Medrano et al., 2019a) or destructive fisheries of benthic communities, such as the case of mussels (Guidetti et al., 2003; Guidetti, 2011). Sea urchin outbreaks can boost overgrazing and cause a shift toward low productive habitats such as turf-forming algae or sea urchin barren grounds (Pinnegar et al., 2000; Airoldi and Beck, 2007; Ling et al., 2015; Maggi et al., 2018; Filbee-Dexter and Wernberg, 2018). Once established, these barren grounds are considered stable alternate states maintained by several feedback mechanisms that prevent the recovery of macroalgal forests. These hysteresis loops affect both sea urchin populations by increasing the probability of recruitment and juvenile survival as more sea urchin adults are found, and algal establishment by reducing the supply of propagules as neighbor algae populations become more scarce (Filbee-Dexter and Scheibling, 2014; Ling et al., 2015).

In the North-Western Mediterranean, the two described ecosystem stable states are found in shallow rocky bottoms in the form of overgrazed communities represented by barren grounds with high abundance of sea urchins and low algal biomass, and highly complex and diverse macroalgal-dominated communities (McClanahan and Sala, 1997; Pinnegar et al., 2000). The highest level of macroalgal complexity in the NW Mediterranean Sea is represented by canopy-forming *Cystoseira sensu lato* species (Fucales). These iconic and fragile Mediterranean *Cystoseira* forests are currently facing a decline, with important losses of the three-dimensional habitat for local species, and important consequences for biodiversity and ecosystem functioning (Bulleri et al., 2002; Thibaut et al., 2005; Mangialajo et al., 2008; Thibaut et al., 2015; Mineur et al., 2015; Blanfuné et al., 2016; Gianni et al., 2017).

Efforts to protect and restore macroalgal forests and similar habitats around the world are based on classical management with Marine Protected Areas (MPAs) and, in particular, No-Take Zones (NTZs). NTZs prevent the overexploitation of key structural species, allowing for the reduction or removal of associated impacts, maintaining and restoring ecosystem function, with the subsequent improvement of trophic regulation on populations of consumers (i.e., trophic cascades). However, not much evidence supports NTZs as effective enough to restore degraded ecosystems (Huntington et al., 2011; Sangil et al., 2012; Toth et al., 2014; Cox et al., 2017). The natural variability on species dynamics and ecological interactions limit recovery benefits only after long term periods (i.e., decades, Babcock et al., 2010; Strain et al., 2019). A more suitable tool for recovery in the shorter term is ecosystem restoration, which involves the active assistance in the recovery of a degraded, damaged, or destroyed habitat (SER 2004). Active restoration speeds up the ecosystem recovery by implementing management techniques (e.g., transplanting) (Perrow and Davy, 2002; Holl and Aide, 2011; Bayraktarov et al., 2016).

Restoration of macroalgal forests has mostly considered transplanting individuals (Vasquez and Tala, 1995; Carney et al., 2005; Falace et al., 2006; Whitaker et al., 2010), but the limited dispersal ability of *Cystoseira* spp. (Mangialajo et al., 2012; Gianni et al., 2013) and the negative impact of transplanting *Cystoseira* individuals from the remaining populations (Verdura et al., 2018) have led to the development of non-destructive restoration techniques such as the enhancement of recruitment potential (Gianni et al., 2013; Falace et al., 2018; Verdura et al., 2018) or the ex situ outplanting (De La Fuente et al., 2019).

Several control measures to reduce sea urchin abundance such as eradication have been also described to actively restore the sea urchin barren grounds

(Leighton et al., 1966; Watanuki et al., 2010; Sangil et al., 2012; Piazzi and Ceccherelli, 2019). Despite these efforts, attempts to recover macroalgal forests from sea urchin barren grounds remain challenging due to the high hysteresis of stable barren state and the difficulty of reestablishing populations of natural predators and consequent trophic cascades (i.e., in old and well-enforced NTZs) that help reduce pervasive sea urchin overgrazing (Ling et al., 2015; 2019).

Combining well-designed active and passive restoration practices has become an indispensable strategy to reverse widespread ecosystem degradation (Lotze et al., 2006; Mitsch, 2014; Possingham et al., 2015). Given that this is a largely unexplored scenario on *Cystoseira* forestation and restoration of degraded barren grounds, the aim of this study was to evaluate the potential success of active restoration techniques alone or combined with marine protection (active and passive restoration) to recover shallow stable degraded habitats. To do this, we tested for the most adequate active restoration techniques to forest shallow sea urchin barren grounds combining the sea urchin eradication with recruitment enhancement techniques to promote the *Cystoseira* forestation inside and outside a Mediterranean NTZ.

## 2. Materials and Methods

### 2.1 Model species

Most *Cystoseira sensu lato* species are endemic to the Mediterranean Sea (Ballesteros, 1990; Cormaci et al., 2012), where they can form forests from the surface to the upper circalittoral zone, play a key functional role (Ballesteros et al., 1998; Mangialajo et al., 2008; Sala et al., 2012; Cheminée et al., 2013).

*Treptacantha elegans* (Sauvageau, 1912) is a Mediterranean endemic species, inhabiting from the infralittoral zone to several meters depth in the Catalan Coast (maximum observed 20 m depth, Capdevila et al., 2019b). Their canopy-forming branches grow in spring, reaching the maximum development and the fertile maturity in early summer when larger specimens may exceed fifty centimeter height (Barceló et al., 2001). The reproductive period of *T. elegans* spans from the end of May to July (individuals of 1 or >1 year old, author's personal observation). Plants are reduced to single axes in autumn and winter with which they remain attached to the substratum perennially (Gómez-Garreta et al., 2001; Rodriguez-Prieto et al., 2013).

## 2.2 Study site: the Montgrí, Illes Medes and Baix Ter (MIMBT) Natural Park

Active forestation tests were carried out in the MIMBT Natural Park in 2018 (Fig. 1). This Natural Park, located in the North-Western Mediterranean Sea, has its core in the NTZ of the Medes Islands, where all fishing activities are prohibited and the abundance of the main sea urchin fish predators and sea urchin predation rates have remained significantly higher than outside the NTZ (Hereu et al., 2012a; Medrano et al., 2019a). Benthic shallow-rocky habitats inside and outside the NTZ are similar and mostly dominated by photophilic algal communities of small erect algae and calcareous algal formations (Ballesteros, 1991; Medrano et al., 2019b). Among these communities, there are conspicuous healthy and well-preserved forests dominated by the fast-growing *T. elegans* species as well as degraded barren grounds dominated by sea urchins (authors, in preparation). The sea urchin *Paracentrotus lividus* (Lamark, 1816) is considered the most abundant and important local herbivore controlling fleshy macroalgal dynamics in these shallow reefs (Sala and Zabala, 1996; Hereu, 2005). Although the black sea urchin *Arbacia lixula* (Linnaeus, 1758) is also common, its abundance is comparatively very low in the Catalan coast (Hereu et al., 2012a).

In the MIMBT Natural Park, 10 locations were selected for this study: six degraded barren grounds to forest (3 inside the NTZ and 3 outside), two dense *T. elegans* forests as reference sites of forest state (1 inside the NTZ and 1 outside), and two degraded barren ground as reference site of degraded habitat (1 inside the NTZ and 1 outside). Given the small extension of the NTZ, we selected a limited number of reference areas. All ten locations were selected in the 5 -10 meter depth range (5 inside the NTZ and 5 outside) covering the most diverse range of barren grounds' sizes and local-scale variability (Table 1) given the limited size of the NTZ area (93 ha).

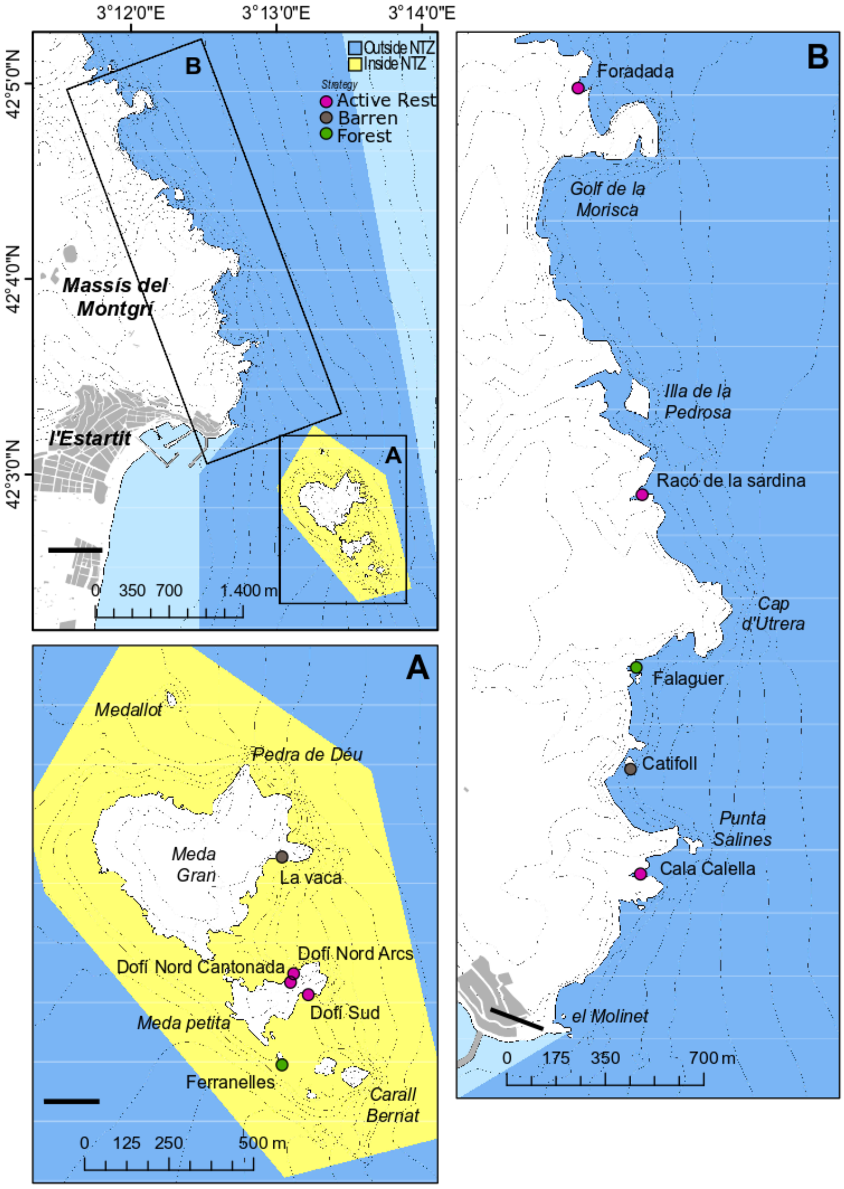

**Figure 1.** Map of the 10 study locations in the Montgrí, Illes Medes and Baix Ter Natural Park in the NW Mediterranean Sea. (A) Locations inside the NTZ. (B) Locations outside the NTZ. Pink dots indicate barren grounds where active restoration strategy was carried out; grey dots indicate reference barren grounds and, green dots represent reference forests.

**Table 1.** Characteristics of the 10 studied locations. On 'Restoration Strategy' column, BC means Barren Control; AR means Active Restoration (i.e. sea urchin eradication and *T. elegans* forestation); and, FC means Forest Control. Values of sea urchin densities and biomass are Mean ± SE.

| Locality | GPS coordinate | Protection | Initial state | Restoration strategy | Depth (m) | Orientation | Eradication extension (m$^2$) | Initial sea urchin density (Ind/m$^2$) | Initial sea urchin biomass (g/m$^2$) |
|---|---|---|---|---|---|---|---|---|---|
| Dofi Sud | 42°2'38.25"N; 3°13'35.48"E | Inside NTZ | Barren | AR | -7.6 | S | 225 | 27.2 ± 2.95 | 208.2 ± 22.6 |
| Dofi_N Arcs | 42°2'40.46"N; 3°13'34.35"E | Inside NTZ | Barren | AR | -6.8 | NW | 90 | 27.1 ± 6.56 | 297.3 ± 59.1 |
| Dofi N Cantonada | 42°2'39.61"N; 3°13'34.15"E | Inside NTZ | Barren | AR | -7 | S | 45 | 25.2 ± 2.67 | 184.3 ± 38.2 |
| Ferranelles | 42°2'31.88"N; 3°13'32.07"E | Inside NTZ | Forest | FC | -5.5 | S | - | 0.67 ± 0.67 | 8.7 ± 8.7 |
| La Vaca | 42°2'51.29"N; 3°13'32.47"E | Inside NTZ | Barren | BC | -7.6 | S | - | 41.1 ± 4.36 | 339.7 ± 50.4 |
| Foradada | 42°4'57.94"N; 3°11'54.46"E | Outside NTZ | Barren | AR | -5.5 | S | 72 | 25.6 ± 3.73 | 181.3 ± 36.6 |
| R. Sardina | 42°4'15.67"N; 3°12'24.65"E | Outside NTZ | Barren | AR | -6 | NW | 660 | 31.2 ± 7.58 | 251.6 ± 34.1 |
| C. Calella | 42°3'35.60"N; 3°12'46.13"E | Outside NTZ | Barren | AR | -6 | S | 24 | 42.4 ± 3.64 | 155.6 ± 33.3 |
| Falaguer | 42°3'57.82"N; 3°12'36.70"E | Outside NTZ | Forest | FC | -7 | S | - | 0.17 ± 0.17 | 8.3 ± 8.3 |
| Catifoll | 42°3'47.90"N; 3°12'38.59"E | Outside NTZ | Barren | BC | -6 | S | - | 42.7 ± 15.09 | 157.3 ± 28.4 |

## 2.3 Forestation techniques

Active forestation of six barren grounds was carried out by a combination of complete sea urchins eradication together with the facilitation of *Treptacantha elegans* forests establishment in the MIMBT Natural Park in 2018.

Macroalgal forestation technique was based on the enhancement of recruitment potential of the new *T. elegans* populations. In spring 2017, we first experimentally tested in situ and ex situ seeding techniques success. To this end, some apical branches with fertile receptacles were collected from a healthy and fertile *T. elegans* donor population inside the NTZ (Ferranelles, Fig. 1) and placed in PVC mesh bags allowing the gametes liberation through the mesh (Choi et al., 2000; Verdura et al., 2018). For in situ recruitment enhancement technique tests, six meshed bags containing about 75-80 g FW of fertile apexes each (77.84 ± 6.49 g FW, Mean ± SE) were placed in a degraded algal community (dominated by barren and turf communities with no *T. elegans* stands) tied to a plastic screw previously fixed in the hard substratum, and 5 flat manufactured limestone plates of 25 cm$^2$ (hereinafter referred to as stone plates) were set directly below the seedling bags as settlement collectors.

To test the same technique in an ex situ experiment, we simultaneously placed 5 identical seedling bags into five 12-L aquaria together with the same settlement stone plates. Aquaria were set to close-fitting the light and temperature natural environmental conditions. Day/night cycles were simulated at 12/12 hours regime and temperature was constant over the two experimental months around 17ºC. *T. elegans* zygotes settled and grew up in aquaria for two months in order to ensure an optimal development of early life stages of *T. elegans* individuals (Falace et al., 2018). After then, the collectors were placed at the same locality than in situ technique collectors. Density of the recruits (new *T. elegans* individuals) in each collector was measured at the transplant moment and was compared between the two techniques 2 months after the transplantation using a generalized linear model (GLM) (McCullagh and Nelder, 1989). The abundance of *T. elegans* recruits was fitted with a negative binomial distribution and a logarithm link function after visually checking the most likely distribution of the data. The variables technique (2 levels: in situ vs. ex situ) and time (2 levels: initial vs. two months later) were included as main factors in the model.

Once the successful technique was estimated in preliminary tests, in spring 2018, in situ seedling technique was implemented within the 6 degraded barren grounds (3 inside the NTZ and 3 outside, Fig. 2A) and the 4 reference sites (Table 1). Taking advantage of this experimental setup, we additionally tested three types of settlement collectors; stone plates (flat manufactured limestone), plots of original substrate (unmanipulated limestone substrate, mainly covered by the representative algal assemblages), and plots of scraped and cleared substrate of approximately 25 cm$^2$ area each. Six collectors of each type were randomly placed or delimited around the 6 seeding bags on each experimental site (Fig. 2B). The density of *T. elegans* recruits was measured at the beginning of the experiment and one year later on the 18 collectors at each site, to assess a) if free and available substrate should be provided to enhance the development of the new recruits, and b) which was the best substrata for initial settlement. The number of initial recruits was fitted in a GLM with the type of collector as explanatory factor (3 levels: stone plates, original substrate, cleared substrate). Differences in the one year mortality rate of recruits between collectors were assessed using the same GLM but fitting the mortality rate as response variable instead of the number of the initial recruits. All GLMs analyses were conducted using the package "MASS" for R software (Venables and Ripley, 2002).

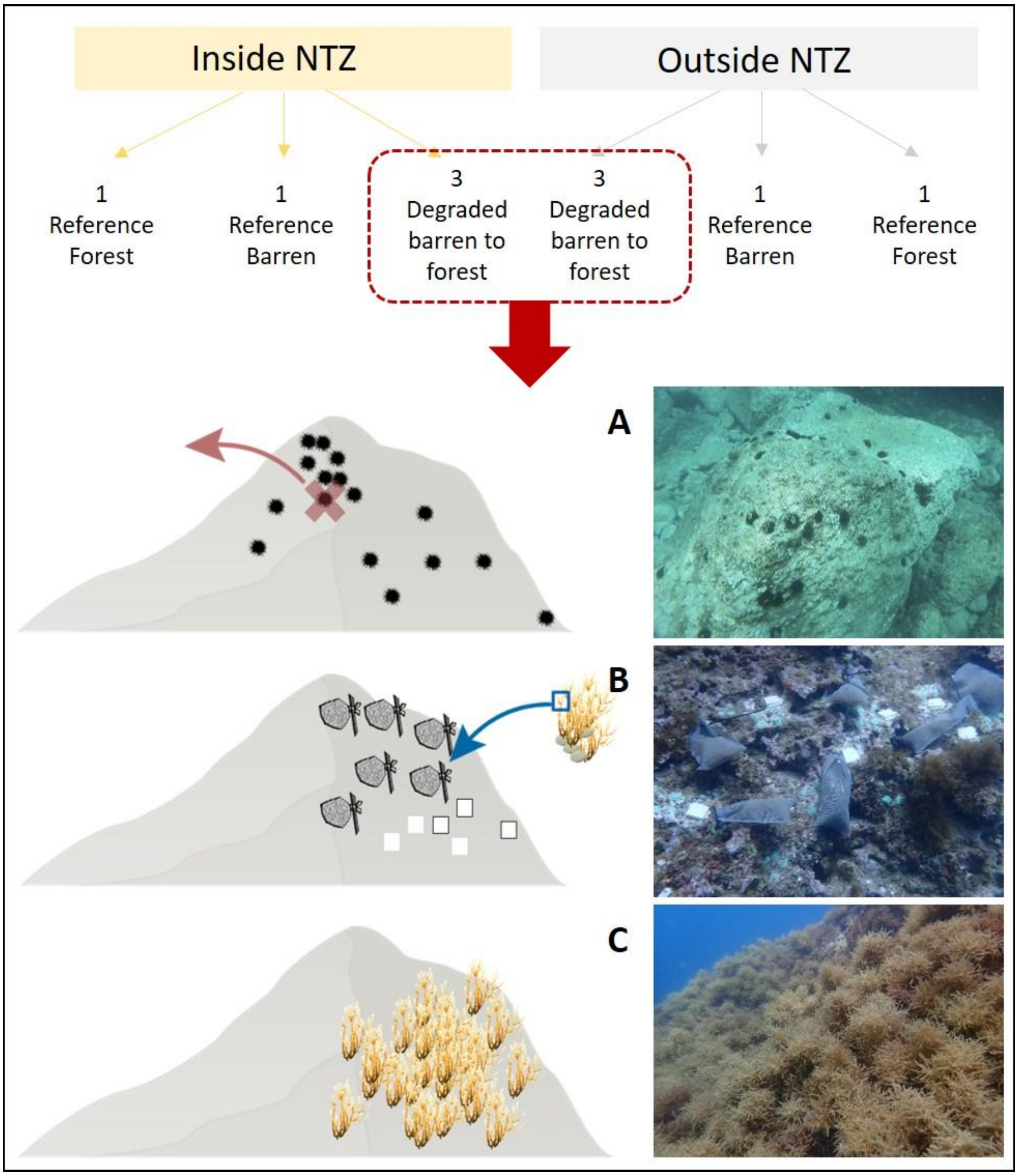

**Figure 2.** Diagram showing the experimental design and the three-step *T. elegans* forestation protocol. A) Complete eradication of sea urchins on the degraded barren grounds. B) Detail of meshed seeding bags (grey) and the three different types of settlement collectors. C) Mature forest of *T. elegans* resulting from restored degraded sea urchin barren grounds.

To remove the sea urchin grazing impact and to ensure the survival of newly settled *T. elegans* populations, we eradicated sea urchin populations on the study sites by manually removing all of them (as Guarnieri et al., 2014). The complete eradication of the sea urchins present in the six selected barrens was performed by SCUBA divers with the permission and following the ethical standards of the Natural Park which ensure that this study did not involve the removal of species listed as endangered. Therefore, in order to promote the forestation of sea urchin barren grounds, we set a three-step protocol involving: 1) the removal of the impact (sea urchins eradication), 2) the seeding with *T. elegans,* and 3) the enhancement of the *T. elegans* recruitment (see video file S6 for detailed protocol and Fig. 2 for the summarized one).

### 2.4 Restoration strategies

To assess the success of active restoration within the two levels of protection (inside a NTZ and outside), we characterized macroalgal and sea urchin communities at the 10 locations by SCUBA diving before the forestation actions (2018; Table 1) and one year later (2019).

Sea urchin abundance and size (diameter) were measured in 10 quadrats of 50x50 cm in order to estimate their biomass and size structure in each location before and one year after the sea urchin eradication. To assess the effectiveness of eradication, the sea urchin biomass in the degraded locations where active eradication was done, was selected as reference level and compared with the mean biomass of reference locations (degraded barren ground and healthy forests) using two generalized linear mixed models (GLMMs) (Bolker et al., 2009) (one for 2018 biomass as the reference level and one for 2019). GLMMs were fitted with sea urchin biomass as independent variable and Restoration Strategy (3 levels: Active restoration, Reference barren ground, and Reference Forest), Protection level (2 levels: inside and outside the NTZ), and Time (2 levels: before (2018) and after eradication (2019)) as fixed factors, and location as random factor nested in Restoration Strategy.

*Treptacantha elegans* cover, number of individuals, and maximum length of the longest axis of each *T. elegans* stand, were measured in 20 randomly placed 50x50 cm quadrats in each location one year after the active restoration (sea urchin eradication and *Cystoseira* seeding). The distance between the *T. elegans* individual further from the seeding bags was measured with a measuring tape and considered as the maximum effective dispersal distance. Percent cover in 2019 (one year after the implementation of the forestation actions) was compared between the different restoration strategies and protection levels using a GLMM as described above.

All GLMMs analyses were conducted using the package "lme4" for R software (Bates et al., 2015). For the fitted models, Type II Wald $\chi 2$ test was used to assess the effect of fixed factors. Wald $\chi 2$ test was performed using the "Anova" function from the CAR package (Fox and Weisberg, 2011).

Macroalgal community was sampled using six replicate 50x50 cm quadrats divided into 25 subquadrats of 10x10 cm. At each quadrat, percent cover of erect algae, turf algae, and bare rock was estimated as the percentage of the number of subquadrats where these different algal types were present (Hereu et al., 2008). In order to explore the relationships between different restoration strategies and benthic community structure, we compared the benthic community structure of the restored locations before and after implementation of the forestation actions (2018 vs. 2019) and the reference ones, using a non-metric multidimensional scaling (nMDS) ordination (Kruskal, 1964). The nMDS was performed with the R package "vegan" (Oksanen et al., 2017) on a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957) calculated from log transformed data of bare rock, erect algae, and turf algae cover. All statistical analysis and plots were run with the software R 3.3.3 (R Core Team, 2017).

Seawater temperature was logged in situ throughout the complete year using Hobo Pendant® autonomous sensors placed at 6 meters depth inside and outside the NTZ to rule out temperature differences between the protection levels. Almost identical temperatures were recorded inside and outside the NTZ during *T. elegans* growing season (Spring), ranging from 11.8 to 15.4 ºC between January and late April of 2019 (Fig. 1 S5).

### 2.5 Costs of forestation actions

To provide an estimate and comparable costs of the combined restoration techniques carried out in this study, the costs of both techniques (complete sea urchin eradication together with the in situ *Treptacantha elegans* forestation) were assessed considering the forestation of 200 $m^2$ sea urchin barren ground. We accounted for the transportation and material costs, and the salary of 3 qualified divers and 1 boat skipper following the methods of previous studies (Verdura et al., 2018; Pagès-Escolà, in press). In terms of time, sea urchin eradication and *T. elegans* forestation of a single barren ground of 200 $m^2$ size should be achieved in a single day involving the staff mentioned above. The staff salary was established according to the guidelines for monitoring protocols in marine protected areas (Generalitat de Catalunya, 2017). All costs were calculated in our study area and they could be variable depending on the country.

## 3. Results

### 3.1 Forestation techniques

In 2017 experiments, density of *T. elegans* recruits was similar in both the in situ and ex situ recruitment enhancement techniques at the beginning (11.84 ± 2.64 and 10.14 ± 5.30 (Mean ± SE) recruits/cm$^2$, respectively). High and similar mortality rates were observed between in situ and ex situ restoration techniques within the firsts 2 months (95.9% for in situ and 94.9% for ex situ, Table 1 S5).

In 2018-2019 experiments, a higher density of newly *T. elegans* recruits was found on the free surface collectors (stone plates and cleared substrate) than on original substrate (Fig. 3A, Table 2 S5). As expected, high mortality rates of recruits were observed one year later within the three collectors tested (80% on ‘cleared substrate’, 86.47% on ‘stone plates, and 92.3% on ‘original substrate’ collectors). The higher survival was reported on the ‘cleared substrate’ collectors (Fig. 3B, Table 3 S5).

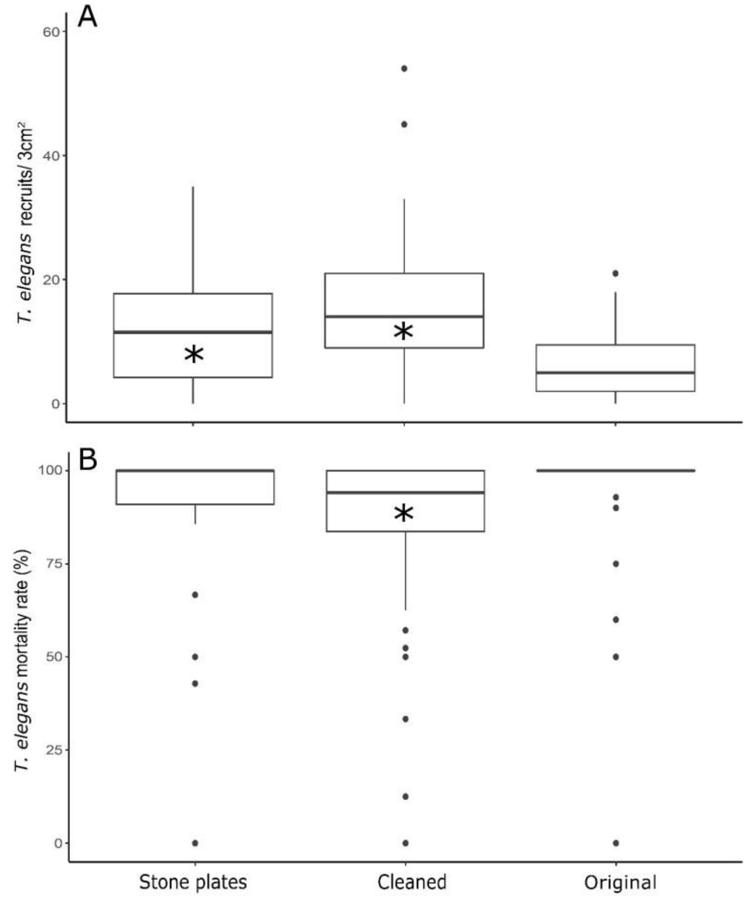

**Figure 3.** Forestation techniques results. (A) Boxplot showing the initial density of recruits within the 3 different collectors. (B) Boxplot showing the mortality rate within the three type of collectors one year after the forestation. In the boxplots, the bold horizontal line indicates the median value (Q2); the box marks the interquartile distances, Q1 and Q3; and the whiskers mark the values that less than Q3+1.5*IQR but greater than Q1–1.5*IQR. '*' indicates significance differences ($p<0.05$) in the GLM analysis

## 3.2 Restoration strategies

High and similar initial sea urchin biomasses were observed in the degraded areas, along with the reference barren grounds, before the eradication actions (2018), whilst lower biomasses defined the reference forests (Fig. 4A, Table 1, and Table 4 S2). Sea urchin biomasses in the degraded barren grounds did not shown differences between levels of protections before the restoration action ($\chi^2_2$ = 1.88, p=0.17).Size structure of the sea urchin populations in the barren grounds before the eradication showed a bimodal distribution characterized by higher densities of small size' sea urchins outside than inside the NTZ (Fig. 4B). One year after the complete eradication, a significant reduction of the sea urchin biomass (involving mainly a reduction of large sized sea urchins) was achieved (Fig. 4, Table 4 S5) leveling the locations selected for restoration actions implementation to the reference forests density values (Fig. 4, Table 4 S5). The same pattern was observed inside than outside the NTZ, meaning null effect of the level of protection on the sea urchin eradication success ($\chi^2_2 = 3.31$, p=0.07, Table 4 S5).

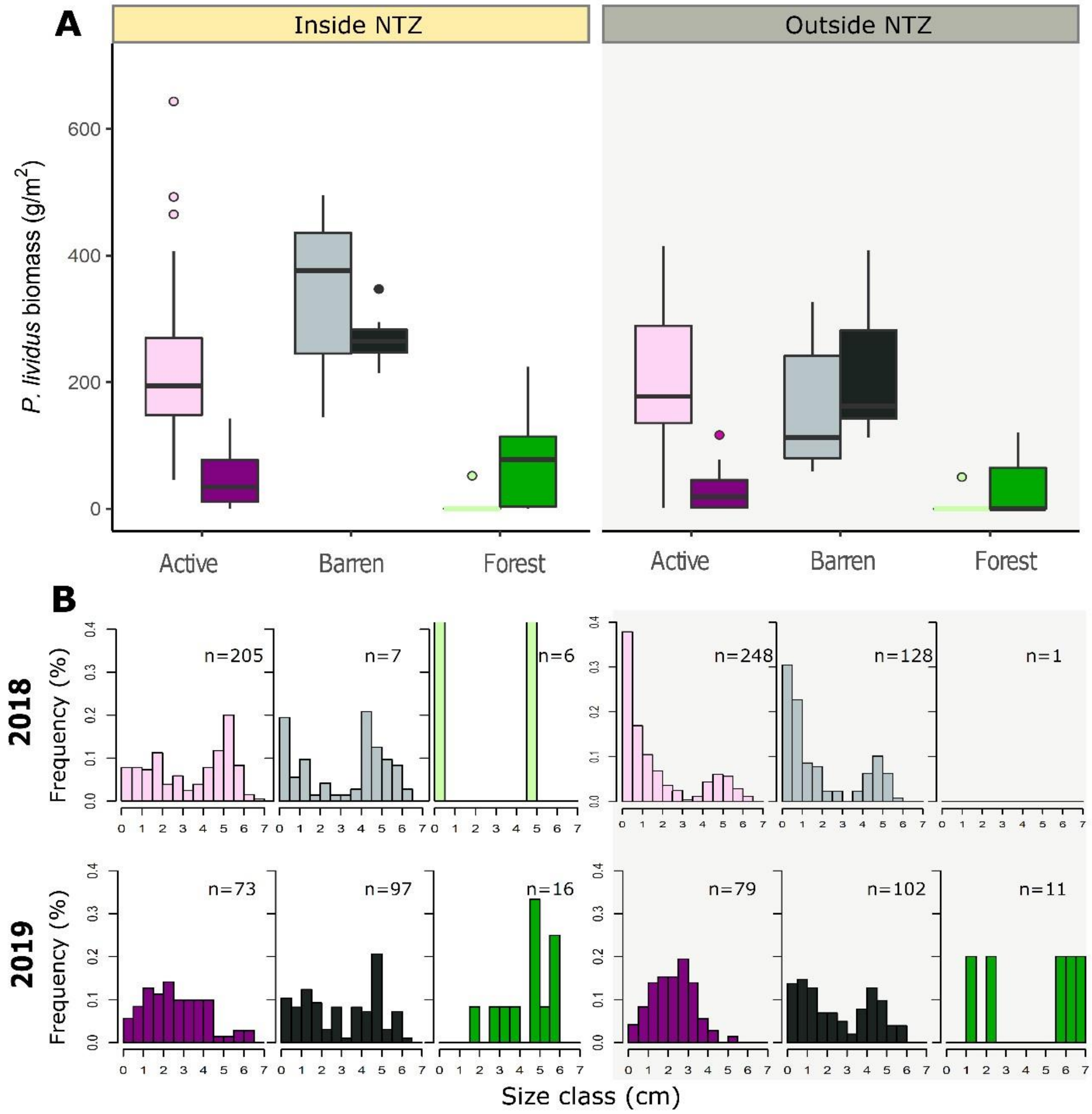

**Figure 4.** Sea urchin biomass (A), and frequency distribution of sea urchins' size-class (B) observed before (light colors) and one year after forestation actions (dark colors) within the different restoration strategies. Pink-colored boxes represent barren grounds in which active forestation was done. Grey-colored boxes represent reference barren grounds and green-colored boxes, reference forests. Left panel represents the strategies carried out inside the NTZ and, right panel, outside the NTZ.

Before the forestation, *T. elegans* populations were practically non-existent within degraded barren grounds whilst they represent about the 50 % of total algal cover of the reference forests (Fig. 5A). One year after forestation, *T. elegans* cover only increased in the locations where the complete sea urchin eradication was performed (Fig. 5A). Although *T. elegans* cover increased in the actively restored grounds, it did not reach the cover observed at the reference forests in the study period (Fig. 5A, Table 5 S5). Comparing *T. elegans* increase on restored locations inside the NTZ with the locations without protection, we observed a higher *T. elegans* cover at equal densities of sea urchins in the locations where both active and passive restoration strategies were combined (Fig. 2 S5). *T. elegans* individuals in the locations where active and passive strategies were combined (active restoration inside the NTZ) reached similar heights to the reference forests ones (7.6 ± 0.25 cm vs. 8.51 ± 0.22 cm (Mean ± SE), respectively), whereas mean height was about half of the wild canopy where active strategies alone were applied compared with the reference forest outside the NTZ (4.25 ± 0.37 cm vs. 8.31 ± 0.22, Mean ± SE, respectively) (Fig. 5B). Limited dispersal ability was observed, which was three times higher inside the NTZ, with a maximum distance of 3 to 10 m away from the seedling bags, and lower outside the NTZ, with a maximum of 1 to 3 m away.

Reference forests were positioned on the right side of the nMDS with a major cover of erect algae in 2018 and 2019 (Fig. 6). Degraded barren grounds were defined by bare rock and turf algae, displaying on the left and the top side before the forestation action (2018). Only the barren grounds forested by combining active and passive restoration strategies (active forestation inside the NTZ) moved close to the reference forests one year after, while the barren grounds actively forested outside the NTZ together with the reference barren remained close to their original position (Fig. 6).

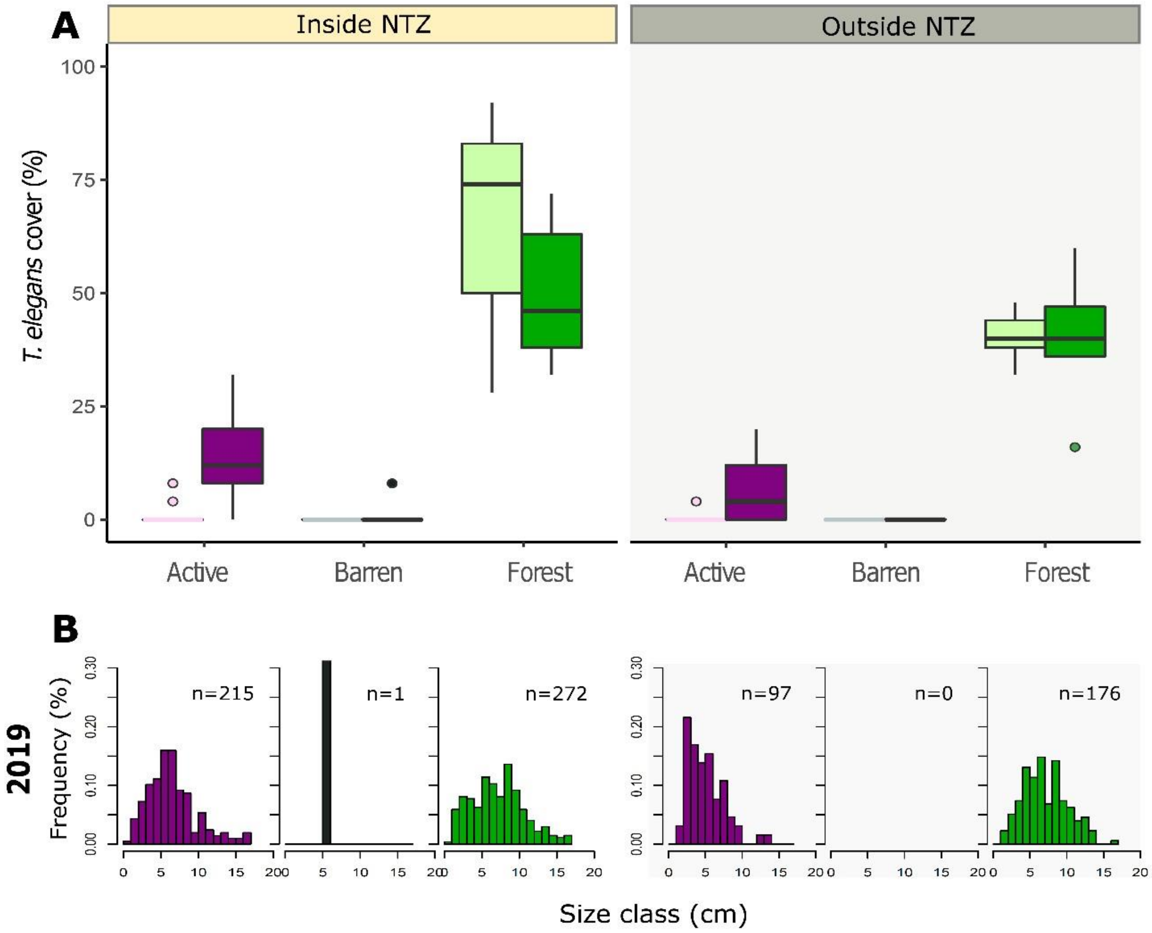

**Figure 5.** Percentage of cover of *Treptacantha elegans* (A), and frequency of distribution of maximum height (B) observed before (light colors) and one year after forestation actions (dark colors) within the different restoration strategies. Pink-colored boxes represent barren grounds in which active forestation was done. Grey-colored boxes represent reference barren grounds and green-colored boxes, reference forests. Left panel represents the strategies carried out inside the NTZ and, right panel, outside the NTZ.

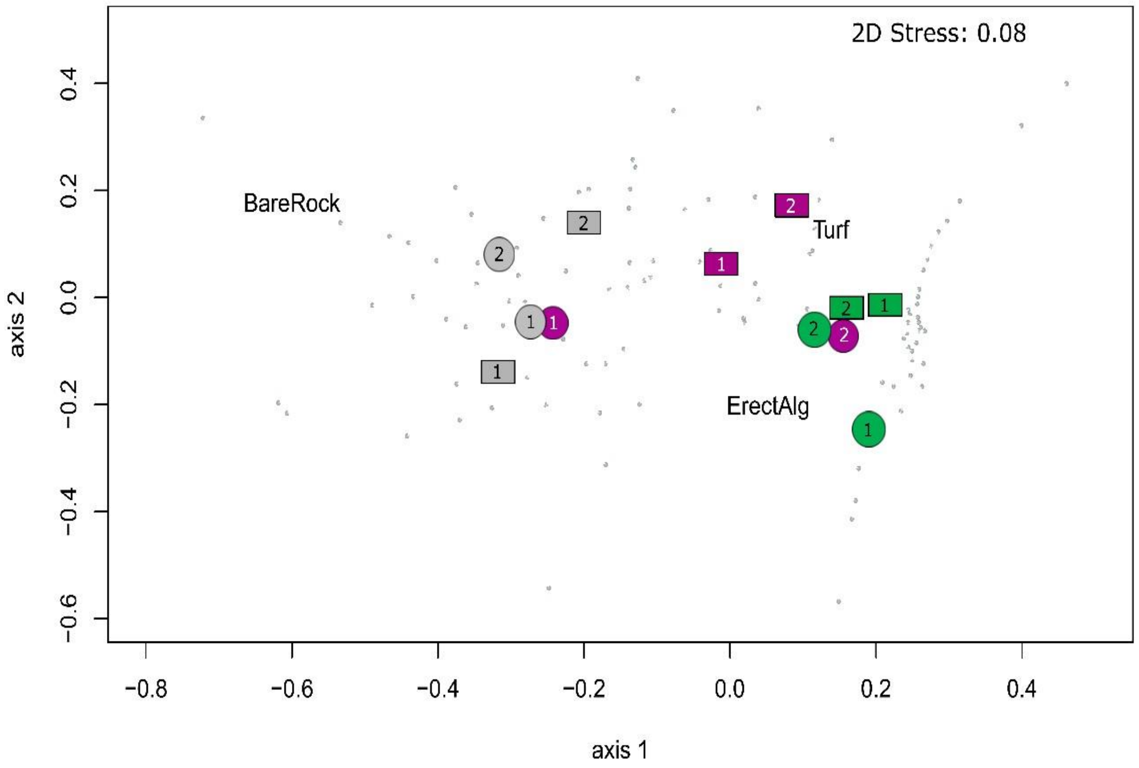

**Figure 6.** Non-metric multidimensional scaling (nMDS) ordination plot of the relationships between different restoration strategies and community structure before (1) and one year after forestation actions (2). The cover of erect algae, turf algae, and bare rock were plotted for each restoration strategy. Boxes indicate non-protected locations (outside the NTZ) and circles indicate locations inside the NTZ. Grey color represents reference barren grounds, green color represents reference forests and, pink, degraded barren ground in which active restoration was done.

## 3.3 Costs of forestation actions

The estimated cost to forest a 200m$^2$ sea urchin barren ground is estimated in 1,140 € (Table 5). While the costs covering the materials used for the active actions accounts for just one tenth of the total costs, staff costs required the majority of the budget. The differences in costs between sea urchins eradication and *Cystoseira* forestation were negligible, encouraging the combination of both actions at the same time.

**Table 2.** Estimated costs of both restoration techniques, the complete sea urchin eradications together with the in situ *Treptacantha elegans* forestation.

| **Concept** | **Rate** | **Cost** | **Total (€)** |
|---|---|---|---|
| **Common costs** | | | |
| *Transport* | | | |
| Boat (gasoil) | 5 km | 1.3/L | 6.5 € |
| Car (gasoil) | 300 km | 0.40€/km | 120 € |
| *Staff* | | | |
| Salary | 3 persons x 1 day | 281.76€/person | 845.28 € |
| Air diving cylinder | 1 dive x 3 persons | 5.85€/dive | 26.55 € |
| Annual diving insurance | 3 persons x 1 day | 4.31 € day/person | 12.93 € |
| | | | **1,011.26 €** |
| **Sea urchins eradication** | | | |
| *Material* | | | |
| Scraper | 3 | 4.5€/unit | 13.5 € |
| Big shopping bags | 3 | 3€/unit | 9 € |
| Safety gloves | 3 pairs | 2.5€/pair | 7.5 € |
| | | | **30 €** |
| ***Treptacantha elegans* forestation** | | | |
| *Material* | | | |
| Epoxy putty | 1 unit of 2 components | 70€/kg | 70 € |
| Plastic screws | 6 units | 12.5€/100 units | 0.75 € |
| Cable ties | 6 units | 1.69€/50 units | 0.21 € |
| Meshed bags | 6 units | 0.15€/unit | 0.9 € |
| Scissors | 1 unit | 1.95€/unit | 1.95 € |
| Hammer | 1 unit | 3.45€/ unit | 3.45 € |
| Cooler box | 1 unit | 20€/ unit | 20 € |
| Zipper plastic bags | 3 units | 1.5€/10 units | 0.45 € |
| | | | **97.71 €** |
| **Total** | | | **~1138.97 €** |

## 4. Discussion

This study presents a novel approach on combined passive and active restoration strategies in a NTZ marine reserve to forest sea urchin barren grounds. A larger forestation success was achieved one year after active and passive restoration strategies were combined, increasing the structural complexity relative to *Cystoseira* forests in barren grounds. A successful three-step protocol to promote forestation of sea urchin barrens was established by combining sea urchin eradication and *Cystoseira* forestation. We evaluated this forestation protocol with 2 different restoration strategies (active, and active + passive). Results presented here supported previous findings about sea urchin grazing or *Cystoseira* recruitment success while reinforcing the role of NTZ marine reserves to improve the ecosystem state and function.

No-take marine reserves have been proposed as an effective tool to control sea urchin abundances due to their potential ability to restore trophic cascades and thus increasing the top-down control on sea urchins (Pinnegar et al., 2000; Guidetti, 2006; Barrett et al., 2009; Sangil et al., 2012). Unfortunately, when large sea urchin individuals are already present, restored trophic cascades may not be strong enough to break hysteresis loops and control sea urchin populations (Medrano et al., 2019a). Beyond this predatory control, recruitment variability, spatial refuge availability, escaping behavior, and adult-juvenile associations can maintain dense sea urchin populations even when predatory fishes are abundant (Nishizaki and Ackerman, 2004; Zhang et al., 2011; Bonaviri et al., 2012; Hereu et al., 2012a; Oliva et al., 2016). Conversely, approaches involving commercial and recreational sea urchin fishery have also been reported in some studies as a potential factor controlling sea urchin abundance (Guidetti et al., 2004; Pais et al., 2007) and can promote *Cystoseira* recruitment as shown in recent studies (Piazzi and Ceccherelli, 2019). Even though sea urchin harvesting is currently highly regulated in our study area, it could be considered as an additional strategy to recover *Cystoseria* forests.

Applied active removal approaches in our study involved the complete eradication of sea urchins to reduce and control their abundance in the barren grounds before forestation. As expected within the short time scale of this study, no reduction of the sea urchin populations was observed within the reference barren inside the NTZ. Conversely, successful control of sea urchin populations was achieved through active eradication, since significantly reduced sea urchin abundance was found after removal both inside and outside the NTZ one year after the eradication, small-sized sea urchins were observed again in the localities due to the arrival of new individuals but larger sea urchins were significantly reduced. Collectively, these observed responses tend to support that fish predation alone cannot successfully control established sea urchin populations with large-sized individuals due the high recruitment and juvenile survival of sea urchins (Medrano et al., 2019a). To date, however, predatory control on sea urchins cannot be completely ruled out, as longer term monitoring is necessary to test the true effectiveness of this single eradication action outside the NTZ. A higher cover of *T. elegans* was observed inside the NTZ than outside at equal sea urchin densities one year after eradication, which could suggest non-lethal effects of sea urchin predators on their prey. Grazing activity of *Paracentrotus lividus* could have been reduced inside the NTZ as a result of predator-induced fear (Hereu, 2005; Manzur and Navarrete, 2011).

Control of sea urchin populations has been generally proposed as individual restoration tools to reestablish habitats such as marine forests. Even though their role reducing overgrazing has been proved, this forestation technique may fail due to limited dispersal capability of natural neighbor algae populations, and the reduced potential of natural ecological succession after sea urchins have been removed (Leinaas and Christie, 1996; Watanuki et al., 2010; Piazzi and Ceccherelli, 2019). To solve this limitation, the combination of both techniques (i.e., combined sea urchin control and enhanced *Cystoseira* recruitment) proposed in our study ensured and accelerated the formation of complex marine forests with low dispersal and recruitment natural potential.

A range of ex situ and in situ seeding techniques have been proposed to enhance the natural limited dispersal ability of *Cystoseria spp.* zygotes and reduce the high mortalities on their first life stages reported here and elsewhere (Mangialajo et al., 2012; Verdura et al., 2018). Conservation efforts to obtain recruits on ex situ hatcheries have been explored on fucoid algae (Hwang et al., 2006; Verdura et al., 2018; de la Fuente et al., 2019) since this technique allows to control the environmental conditions for an optimal development obtaining large densities of new recruits (Falace et al., 2018). These techniques are limited due to the high costs to maintain infrastructures, transportation, and the limited range of reproducible environmental conditions (usually only light and temperature). Therefore, ex situ approaches are only cost-effective when the density of adults and recruits of *Cystoseira* species in the field are very low and the necessary recruit densities can only be obtained in laboratory conditions. Since we found similar densities and mortality rates of *T. elegans* recruits with both in situ and ex situ seeding techniques, we selected the in situ approach due to the much lower economic costs and avoiding any acclimation steps to natural environmental conditions in the development stages.

The mentioned limited natural recruitment in macroalgae also relies on their ability to colonize the substrate upon arrival which can be driven by light availability (Reed and Foster, 1984), competition (Steen and Scrosati, 2004), or microhabitat characteristics such as substrate type (Benedetti-Cecchi and Cinelli, 1992; Mangialajo et al., 2012). Similar limitations are also found in *Cystoseira* species (Capdevila et al., 2015; Verdura et al., 2018), and our study shows differences in the ability of *T. elegans* to recruit on occupied and cleaned substrata. We found that recruitment success could be improved by providing adequate cleared (i.e., from both organisms and sediment) substrate available to the new recruits. Although no major differences were found in recruit densities between stone plates or natural cleared collectors, recruiting on natural substrates ensures long-term viability as stone plates may breakdown due to deterioration. Besides, preserving the natural heterogeneity and complexity of the substrate such as in cleared collectors may favor the survival and growth of *Cystoseira* early life stages. These observations suggest that cleared natural collectors are a promising technique to enhance recruitment for *T. elegans*.

Following general approaches, we evaluated the success of this restoration action based on high survival and/or density sustained over time (Gianni et al., 2013). *T. elegans* cover increased one year after forestation, reaching cover levels high enough to consider results from the combined active with passive strategy very successful. As suggested in previous studies (Verdura et al., 2018), we also evaluated the *T. elegans* size-class structure to complement this result with other recovery attributes. *T. elegans* individuals of restored forests inside the NTZ reached similar size-class structure to the reference forest only a year after the active restoration. The recovery time of degraded habitats with mature *T. elegans* forests observed in this study was shorter than the observed on previous successful *Cystoseira* restoration actions (e.g., Verdura et al., 2018). In addition, we compared the benthic community structure on restored locations which became similar to the reference forests one where active and passive restoration was combined, replacing the low productive barren grounds with more diverse and functional forests. However, cover levels in these restored forests did not reach reference levels in the mature *T. elegans* forests for any of the restoration strategies evaluated. Other successful restoration cases tend to indicate that complete functional and diversity restoration of macroalgal habitats is a complex process only achievable in the longer term (Marzinelli et al., 2016).

The small extension of the NTZ marine reserve in our study limited the design for replicated locations, but allowed the rapid assessment of local restoration efforts. Only one location inside the NTZ could be included into the assessment for the passive restoration strategy as no more locations were eligible, and thus the success of this approach in restoration in our study site remains not fully explored. Our design, however, allowed for a rapid (in one year) and low-cost forestation of degraded shallow barrens occurring at local scales of hundreds of square meters. Low economic costs of combining forestation actions with sea urchins eradication in our study were comparable to other in situ actions carried out to restore *Cystoseira barbata* populations at local scales (Verdura et al., 2018), indicating the economic feasibility of these local forestation actions.

In light of the global decline of canopy-forming macroalgal forests, forestation of sea urchin barren grounds is encouraged in this study as a tool to shift from low productive and structurally poor barren grounds to high productive and diverse marine forests. Finally, this successful restoration case shows that not only do combined passive and active restoration techniques provide better results than isolated approaches, but also indicates that more effectively-managed No-Take marine reserves can be essential for both management purposes and ecological restoration.

## Acknowledgements

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# General discussion and conclusions

# Discussion

This thesis addressed marine vegetation changes in the shallow rocky shores of the Montgrí, Illes Medes and Baix Ter Natural Park (NW Mediterranean Sea) integrating the macroalgal and sea urchin dynamics in front of natural and human-related impacts, and the role and effectiveness of Marine Protected Areas and restoration actions as conservation tools at lower trophic levels. Besides, since most of this thesis is based on long-term monitoring data, a valuable baseline of the algal community's dynamics and current status was provided here which could be vital to predict and detect future ecological changes that could jeopardize the preservation of seaweed forests.

Loss of marine forests due to sea urchin outbreaks has been often reported within temperate seas (reviewed by Filbee-Dexter and Scheibling, 2014). Once the forests shift to barren states, the rapid transition from macroalgal beds to the sea urchin high-density state increase the feedbacks that reinforce the resilience of the degraded state (Filbee-Dexter and Scheibling, 2014; Melis et al., 2019). Facing the ongoing global change, potential synergistic interactions of multiple stressors may further increase the hysteresis of stable degraded ecosystems which would hinder the future conservation of marine forests and important losses of ecosystems services (Rogers-Bennett and Catton, 2019). As human threats are expected to increase on the marine environments (IPCC, 2014), it is likely that regime shifts will become more frequent, presenting major challenges for ecosystem management (Conversi et al., 2015). Due to their worldwide concern and their expected upward trend, an emphasized approach to overgrazing threat on macroalgae and consequent regime-shifts between marine forests and barren grounds were addressed here from a management perspective.

## 1. Understanding long-term dynamics of macroalgae and keystone herbivores

### 1.1. Some optimism face to global decline of algal communities

Seaweed beds formed by macroalgae on rocky bottoms have been recognized to have a key role in temperate coastal ecosystems. Macroalgal beds are threatened by multiple and diverse stressors, ranging from local to global threats, as a result of human activities or natural fluctuations (Mineur et al., 2015). Although several studies reported dramatic declines of marine forests as consequence of several ongoing stressors impacting these communities at local and global scales (Airoldi and Beck, 2007; Connell et al., 2008; Raybaud et al., 2013; Mineur et al., 2015), this thesis exposes two encouraging stories. First, macroalgal assemblages in the shallow rocky shores of the Montgrí, Illes Medes and Baix Ter (MIMBT) Natural Park were stable over the last fifteen years contrasting with important declines of algal forests over the world (Wernberg et al., 2011a; Filbee-Dexter and Wernberg, 2018) and also in the Mediterranean Sea (Perkol-Finkel and Airoldi, 2010). Some variability were observed thought the past years but the absence of major shifts in the most representative macroalgal species abundance was highlighted in this dissertation. Secondly, this thesis provides evidence of a recent expansion of the canopy-forming *Treptacantha elegans* in the Catalan coast. Bearing in mind that this species was reported in decline in nearby rocky shores (Thibaut et al., 2005; 2015) and listed as threatened species in the Mediterranean Sea mainly as a result of coastal development and sea urchin overgrazing (Verlaque et al., 2019), ours results provide some hope on the capacity of these algal forests to persist or even widen their geographical distributions under global change scenarios (see also Thibaut et al., 2014b).

Redistribution of the species is a common response to stressors on land and ocean, with shifts in the latitude and depth to keep pace with preferred environmental conditions (Pecl et al., 2017). Recent changes in the distribution of many marine taxa have been documented on all continents (Perry et al., 2005; Wernberg et al., 2011b; Poloczanska et al., 2013). Changes in macroalgal distribution, which may be particularly sensitive to human threats (Harley et al., 2012; Poloczanska et al., 2013), include both range extensions, where species colonize new, usually adjacent habitats, and range contractions, where species lose previously occupied areas (Wernberg et al., 2011b; Bates et al., 2014). The spreading of the canopy forming *T. elegans* could be explained by the interaction of favorable traits to colonize and compete for the substrata detailed in this thesis, such as their fast-growing dynamics, their early fertile maturity -within their first years of life- and their high turnover rate, compared with other canopy-forming species.

## 1.2. Lessons learned from long-term monitoring: understanding temporal shifts of keystone species and the role of extreme events in algal-dominated communities

Sea urchins, and especially *Paracentrotus lividus,* are the main herbivore structuring the algal communities in the NW Mediterranean Sea (Hereu et al., 2012a). Recruitment variability, spatial refuge availability, escaping behavior, and adult-juvenile associations can act as feedback mechanisms that maintain dense sea urchin populations even when predatory fish are abundant (Nishizaki and Ackerman, 2004; Zhang et al., 2011; Bonaviri et al., 2012; Oliva et al., 2016). Due to the combination of these factors, the long-term monitoring of sea urchins in the studied Natural Park have showed a large variability on long-term trends of *P. lividus* density since 1991 regardless of the high densities of predatory fish observed inside the NTZ (Hereu et al., 2012a). Beyond this variability, the long-term dataset studied in this thesis has allowed to report the rebuilding capacity of collapsed sea urchin stocks after extraordinary mass mortalities, which have been poorly studied until now due to the lack of robust data (but see Edmuns and Carpenter, 2001; Miller et al., 2003; Girard et al., 2012; Clemente et al., 2014; Lessios, 2016).

The black sea urchin *Arbacia lixula* is also common in the Mediterranean Sea but is much more abundant in the southern and eastern areas of the Mediterranean Sea than in the NW where this thesis took place, with a low density in the MIMBT Natural Park over the last decades (Hereu et al., 2012a). *A. lixula* is considered a thermophilic species (Privitera et al., 2011; Wangensteen et al., 2013) and it has been suggested that climate change can increase its abundance and distribution (Francour et al., 1994; Wangensteen et al., 2012). Nevertheless, there is little quantitative evidence of these increasing patterns in the field to date. As a result of the long-term monitoring dataset studied, this thesis reported an increase of densities and biomass over the last years in the MIMBT Natural Park, reaching values never observed before. The upward trend seems to be linked to the fast-growing rate of *A. lixula* sea urchin (Barrera, 2018) and the warming trends observed on the Catalan coast in the recent decades, where the temperature has increased 0.26 ± 0.08 °C/decade from 1985 to 2015 (Vargas-Yáñez et al., 2017).

Several studies to date have demonstrated that extreme climate events can have important effects on rocky shore communities (Denny et al., 2009; Micheli et al., 2016; Borja et al., 2018). Severe storms and extreme waves can trigger mass mortalities in sea urchins populations (Scheibling and Lauzon-Guay, 2010; Scheibling et al., 2010) and have been also considered as potential drivers eroding macroalgal beds (Navarro et al., 2011; Borja et al., 2018; Capdevila et al., 2019a). The extraordinary storm occurred in the MIMBT Natural Park in 2008 give us the opportunity to address partly this issue understanding how benthic communities could respond to single catastrophic events in the framework of a long-term monitoring data. Benthic shallow-rocky habitats in the MIMBT Natural Park are mostly represented by photophilic algal communities of small and erect algae (e.g. *Dyctiota spp.*) and calcareous algal formations (Ballesteros, 1991). Right after the 2008 storm, and accordingly with similar studies (Navarro et al., 2011; Michelli et al., 2016; Maggi et al., 2018), macroalgal assemblages in the MIMBT Natural Park changed abruptly (Fig. 1). Most of the perennial macroalgal species were swept away while the seasonal species took advantage of the available space. However, the abundance and structure of the main macroalgal assemblages recovered swiftly to pre-storm values and no long-term impacts were observed over the following years, indicating that impacts of a single extraordinary storm on rocky-shallow algal communities may be reversed relatively quickly through the recovery of the main canopy-forming species (Fig. 1). Contrarily, sea urchin populations were affected after the 2008 severe storm. After the almost complete depletion of sea urchin populations, they needed more time to recover than the observed for macroalgal assemblages. Besides, clear differences in recovery related to the marine protection were found here, which will be addressed in the next section of this discussion (Fig. 1).

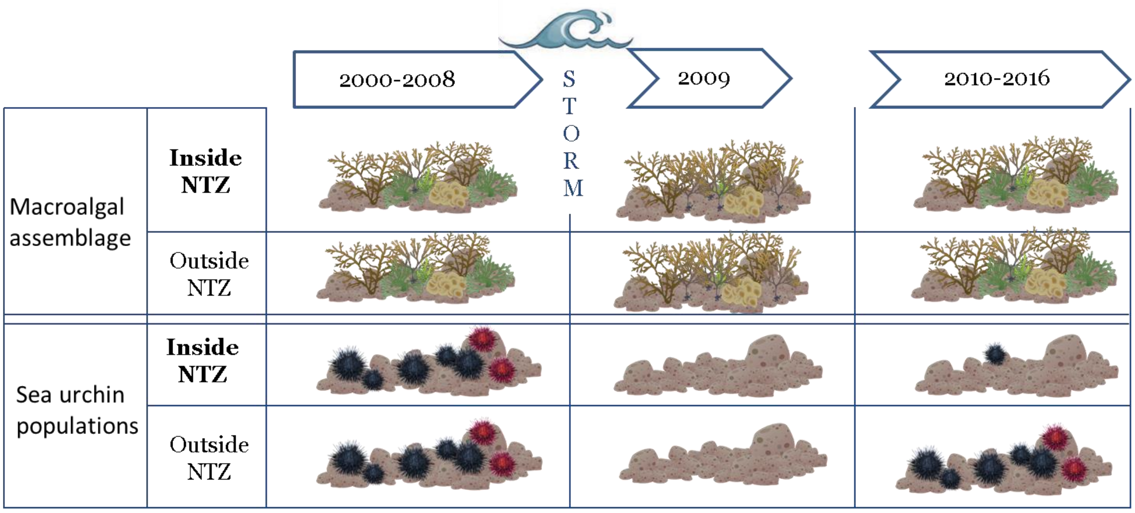

**Figure 1.** Schematic illustration of the effects of the 2008 severe storm on macroalgal assemblage and sea urchin populations inside and outside the NTZ.

Notwithstanding that, we should consider that these recovery patterns correspond to a single extreme event and an increase in their recurrence or frequency could modify the observed patterns. An increase in the intensity and frequency of extreme climatic events, such as extraordinary storms has been observed since 1950 and is expected to increase in the future (IPCC, 2014; Reguero et al., 2019), especially in the Mediterranean Sea, which has been highlighted as a hotspot of ongoing climate change. Facing these environmental changes future research exploring the thermotolerance of studied species may be addressed to properly manage and conserve seaweed marine forests in our changing sea.

## 2. The role of Marine Protected Areas in benthic communities

Facing all the exposed local and global threats, marine protected areas may mitigate the total sum of local disturbances affecting the marine ecosystems, hence enhancing the resilience of populations toward global factors away from the umbrella of the local management such as climate change (McLeod et al., 2009; Brown et al., 2013; Lamb et al., 2015).

The benefits of MPAs and NTZs are universally known, being the increase in biodiversity and species biomass the most repeatedly reported. Although Marine Protected Areas (MPAs) have been historically conceived to conserve commercial and harvested species from the human impacts, especially from fishing (No Take Zones -NTZs-) and coastal development, including species that play a key ecological role in the design of MPAs (e.g., canopy forming algae) may enhance the conservation of the biodiversity and of the complex services that they provide to the ecosystem functioning (Palumbi et al., 2009; Gianni et al., 2013). NTZs prevent the overexploitation of key structural species, allowing for the reduction or removal of associated impacts, maintaining and restoring ecosystem functioning, with the subsequent improvement of trophic regulation on populations of consumers (i.e. trophic cascades).

No-take areas (those fully protected from fishing) have been proposed as an effective tool to control sea urchin abundances due to their potential ability to restore trophic cascades and thus increasing the top-down control on sea urchins (Fig. 2) (Pinnegar et al., 2000; Guidetti, 2006; Barrett et al., 2009; Sangil et al., 2012).

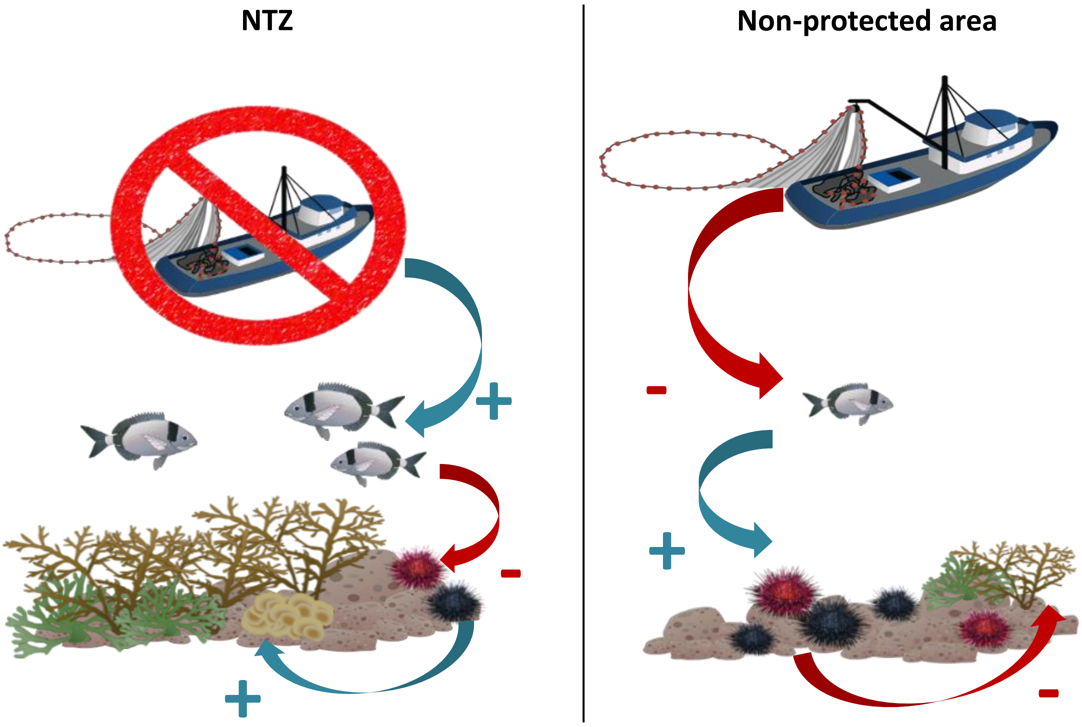

**Figure 2.** Schematic illustration of a trophic cascade in Mediterranean rocky shallow shores and the role of marine protected areas on it. Blue arrows indicate positive effects and red ones, negative effects. Symbols extracted from IAN Symbol Libraries.

Unfortunately, we have seen in the chapter 2 that, if large sea urchin individuals were already established when the protected area was created (such as in the MIMBT Natural Park), restored predator fish populations may not be strong enough to break the stability of sea urchin barren community. This is consistent with the presence of feedback mechanisms that maintain the high stability of high-density sea urchins state. For example, the presence of adult individuals facilitate the juvenile survival by offering refuge under their spiny canopy for avoiding predation (Zhang et al., 2011; Hereu et al., 2012a) and reducing micro-predator abundance (Bonaviri et al., 2012). The 2008 dramatic storm reset these feedback mechanisms by removing the sea urchin adult individuals. After the almost complete depletion of adult *P. lividus* individuals the trophic cascades inside the NTZ became reestablished which contributed to explaining why sea urchin populations outside the NTZ recovered faster than the populations inside the NTZ. The results of this natural and destructive event shown that predation was the main factor controlling the recovery of sea urchin populations in the NTZ.

Macroalgal assemblages in the MIMBT Natural Park were similar in algal composition and total cover inside and outside the protected NTZ over the last fifteen years. Therefore, a negligible effect of protection was observed on the abundance of the main macroalgal assemblages. However, we observed differences in the abundance of the canopy-forming alga *Treptacantha elegans* which was larger inside the protected NTZ. Given that, NTZs could be particularly relevant as conservation tools for canopy-forming algae as was also suggested by Sala et al. (2012). The combined results of the long-term monitoring of both benthic communities in the rocky-shallow infralittoral zone of the MIMBT Natural Park (macroalgal beds and sea urchin populations) stress out the important potential role of NTZs in the structure of benthic communities (Sangil et al., 2012; Sala and Giakoumi, 2017).

We must not lose sight of the local scale of this thesis in which, the obvious logistics constraints, limit this study to a single marine protected area with no replication of the effect of protection. A perfect design would imply to work simultaneously in different MPAs, however taking into account the intensive fieldwork that implies all the sampling performed in this Ph.D. as well as the inherent differences between MPAs due to the biotic and abiotic factors and the difficulty to perform long-term studies to understand the community dynamics, perform this kind of studies at large spatial scales become really difficult. Notwithstanding that, comparing communities at different regional scales requires a deep understanding of the natural variability of local communities, which was the aim of this thesis for the macroalgal communities on the MIMBT Natural Park.

## 3. Restoration actions in macroalgal beds: a promising but challenging conservation tool

Passive conservation strategies such as NTZs could not be enough to restore degraded benthic ecosystems (Huntington et al., 2011; Sangil et al., 2012; Toth et al., 2014; Cox et al., 2017). Even in NTZs, the natural variability of species dynamics and ecological interactions prevent the recovery or limit it only to long term periods (i.e. decades Babcock et al., 2010; Strain et al., 2019). A more suitable tool for recovery in the shorter term is ecosystem restoration, which involves the active assistance in the recovery of a degraded, damaged, or destroyed habitat (SER 2004).

In the case of degraded macroalgal and seagrass beds, even if there is mitigation or full removal of environmental stressors due to the passive conservation management strategies such as NTZs, it is difficult to recover due to lack of nearby healthy populations which can produce propagules and hence restore the damaged site. This is really relevant for these species with low dispersal ability such as the canopy-forming fucoids (Nyström et al., 2012; Basconi et al., 2020). In these conditions, active restoration can help to recover ecosystems and habitats by management techniques (Perrow and Davy, 2002; Elliott et al., 2007; Holl and Aide, 2011; Bayraktarov et al., 2016). However and despite their importance, macroalgal ecosystems receive little attention and research funding compared with other marine habitats (Bennet et al., 2016; Wood et al., 2019). In top of that, lower success of the few active restoration actions carried out in macroalgal ecosystems were reported in contrast with restoration success of the most restored marine habitats, such as coral reefs, mangroves or salt marshes (Fig. 3) (Basconi et al., 2020).

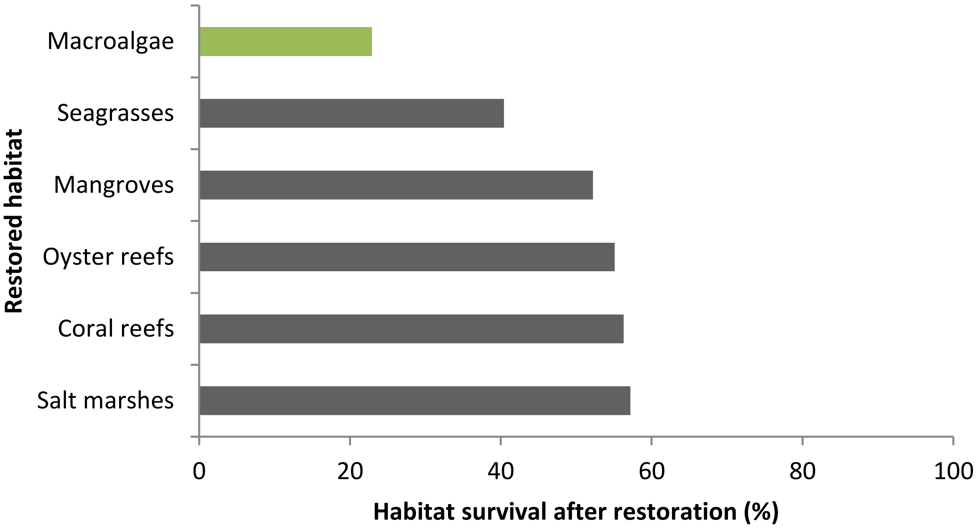

**Figure 3**. Average survival per habitat after restoration. Data extracted from Basconi et al., 2020.

Combining well-designed active and passive restoration practices have become indispensable to reverse widespread ecosystem degradation (Lotze et al., 2006; Mitsch, 2014; Possingham et al., 2015). However, the combination of active (i.e., transplantation) and passive restoration (i.e., NTZs) is a largely unexplored opportunity for macroalgal forests. Therefore, this thesis presents a novel approach on combined passive and active restoration strategies as a tool to shift from degraded sea urchin barren grounds to functional and productive marine forests.

By integrating the acquired knowledge in this thesis about the dynamics of macroalgal beds and sea urchin populations in the MIMBT Natural Park, a three step restoration protocol was assessed in the last chapter to promote the forestation of sea urchin barrens combining the complete eradication of sea urchin populations and *Treptacantha elegans* forestation. Successful forestation of barren grounds was only achieved when this restoration protocol was conducted inside the NTZ. In other words, restoration success was achieved only when passive and active restoration tools were combined, highlighting the important role of marine protected areas not only in conservation but also in ecological restoration.

The majority of restoration studies reported success in terms of survival of the reintroduced species but, in so doing, is difficult to assess the recovery of the ecosystem functionality (Basconi et al., 2020). Here, we went one step further while considering the *Treptacantha elegans* recruitment success of the next generation when foresting but, if this thesis could last more than 3 years, restoration success would be ideally assessed by other measurements evaluating the recovery of ecosystem services and functionality. The incorporation of the study of ecological networks on healthy marine forests against reforested ones may provide a good assessment of the full recovery of the community beyond the effects on single species.

I want to highlight that the use of the word 'forestation' may be more accurate in this thesis than the 'restoration' one. Although degraded and low productive barren areas were transformed here to highly diverse and functional canopy-forming forests, we ignore if the previous state to these degraded habitats were degraded or damaged macroalgal forests or any other habitat.

## 4. Insights into the management and conservation of algal forests

Our forestation results could lead to fisheries agreements that unify the restoration actions with the efforts already done by the sea urchins' fishermen instead of removing high densities of sea urchins only for restoration, especially on barren grounds dominated by commercial species such as *Paracentrotus lividus*. Similar approaches involving commercial and recreational sea urchin fishery have also been reported as potential factor controlling sea urchin abundance (Guidetti et al., 2004; Pais et al., 2007) and can promote *Cystoseira* recruitment as showed in recent studies (Piazzi and Ceccherelli, 2019). Sea urchin fishery policy is highly restrictive on our coast, which could favor the expansion of sea urchin barren grounds. Given the limited number of sea urchin fishery licenses and the reported results of this thesis, I would like to propose that the sea urchin fisheries may be restricted to sea urchin barren grounds as a win-win fishery activity. On one side, fishermen's efforts will be reduced as the densities of sea urchins in the barren grounds are higher than in the common sea urchin fishery grounds. On the other side, algal seeding transplantation actions could be combined with the fishing activities to promote marine forests instead of desert grounds when sea urchins where fished from the restricted grounds. Furthermore, non-commercial species such as *Arbacia lixula* may be controlled by active removal actions such as the ones presented in this thesis.

This dissertation demonstrates the valuable role of the long-term ecological monitoring to provide fundamental knowledge of ecosystems functioning but also as a key tool to detect changes in marine ecosystems (e.g., regime shifts), to validate ecological models, and to design effective management actions (Lindenmayer et al., 2012; Conversi et al., 2015). However, the establishment and maintenance of long-term monitoring have been limited by deficient financial support whereby funding cycles typically support 1-4 years-long projects and that is why ecological research recently emphasize studies using meta-analysis and mathematical models (Day, 2008; Lindenmayer et al., 2012; Lamb, 2017). The lack of long-term studies in macroalgal forests was highlighted in the introduction and should be addressed in order to understand future changes inside but also outside MPAs. In this sense, Citizen Science could be considered an interesting tool to preserve long-term rigorous monitoring. Citizen science projects involve public in scientific research with the added benefit of increase their knowledge and sensitivity to not only study but also protect the natural systems (Bonney et al., 2009).

# Conclusions

## Macroalgal and sea urchins long-term dynamics in the Montgrí, Illes Medes and Baix Ter (MIMBT) Natural Park

- From a well-designed long-term monitoring, this thesis provides current baselines of the rocky-shallow macroalgal assemblages and sea urchin populations in the NW Mediterranean Sea (CHAPTER I and CHAPTER II).

- Coastal macroalgal assemblages in the MIMBT Natural Park were stable and similar in total algal cover over the fifteen years of study with no species replacement, although some variability were observed between years (CHAPTER I).

- Impacts of a single extraordinary storm on rocky-shallow macroalgal communities may be reversed relatively quickly as no long-term impacts were observed in the abundance and composition of the main macroalgal assemblages after the severe storm of 2008. However, we should consider that an increase in the intensity and frequency of extreme climatic events is expected in the future (CHAPTER I).

- Bottom-up (resources control) and top-down (herbivorous control) processes interactively influence the structure of macroalgal benthic communities in the MIMBT Natural Park. Bottom-up forces such as spring nutrient concentration and water temperature influence the abundance of most of the seasonal algal species while top-down forces may play a role in determining the abundance of fucoids such as the canopy-forming *Cystoseira sensu lato* species and the encrusting coralline algae species (CHAPTER I).

- Predation (top-down) is the main force controlling the recovery of depleted sea urchin populations since, after the observed mortality event, sea urchin populations inside the NTZ recovered slowly than the populations outside the NTZ (CHAPTER II).

- The canopy-forming alga *Treptacantha elegans* has substantially increased their depth range and spatial distribution over the last two decades in the Catalan coast (CHAPTER III).

- In the Medes Islands NTZ, *T. elegans* forests showed a fast growing dynamics, and their stands were able to reach their fertile maturity within their first year of life, which could represent an advantage to colonize the available substrate (CHAPTER III).

- No differences were observed between *T. elegans* annual mortality rates and the percentage of new individuals observed within the same year. This turnover rate may support their recently expansion (CHAPTER III).

- All the *T. elegans* populations analyzed along the Catalan constitute a single genetic group with little differentiation of populations (CHAPTER III).

## Management outcomes for macroalgal forests and sea urchin barrens

- Marine protection does not affect the composition and total algal cover of the coastal macroalgal assemblages in the rocky shores in the MIMBT Natural Park. However, protection is particularly relevant to canopy-forming *T. elegans,* which was more abundant inside the NTZ than in unprotected areas (CHAPTER I).

- Restored trophic cascades may not be strong enough to break hysteresis loops and control sea urchin populations once they are established. However, fully protected areas (NTZs) have an important role controlling the recovery patterns of sea urchins populations (CHAPTER II, CHAPTER IV).

- Due to the fast and stable population dynamics of the canopy-forming *T. elegans*, forestation of sea urchin barren grounds using *T. elegans* seeding is encouraged in this thesis as a potential technique to restore degraded ecosystems in rocky shallow habitats (CHAPTER III and CHAPTER IV).

- A successful three-step protocol to reverse degraded shallow hard-bottoms into high productive and diverse marine forests has been achieved. This protocol includes: 1) sea urchin population eradication, 2) seeding with *T. elegans*, and 3) enhancement of *T. elegans* recruitment (CHAPTER IV).

- Our results showed that combined active and passive restoration (e.g., MPAs or NTZs) strategies increase the success of restoration actions (CHAPTER IV).

- More effectively-managed No-Take marine reserves can be essential for both management purposes and ecological restoration (CHAPER II and CHAPTER IV).

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# Supplementary material

## Supplementary material Introduction (S1)

Bahlai, C. A., Colunga-Garcia, M., Gage, S. H., and Landis, D. A. (2015). The role of exotic ladybeetles in the decline of native ladybeetle populations: evidence from long-term monitoring. *Biological Invasions*, 17(4), 1005-1024.

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## Supplementary material Chapter I (S2)

**Table 1 S2**. Results from the Tukey pairwise comparison on the CVs between protection levels (inside/outside NTZ). Bolded font indicate significant differences between pairs. Species code in Table 1.

| Pairs (inside/outside NTZ) | Pr(F) |
|---|---|
| Inside/Outside NTZ | **0.749** |
| | |
| *Asparagopsis* | **0.005** |
| *Dictyota* | 0.011 |
| *T. elegans* | 0.125 |
| *C. vermilara* | 0.231 |
| *C. bursa* | 0.516 |
| *Halopteris* | **0.030** |
| *Laurencia* | 0.526 |
| *Padina* | 0.156 |
| *Sphaerococcus* | 0.319 |
| *Wrangelia* | **<0.001** |
| *Corallina* | 0.897 |
| *Jania* | 0.190 |
| *L. incrustans* | 0.019 |
| *M. alternans* | **0.043** |

## Supplementary material Chapter II (S3)

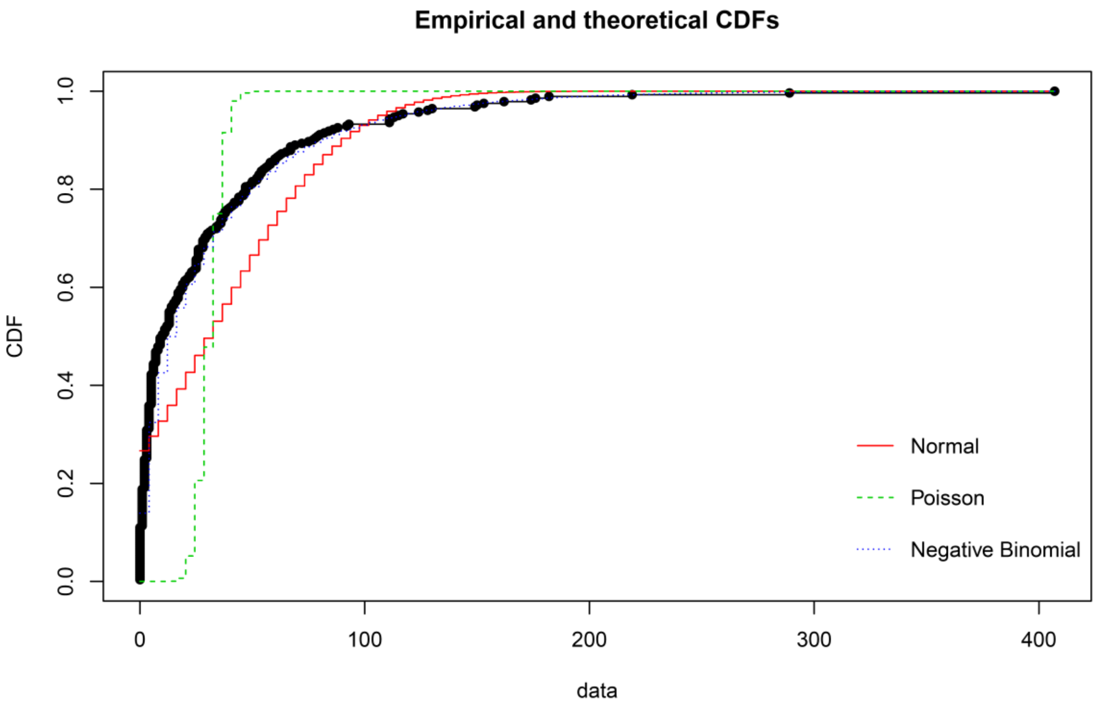

**Fig. 1 S3.** Empirical cumulative distribution of *Paracentrotus lividus* abundance between 2009 and 2013 (the studied recovery period) against fitted distribution functions. The negative binomial distribution matches more closely.

# Supplementary material Chapter III (S4)

**Table 1 S4.** Pearson correlation coefficients between the length of the longest axis of *T. elegans* and other morphometric variables

| Variable | r2 | p |
|---|---|---|
| Biomass of branchlets (g fw) | 0.61 | **<0.01** |
| Holdfast diameter (cm) | 0.40 | **0.04** |
| Main axis length (cm) | 0.48 | **0.01** |
| Number of tophules | 0.48 | **0.01** |
| Number of primary axes | 0.20 | 0.33 |

**Table 2 S4.** AMOVA analyses results for differentiation between the three populations. P-value significance for 10,100 permutations.

| **Source of Variance** | **df** | **Sum of Squares** | **Variance Components** | **% Variation** | **F-$_{ST}$** | **P-value** |
|---|---|---|---|---|---|---|
| Among populations | 2 | 6.41 | 0.074 | 6.7 | 0.07 | **<0.001** |
| Within populations | 85 | 87.84 | 1.033 | 93.3 | | |

# Supplementary material Chapter IV (S5)

**Table 1 S5**. Results of the generalized linear model (GLM) testing differences in density of *T. elegans* recruits between different forestation techniques (in situ and ex situ) across time.

| **Model** | **Variables** | **Coefficients** | | | | **AIC** |
|---|---|---|---|---|---|---|
| | | Estimate | Std. Error | *z value* | *p* | |
| Recruits density ~ Technique + Time | Intercept | 3.4144 | 0.4349 | 7.852 | <0.001 | 137.01 |
| | Technique (*in situ*) | 0.1579 | 0.6142 | 0.257 | 0.797 | |
| | Time (2 months) | -2.5390 | 0.6745 | -3.764 | **<0.001** | |
| | Technique*Time | -0.6969 | 0.9841 | -0.708 | 0.479 | |

**Table 2 S5**. Results of the generalized linear model (GLM) comparing the initial density of *T. elegans* recruits between the different tested collectors.

| **Model** | **Variables** | **Coefficients** | | | | **AIC** |
|---|---|---|---|---|---|---|
| | | Estimate | Std. Error | *z value* | *p* | |
| Recruits density ~ Collector | Intercept | 1.9938 | 0.1442 | 13.829 | **<0.001** | 682.17 |
| | Stone plates | 0.6585 | 0.1988 | 3.312 | **<0.001** | |
| | Cleared substrate | 0.8570 | 0.1964 | 4.364 | **<0.001** | |

**Table 3 S5**. Results of the generalized linear model (GLM) comparing the *T. elegans* recruits mortality two months after transplantation in relation to the type of collector tested.

| **Model** | **Variables** | **Coefficients** | | | | **AIC** |
|---|---|---|---|---|---|---|
| | | Estimate | Std. Error | *z value* | *p* | |
| Mortality rate ~ Collector | Intercept | 0.92357 | 0.03855 | 23.956 | **<0.001** | 44.083 |
| | Stone plates | -0.05887 | 0.05304 | -1.110 | 0.2687 | |
| | Cleared substrate | -0.11685 | 0.05261 | -2.221 | **0.0277** | |

**Table 4 S5.** Results of the generalized linear mixed models (GLMMs) testing differences in the sea urchin biomass before and after the eradication between the active restoration strategy and the reference sites and the role of protection.

| **Models** | | **Coefficients** | | | | |
|---|---|---|---|---|---|---|
| | **Fixed effects** | Estimate | Std. Error | *z value* | *p* | **AICs** |
| Sea urchin biomass ~ Strategy * Protection*Year + (1\|Strategy:Location) | Intercept | 5.27896 | 0.20484 | 25.771 | **<0.001** | 2163.8 |
| | Strategy Barren | -0.22075 | 0.38335 | -0.576 | 0.565 | |
| | Strategy Forest | -3.15869 | 0.52052 | -6.068 | **<0.001** | |
| | NTZ | 0.13598 | 0.281 | 0.484 | 0.628 | |
| | Year 2019 | -1.89847 | 0.29135 | -6.516 | **<0.001** | |
| | NTZ: Strategy Barren | 0.63339 | 0.60288 | 1.051 | 0.293 | |
| | NTZ: Strategy Forest | -0.09471 | 0.73219 | -0.129 | 0.897 | |
| | Strategy Barren:2019 | 2.20833 | 0.56189 | 3.93 | **<0.001** | |
| | Strategy Forest:2019 | 3.22407 | 0.66516 | 4.847 | **<0.001** | |
| | NTZ:2019 | 0.32097 | 0.40546 | 0.792 | 0.429 | |
| | Strategy Barren:NTZ:2019 | -0.86416 | 0.83694 | -1.033 | 0.302 | |
| | Strategy Forest:NTZ:2019 | 0.4878 | 0.93641 | 0.521 | 0.602 | |
| | **Random effects** | **Variance** | **Std. Deviation** | | | |
| | Strategy: Location | <0.001 | <0.001 | | | |
| Sea urchin biomass 2019 ~ Protection*Strategy + (1\|Strategy:Location) | Intercept | 3.38049 | 0.24608 | 13.737 | **<0.001** | 1007.8 |
| | NTZ | 0.45695 | 0.34741 | 1.315 | 0.188 | |
| | Strategy Barren | 1.98758 | 0.48915 | 4.063 | **<0.001** | |
| | Strategy Forest | 0.06538 | 0.49196 | 0.133 | 0.894 | |
| | NTZ: Strategy Barren | -0.23026 | 0.69138 | -0.333 | 0.739 | |
| | NTZ: Strategy Forest | 0.3931 | 0.69412 | 0.566 | 0.571 | |

**Table 5 S5.** Results of the generalized linear mixed model (GLMM) testing differences in the final *T. elegans* total cover between the active restoration strategy and the reference locations and the protection role.

| **Models** | **Fixed Effects** | **Coefficients** | | | | **AIC** |
|---|---|---|---|---|---|---|
| | | Estimate | Std. Error | *z value* | *p* | |
| *T. elegans* cover 2019 ~ Protection*Strategy + (1\|Strategy:Location) | Intercept | 14.697 | 0.4085 | 3.597 | **<0.001** | 378.4 |
| | NTZ | 1.071 | 0.5543 | 1.932 | 0.05 | |
| | Strategy Barren | -30.458 | 362.039 | -0.084 | 0.9329 | |
| | Strategy Forest | 22.177 | 0.7607 | 2.915 | **<0.001** | |
| | NTZ: Strategy Barren | 281.712 | 362.039 | 0.078 | 0.9379 | |
| | NTZ: Strategy Forest | -0.8476 | 10.631 | -0.797 | 0.4253 | |
| | **Random effects** | **Variance** | **Std. Deviation** | | | |
| | Strategy: Location | 0.2866 | 0.5353 | | | |

**Figures**

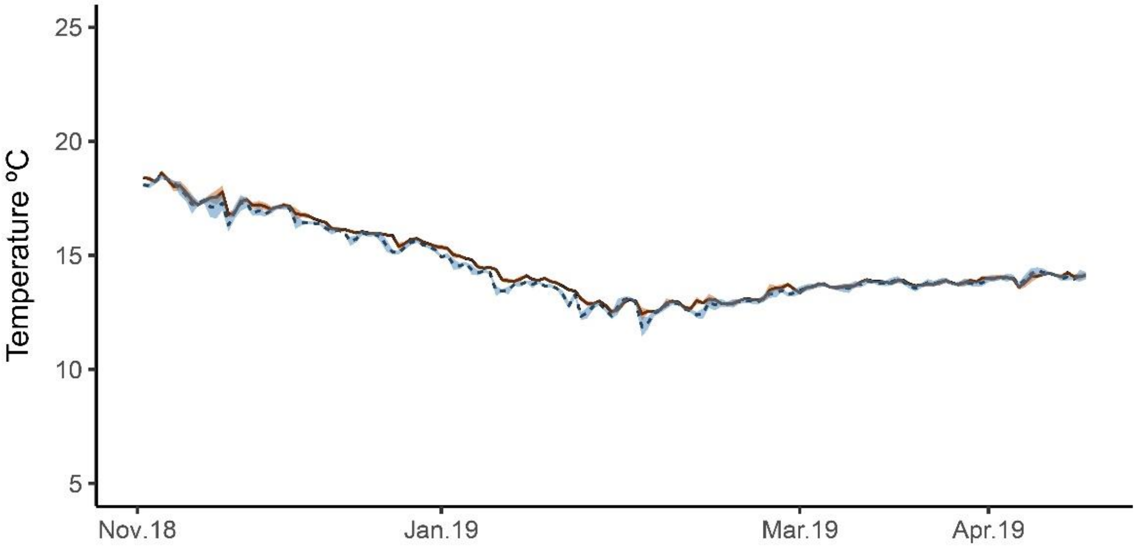

**Fig. 1 S5**. Thermal regime inside and outside the NTZ over the studied period at 6 m depth. Lines correspond to daily mean temperature (ºC) and the colored areas to the SD. Orange color represents the values outside the NTZ and blue color represents the values inside the NTZ.

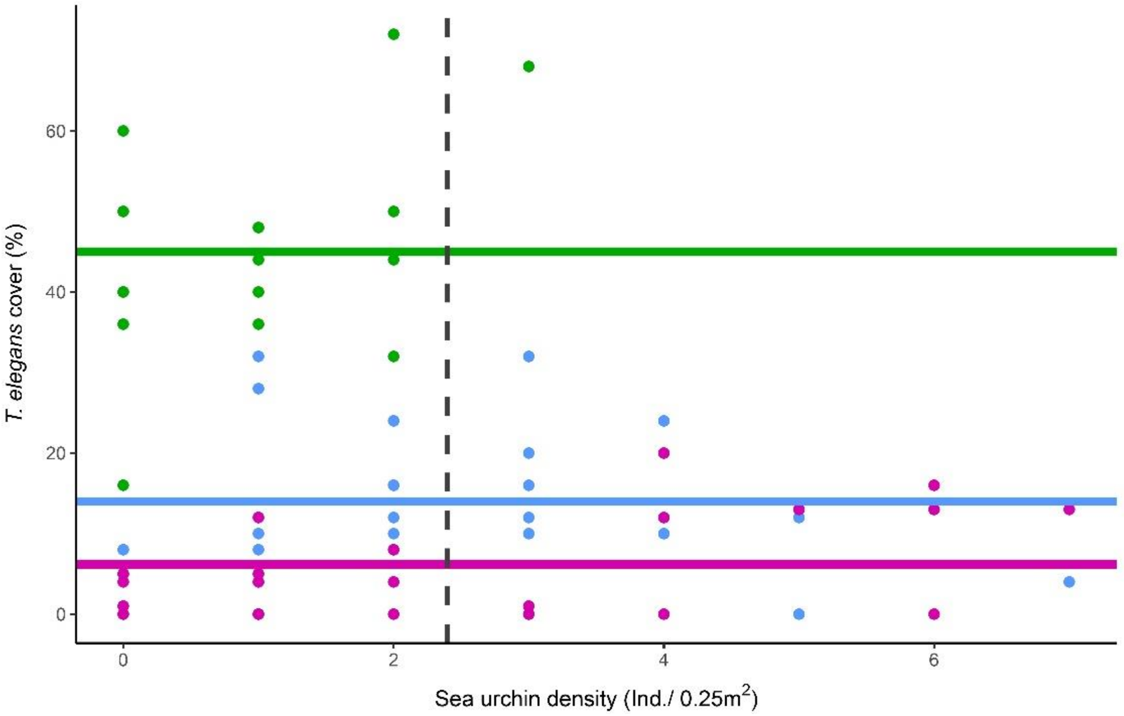

**Fig. 2 S5.** Relationship between *T. elegans* cover and sea urchin abundance one year after forestation within the different restoration strategies. Horizontal continuous and colored lines represent the mean cover of *T. elegans* within the different restoration strategies (pink: A, blue: A+P, and green: reference forest). Vertical dotted line point out the mean density of sea urchins observed one year after the complete eradication.

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# Long-term monitoring of temperate macroalgal assemblages inside and outside a No take marine reserve

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ABSTRACT

Macroalgal communities have an essential role in the shallow benthic habitats of temperate seas, where changes in their composition can resonate through entire coastal ecosystems. As all major ecosystems on Earth, algal beds have already been affected by multiple disturbances. Passive conservation tools, such as marine protected areas or No-take zones, have the potential to reduce some of the anthropogenic impacts by limiting human activity. However, without a good knowledge of the natural community dynamics, it is not easy to discern between changes fruit of the intrinsic variability of biological communities and the ones caused by human-related stressors. In this study, we evaluated the natural variability of macroalgal communities' composition inside and outside a Mediterranean No-Take marine reserve during 15 years. We described their temporal dynamics considering their main drivers and we tested the effect of protection in seaweed beds. We did not find differences either in the composition of the macroalgal assemblages or the total algal cover between protected and non-protected locations over the fifteen years of study. Nevertheless, we observed a positive effect of the protection increasing the cover of some specific species, such as the canopy-forming *Treptacantha elegans*. Our results highlight the importance of obtaining long-term data in ecological studies to better understand the natural variability of marine communities. Accordingly, a robust understanding of the community dynamics would help us to avoid misinterpretations between 'impacted' or 'in-recovery' communities when recovery times are longer than the study periods.

## 1. Introduction

Global and local human disturbances have affected all major ecosystems on Earth, including coastal algal communities (Halpern et al., 2007; Gianni et al., 2017), the dominant primary producers in the coastal zone (Krause-Jensen and Duarte, 2016). Overfishing, global warming, and exceptional storms further contribute to transforming rocky infralittoral algal habitats (Ling et al., 2009; Smale and Vance, 2016; Wernberg et al., 2016; Maggi et al., 2018). Conservation tools such as Marine Reserves or No-Take Zones (NTZ) have the potential to reduce some of these anthropogenic disturbances (i.e, banning fishery activities) and to restore benthic habitats through trophic cascade effects (Shears and Babcock, 2002; Guidetti, 2006; Babcock et al., 2010).

Macroalgae play a key role in the structure of temperate benthic ecosystems (Jones et al., 1994; Duarte and Cebrian, 1996; Teagle et al., 2017), representing an important source of carbon sequestration (Krause-Jensen and Duarte, 2016) and providing invaluable ecosystem services in many shallow coastal systems (Blamey and Bolton, 2018). Consequently, changes in macroalgal communities may sway in the whole coastal ecosystem (Mineur et al., 2015). In the Mediterranean Sea, algal communities dominate the shallow benthic habitats (Zabala and Ballesteros, 1989). There, the highest level of structural complexity is represented by canopies of fucoid algae, mostly *Cystoseira sensu lato* populations, providing habitat, shelter, and food to many associated organisms and harboring a high diversity and productivity (Ballesteros et al., 1998; Mangialajo et al., 2008; Sala et al., 2012; Cheminée et al., 2013). Water pollution, modifications of natural rocky coastline, and sea urchin overgrazing as a consequence of overfishing are the major drivers

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of the loss of fucoids in the Mediterranean Sea (Airoldi and Beck, 2007; Mineur et al., 2015). Consequently, severe declines of macroalgal forests (i.e. *Cystoseira spp.*) have been documented, with the consequent changes in the associated species, and effects cascading up to higher trophic levels (Ling et al., 2015; Cheminée et al., 2013).

Long-term ecological studies observing natural communities dynamics under the human influence are essential to understand the operation of natural systems, and most especially, to know the communities' baseline before describing pressures or impacts. Changes in marine benthic communities and conservation effects are usually reported in 'before-after' studies or with 'snapshots' of the protected area vs. the unprotected one, while long-term monitoring programs are focused on specific species or populations, such as corals, seagrasses, sea urchins or kelp species among others (e.g. Kirkman and Kirkman, 2000; Steneck et al., 2002; Babcock et al., 2010; Gross and Edmunds, 2015). Despite the essential role of macroalgal beds in benthic communities, there is an important lack of continuous and long-term studies that integrate community changes together with their long-term dynamics and with the conservation role, without which we can misinterpret communities alterations.

In this study, we annually monitored infralittoral macroalgal communities inside and outside a NTZ located in the North-Western Mediterranean Sea during 15 years. Benthic shallow-rocky habitats in the study area are mostly represented by photophilic algal communities of small and erect algae (e.g., *Dyctiota spp.*) and calcareous algal formations (Ballesteros, 1991). The sea urchin *Paracentrotus lividus* is the main herbivorous species that control algal biomass (Hereu et al., 2012; Medrano et al., 2019). Abundance and temporal trends on sea urchin populations were similar inside and outside the studied NTZ (Hereu et al., 2012) until an exceptionally violent storm occurred in 2008 that drastically reduced sea urchin densities. After that, the populations of the sea urchin *P. lividus* outside the NTZ recovered faster than the population inside the NTZ (Medrano et al., 2019). The present study aims to describe the temporal dynamics of the macroalgal communities considering their main drivers and environmental stressors and to evaluate the effect of protection in macroalgal beds over time by analyzing long-term monitoring data.

## 2. Methods

### 2.1. Monitoring and study area

Shallow rocky infralittoral macroalgal communities were monitored at eight localities in the Montgrí, the Illes Medes and the Baix Ter Natural Park, located in the North-Western Mediterranean Sea (Fig. 1). Within the Natural Park, four out of the eight sampled localities were placed inside the Medes Islands No-Take Zone (NTZ) and, the other four, outside the boundaries of the NTZ (Fig. 1).

To monitor macroalgal communities, we annually sampled the algal assemblage structure based on the main seaweed species (Table 1) from 2001 to 2016, excluding the years 2006, 2007, 2011 and 2015. The algal percent cover was visually quantified using 50 × 50 cm quadrats divided into 25 subquadrats of 10 × 10 cm (Hereu et al., 2008). Twenty replicate quadrats were randomly counted at each location at depths between 5 and 10 m by SCUBA divers. At each quadrat, each species percentage cover was estimated as the percentage of the numbers of subquadrats where the species was present relative to the total number of subquadrats sampled (as described in Sala and Ballesteros, 1997 and Hereu et al., 2008). To avoid the effect of seasonality, sampling was always performed at the end of May, when the most representative macroalgae (perennial and seasonal species) reach the maximum biomass in the study area (Sala and Boudouresque, 1997).

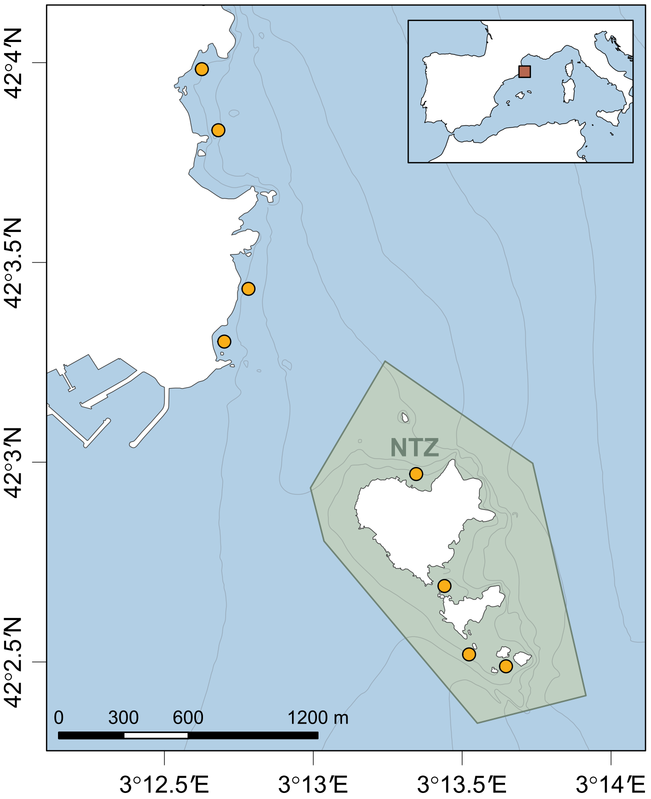

**Fig. 1.** Map of the study sites in the Montgrí, the Illes Medes, and the Baix Ter Natural Park (NW Mediterranean Sea). Orange dots represent the long-term monitoring sites: four are located outside the NTZ, and four more are inside the Medes Islands NTZ. The perimeter of the Medes Islands NTZ is delimited by the green polygon. Unprotected zone is outside the green polygon. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### 2.2. Environmental drivers and stressors determining algal assemblage structure

To investigate the relation between the composition of the macroalgal assemblages and the effect of the environmental drivers inside and outside the NTZ, we used Redundancy Analysis (RDA) (Van Den Wollenberg, 1977) on fourth-root transformed percentage cover data of all the monitored macroalgal species (Table 1). The RDA is a multivariate analysis technique which allows to introduce explanatory (environmental) variables considering multiple regressions to determine linear combinations of these variables with the dependent variables (seaweeds). Water nutrients, sea-surface temperature, sea urchin abundance, and level of protection (as categorical variable) were included as environmental variables (adapted from Hereu et al., 2008).

Specifically, Chlorophyll-a (Chl-a) levels are a good proxy for nutrient levels in coastal waters (Brodie et al., 2007). Therefore, Chl-a data from the MODIS-Aqua sensor were extracted for the monitored area with a 4 km resolution from NASA Giovanni (MODISA_L3m_CHL_v2018; https://giovanni.gsfc.nasa.gov/giovanni/) from 2002 (starting date of this time-series) to 2016. In order to relate the nutrient concentration with the sampled algae cover, the average concentration of Chl-a (mg/m$^3$) was calculated for the spring season of each sampled year (March to May).

Sea-surface temperature (SST) data were obtained from the L'Estartit Meteorological Station (http://meteolestartit.cat/mar/temperatura/), where temperature has been measured 1.7 km offshore of the Medes Islands NTZ (the northwestern Mediterranean, 42°03′N 3°15′15″E) since 1974. Like Chl-a, we have considered here the mean SST of the spring season (March to May) for each year from 2001 to 2016.

**Table 1**
List of the main macroalgal species present in the study area and included in the anual monitoring: full names, abbreviations, and authorities. The species in bold represent about 90% of total algal cover and were considered here as the fourteen major species.

| | | |
|---|---|---|
| *Rhodophyta* | | |
| *Amphiroa rigida* | Amphiroa | Lamouroux |
| ***Asparagopsis armata*** | **Asparagopsis** | **Harvey** |
| *Bonnemaisonia asparagoides* | Bonnemaisonia | (Woodward) C. Agardh |
| *Ceramium ciliatum* | Ceramium | Ducluzeau |
| ***Corallina elongata*** | **Corallina** | **Ellis and Solander (also accepted as *Ellisolandia elongata*)** |
| *Gelidium spinosum* | Gelidium | (Gmelin) Silva |
| ***Jania rubens*** | **Jania** | **(Linnaeus) Lamouroux** |
| ***Laurencia obtusa*** | **Laurencia** | **(Hudson) Lamouroux** |
| *Liagora viscida* | Liagora | (Forsskal) C. Argardh |
| ***Lithophyllum incrustans*** | **L.incrustans** | **Philippi** |
| ***Mesophyllum alternans*** | **M.alternans** | **(Foslie) Cabioch and Mendoza** |
| *Peyssonnelia bornetii* | Peysonnelia | Bourderesque and Denizot |
| ***Sphaerococcus coronopifolius*** | **Sphaerococcus** | **(Goodenough and Woodward) Stackhouse** |
| ***Wrangelia penicillata*** | **Wrangelia** | **C. Agardh** |
| *Phaeophyta* | | |
| *Cladostephus spongiosus* | Cladostephus | (Hudson) C. Agardh |
| *Colpomenia sinuosa* | Colpomenia | (Mertens ex Roth) Derbès and Solier |
| *Cystoseira compressa* | C.compressa | (Esper) Gerloff and Nizamuddin |
| ***Treptacantha elegans*** | **T.elegans** | **Sauvageau** |
| ***Dictyota spp*** | **Dictyota** | |
| ***Halopteris scoparia*** | **Halopteris** | **(Linneaus) Sauvageau** |
| ***Padina pavonica*** | **Padina** | **(Linneaus) Thivy** |
| *Zanardinia typus* | Zanardinia | (Nardo) Furnari |
| *Chlorophyta* | | |
| *Acetabularia acetabulum* | Acetabularia | (Linnaeus) Silva |
| ***Codium bursa*** | **C.bursa** | **(Linnaeus) C. Agardh** |
| *Codium effusum* | C.effusum | (Rafinesque) Delle Chiaje |
| ***Codium vermilara*** | **C.vermilara** | **(Olivi) Delle Chiaje** |
| *Flabellia petiolata* | Flabellia | (Turra) Nizamuddin |
| *Halimeda tuna* | Halimeda | (Ellis and Solander) Lamouroux |
| *Ulva rigida* | Ulva | C. Agardh |

We used the same physical environmental variables (Chla- and SST) for locations inside and outside the NTZ, given that the average distance between the locations is lower than the spatial resolution of the described physical environmental variables (2 km).

The effect of protection on fish communities and the sea urchin abundance inside and outside the Medes Islands NTZ over the studied period were obtained from the long-term monitoring program of the Montgrí, the Illes Medes and the Baix Ter Natural Park (Medrano et al., 2019). Sea urchins missing data of the years 2006, 2007, 2014 and 2015 were linearly interpolated from the known values in the time-series. It is important to note that higher fish abundance and biomass of the main sea urchin predator species have remained significantly larger inside the NTZ than outside (García-Rubies and Zabala 1990; Sala, 1997; Hereu, 2005; Sala et al., 2012), with higher predation rates of sea urchins observed inside the NTZ over the last years (Sala, 1997; Hereu, 2005, 2012). Contrarily, no clear effect of protection on the unique herbivorous fish in the area, *Sarpa salpa*, was observed in rocky bottoms (Macpherson et al., 2002; Hereu et al. non pub. data).

The collinearity of the four variables was checked. Although the maximum correlation was found between the variables Sea urchin abundance and Protection level (Spearman correlation: −0.7), we considered and included all four variables as reasonably explanatory in this study.

### 2.3. Long-term trends of the main macroalgal species inside and outside the NTZ

The fourteen most representative algal species (representing a total cover of 89 ± 0.06% (Mean ± SD) in the studied monitoring period, Table 1 bolded species) were selected to describe the temporal patterns of the macroalgal dynamics across protection levels and time. To show a clearer global view rare species were excluded. First, kite diagrams of the percentage of the main algal cover were plotted to visualize the changes of these algal assemblages over the studied period inside and outside the NTZ. Second, to assess the abundance variability throughout the studied period inside and outside the NTZ, we calculated the coefficient of variation (CV) of the algal relative abundance in each location. CVs of the fourteen most representative macroalgae were estimated as the mean CV of each species between the monitoring sites and CVs of the assemblages between protection levels were estimated with the fourteen CVs mean of each site. To test pairwise comparisons among CV, we used Tukey's Honestly Significant Differences (HSD) test in each pair of means (inside/outside NTZ). Finally, to test for differences in overall macroalgal assemblage structure among protection and time, we used multivariate generalized linear models implemented in the *manyglm* function of the *mvabund* R package (Wang et al., 2017). The macroalgal abundance in each monitored site was fitted as the response variable with a negative binomial distribution after visually checked the most likely distribution of the data. The variables protection (inside/outside NTZ) and time (years) were included as main fixed effects in the model. We then ran a univariate analysis of variance to test the significance of the effects in every algal species (Wang et al., 2017).

All statistical analysis and plots were run with the software R 3.3.3 (R Core Team, 2017). Multivariate analyses were performed with the R package 'vegan' (Oksanen et al., 2017) and R Package 'mvabund' (Wang et al., 2017).

## 3. Results

### 3.1. Environmental drivers and stressors determining algal assemblage strucure

The first two axes of the RDA explained the 68% of the species-environment relationship (Fig. 2A RDA, Table 2). The four biotic and abiotic variables were significantly correlated with the RDA axes. Protection and Sea urchins were strongly correlated with the first axis, while environmental variables related to seasonality (SST and Chl-a) were significant correlated with the second axis (Table 3).

Along the first axis, species positioned at the far right end were more abundant in protected sites with low abundance of sea urchins (e.g., *Asparagopsis armata* and *Treptacantha elegans*). These species were separated from those most commonly observed in non-protected sites, which were correlated with higher abundances of sea urchins (e.g., *Wrangelia penicillata, Lithophyllum incrustans, Peyssonnelia bornetii).* In line with that, the species identified as canopy-forming (*Cystoseira sensu lato* species) were positioned in the right side of the axis, while the encrusting coralline algae were on the left side (e.g, *Lithophyllum incrustans* and *M. alternans*). Along the second axis, the macroalgal seasonal species (spring-growing) related to the spring nutrient peak and cold waters were positioned in the top of the RDA biplot (e.g., *Dictyota spp., Bonnemaisonia asparagoides,* and *Ulva rigida*). Despite being a seasonal growing alga, since *Laurencia obtusa* biomass peak takes place during the late spring-early summer, it was mostly related to warm waters as well as the perennial *Codium* species (Fig. 2A).

Macroalgal assemblages inside and outside the NTZ shifted towards the same ordination space over time, highlighting inter-annual variability of the assemblage, and homogeneous patterns of change in both protection levels (Fig. 2B). Despite this observed variability, the last dot (representing 2016 data) was located close to the origin dot (representing 2003 data), indicating that no major shifts in the algal

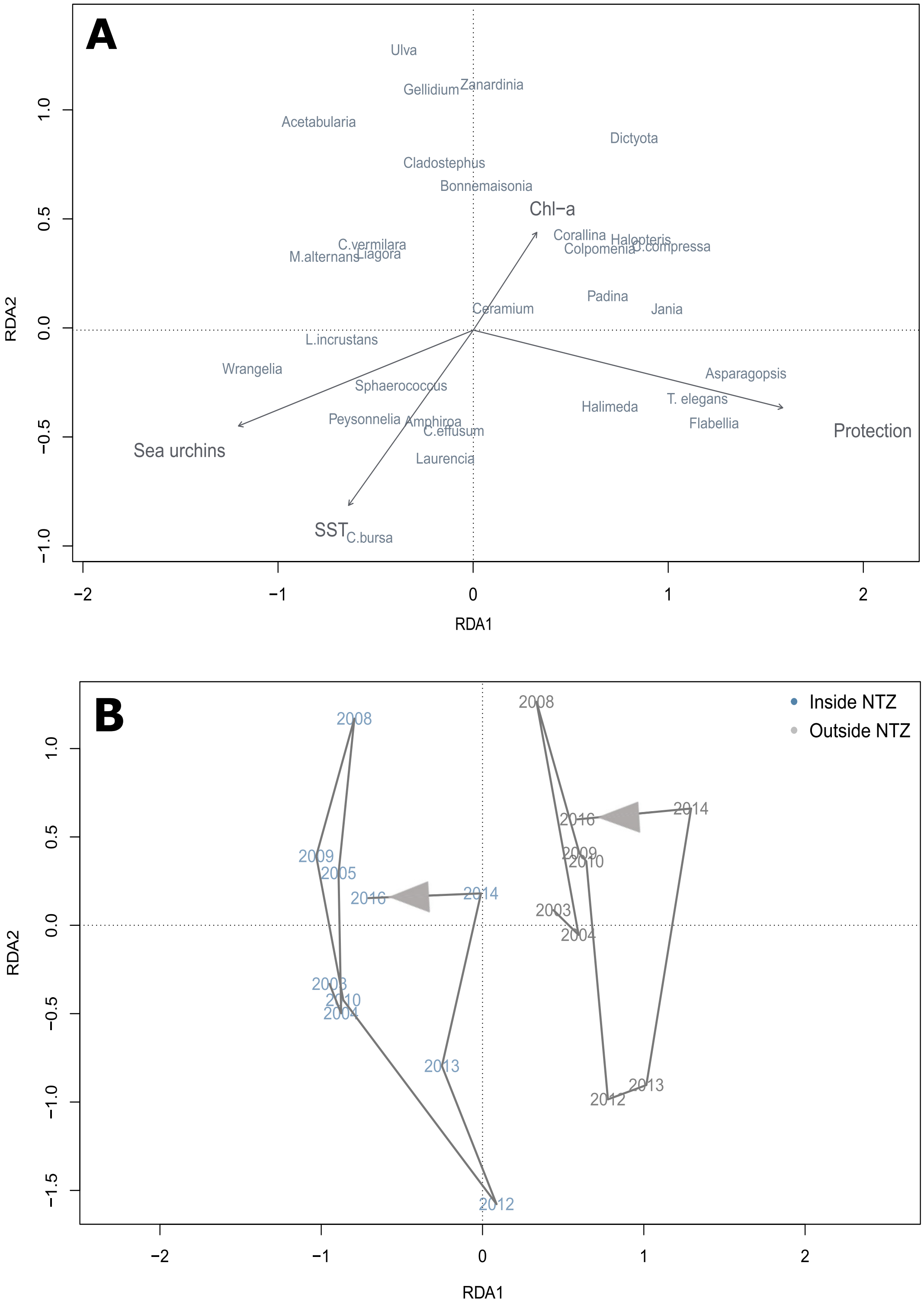

**Fig. 2.** (A) Redundancy Analysis ordination for macroalgal cover data over time. Blue dots are sampling sites inside the NTZ and grey dots, outside the NTZ. Species code in Table 1 (B) Biplot of the Redundancy Analysis connecting the monitored years with grey lines within the protection levels. Font colors indicate the protection level (Blue = inside the NTZ, Grey = outside the NTZ). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

assemblages occurred over this long-term study (Fig. 2B).

### *3.2. Long-term trends of the main macroalgal species inside and outside the NTZ*

The cover of the main macroalgal communities was homogeneous between the protected and the non-protected locations (inside/outside NTZ) (Fig. 3). The seasonal species of the genus *Dictyota* and the perennial *Corallina elongata* (today also accepted as *Ellisolandia elongata*) were the dominant seaweed in the study area during Spring, with a mean cover of 60 ± 15% and 52 ± 17.3% (Mean ± SD), respectively, across the eight monitored sites over time (Fig. 3). High stability of seaweeds cover

**Table 2**
Results of redundancy analysis (RDA) on fourth-root transformed data.

| Axes | RDA 1 | RDA 2 | RDA 3 | RDA 4 |
|---|---|---|---|---|
| Eigenvalues | 2.8765 | 1.4672 | 1.2062 | 0.8398 |
| Cumulative percentage variance | | | | |
| of species data | 9.92 | 14.98 | 19.14 | 22.03 |
| of species-environment relation | 45.02 | 67.98 | 86.86 | 100 |

**Table 3**
Biplot scores for environmental variables, the coefficient of determination (R2), and their significance using 999 permutations on the RDA results.

| Variable | RDA 1 | RDA 2 | $R^2$ | p (>r) |
|---|---|---|---|---|
| Protection | 0.97570 | −0.21911 | 0.7393 | 0.001 |
| Sea-urchins | −0.93935 | −0.34297 | 0.4598 | 0.001 |
| SST | −0.62272 | −0.78244 | 0.2954 | 0.001 |
| Chl-a | 0.58762 | 0.80913 | 0.0859 | 0.043 |

SST:Surface Seawater Temperature. Chl-a: Chlorophyll-a as proxy of nutrients.

over the fifteen years was found in most of the species in both protection levels. Annual changes in the species cover were detected simultaneously throughout the fifteen years in the protected NTZ and unprotected zones, suggesting the same variability on algal community drivers and the similar impact of stressors in both levels of protection (Fig. 3).

The coefficient of variation showed a consistency of the total macroalgal cover estimates over time in both protection levels (Fig. 4A). For the particular species *A. armata*, *Halopteris scoparia*, *W. penicillata*, and *M. alternans,* CV pairwise comparisons showed differences within levels of protection being *A. armata* and *H. scoparia* more variable outside the NTZ while the abundances of *W. pencillata* and *M. alternans* were more variable inside the protected area (Fig. 4B, Table 1 supplementary). Among all the most abundant species, the canopy-forming species *Treptacantha elegans* showed the highest variability (Fig. 4B).

Short-term changes in macroalgal abundance such as the observed after the 2008 extraordinary storm occurred in the studied period (Fig. 3), but these were not significant over all the multi-year monitoring for the majority of the species (Table 4). Only three of the fourteen dominant species showed differences in their cover across protection levels (Table 4). In spite of the very low cover, the seasonal growing naturalized alga *A. armata* and the perennial canopy-forming *T. elegans* were more abundant inside the NTZ than outside. In contrast, the seasonal growing species *W. penicillata* was the most characteristic species outside the NTZ (Fig. 3, Table 4). While *L. obtusa* increased its relative cover over the study period (Fig. 3, Table 4) the calcareous algae *C. elongata* and *Jania rubens* decreased over the study period but only outside the NTZ (Fig. 3, Table 4).

## 4. Discussion

Our results revealed that the coastal macroalgal assemblages of the

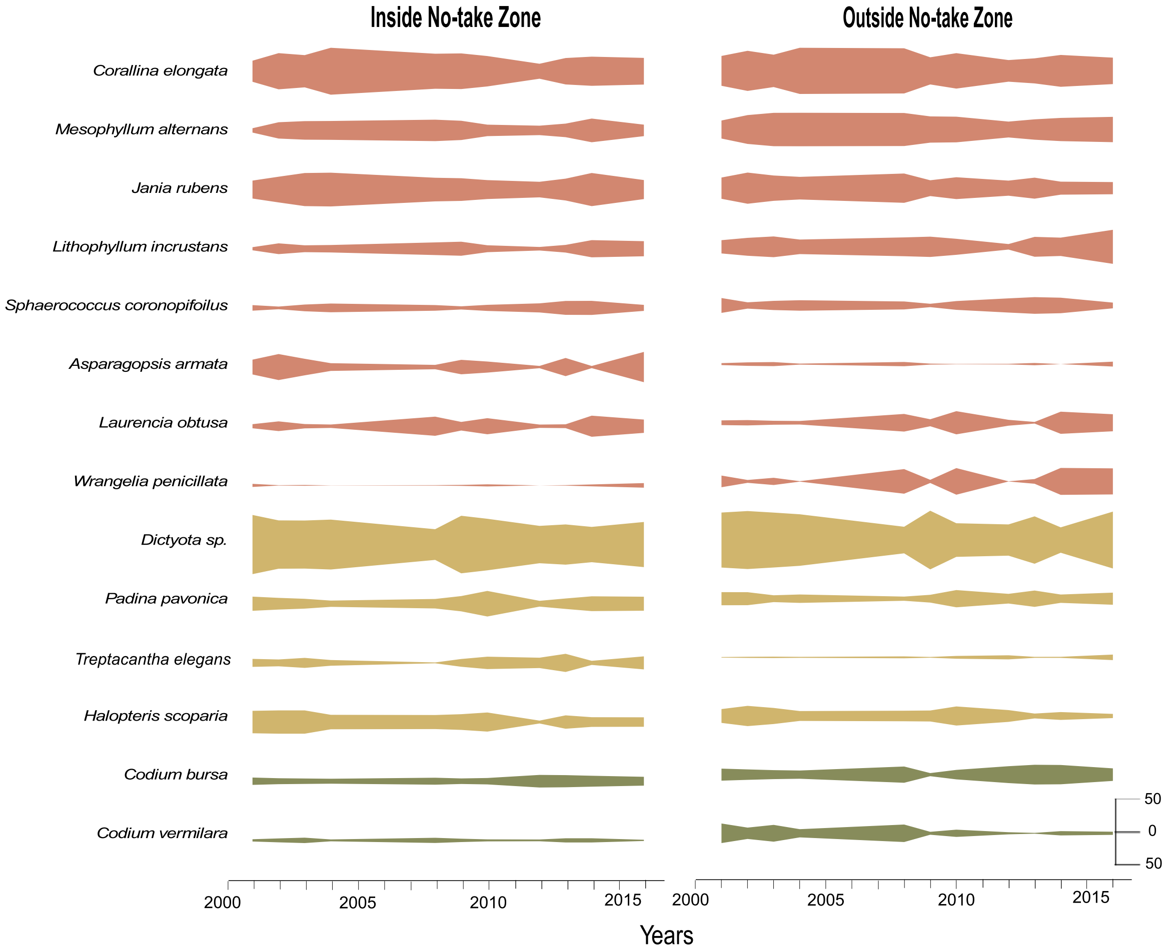

**Fig. 3.** Kite diagram representing the cover of the fourteen most representative macroalgal species in the study area over the fifteen years. Left panel corresponds to the percentage cover within NTZ localities and right panel to the percentage cover of the localities outside the NTZ. Percentage cover was scaled to the maximum value of 50 and the colored diagrams show spectral values from 0 to 50 for each species. The color code indicates the corresponding macroalgae phyllum (Rhodophyta: red, Phaeophyta: yellow, and Chlorophyta: green). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

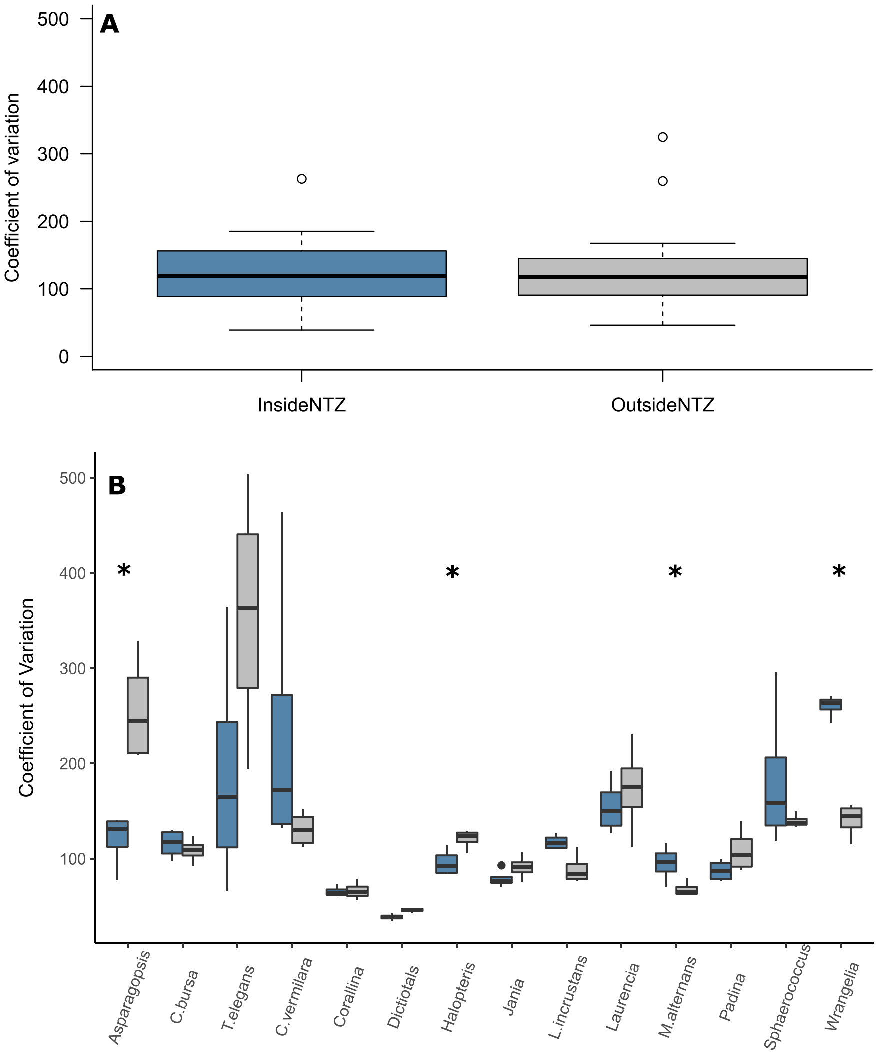

**Fig. 4.** Coefficients of Variation (CVs) over time. Blue color represents the values inside the NTZ and grey color the values outside the NTZ. (A) Boxplot showing within-protection CVs in total macroalgae cover (inside/outside the NTZ). (B) Boxplot showing CVs in the cover of the fourteen main macroalgae species within protection levels. '*' indicates significance differences ($p < 0.05$) in pair means using Tukey pairwise comparison. Species code in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 4**
Generalized Linear Model manyglm results (Deviation test statistic, p-value) of macroalgal cover (Percentage/0.25 $m^2$) Df: degrees of freedom. Bold indicates $p < 0.05$. ':' indicates interaction. Species code in Table 1.

| Source | Df | *Asparagopsis* | *Dictyota* | *T. elegans* | *C. vermilara* | *C. bursa* |
|---|---|---|---|---|---|---|
| Protection | 1 | **25.093, 0.001** | 2.45, 0.596 | **23.324, 0.001** | 5.545, 0.209 | 2.26, 0.607 |
| Time | 10 | 31.461, 0.343 | 30.58, 0.343 | 25.542, 0.547 | 26.429, 0.531 | 39.415, 0.112 |
| Protection:Time | 9 | 17.314, 0.088 | 16.605, 0.088 | 16.276, 0.088 | **28.503, 0.020** | 6.463, 0.088 |
| **Source** | **Df** | ***Halopteris*** | ***Laurencia*** | ***Padina*** | ***Sphaerococcus*** | ***Wrangelia*** |
| Protection | 1 | 0.001, 0.993 | 0.14, 0.993 | 0.108, 0.993 | 0.089, 0.993 | **14.214, 0.006** |
| Time | 10 | 29.86, 0.354 | **47.096,0.010** | 27.475, 0.489 | 26.928, 0.510 | 40.56, 0.112 |
| Protection:Time | 9 | 18.854, 0.061 | 13.157, 0.088 | 19.008, 0.061 | 13.115, 0.088 | 11.207, 0.088 |
| **Source** | **Df** | ***Coralina*** | ***Jania*** | ***L. Incrustans*** | ***M. Alternans*** | |
| Protection | 1 | 0.933, 0.889 | 1.62, 0.744 | 8.749, 0.052 | 3.736, 0.391 | |
| Time | 10 | 34.044, 0.228 | 21.578, 0.547 | 33.851, 0.228 | 35.31, 0.169 | |
| Protection:Time | 9 | **26.231, 0.025** | **24.497, 0.025** | 18.032, 0.077 | 15.289, 0.088 | |

Montgrí, the Illes Medes and the Baix Ter Natural Park were stable and similar in total algal cover inside and outside the protected NTZ over the fifteen years of study with no species replacement, although some variability were observed between years. The communities were dominated by the perennial species *C. elongata* and the seasonal *Dictyota* species. *Corallina elongata* has been previously described as predominant species subjected to moderate pollution (Díez et al., 1999; Soltan et al., 2001). Species related to high-quality environmental requirements, such as *Cystoseira sensu lato* species (Thibaut et al., 2005; Mangialajo et al., 2008). were less abundant in the study area. In addition to environmental quality, bottom-up (herbivorous control) and top-down (resources control) processes interactively influence the structure of macroalgal benthic communities (Korpinen et al., 2007; Smith et al., 2010). As we expected, bottom-up forces such as spring nutrient concentration and the ordination of the majority of seasonal algal species were associated to water temperature, in which herbivory showed the weakest influence (except for *A. armata* and *W. penicillata*). Contrarily, top-down forces may play a role in determining the abundance of fucoids such as the canopy-forming *Cystoseira sensu lato* species and the encrusting coralline algae species. It has been extensively documented that increased abundance of grazers feeding on algal beds can significantly change the structure of shallow benthic communities (Airoldi and Beck, 2007; Filbee-Dexter and Scheibling, 2014; Mineur et al., 2015).

The sea urchin *Paracentrotus lividus* is the most important herbivore in the study area, playing an important role in structuring algal communities (Sala and Zabala, 1996; Palacín et al., 1998; Hereu et al., 2008). As *P. lividus* preferentially graze on fleshy algae (Privitera et al., 2008), encrusting algae showed a positive relationship to the presence of sea urchins. Although many studies have been demonstrate that herbivorous fishes may also influence the structure of the benthic assemblages (Vergés et al., 2009; Gianni et al., 2017), we did not consider them because there are not evidences of the effect of protection on the abundance of the unique herbivore fish in this area, *Sarpa salpa* (Prado et al., 2007). Moreover, their effects on macroalgal beds in the studied area are weaker than the sea urchins one (Hereu, 2006; Hereu et al., 2008). This could be explained by the feeding preference and behavior of *Sarpa salpa*, the only strictly herbivorous fish in this area (Sala and Boudouresque, 1997). *S. salpa* has shown preference for high palatable plants such as seagrasses or fleshy algae (Vergés et al., 2009; Ali et al., 2017). In addition, fishes feeding behavior differ from the sea urchins as fishes bite the leaf while sea urchins graze the entire thalli which could deplet large algal extensions (Hereu, 2006; Jadot et al., 2006).

No-take marine reserves can indirectly restore the original trophic cascades recovering the abundance of herbivores' predators (Sala and Giakoumi, 2017) and controlling the herbivore populations (Medrano et al., 2019). Results of this study go one step further in the trophic cascade of the studied area and also demonstrate that No-Take marine reserves can influence the abundance of the canopy-forming *Cystoseira sensu lato* species, being more abundant in protected areas (as reported for the same studied NTZ in Sala et al., 2012). Without losing sight of the large variability observed in the cover of perennial canopy-forming *T. elegans* over time suggesting a high influence of local conditions on this species, this results reinforces the usefulness of marine reserves as conservation tools also at lower trophic levels, which is particularly relevant when considering the global decline of *Cystoseira sensu lato* species. in the Mediterranean Sea (Thibaut et al., 2005, 2014). Despite *Cystoseira sensu lato* species were not the dominant species in the macroalgal beds of the Natural Park, their loss or replacement could have major consequences for many associate organisms (e.g., lowering fish recruitment, Cheminée et al., 2013). On the other hand, *Codium vermilara* showed completely opposite ordination relative to the main drivers and stressors than *Cystoseira spp*. This result support the hypothesis of *C. vermilara* could have been replaced by *Cystoseira sensu lato* species assemblages after their historical decline in the NW Mediterranean Sea (Ricart et al., 2018). Regarding the less abundant species, our study confirms the effect of protection for *A. armata, which* was already suggested by Sala and Boudouresque (1997). This unpalatable red alga was practically absent outside the NTZ, but showed a high variability over the fifteen years. The reverse pattern was observed for *W. penicillata* species, more abundant and less variable outside the NTZ. We did not detect the influence of any of the studied drivers in the filamentous turf forming *Ceramium ciliatum*, probably because of their short life cycle (Bologa et al., 1995).

Extreme climate events such as severe storms have been also considered as potential drivers eroding macroalgal beds (Navarro et al., 2011; Borja et al., 2018; Capdevila et al., 2019). An exceptionally storm with drastic consequences on benthic communities occurred in the study area in 2008 (Mateo and Garcia-Rubies, 2012; Sanchez-Vidal et al., 2012), where we observed a short-term effect in the macroalgal communities exerted by the storm like those previously reported in other studies (Navarro et al., 2011; Micheli et al., 2016; Maggi et al., 2018). Benefiting from the available space that resulted from the storm, a rapid increase of highly seasonal *Dictyota* species were observed right after the storm (the year 2009), while most of the perennial species decreased. Over the following years, the abundance and structure of the main macroalgal assemblages recovered swiftly to pre-storm values and no long-term impacts were observed, indicating that impacts of a single extraordinary storm on rocky-shallow algal communities may be reversed relatively quickly. However, we should consider that an increase in the intensity and frequency of extreme climatic events, such as extraordinary storms, has been observed since 1950 and is expected to increase in the future (IPCC et al., 2014; Reguero et al., 2019), especially in the Mediterranean, which has been highlighted as a hotspot of ongoing climate change (IPCC et al., 2014; Cramer et al., 2018). Our results stress the importance of increasing spatial and temporal scales to better understand the natural variability of the marine communities and do not misunderstand the changes observed in algal assemblages (Lindenmayer et al., 2012). If the same dataset of this study would have been used to describe the consequences of this extraordinary storm right after the impact, notable differences could have been described.

Despite the relevance of monitoring algal communities, it is important to highlight some limitations of this study. In order to effectively document long-term changes of the macroalgal assemblages structure over time, we prioritize a broad view of the community by monitoring the algal cover of the main species in our study area and missing the minority species. This methodology allows to maintain long-term monitoring programs, involving different observers due to the easy identification of a reduced number of species but it is not the optimal ecological design in order to analyze other community indexes such as biodiversity or richness. Many studies pool species into functional groups, as a way to predict algal community composition (Steneck and Dethier, 1994). This reduction of species-specific information is generally accepted at the expense of a broader view of the changes in community structure. However, it is important to stress that this approach hinders to detect some important changes at the species level because different algal species have different responses to herbivores and other perturbations independently on their functional group (Hereu et al., 2008). Most of the results exposed here would have been lost or misinterpreted working with functional groups such as the high variability of *T. elegans*, which would have been pooled in the erect algae group.

A major insight of this study is the absence of major shifts in species replacement and abundance and the null effect of protection in the most important macroalgal communities in the rocky-shallow infralittoral zone of the studied area. Differences only regard the less abundant species, among them the canopy-forming *Treptacantha elegans* which took preference inside the protected NTZ. These results provide a current baseline of algal communities and contribute to the literature on the role of NTZs marine reserves in the benthic communities.

## Authors' contributions

MZ, BH, and CL designed and began the long-term monitoring. MZ, BH, CL, EA, AM, PC MPE, and IMS conducted most of the fieldwork presented in this study. AM analyzed the data and wrote the first version of the manuscript with reviews and editing from all the authors.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.104826.

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# No-take marine reserves control the recovery of sea urchin populations after mass mortality events

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ABSTRACT

Understanding how no-take zones (NTZs) shape the population dynamics of key herbivores is crucial for the conservation and management of temperate benthic communities. Here, we examine the recovery patterns of sea urchin populations following a high-intensity storm under contrasting protection regimes in the NW Mediterranean Sea. We found significant differences in the recovery trends of *Paracentrotus lividus* abundance and biomass in the five years following the storm. The *P. lividus* populations outside the NTZ recovered faster than the populations inside the NTZ, revealing that predation was the main factor controlling the sea urchin populations inside the NTZ during the study period. *Arbacia lixula* reached the highest abundance and biomass values ever observed outside the NTZ in 2016. Our findings reveal that predation can control the establishment of new sea urchin populations and emphasize top-down control in NTZs, confirming the important role of fully protected areas in the structure of benthic communities.

## 1. Introduction

Within marine protected areas, no-take zones (NTZs), where the exploitation of marine resources is not allowed, provide large-scale controlled areas for examining trophic interactions and cascade effects compared to natural ecosystems (Pinnegar et al., 2000; Guidetti, 2006). Previous empirical and observational studies within NTZs have demonstrated the role of herbivores, such as sea urchins, and their predators as major structuring forces in marine temperate benthic communities (Castilla and Durán, 1985; Shears and Babcock, 2002). As a result of prohibiting extractive activities, predation and top-down control increases as lower trophic levels decline, including sea urchins (Guidetti, 2006; Barrett et al., 2009; but see also Halpern, 2003).

Beyond the top-down control of predatory fishes, many processes, such as recruitment variability, spatial refuge availability and their escaping behaviour, influence sea urchin abundances (Sala and Zabala, 1996; Nishizaki and Ackerman, 2004; Hereu et al., 2012a; Oliva et al., 2016). Moreover, when sea urchin populations are established, adult individuals can also facilitate juvenile survival by reducing micro-predator abundance (Bonaviri et al., 2012) and offering refuge under their spine canopy for avoiding predation (Tegner and Dayton, 1977; Zhang et al., 2011; Hereu et al., 2012a).

A favourable combination of such processes can maintain dense sea urchin populations, even when they coexist with abundant fish predators within NTZs; this provides evidence of the existence of strong feed-back processes that provide resilience for sea urchin populations once they are established (Bonaviri et al., 2012; Ling and Johnson, 2012). In addition to biological interactions, other factors, such as hydrodynamic forces (Micheli et al., 2016), extraordinary storms (Sanchez-Vidal et al., 2012), sea urchin diseases (Girard et al., 2012; Clemente et al., 2014), or even human harvesting (Pais et al., 2011), can also determine the abundance of sea urchins at local scales.

To date, several studies have examined the long-term natural trends of established sea urchin populations (Lessios et al., 1984; Sala et al., 1998; Hereu et al., 2012a), where all of the processes affecting their dynamics occur together. Nevertheless, few studies have reported the rebuilding capacity of collapsed sea urchin stocks after extraordinary mass mortalities (Edmuns and Carpenter, 2001; Miller et al., 2003; Girard et al., 2012; Clemente et al., 2014; Lessios, 2016). Hence, little is known about the underlying mechanisms that drive their recovery and how conservation tools such as NTZs can influence and modulate these processes (Hunte and Younglao, 1988; Guidetti, 2006; Hamilton and Caselle, 2015).

To investigate the underlying mechanisms behind the recovery sea

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urchin populations and the role of fully protected areas in such recovery patterns, we used long-term monitoring data (nearly three decades) of sea urchin populations inside and outside the NTZ of Montgrí, Illes Medes and Baix Ter Natural Park in the NW Mediterranean Sea.

In this area, the purple sea urchin, *Paracentrotus lividus* (Lamark), is the most abundant species and is considered the most important herbivore controlling the macroalgal dynamics on the shallow reefs (Sala and Zabala, 1996; McClanahan and Sala, 1997; Bulleri et al., 1999; Hereu, 2005). Although the black sea urchin, *Arbacia lixula* (Linnaeus), is also common, it is approximately one order magnitude less abundant than *P. lividus* on the Catalan coast (Hereu et al., 2012a). Despite its low density, *A. lixula* is considered a thermophilic species (Privitera et al., 2011; Wangensteen et al., 2013), and it has been suggested that climate change can increase its abundance and distribution (Francour et al., 1994; Wangensteen et al., 2012). Nevertheless, there is little quantitative evidence of these increasing patterns in the field to date. The abundance of both species did not show significant differences between contrasting protection levels from 1991 to 2010 (Hereu et al., 2012a). In December 2008, when an extraordinary storm occurred in this area (Mateo and Garcia-Rubies, 2012) that had drastic effects on the benthic communities (Sanchez-Vidal et al., 2012), a sea urchin population mass mortality event occurred (Hereu et al., 2012b; Pagès et al., 2013). The main goal of this study was to examine the recovery patterns of the devastated sea urchin populations under contrasting protection regimes. We hypothesize that top-down control could play a major role restricting the recovery of depleted sea urchin populations within the NTZ, while a fast recovery was expected outside the NTZ where the predation pressure is lower (García-Rubies et al., 2013). Since almost all of the adult individuals were lost after the storm, we anticipated that the population recovery would rely on recruitment and juvenile survival.

Due to the key role of sea urchins in structuring sublittoral communities in the Mediterranean Sea, understanding how fully protected areas can shape their population dynamics may be crucial for enhancing the effectiveness of conservation actions for temperate benthic communities.

## 2. Materials and methods

### 2.1. Study site

The Montgrí, the Illes Medes and the Baix Ter Natural Park is located on the north-eastern coast of Catalonia, Spain (NW Mediterranean Sea, Fig. 1), covering a marine area of 2.037 ha. The park has its core in the no-take zone (NTZ) of the Medes Islands, which comprises the Archipelago of the Medes Islands (93 ha), where all fishing and harvesting activities have been prohibited since 1983 (García-Rubies and Zabala, 1990). Outside the NTZ, the nearby Montgrí coast is divided into a peripheral zone, a contiguous beltway zone to the Medes Islands that was established in 1990 where artisanal fishing and recreational angling are allowed under restriction, and a no reserve zone, where artisanal, recreational and spearfishing are allowed (Fig. 1).

The total fish biomass and abundance of the apex predators and carnivores have remained significantly higher in the NTZ than in the two areas where some type of fishing is allowed, with no differences between them (Sala et al., 2012; García-Rubies et al., 2013; Hereu et al., 2017). Because there were no differences in the fish abundance and biomass between the peripheral zone and the no-reserve zone, both of the areas were considered outside the NTZ in this study. Accordingly, a higher fish biomass of the main sea urchin predatory species (Table 1) and higher sea urchin predation rates have been observed inside the NTZ in recent years (Sala, 1997; Hereu et al., 2005, 2012a).

### 2.2. Long-term monitoring

We monitored four sea urchin populations inhabiting extensive reef areas (150 $m^2$) with the same topography that is dominated by large limestone boulders in the natural park yearly beginning in 1991 (Fig. 1). Two of the four studied populations were located in the Medes Islands NTZ (sites coordinates: 42°2.497′N; 3°13.629′E and 42°2.658′N; 3°13.479′E), and the other two were located outside the NTZ in the nearby coast: one located inside the peripheral zone (42°3.313′N; 3°12.721′E) and one in the no-reserve zone (42°3.952′N; 3°12.591′E).

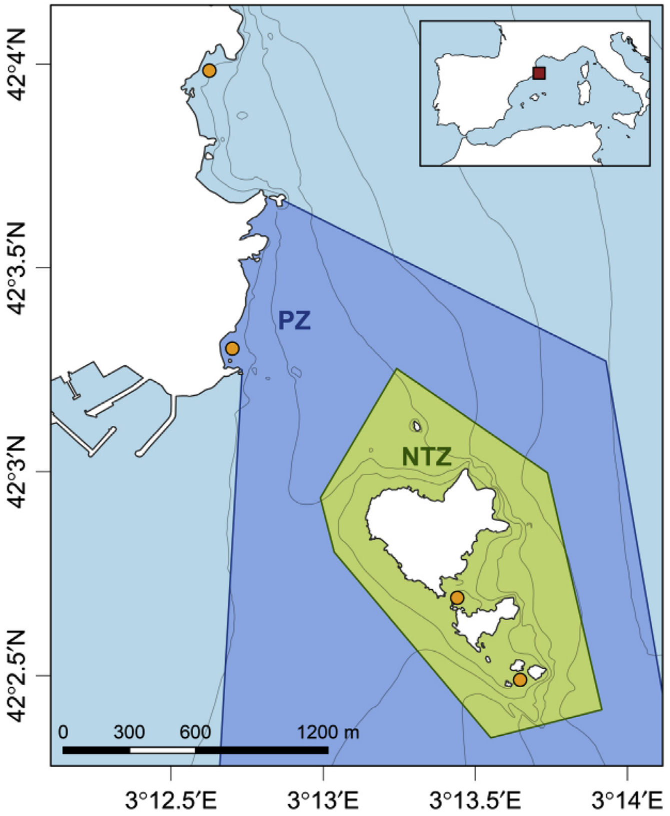

**Fig. 1.** Map of the study locations in the Montgrí, the Illes Medes and the Baix Ter Natural Park in the NW Mediterranean Sea. Orange dots represent the long-term monitoring sites: two are located outside the NTZ, and two more are inside the NTZ. The perimeter of the Medes Islands NTZ is delimited by the green polygon, and the peripheral zone (PZ) is delimited by the blue polygon. No reserve zone is outside the green and blue polygons. (For the interpretation of the references to colour in this figure legend, please refer to the web version of this article).

During the first monitoring years, the sea urchin population densities remained similar inside and outside the NTZ despite the higher fish abundances observed in the NTZ throughout the study period (Hereu et al., 2012a). In December 2008, an extraordinary storm struck the Catalan coast, with maximum wind speeds up to 20 m/s, significant wave heights as great as 8 m, record maximum wave heights in excess of 14 m, and wave periods up to 14 s. This storm was categorized as a highly extreme event with a return period of more than 100 years (Sanchez-Vidal et al., 2012). Substantial negative effects on the benthic populations and communities inhabiting soft and hard bottoms (e.g., macroalgae, gorgonians, seagrass meadows and sea urchins) from shallow to deep waters were documented after the storm (Mateo and García-Rubies, 2012; Sanchez-Vidal et al., 2012; Pagès et al., 2013). The impact of these forces on the sea urchin populations rendered a more than 80% loss of individuals and 90% of the biomass and levelled the sea urchin populations between the areas inside and outside the NTZ. The larger specimens of *P. lividus* were the most affected, with consequent changes in their size frequency distribution (Hereu et al., 2012a, 2012b).

**Table 1**
Density and biomass (Mean ± SE) of the main *P. lividus* predators vulnerable to fisheries inside and outside the Medes Islands NTZ from 2009 to 2016 (Hereu et al., 2017).

| Species | Density (Ind/500 $m^2$) | | Biomass (kg wet mass/500 $m^2$) | |
|---|---|---|---|---|
| | NTZ | No reserve | NTZ | No reserve |
| ***Sparidae*** | | | | |
| *Diplodus sargus** | 12.4 ± 1.7 | 7.9 ± 1.9 | 3.1 ± 0.5 | 1.5 ± 0.4 |
| *main predator of juveniles and adult sea urchins (> 10 mm) (Sala and Zabala, 1996; Sala, 1997; Hereu et al., 2005) | | | | |
| *Diplodus vulgaris* | 29.9 ± 6.2 | 17.2 ± 3.7 | 3.6 ± 0.9 | 1.3 ± 0.4 |

### 2.3. Sampling methodology

To describe the medium-term (5 yrs) recovery patterns of sea urchin populations inside and outside the NTZ after the 2008 mass mortality event on the *P. lividus* and *A. lixula* populations under different protection regimes, we extended the annual monitoring until 2016. Although the abundances of *Arbacia lixula* were very low in our study area for the duration of the monitoring, we also evaluated their temporal trend to detect any clear pattern in this species.

The sea urchin species, abundance and size were recorded in three 50 $m^2$ transects at 6 m depth in each study site. As described in Hereu et al. (2012a), the transects were divided into five 10 $m^2$ sub-transects, and within each transect, we counted and measured the diameter of all the present sea urchins. The sea urchin biomass was calculated using the allometric length-weight relationship, $W = a(TD)^b$, where W is the wet weight (in g) and TD (in cm) is the measured sea urchin test diameter. Biomass of *P. lividus* was estimated as $W = 0.00319(TD)^{2.479}$(Ling et al., 2015) and, *A. lixula* biomass was estimated as $W = 0.8467(TD)^{2.6042}$ (Ballesteros, 1981).

### 2.4. Data analysis

To detect potential structural changes in the *P. lividus* populations due to the 2008 storm, the mean abundance per year was fitted into sequential linear regressions. Every potential change point in the data series was assessed by computing the residuals of the linear models and analysing the F statistics using the R package 'strucchange' for R (Zeileis et al., 2003).

We used generalized linear mixed models (GLMMs) to determine differences in the medium-term (5 yr) recovery patterns of the sea urchin populations between the NTZ and the non-protected area. The *P. lividus* densities and biomass in the five subsequent years (2009–2013) were fitted as the dependent variables. The explanatory variables time (years) and protection level (inside/outside the NTZ) were included as fixed effects, and the sample sites nested to the protection level were a random effect. The data distribution was visually inspected by fitting the response variable to the most likely distribution (Fig. 1, supplementary material), and the negative binomial GLMMs with a logarithm link function were conducted using the package 'lme4' for R (Bates et al., 2015).

Because of the low abundance and biomass of *Arbacia lixula*, which were an order of magnitude lower than those of *P. lividus*, we did not statistically examine the recovery patterns of this species.

All the statistical analyses were performed with R version 3.3.3 (R Core Team, 2017).

## 3. Results

The abundance and biomass of *P. lividus* reached the lowest values ever recorded after the storm of 2008 (Figs. 2 and 3). Despite the short-term effects of the mortality event, we found significant differences in the recovery trends of the *P. lividus* abundance and biomass over the following five years under the different protection regimes (Table 2 and Fig. 3). Sequential F tests and the critical F values identified a shift in the *P. lividus* populations inside the NTZ after the 2008 storm, resulting in a significant reduction in the population abundance from that time on (Fig. 2).

In contrast, outside the NTZ, both the *P. lividus* abundance and biomass started to recover one year after the mortality caused by the storm, and the biomass increased fourfold in two years, from the lowest value of 119.7 ± 28.3 g/10 $m^2$ in 2009 to 445.6 ± 83.7 g/10 $m^2$ (mean ± SE) in 2011 (Fig. 3). The non-protected populations displayed a fast recovery in density in 2010 (Fig. 3), which was consistent with the high recruitment peak observed that year (61.6% of the population, Fig. 4). From these new sea urchins, the non-protected populations began to grow, reaching a similar size structure as that before the storm in 2013 (Fig. 4).

In 2016, eight years after the storm, the *P. lividus* abundance and biomass in the non-protected sites were higher than the pre-storm values in 2008 and higher than those observed inside the NTZ (Figs. 2 and 3).

In contrast, inside the NTZ, the *P. lividus* abundance and biomass continued decreasing for some of the years after the 2008 mass mortality event, reaching the lowest biomass values three years after the storm (40.8 ± 15 g/10 $m^2$ in 2011). The sea urchin biomass in the NTZ started to recover in 2013 (255.2 ± 59.6 g/10 $m^2$), and 5 years after the storm, the biomass and density values were far from the pre-storm year values (Fig. 2). No recruitment peak was observed inside the NTZ until 2011, when the populations were dominated by small individuals (> 80% of the sea urchins were < 4 cm in diameter, Fig. 4).

The abundance of the *Arbacia lixula* populations was lower than that of *P. lividus*, but it also dropped as a consequence of the 2008 storm. Nevertheless, the *A. lixula* populations did not show significant contrasting short-term recovery patterns under different protection regimes, which was observed in *P. lividus*. The abundance and biomass of *A. lixula* outside the NTZ reached the highest values ever observed in this area in 2016. In contrast, inside the NTZ, the recovery was slower, and in 2016, the density values were similar to those observed before the mass mortality event (Fig. 5a–b).

## 4. Discussion

In this study, we tested the effects of a NTZ on the recovery process of a key herbivore following a high-intensity storm in a temperate benthic community. We conclude that the sea urchin *Paracentrotus lividus* populations outside the NTZ recovered faster than the populations inside the NTZ, revealing that predation is the main factor controlling the recovery of sea urchin populations in the NTZ. This is consistent with many studies that also demonstrated the role of top-down control as a major structuring force in benthic communities in other temperate systems (Shears and Babcock, 2002; Guidetti, 2006; Halpern et al., 2006; Clemente et al., 2011).

After the almost complete depletion of adult *P. lividus* populations due to the dramatic storm in 2008 and given that the foundation and recovery of benthic populations relies on the interaction between settlement and post-settlement mortality, we expected differences in one

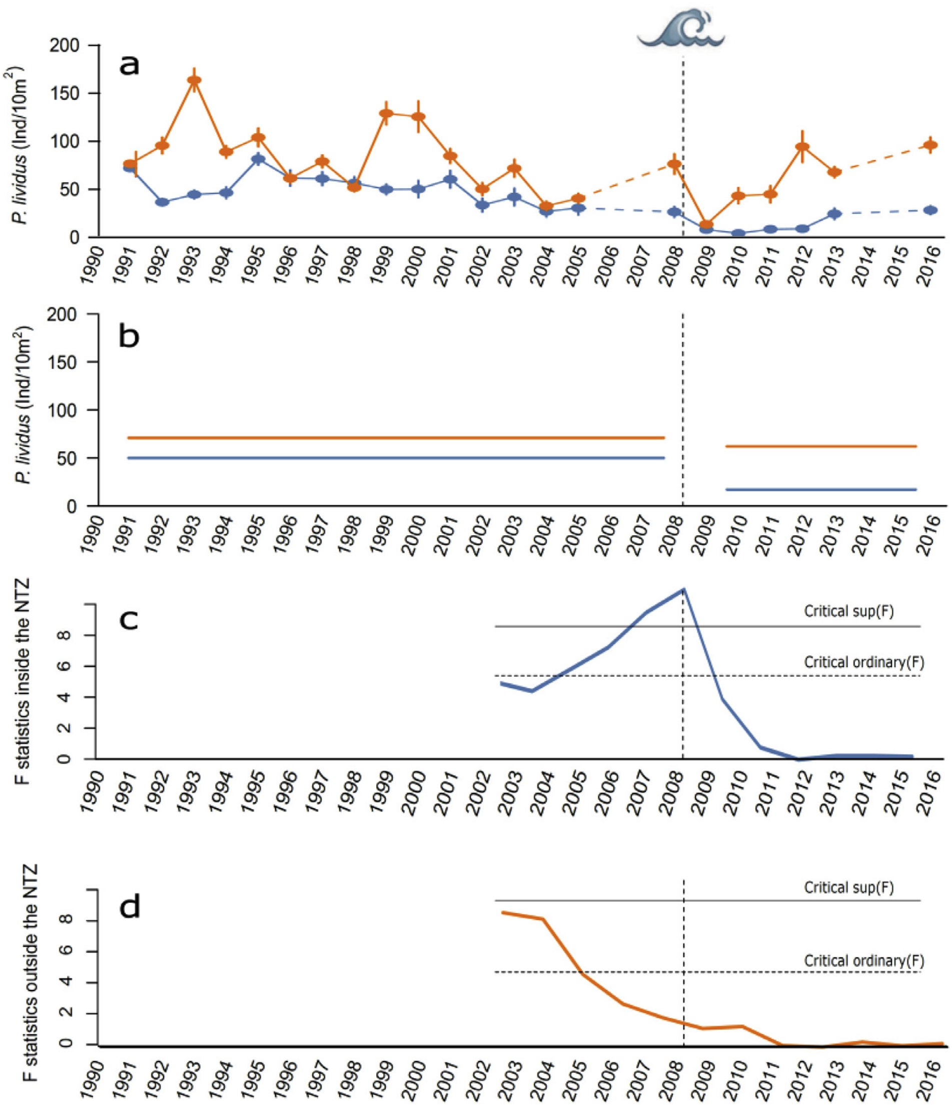

**Fig. 2.** *Paracentrotus lividus* abundance over time. Blue lines represent the values inside the NTZ, and orange lines represent the values outside the NTZ. (a) The density of *P. lividus* per $10\,m^2$ (Mean ± SE) since 1990. (b) Mean values of the *P. lividus* density before (average density from 1991 to 2008) and after the extraordinary storm (average density from 2010 to 2016). (c–d) Change-point analysis by sequential F tests with the proper critical F levels. (c) Identified change point after the 2008 storm inside the NTZ, and (d) the absence of a change point after the storm outside the NTZ. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

or both of the processes between the protected and non-protected areas to fully understand the contrasting recovery patterns observed.

The settlement of *P. lividus* is decoupled from local populations and can be highly variable at small scales given the long planktonic stage of sea urchin larvae (Hereu et al., 2004, 2012a). Therefore, the arrival of new individuals is not expected to explain the differences between the protected and non-protected areas, suggesting a key role of post-settlement mortality to fully understand the contrasting recovery patterns observed between the different protection levels.

In fact, the high juvenile predation rate (Hereu et al., 2005) and the absence of adult individuals that facilitate juvenile survival inside the NTZ may explain the slow sea urchin population recovery within the fully protected area. After the depletion of sea urchin populations in 2008, the size structure of the *P. lividus* populations inside and outside the NTZ changed dramatically. When the recovery began, unimodal size distributions dominated by small sizes were observed in both areas because of recruitment pulses. Although the settlement episodes were simultaneous both inside and outside the NTZ, the recruitment peak observed in 2010 outside the NTZ was not observed in the NTZ, suggesting high predation control within the NTZ. After the 2010 recruitment peak, the sea urchin populations outside the NTZ began to recover, showing immediate increases in their abundance, size and biomass according to the reported growth rates for this species (Turon et al., 1995; Ouréns et al., 2013). In addition, the annual arrival of new settlers and the low predation rate maintained the smallest size class as the most frequent class outside the NTZ over time (Sala and Zabala, 1996; Hereu et al., 2012a). Inside the NTZ, the recovery of adult individuals was slower, as it was determined by the lower survival of juveniles.

Adult *P. lividus* specimens were established in populations in both protection regimes, i.e., inside and outside the NTZ, at different times: in 2013 outside the NTZ and in 2016 inside the NTZ. In 2016, the population structures were characterized by a bimodal distribution, with one mode in the adult sea urchin size class in both areas and another in the juvenile size class, which is typical of sea urchin populations under a certain degree of predation pressure (Sala and Zabala, 1996), but with the majority of the smallest size classes in the non-protected area due to the highest post-settlement mortality inside the NTZ. These observed patterns support that top-down control is crucial for determining the sea urchin population structure within the NTZ and reaffirm the importance of adults in maintaining sea urchin populations. When sea urchins reach the refuge size from their predators (Sala and Zabala, 1996), they facilitate post-settlement survival by conferring protection from predation (Tegner and Dayton, 1977; Ouréns et al., 2014), eliminating micropredator grazing (Bonaviri et al., 2012), and protecting them from environmental and hydrodynamic forces (Nishizaki and Ackerman, 2004).

Before the extraordinary storm, the similar abundance and biomass

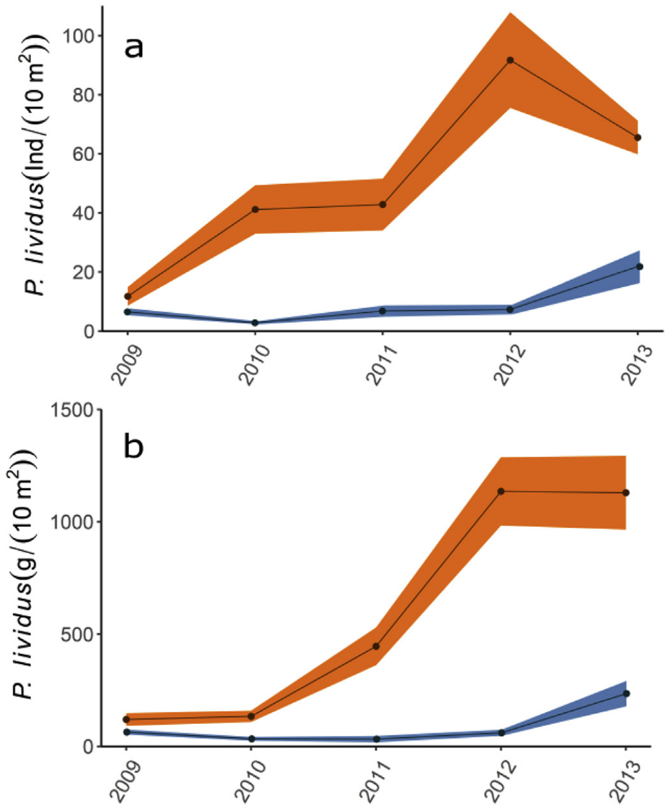

**Fig. 3.** Recovery patterns of *Paracentrotus lividus* in abundance (a) and biomass (b). Black points correspond to the mean, and the coloured areas correspond to the SE. The blue colour represents the values inside the NTZ, and orange represents the values outside the NTZ. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

trajectories observed over the years between the sea urchin populations of both protection regimes reinforce that factors other than top-down control can be important over large scales, as was also revealed in Guidetti and Dulčić (2007) and in Bonaviri et al. (2012).

In 2008, the *P. lividus* densities reached the lowest values inside the NTZ throughout the long-term monitoring until the storm. This together with the time scale could be crucial in the observed responses of long-term studies (Babcock et al., 2010), and we think that predation could have become the major controlling force of sea urchin populations inside the NTZ regardless of the storm on a timescale longer than the one studied.

The *Arbacia lixula* populations showed a similar recovery pattern to that of the *P. lividus* populations, as the density and biomass values outside the NTZ increased in 2012. In addition, *A. lixula* reached densities and biomasses never observed in this area outside the NTZ in 2016. *A. lixula* is a thermophilic species that is more abundant in the

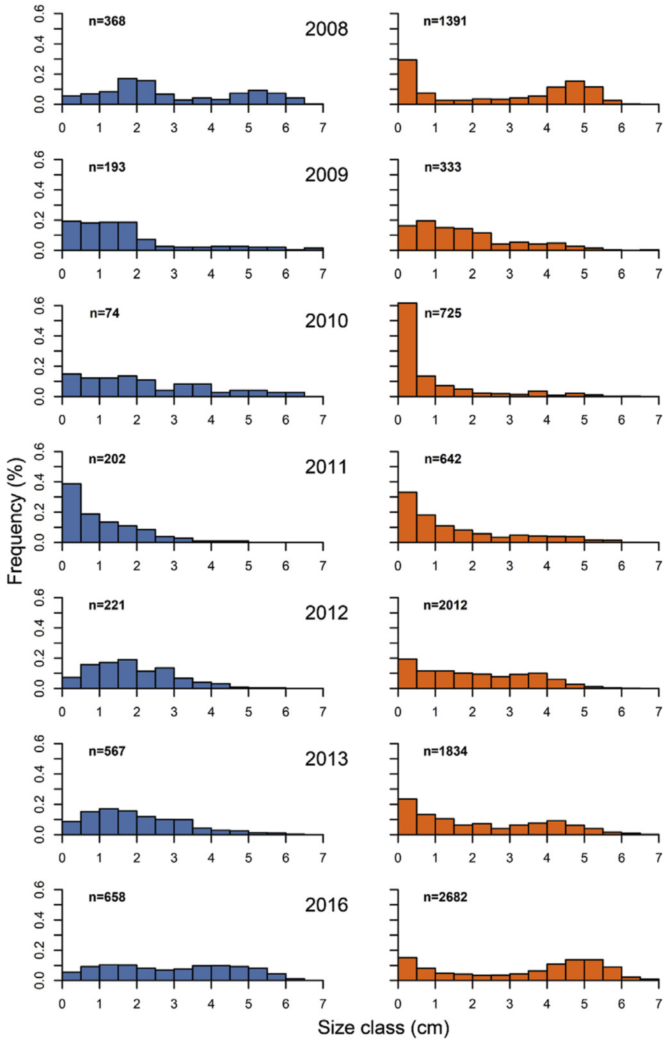

**Fig. 4.** Size class distribution of *Paracentrotus lividus* before the extraordinary storm (2008) and in the years following the storm (2009–2016). The blue colour represents the frequency distribution inside the NTZ, and orange represents the frequency distribution outside the NTZ. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**

Results of the generalized linear mixed models (GLMMs) testing differences in the *P. lividus* density and biomass between different protection levels (inside and outside the NTZ) and in the five subsequent years after the storm (2009–2013). To select the maximum likelihood model, we used Akaike's information criteria (AIC). According to Burnham and Anderson (2003), the model with the lowest AIC value was considered the most appropriate, which is indicated in bold.

| Models | Variable | Coefficients | | | | AICc |
|---|---|---|---|---|---|---|
| | | Estimate | Std. Error | z value | p | |
| **Density ~ Protection*Years + (1\|Protection:Site)** | Intercept | 3.7138 | 0.5421 | 6.851 | < 0.001 | 2104.3 |
| | Protection (MPA) | −2.0464 | 0.7673 | −2.667 | 0.0076 | |
| | Year | 0.6540 | 0.0842 | 7.768 | < 0.001 | |
| | Protection*Year | −0.3182 | 0.1132 | −2.811 | 0.0049 | |
| Density ~ (1\|Protection:Site) | Intercept | 2.8035 | 0.6739 | 4.16 | < 0.001 | 2169.3 |
| **Biomass ~ Protection*Years + (1\|Protection:Site)** | Intercept | 5.9833 | 0.259 | 23.101 | < 0.001 | 3447.3 |
| | Protection (MPA) | −1.6393 | 0.3645 | −4.497 | < 0.001 | |
| | Year | 0.9989 | 0.1103 | 9.057 | < 0.001 | |
| | Protection*Year | −0.7008 | 0.1454 | −4.820 | < 0.001 | |
| Biomass ~ (1\|Protection:Site) | Intercept | 5.3857 | 0.5519 | 9.759 | < 0.001 | 3520.8 |

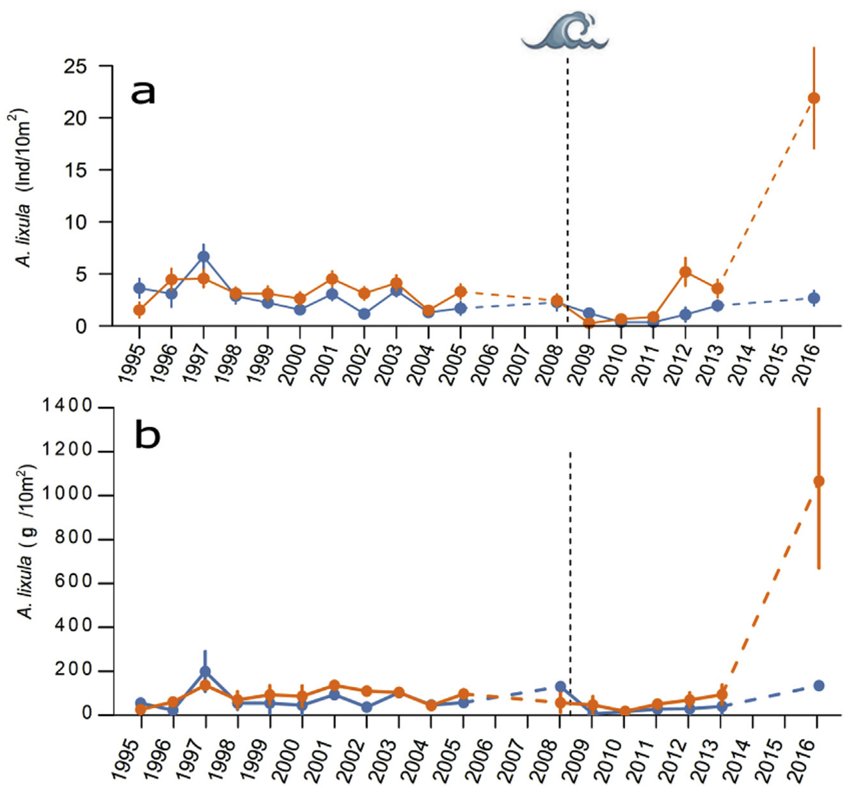

**Fig. 5.** *Arbacia lixula* abundance (a) and biomass (b) since 1995. Coloured dots represent the mean per 10 $m^2$, and bars represent the SE. The blue colour represents the values inside the NTZ, and orange represents the values outside the NTZ. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

southern and eastern areas of the Mediterranean Sea. Thus, such an upward trend seems to be linked to the warming trends observed on the Catalan coast in recent decades, where the temperature has increased 0.26 ± 0.08 °C/decade from 1985 to 2015 (Vargas-Yáñez et al., 2017), and is probably enhanced by the lack of predation pressure when fishing is allowed. In addition, the preliminary results on the *A. lixula* growth rate (Barrera, 2018) have shown the faster growth of newborn *A. lixula* compared with *P. lividus*, which is suggested as a win-win strategy for *A. lixula* under expected global change conditions.

The coexisting sea urchins *P. lividus* and *A. lixula* have different diets and foraging activities (Wangensteen et al., 2011; Agnetta et al., 2013), and it has been described that *P. lividus* has a preference for fleshy algae and that *A. lixula* preferentially graze on encrusting coralline algae (Privitera et al., 2008), having an important role in the maintenance of sea urchin barrens once they are established (Agnetta et al., 2015). In addition, a wider area can be impacted by *A. lixula* grazing activity than by *P. lividus* activity due to its higher mobility on barren zones (Bonaviri et al., 2011). Therefore, the increase of this species may have large consequences for macrophyte-dominated communities.

Physical disturbances and interactions among multiple stressors acting at local and global scales can drive important changes in the structure and function of marine populations and communities. Several studies to date have demonstrated that severe storms and extreme waves can trigger mass mortalities in sea urchins populations (Scheibling and Lauzon-Guay, 2010; Scheibling et al., 2010) and have important effects on rocky shore communities in general (Denny et al., 2009; Micheli et al., 2016; Borja et al., 2018). An increase in the intensity and frequency of extreme climatic events, such as extraordinary storms (as observed in our study), has been observed since 1950 and is expected to increase in the future (IPCC, 2014; Reguero et al., 2019), especially in the Mediterranean, which has been highlighted as a hotspot of ongoing climate change (IPCC, 2014, Cramer et al., 2018).

Our findings reveal that predation can control the establishment of new sea urchin populations and emphasize top-down control in NTZs. These results confirm the important potential role of protected zones, those areas fully protected from fishing, in the structure of benthic communities (Sangil et al., 2012; Sala and Giakoumi, 2017). Additionally, the contrasting patterns regarding different levels of protection observed in this study highlight the relevance of well-designed long-term monitoring to better understand the natural variability of sea urchin populations and to discern the underlying mechanisms when mass mortality events occur. Long-term monitoring also provides useful insights into the management and conservation of algal-dominated benthic ecosystems.

## Author contributions

BH and CL designed and began the long-term monitoring. BH, CL, AM, EA, PC, IMS and MPE conducted the fieldwork after the 2008 storm. AM performed the statistical analyses with contributions from CL, BH, IMS and EA, and all the authors designed the figures. AM wrote the first version of the manuscript with reviews and editing from all the authors.

## Declaration of interests

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.02.013.

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