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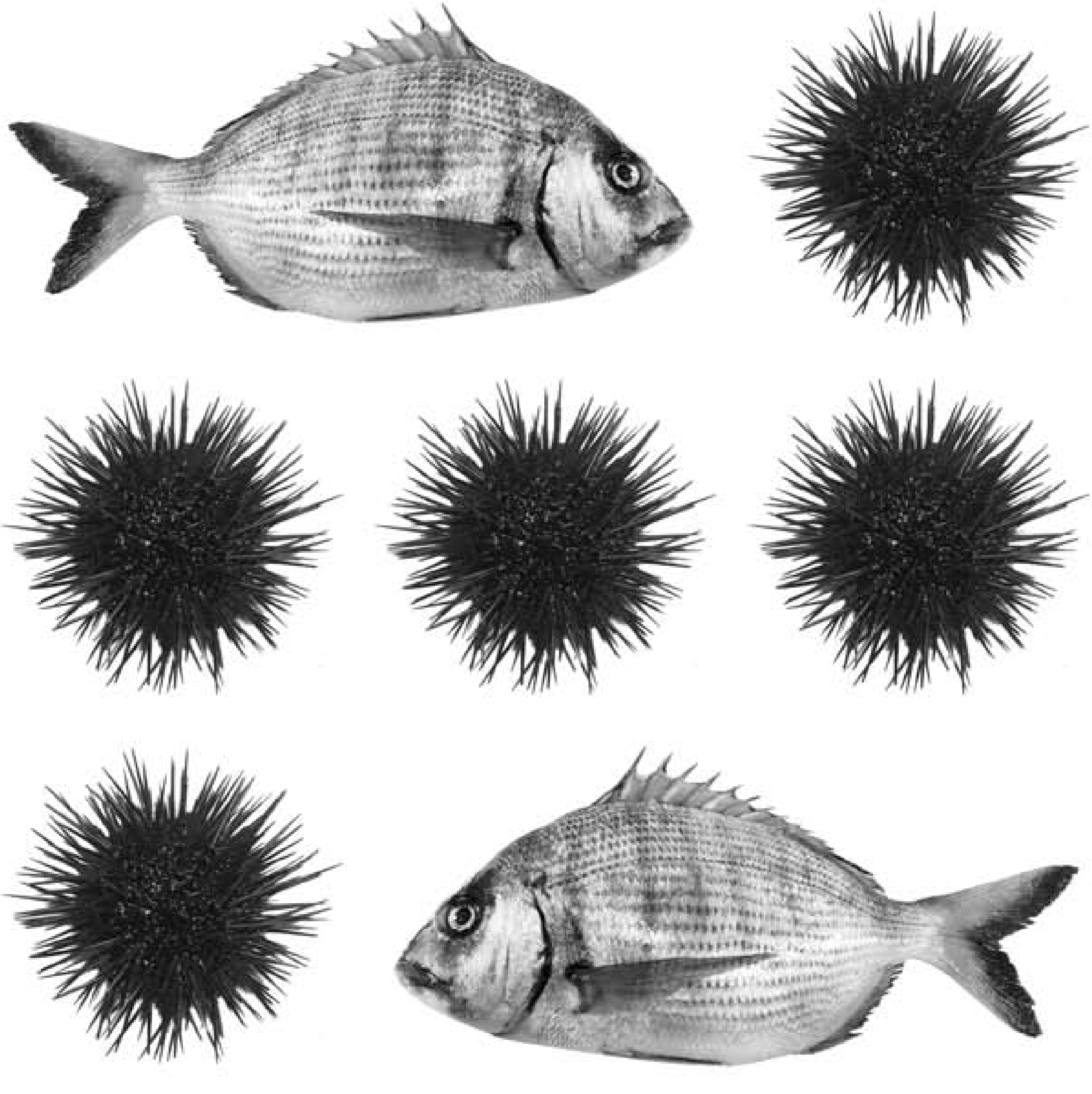

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Jordi Boada Garcia

Tesi Doctoral

Universitat de Barcelona
Factultat de Biologia
Programa de Doctorat d'Ecologia Fonamental i Aplicada

Change agents: sea urchin regulation and state shifts in Mediterranean macrophyte systems

"El control de les garotes i els canvis d'estat en els ecosistemes de macròfits de la Mediterrània"

Memòria presentada per Jordi Boada Garcia per optar al
Grau de Doctor per la Universitat de Barcelona

Jordi Boada Garcia
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A la Valèria

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INFORME DELS DIRECTORS

Els directors de la present tesi, la Dra. Teresa Alcoverro Pedrola i el Dr. Javier Romero Martinengo certifiquen que en Jordi Boada Garcia ha participat activament en el desenvolupament de la feina associada a cadascun dels articles presentats en aquesta Tesi Doctoral, així com en la seva elaboració.

En concret, la seva participació en cadascun dels articles ha consistit en:

- Plantejament dels objectius
- Planificació i execució dels experiment, tant pel què fa a feina de camp com al laboratori.
- Processat i anàlisi de les mostres obtingudes.
- Redacció dels articles i seguiment del procés de revisió dels mateixos.

Finalment, certifiquen que cap dels coautors dels articles presentats a continuació i que conformen la present Tesi Doctoral utilitzarà implícitament o explicita aquests treballs per a l'elaboració d'una Tesi Doctoral.

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LLISTAT D'ARTICLES PUBLICATS, ENVIATS O EN PREPARACIÓ

Capítol 3

Boada, J., Arthur, R., Pagès, J., Pessarrodona, A., Oliva, S., Ceccherelli, G., Romero, J., Alcoverro, T. (en revisió) Immanent conditions determine imminent collapses: nutrient regimes define the grazing resilience of macroalgal communities. *Ecology letters*. **Factor d'impacte:** 10.689

Capítol 4

Boada, J., Sanmartí, N., Selden, R. L., Lucas, A., Pérez, M., Alcoverro, T., Romero, J. (2015) Evaluating potential artifacts of tethering techniques to estimate predation on sea urchins. *Journal of Experimental Marine Biology and Ecology*. **Factor d'impacte:** 1.866

Capítol 5

Boada, J., Farina, S., Romero, J., Alcoverro, T. (en preparació) Herbivore control in connected seascapes: habitat determines when in the life history of a key herbivore population regulation occurs.

Capítol 6

Boada, J., Arthur, R., Farina, S., Santana, Y., Mascaró, O., Romero, J., Alcoverro, T. (2015) Hotspots of predation persist outside marine reserves in the historically fished Mediterranean Sea. *Biological Conservation*. **Factor d'impacte:** 3.762

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CONTENTS

1

INTRODUCTION

1.1 THE ECOLOGY OF STATE SHIFTS

Ecological communities are dynamic entities subject to frequent changes that can occur either naturally or caused by human pressures. Understanding these changes has been central to ecological science and many ecologists have endeavoured to reduce the uncertainty that appears inherent to them. Changes in ecosystem state can be either gradual (Fig.1. 1a) or abrupt. If the ecosystem state changes abruptly, it can take the system down two very (Scheffer *et al.* 2001). different trajectories. In the first, the ecosystem state changes suddenly when the stressor crosses a given threshold limit and the original state is recovered if the stressor attenuates, with recovery (backward path) following the same trajectory as deterioration (forward path; continuous, Fig.1. 1b). In the second scenario, the ecosystem state changes suddenly as well, but restoring the stressor to levels before the abrupt change it is not sufficient to recover ecosystem state; instead the stressor has to be reduced well below pre-deterioration levels (discontinuous, Fig.1. 1c). The difference in stressor levels between the forward and backward paths is known as hysteresis (see Box 1 for this and other definitions). Hysteretic transitions in ecology have been termed 'phase shifts', 'regime shifts' or 'catastrophic transitions' among others (Conversi *et al.* 2015). When this occurs, the ecosystem has more than one alternative stable states and transitions between them are presumed to be less predictable than when changes take place in a continuous manner, which has profound implications for the way the system is likely to respond to changes in the environment. Key structural species play essential roles in 'ecological regime shifts'; the presence, absence or variation in their numbers can critically modify community structure triggering ecosystems to the thresholds of critical transitions (Conversi *et al.* 2015). Often what drives shifts in natural communities are changes in species interactions, with top-down processes playing a central role. However, other stochastic phenomena such as extreme storm events, fires and disease outbreaks can also trigger ecosystems to shift to highly stable depleted states. Overall, the dynamic of an ecosystem and its susceptibility to catastrophic shifts depends on its trophic interactions and is influenced by multiple and interacting external factors either physical or biological (Fig.1. 2, Conversi *et al.* 2015).

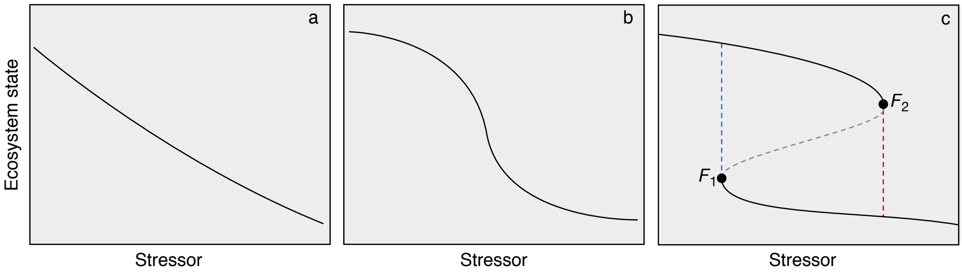


FIGURE 1.1. Ecosystem state pathways of change according to stressor levels a) the ecosystem state is reduced linearly with an increase in stressor, b) the ecosystem state suddenly depletes in a continuous mood when certain stressor levels are achieved and c) the ecosystem state abruptly changes discontinuously when reaching the tipping point (F_2) indicating the presence of alternative attractors and hysteresis (F_1) indicates the tipping point for recovery. From Scheffer *et al.* (2001).

examples of regime shifts exist from both terrestrial and aquatic environments. Woodlands often give way to open grassland ecosystems when conditions change. These grasslands may be highly stable as herbivore pressure prevents colonisation by woody plants, preventing woodlands from growing once again (Box 2A). When herbivores decline because of human harvesting or due to the direct or indirect effects of predator control, woody vegetation is no longer limited, completely changing the ecosystem (Wolf et al. 2007). Another well-known example of ecosystem shifts occurs in lakes when a sudden loss of transparency related to increased nutrient loads by human activities can precipitate one state to shift to another (Box 2B, Scheffer et al. 1993). When nutrients exceed critical values, lake waters shift from clear to turbid, with benthic vegetation disappearing (together with a host of associated fauna) and phytoplankton dominating the new ecosystem state. The presence of zooplankton predators like fish enhances the stability of the turbid state by reducing phytoplankton control. The recovery of the original clarity of waters requires the reduction of nutrients to substantially lower levels than the thresholds that caused the shift. A temporary reduction in fish biomass has been shown to work as a successful measure to recover the original state. In coral reefs, a similar process occurs, where the simultaneous effect of increased nutrient loads from human activities together with reduction in fish herbivores because to overfishing results in catastrophic shifts (Box 2C, McCook 1999). When this happens, coral structures are overgrown by macroalgae if no alternative top-down control by herbivores exists (e.g. sea urchins). The lower palatability of adult algae, the inhibition of corals recruitment in algal dominated substrates and the depressed recovery of herbivore guilds make returning to the original coral-dominated state very difficult. Other marine systems that are clearly prone to catastrophic shifts are the macrophyte dominated ecosystems that dominate temperate rocky reefs (i.e. macroalgal habitats and kelp forest, Box 2D, Filbee-Dexter & Scheibling 2014). Predator release, often related to overfishing, produces massive herbivore outbreaks (i.e. sea urchins) that trigger catastrophic shift from the well-structured habitat of kelp or other macroalgal species to bare rock extensions. These altered states are less diverse and productive and very stable, making the recovery to the original macroalgal state extremely difficult.

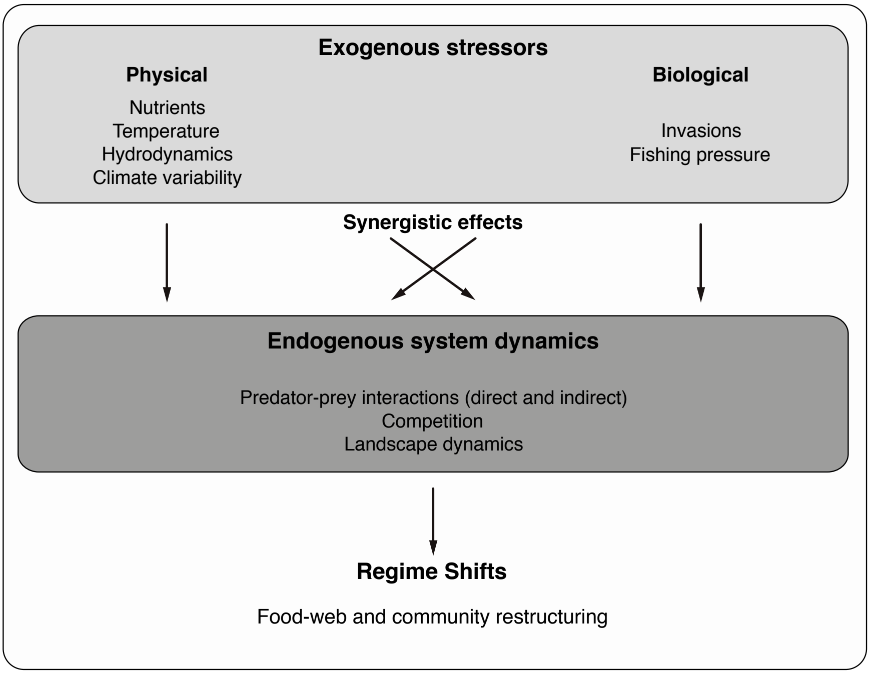


FIGURE 1.2. Schematic description of factors driving regime shifts in ecosystems; exogenous stressors either physical or biological (light grey box) and ecosystem endogenous factors (dark grey box) from Conversi (2015).

Identifying critical stressor levels (thresholds) in ecosystems characterized by discontinuous transitions is a fundamental problem in ecology, and the first step in reducing the uncertainty behind state shifts. Furthermore, unpacking the mechanisms regulating stressor levels is crucial. In attempting to anticipate catastrophic thresholds, a set of essentially phenomenological early warning signals have been identified which may serve as indicators that the system is primed for a sudden change in state. As a system approaches a catastrophic threshold it will begin to show subtle changes in variance in ecosystem state, critical slowing down in ecosystem dynamics and an increase in self-organized patchiness – if identified in time, these indicators could be used to pull ecosystems back from the brink of collapse (Andersen *et al.* 2009; Dakos *et al.* 2015). However, identifying these early warning signals strongly depends on long-term monitoring and is based on an understanding of baseline ecosystem dynamics, which is sometimes very difficult to achieve. Additionally, such approaches do not clearly outline the mechanistic processes underlying transition dynamics, which are, in fact, its key predictors. An alternative approach requires a specific understanding of the mechanisms that determine why and when thresholds occur, which can help in assessing the overall vulnerability of a community to catastrophic shifts as well as in locating where, along a gradient of stressors, these thresholds may lie.

Terrestrial carnivores have been on a path of steady decline as their habitats fragment and their populations succumb to hunting pressures (Ripple *et al.* 2014). The world's oceans have gone through their own critical trophic downgrading as a consequence of overfishing (Box 3, Pauly *et al.* 1998) and habitat loss. The loss of top predators has cascading effects across the trophic network, ultimately affecting the primary producers (top-down control), (Ripple *et al.* 2014). In fact, the release from top-down forces is one of the principal de-structuring agents in ecosystems, and in the regions that have suffered the greatest trophic downgrading the continued functionality of the ecosystems is seriously compromised (Estes *et al.* 2011). While predation is clearly critical for healthy ecosystems, bottom-up processes acting on primary producers also play vital roles in structuring terrestrial and marine ecosystems. These include conditions of light, temperature, precipitation and nutrients, among others that together influence the biomass of producers that sustain the trophic network (bottom-up control). Determining the relative importance of top-down and bottom-up processes in ecosystem structuring is critical to predict variations of the main stressor values, particularly when the stressor is a key structuring species. Perhaps the most powerful way to do this is with ecological experiments, which allow you to carefully control stressors and measure ecosystem responses to them (Box 4). First, obtaining relative estimates of different sets of potential stressors in the field can serve, at the very least, to track variations across space and time and to help identify the main control agents (top-down or bottom up). Second, mesocosms experiments in the laboratory are necessary to identify the mechanisms behind ecological interactions. These can form the basis from which to build up accurate qualitative and quantitative models of ecosystem functioning to predict and, eventually, prevent, regime shifts (Dambacher *et al.* 2009; Marzloff *et al.* 2011).

Understanding alternative states dynamics has critical consequences for management. Unfortunately, when shifts occur ecosystems revert to significantly poorer states in which productivity and diversity, as well as other ecological services, are heavily depleted. Additionally, these new pauperized states are commonly maintained with a series of feedbacks that enhance the persistence of the new ecosystem organisation. Altogether, this means that collapsed states are normally more resilient than more 'healthy' states. The shift to undesired states of ecosystems can have dramatic effects not only on biodiversity but also on economies and societies. When an ecosystem collapses, it loses (or, at best, reduces) most, if not all, of its associated ecosystem goods and services that support human communities. Hence, knowledge of the drivers and mechanisms underlying regime shifts is of fundamental importance for managers as well as for policy makers (Scheffer *et al.* 2001; Conversi *et al.* 2015). Finding critical indicators of alternative states that can reduce uncertainty of when or why these shifts occur, becomes a management imperative to protect ecosystem functionality, diversity and services. Once these indicators are obtained, mapping ecosystems according to their potential resilience to ecosystem shifts will help managers to prioritise areas to protect or to identify areas where intervention is needed.

1.2. Macrophyte overgrazing and sea urchin barrens

In the early seventies Estes and. Palmisano (1974) described one of the first and most famous observations of regime shift dynamics in marine ecosystems. By that time, the destruction of benthic macrophyte ecosystems because of overgrazing by sea urchins population outbreaks had already been detected in some places around the world. However, in their seminal work, Estes and Palmisano identified that the maintenance of the kelp state was dependent on the top-down control of sea urchins by their dominant predators – sea otters. Similar findings of barren creation after sea urchin release from the predator pressure have been further reported elsewhere (Pinnegar *et al.* 2000; Guidetti 2004; Clemente *et al.* 2008; Ling *et al.* 2009a). Sea urchin grazing has been identified as a major agent of ecosystem shifts from, seaweed to coralline algae dominated barrens. These shifts have been described from locations around the world (Box 5), being predominant in the temperate waters of the Northern

Hemisphere (Filbee-Dexter & Scheibling 2014). Together with sea urchins, other invertebrate species like starfish, brittle stars, limpets or mussels dominate barrens and have been widely shown to enhance their stability. In addition, adult urchins also facilitate new urchin settlement, further ensuring the maintenance of this new state. To return to the initial macroalgal dominated state, sea urchin density (the principal stressor) has to decrease to values well below those in the initial, macroalgal-dominated state. Therefore, marine reserves have been proposed as a central management tool to maintain (or, potentially restoring) macroalgal states, by conserving (or, eventually, recovering) the predation function (Pinnegar *et al.* 2000, Fig. 1. 3) However, most of studies supporting these views are based on comparisons between MPAs and unprotected areas, whereas there is a significant lack of studies attempting to frame the role of predation into a more comprehensive conceptual model that accounts for sea urchin settlement, recruitment, as well as other demographic processes. This type of approach needs large spatial scales to be properly attempted.

Grazing on seagrasses is in general more prevalent that previously thought in tropical and temperate areas (Heck & Valentine 2006; Prado *et al.* 2007; Kelkar *et al.* 2013). The main herbivores are green turtles and dugongs in tropical areas and sea urchins and fish herbivores in temperate ones. This high grazing pressure is relatively well tolerated by seagrasses, thanks to a series of adaptations including compensatory growth, deterrent and mechanical defences, protection of apical rhizomes or meristematic growth, among others (Vergés *et al.* 2008). Despite these defences however, there have been instances of seagrass meadows subject to important overgrazing events (Eklöf *et al.* 2008a; Planes *et al.* 2011). After (Camp *et al.* 1973) and collaborators first reported sea urchin overgrazing in *Thalassia testudinum* seagrass beds in the Gulf of Mexico, several other reports have emerged of similar events around the world (Box 5, Eklöf *et al.* 2008b). One of the, perhaps, best-studied examples was documented in the seagrass beds of the Mombasa Marine National Park in Kenya. Several massive sea urchin (*Tripneustes gratilla*) aggregations (~50m^{-2}) were found in Mombasa lagoon which formed fronts that advanced on the seagrass meadow, leaving large tracts of dead seagrasses (mostly *Thalassodendron ciliatum*) in their wake (Alcoverro & Mariani 2002). Overgrazing events have also been reported in tropical seagrass ecosystems after the massive arrival of green turtles or dugongs (Heithaus et al. 2014). Interestingly, recent studies show that urchins can migrate from macroalgal habitats after reducing them to barrens, moving to adjacent seagrass meadows, precipitating overgrazing events in them as well (Ling et al. unpublished results).

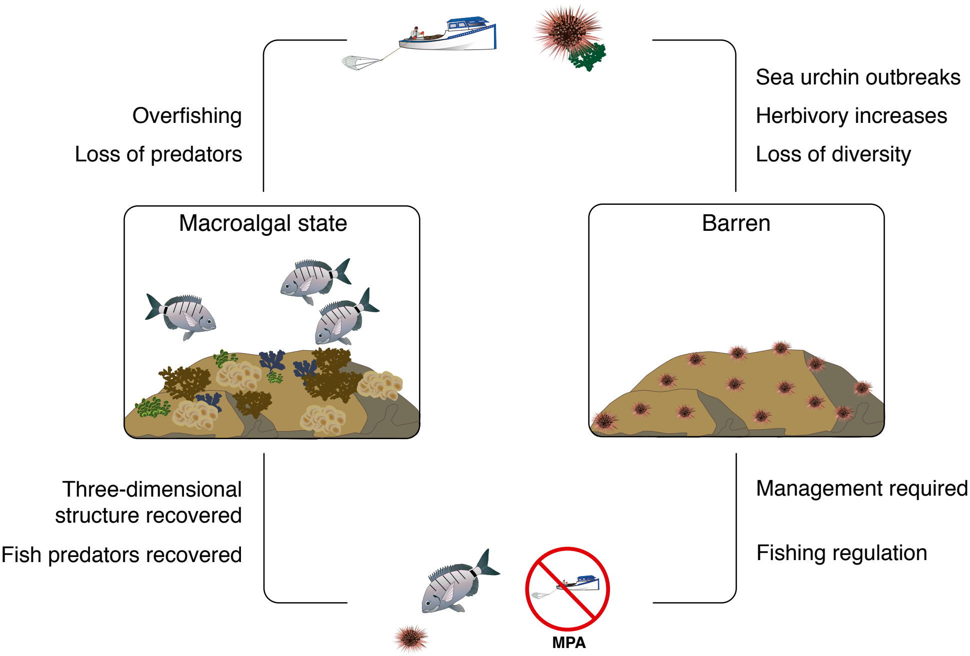


Figure 1.3. Alternative stable states in Mediterranean macroalgal communities (Pinnegar 2000). The predator release related to fishing activity causes major sea urchin outbreaks. Establishing Marine Protected Areas enhance the recovery of predatory fish species and the consecutive recovery of the structured macroalgal communities.

1.3. The Mediterranean macrophyte ecosystems

The Mediterranean Sea is considered a hotspot of biodiversity (Coll *et al.* 2010). Mediterranean waters are oligotrophic in general, although significant regional differences exist in nutrient regimes (e.g. Western vs. Eastern basin, continental vs. insular, Bosc *et al.* 2004). Shallow rocky bottoms in the Mediterranean are dominated by several species of canopy forming algae that constitute a highly diverse and structured habitat (Sales & Ballesteros 2009; Sala *et al.* 2012). Species of the order *Fucales*, especially *Cystoseira* spp are representative of a low impacted rocky shore (Sales & Ballesteros 2009). Other species of fleshy algae (i.e. of genus *Dictyota*, *Halopteris* and *Padina*) dominate shallow rocky bottoms giving three-dimensional structure to these habitats. Seagrass meadows (mainly *Posidonia oceanica*) mostly dominate the soft sandy bottoms (also sometimes found on rocky substrates) in Mediterranean shallow subtidal shores from 1 to 25-40 m depth depending on water transparency. Seagrass meadows and macroalgal communities are habitats of key importance as their main structural species are habitat engineers that provide refuge to a large number of fish and invertebrate species of major interest for fisheries (Garcia-Rubies & Macpherson 1995).

The sea urchin *Paracentrotus z* and the fish *Sarpa salpa* are the main herbivores feeding on benthic Mediterranean vegetation (Boudouresque & Verlaque 2001; Prado *et al.* 2007). The vertical distribution of *P. lividus* ranges from near the surface to 20m depth, reaching its maximum abundance at intermediate depths (4-8 m). The regulation of Mediterranean sea urchin population dynamics appears to rely on a set of interacting factors at multiple scales (Hereu *et al.* 2012; Prado *et al.* 2012). Several studies suggest that predator release is the principal process causing major *P. lividus* outbreaks that trigger shifts in macrophyte communities (particularly macroalgal communities, Sala 1997). However, most of these studies have been limited to few marine reserves where the main focus has been on macroalgal habitat, and few studies have examined these processes across the larger seascape where these habitats are often interspersed (Fig. 1.4). Habitat-specific traits can determine life-history processes of settlement (Prado *et al.* 2012), recruitment (Tomas *et al.* 2004) and protection against predators (Farina *et al.* 2009), and their effects on the dynamics of populations should be further investigated. Altogether, the overall understanding of the population dynamics of herbivores and their controls, with the aim of predicting critical thresholds, appears a challenging and complex task. Yet protecting rich benthic vegetation from shifting to depauperate states is an important management imperative to maintain ecosystem functionality, diversity and services.

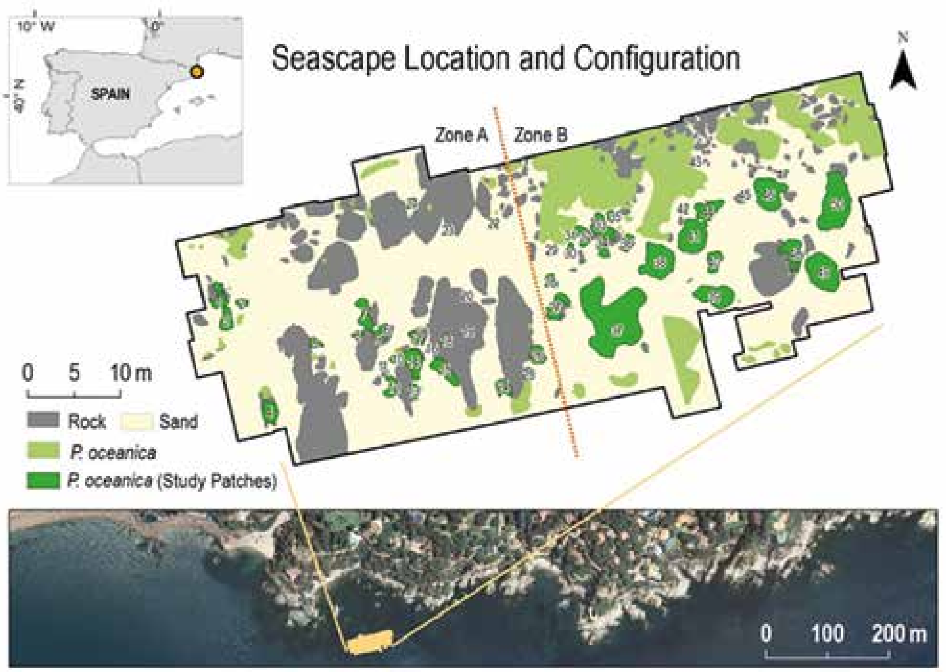


Figure 1.4. A map of Cala Frares at Lloret de Mar (Western Mediterranean Sea; 41°41′54″N, 2°51′38″E, 75mx35m). Seascapes are characterized by macroalgal communities on rocky substrates (grey) and *Posidonia oceanica* meadows (green).

1.4. General aims

In this thesis I attempt to address the uncertainty associated with regime shift transitions in macrophyte ecosystems in the Mediterranean (i.e. macroalgal rocky habitats and *Posidonia oceanica* seagrass meadows). I endeavour to understand and reduce this uncertainty with the following approaches, summarized graphically in Fig. 1. 5:

1. Identify where along the gradient of stressors (i.e. sea urchin biomass) critical ecosystem thresholds exist in Mediterranean macrophyte communities. **Chapter 3**.
2. Understand the mechanisms that trigger critical ecosystems transitions. **Chapter 3**.
3. Identify the principal factors driving population dynamics of the key herbivore in these systems (the main stressor). **Chapter 5**.

After addressing these objectives, I proceed to identify hotspots of active functional predation (the main structuring agent) to map potential ecosystem resilience across the seascape and to provide managers with a potential means of prioritising areas to protect or intervene as necessary (**Chapter 6**). In addition, the thesis includes a chapter testing and developing the most appropriate methodology to assess predation on sea urchins (**Chapter 4**).

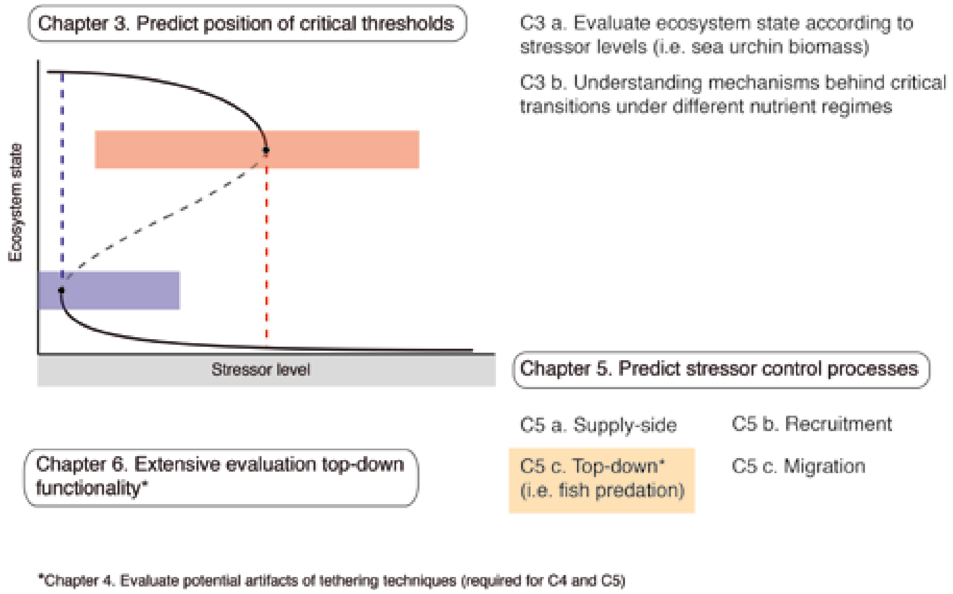


FIGURE 1.5. Main objectives of this thesis, schematic description.

Most chapters use a combination of correlational large-scale studies, experimental approaches and conceptual and theoretical insights. The data presented here will further serve to provide mathematical tools to assess stable states of macrophyte ecosystems in the Mediterranean. Taken together, I intend to improve the understanding and predictability of critical regime shifts and provide important clues to effectively managing the sustainability of shallow macrophyte ecosystem services.

Definitions

BOX 1

Regime shift - Abrupt change in the community organisation that encompasses multiple variables including key species. Regime shifts have been given several synonyms including ***Phase shifts***, ***state shifts*** and ***ecosystem reorganisations***. When shifts involve a clear tipping point and the system is known to have alternative stable states they are also known as ***critical transitions***.

Domain of attraction - The set of values for variables from which a system returns to a particular equilibrium state or dynamic regime. Also known as ***attractor***.

Alternative stable states - The different attractors to which a system may gravitate. In systems with alternative stable states, the depth or size of the basin of attraction is a measure of its ***resilience***.

Critical threshold - The limiting conditions beyond which the qualitative behaviour of a community suddenly changes. Also referred to as ***tipping point*** or ***bifurcation***. It is generally associated with shifts between alternative stable states.

Hysteresis - In discontinuous regime shifts, the phenomenon by which the recovery path from altered conditions to the original state requires stressor values below those that triggered the altered state.

Resistance - The degree to which a variable of the system is changed following a perturbation.

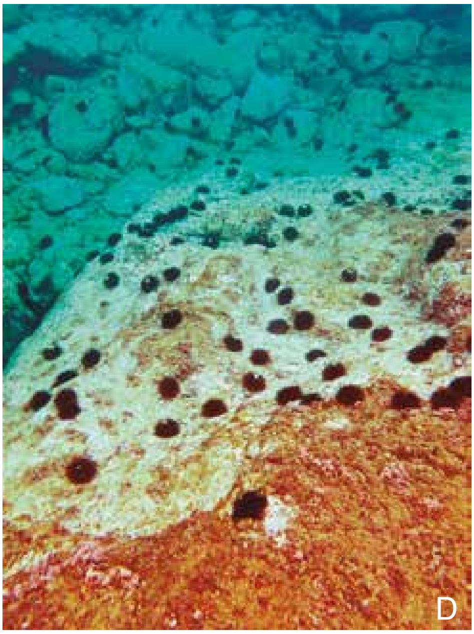
Resilience - The capacity of a system to absorb disturbances and reorganize to maintain essentially the same functions, structure, identity and feedbacks.

Feedback - Processes or mechanism with the potential to stabilise or destabilise system states. ***Negative feedbacks*** buffer changes and stabilise a particular state of a system contributing to maintaining it. ***Positive feedbacks*** are destabilizing mechanisms, which are necessary to move from one system state to another.

These definitions are adapted from Conversi (2015) and Pimm (1984)

Ecosystem shifts - Classical examples

BOX 2



Changes in environmental conditions or species interactions can drive major changes in ecosystem structure. Here I discuss some of the most classical examples of alternative stable states in ecosystems. Picture A shows a herbivore exclusion area in Scotland; the grassland state outside the fenced area is maintained by deer while in inside the exclosure, woody plants are able to grow. Eutrophic lakes B, are a good example of degraded ecosystems where in turbid waters, phytoplankton is dominant. In tropical coral reefs (C) macroalgae can quickly overtake the benthos when herbivory fish populations are depleted due to overfishing. Picture D illustrates the case study I address in this thesis which release from fish predation causes massive sea urchin outbreaks that trigger macroalgal communities to shift to barren states.

Photo credits: A. Jordi F. Pagès, B. Marc Ventura, C. Jordi Boada and D. Jordi Boada & Teresa Alcoverro

Trophic downgrading

BOX 3

Harvesting and intense land use by humans is responsible for large-scale habitat destruction and the extinction of large predators in ecosystems. Although this species loss is mostly considered as a biodiversity issue, the loss of top consumers has dramatic consequences for the whole ecosystem. In marine environments Pauly et al. (1998) traced how global fisheries catches were rapidly shifting towards lower mean trophic levels in what was described as 'fishing down the food web'. The world's oceans continue to be extremely overfished and the continued loss of top predators raises important questions around the continued functionality of certain ecosystems. For instance, the loss of predatory sharks has led to major overgrazing events by sea turtles in several seagrass ecosystems. Similarly, sea otter population collapses due to harvesting produced uncontrolled sea urchin outbreaks causing massive pauperization of kelp beds to bare rock extensions. The illustration in this box shows the decrease in mean trophic level first described by Pauly et al. (1998) for the Mediterranean Sea.

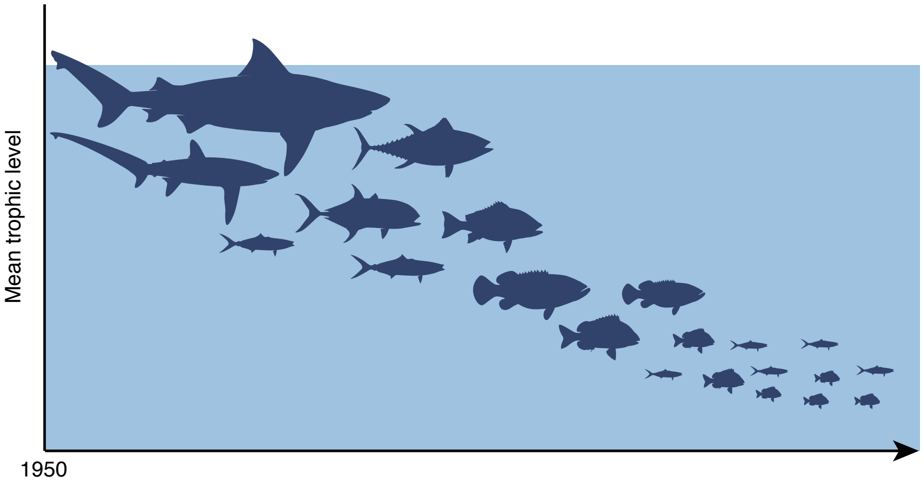
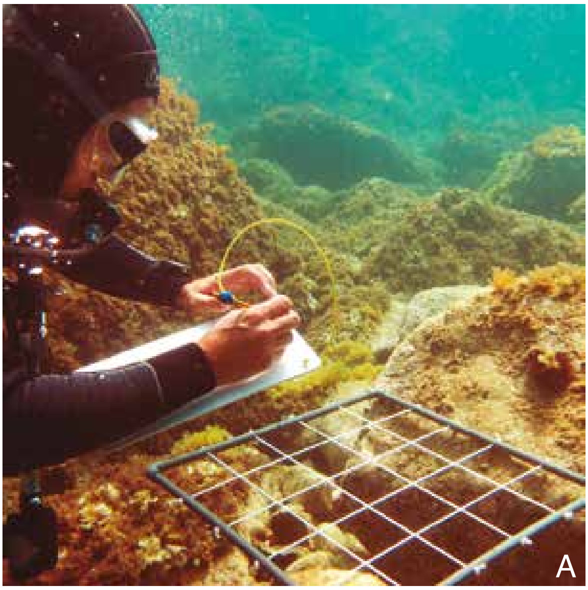


Illustration credits: This illustration was redraw from Pauly et al. 1998 by Jordi Boada using Nick Botner's open access vector images

Experiments in ecology

BOX 4

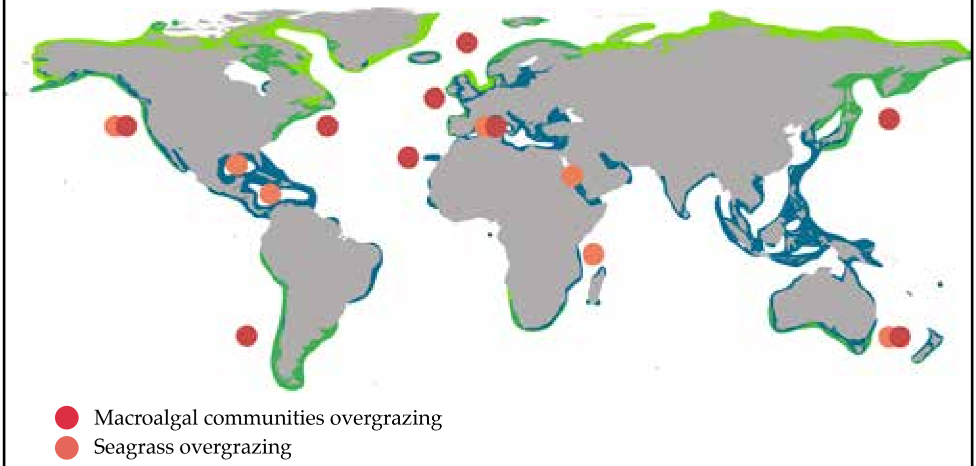


Measuring process in nature is central to ecological science. In 1989, Hairston published an entire volume addressing the principal questions relevant to experimental marine and terrestrial ecology. Testing ecological hypotheses requires a toolbox of field observations together with carefully designed field and laboratory experiments. Field surveys (A) are useful to assess the state of ecosystems or abundances of species and account for their variance in time or space. Manipulative experiments either in the field (B) or in the laboratory are essential to isolate the effect of ecological processes. Advances in technology like the use of underwater cameras in fish surveys often serve to improve experimental techniques (C). However, testing its applicability and robustness as well as assessing its potential artifacts is essential. This thesis relies heavily on experimental ecology to explore the mechanisms driving state transitions (field and laboratory experiments) and to evaluate ecological processes in the field. Additionally we have explored the potential artifacts of the most common techniques to assess predation impacts on sea urchins.

Photo credits: A, Yaiza Santana & Valèria Mayoral, B and C, Jordi Boada & Valèria Mayoral

Macrophyte overgrazing

BOX 5



Sea urchin overgrazing events have been described from coastal ecosystems across the world. Well-structured kelp beds or other macroalgal communities shift rapidly to barren formations when sea urchin outbreaks occur. Similarly, seagrass habitats suffer overgrazing events by several herbivore species ranging from small invertebrates (i.e. sea urchins) to sea turtles and dugongs. In the map above the worldwide distribution range of kelp beds (known range, dark green and potential range, light green) are shown together with the distribution range of seagrass meadows (dark blue). Red dots (macroalgae) and orange dots (seagrass) show regions where sea urchin overgrazing events have been described. Sea urchin barrens in macroalgal communities mostly dominate temperate seas in the Northern Hemisphere. Additionally, although less instances of seagrass meadow overgrazing have been reported (possibly because they are naturally more resistant to herbivore pressure) these instances are globally distributed.

This illustration was assembled based on available published reports of overgrazing events mainly, from (Eklöf et al., 2008 and Filbee-Dexter & Scheibling, 2014)

2

OBJECTIVES

OBJECTIVES

This thesis is structured in four main chapters. In each chapter I aim responding for particular key objectives / hypotheses in my general intention to understand the mechanisms behind critical transitions from macrophyte ecosystems to barren areas. Particularly, I focus on regime shifts to identify the position of critical thresholds and the mechanisms behind (**Chapter 3**). A special chapter in methodology (**Chapter 4**) is included to confirm the efficiency of the sea urchin tethering techniques used in **Chapter 5** and **Chapter 6**. Then, top-down and supply-side controls together with proxies of early post-settlement mortality and migration effect are explored to try to determine regulation on adult populations (**Chapter 5**). Finally, in the last chapter I try to determine areas using functional attributes (predation) across a wide stretch of the Mediterranean (**Chapter 6**). The results exposed in this thesis are the product of field surveys (**Chapter 3, 5** and **6**), manipulative field experiments (**Chapter 3, 4, 5** and **6**) and controlled laboratory experiments (**Chapter 3**).

The detailed objectives of each chapter are described below:

- **Chapter 3.** *Immanent conditions determine imminent collapses: nutrient regimes define the grazing resilience of macroalgal communities.* In this chapter I focus on determining the position of critical threshold stressor levels (sea urchin abundance) that produce ecosystem shifts in Mediterranean macroalgal habitats in two different nutrient regimes. Our hypothesis to test is that lower levels of herbivory are required in low nutrient regimes to shift macroalgal communities to barrens. Furthermore, I experimentally explore potential mechanisms driving differences in the threshold position: nutrient-dependent changes on sea urchin feeding behaviour and changes on plant growth driven by nutrient availability.

- **Chapter 4.** *Evaluating potential artifacts of tethering techniques to estimate predation on sea urchins.* In this methodological chapter I test the effectiveness of the most extended technique to mark invertebrate species to account predation rates (Aronson & Heck 1995), that will be used in **Chapter 5** and **Chapter 6**. As tethering techniques used to tag sea urchins are generally invasive methods, I explore the potential first (mortality) and second (behaviour) order artifacts associated (Peterson

& Black 1994). In specific, I measure the mortality rates of marked urchins in i) two different confinement conditions and ii) two different temperature conditions. In addition, I measure i) prey detectability by fish predators of pierced and unpierced urchins of different size classes and ii) prey detectability of pierced *versus* unpierced sea urchins by two common representative benthic predators (i.e. the gastropod *Hexaplex trunculus* and the starfish *Marthasterias glacialis*).

- **Chapter 5.** *Herbivore control in connected seascapes: habitat determines when in the life history of a key herbivore population regulation occurs.* In this chapter I explore which are the main factors controlling the population dynamics of the sea urchins, which I believe to be likely highly dependent on the habitats. In specific, I try to identify habitat-specific regulating agents of this herbivore's populations by comparing the strength of supply-side, top-down control and migration processes in macroalgal ecosystems and seagrass meadows of *Posidonia oceanica*.

- **Chapter 6.** *Hotspots of predation persist outside marine reserves in the historically fished Mediterranean Sea.* In **Chapter 5** I intend to evaluate predation in the main macrophyte habitats and I also aim to determine areas where predation is still functional. I did this by evaluating predation impact on sea urchins along a wide stretch of the Mediterranean Sea in macroalgal and seagrass habitats. Moreover, I attempt to elucidate which are the main mechanisms determining those hotspots: i) predator habitat use (related to abundance) and ii) habitat-specific factors (i.e. presence of refuges).

3

IMMANENT CONDITIONS DETERMINE IMMINENT COLLAPSES: NUTRIENT REGIMES DEFINE THE GRAZING RESILIENCE OF MACROALGAL COMMUNITIES

ABSTRACT

Ecosystems characterized by non-linear dynamics are inherently surprising, making very difficult to predict where state-changing thresholds lie. Unpacking the mechanisms underlying these state shifts can help considerably reduce this unpredictability. We examined how differences in nutrient regimes mediated the capacity of temperate macrophyte communities to sustain sea urchin grazing. In relatively nutrient-rich conditions, macrophyte systems were considerably more resilient to urchin grazing, shifting to barrens beyond 1800 g/m^2 (urchin biomass), more than twice the threshold biomass of more nutrient-poor conditions. The mechanisms driving these differences are linked to how nutrients mediate urchin foraging and algal growth: controlled experiments showed that low nutrient conditions trigger compensatory feeding in urchins and significantly reduce plant growth. These mechanisms act together to halve macrophyte community resilience in relatively oligotrophic conditions. Understanding how context-specific conditions modify non-linear ecosystem dynamics can significantly improve our ability to predict where and why ecosystem thresholds occur.

INTRODUCTION

Identifying where critical thresholds occur in systems characterised by non-linear dynamics is fundamental to objectively quantify their resilience (May 1977; Scheffer & Carpenter 2003; Andersen et al. 2009). Hysteretic behaviour has been observed in several ecosystems as diverse as freshwater lakes, grasslands, coral reefs, kelp forests and macroalgal communities, among others (Scheffer et al. 2001); after collapse, these systems may not recover their initial state, even when the responsible stressors have been reduced. These altered states are typically maintained by increases in the abundance of a key species that reinforce stabilizing feedbacks (Petraitis & Dudgeon 2004). A considerable body of research has focused on describing the role of external stressors in triggering these sudden shifts in functional state and the mechanisms underlying these collapses (Terborgh et al. 2001; Estes et al. 2011; Conversi et al. 2015). Several stressors have been identified as critical state-changing agents including overfishing, pollution and abnormal nutrient loading, population outbreaks of grazers, and large infrequent disturbances like storms, fires, temperature anomalies and other stochastic events (Scheffer & Carpenter 2003; Folke et al. 2004). Among the best described of these shifts occurs when herbivorous sea urchins, released from predation, quickly overtake nearshore macrophyte communities, overgrazing kelp forests and macroalgal beds, reducing them to functionally depauperate rocky barrens (Filbee-Dexter & Scheibling 2014). These altered states are maintained by a series of feedbacks that prevent recovery even when herbivore populations are brought under subsequent control. While in most ecosystems the drivers of these thresholds are relatively well understood, precisely predicting where these thresholds lie is a frontier in ecology, especially since they often vary considerably between regions. While external stressors are clearly responsible for precipitating catastrophic ecosystems shifts, how systems respond to these stressors may differ considerably, highly dependent on context-specific conditions. Most studies on regime shifts only consider a single major driver, due to the difficulty of controlling all potential stressors that could influence system behaviour (Conversi et al. 2015). Nevertheless, other apparently insignificant factors also trigger ecosystems to less diverse states and sometimes those strengths are inherent to the community.

The inherent conditions that determine the overall buffer capacity of each ecosystem include a range of structuring environmental and ecological regimes within which the system finds itself (Scheffer et al. 2001). These include a suite of environmental gradients such as rainfall or fire regimes, natural nutrient loads, local hydrodynamics, among others (Folke et al. 2004). These factors may interact in complex ways with ecosystem processes, mediating both the stabilising feedbacks as well as the mechanisms that trigger system shifts. For instance, under intense grazing pressure, Sahelian grasslands can shift from perennial grasses either to annual grasses (from which recovery can be fairly rapid) or to annual herbs (an altered stable state). Which trajectory the system takes appears to be dictated largely by rainfall regimes, with drought conditions predisposing communities to sudden hysteretic shifts to shrub-dominated vegetation (Rietkerk et al. 1996). Similarly, the resilience of many marine systems such as coral reefs and kelp forests can also be strongly mediated by natural nutrient regimes (Witman & Roy 2009); post-collapse, the recovery of these systems can be significantly retarded when nutrients facilitate the recruitment, growth and space-occupation of competitors (Airoldi 1998; Worm et al. 1999). These conditions can vary considerably with location, making ecosystem trajectories intrinsically difficult to predict (Holling 1973).

Reducing uncertainty in complex systems requires a much better handle of how ecosystem processes are modified by context-specific underlying conditions. Attempts to anticipate thresholds have focused on looking for characteristics of boundary conditions as a signal of impending system change (Scheffer & Carpenter 2003; Andersen et al. 2009; Scheffer et al. 2009; Hastings & Wysham 2010). These signals may manifest as subtle changes in the variance and skew of key ecosystem variables, self-organised patchiness or as a slowing down in ecosystem dynamics (Rietkerk et al. 2004; Carpenter & Brock 2006; Guttal & Jayaprakash 2008; Dakos et al. 2015). It has been argued that these changes in system behaviour can serve as powerful early-warning indicators presaging a major shift in state – either catastrophically (Scheffer et al. 2009) or more continuously (Kéfi et al. 2012). In most instances, signals have been derived from ecosystem models or by hind-casting of systems that have already experienced shifts; finding meaningful predictive metrics that work in real-world situations is still elusive. In addition, these indicators are essentially phenomenological in their nature and dependent on long-term monitoring based on an understanding of baseline ecosystem dynamics from which the variance and autocorrelation signals of critical slowing can be derived (Scheffer et al. 2009). While useful, such correlative approaches are not geared to illuminating the mechanistic processes underlying these transitions. Identifying causal mechanisms may be unrealistically complex, particularly in high diversity ecosystems, where a suite of interdependent biotic and abiotic processes likely drives the functioning of the system. However, where it is possible, understanding the mechanisms of these transitions would allow a clearer evaluation of their resilience potential. In addition, it would provide managers with clear directions of where to prioritize ameliorative measures in order to maximize ecosystem resilience or avoid tipping points.

In this context, Mediterranean rocky macroalgal communities are useful models to explore how regional conditions can mediate the mechanisms of ecosystem transitions since they are characterised by non-linear responses, are relatively simple, and occur in conditions that differ considerably in their inherent nutrient regimes (Pinnegar et al. 2000; Filbee-Dexter & Scheibling 2014; Ling et al. 2015). When overfished, these systems often shift to urchin-dominated barrens when their populations cross critical thresholds (Sala & Zabala 1996; Pinnegar et al. 2000; Filbee-Dexter & Scheibling 2014). These thresholds are breached when rates of consumption (herbivory) surpass plant growth rates (see Fig. 3.1). In this study we examined if inherent nutrient regimes could mediate where these thresholds occur in response to sea urchin biomass (Ling et al. 2015). In addition, we experimentally examined the potential mechanistic pathways by which nutrients could modify these thresholds. Our hypothesis is that nutrient regimes can substantially determine the relationship between growth rate and consumption by i) influencing herbivore consumption rates based to food quality (compensatory feeding) and / or ii) modifying macroalgal growth based on the nutrient availability. Given these mechanisms, we expect that macroalgal communities in low nutrient regimes will experience sudden shifts to algal barrens at lower urchin biomasses compared to communities in high nutrient regimes. This study provides insights of how immanent conditions can influence the location of critical thresholds in the capacity of temperate macroalgal systems to buffer grazing pressure.

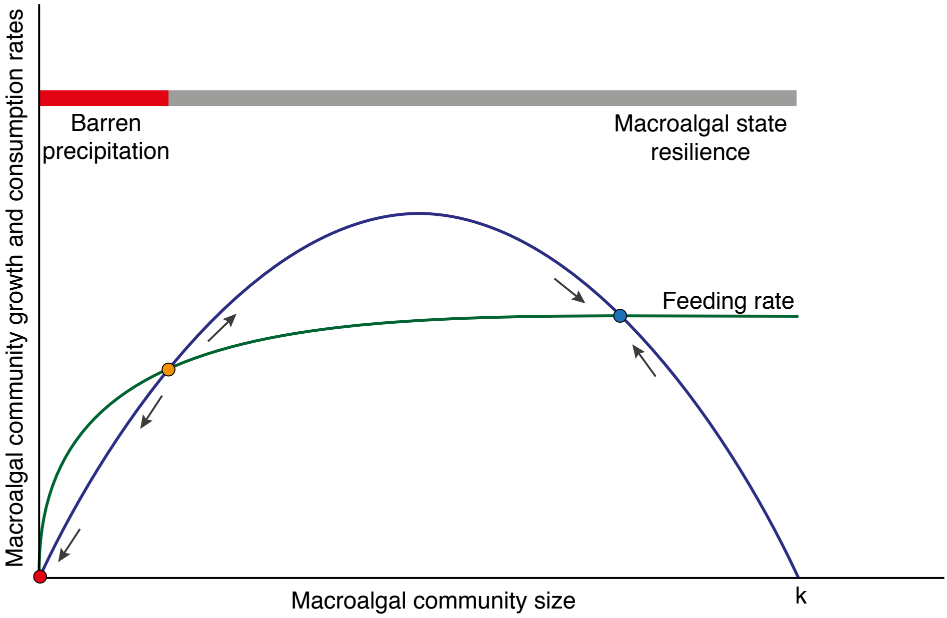


Figure 3.1. Consider a macroalgal community limited in growth by the space available to a carrying capacity *k*, which grows following the solid blue line in the absence of herbivores. A population of herbivores consumes this macroalgae community at a rate represented by the solid green line (intermediate pressure, May 1977). Under this consumption curve, two stable states can exist; a barren state (red point) and a well-structured macroalgal state (blue point). One unstable state exists (orange point) in which situations on the left precipitate barren formation (consumption > growth) and situations on the right enhance the macroalgal community stability (consumption < growth). Thus, the distance between the unstable point and red points represents the barren precipitation state and the distance between the unstable point and the carrying capacity k represents the macroalgal state resilience.

Materials and Methods

Study System and main objectives

Shallow rocky shores of the Mediterranean are dominated by several species of erect algae that constitute a very structured and diverse community (Sales & Ballesteros 2009; Sala et al. 2012). These communities grow under the oligotrophic conditions characteristic of the Mediterranean basin. However, there is considerable variation in nutrient conditions within the basin: the Western Mediterranean continental shores typically have relatively high nutrient inputs (i.e. riverine), while the Eastern Mediterranean and the Islands are comparatively nutrient poor (Bosc et al. 2004). The sea urchin *Paracentrotus lividus* is the principal herbivore in this system, known to precipitate regime shifts when abundances increase (Pinnegar et al. 2000; Boudouresque & Verlaque 2001). Unable to sustain urchin grazing pressure, macroalgal systems collapse, giving way to rocky barrens, characterised by vast stretches of bare rock covered with coralline algae and thin turfs. This typically occurs when urchin populations are released from predation pressure, often associated with overfishing of their dominant fish predators (Pinnegar *et al.* 2000; Boada *et al.* 2015a). Less commonly, banner years of sea urchin recruitment have also been associated with overgrazing events (Cardona et al. 2013). Once the barren is created, several positive feedbacks maintain the system in this state. These include enhanced post-settlement survival of sea urchins, reduced potential for algal recruitment, reductions in the recruitment of predatory fish, and the facilitation of new herbivore species (i.e. *Arbacia lixula*) (Bulleri et al. 1999; Guidetti et al. 2003; Bulleri 2013; Cheminee et al. 2013). In this study we assessed (1) the role of nutrients in mediating critical transitions (thresholds) in macrophyte communities and (2) determined the mechanisms underlying these transitions in order to explain differences in the resilience of the macroalgal system subjected to contrasting nutrient regimes. The first objective was assessed indirectly using correlative data on barren extensions with herbivore pressure, taken at regions with different nutrient status. Regarding the second objective, we assessed two hypotheses (i) resilience in nutrient-poor areas is lower due to compensatory feeding by herbivores or (ii) resilience in nutrient-poor areas is lower given a lower algal growth. We examined both hypothesis experimentally by measuring feeding rates and plant growth rates under contrasting nutrient conditions.

1) Determining thresholds under different nutrient regimes

To determine if nutrient regimes could influence ecosystem thresholds we surveyed shallow macroalgal rocky communities in two regions within the Mediterranean Sea characterised by significantly different nutrient conditions (i.e. Catalan coast in Spain and Sardinia Island in Italy respectively; Bosc et al. 2004). To further confirm the contrasting nutrient regimes in both regions, we measured nutrient content from Posidonia oceanica seagrass shoots ($n = 5$) in several localities at the two regions (see below). *P. oceanica* seagrass shoot nutrient content has extensively been used as an indicator of water quality within the European Water Framework Directive (Roca et al. 2015). Our results corroborate the differences in nutrient availability between the selected regions (n = 20 in the Catalan coast and n = 9 in Sardinia, Fig. S1, p-value < 0.03). In contrast, the two selected regions in the North Western Mediterranean have similar values regarding seawater temperature and salinity. The algal communities found in these locations are both dominated by photophilic canopy-forming algae of the genus *Dictyota, Halopteris* and *Padina* among others. To assess the thresholds of urchin biomasses that these systems could sustain, we selected a total of four different localities (10s of km apart) in each region (i.e. l'Ametlla de Mar 40.9ºN 0.8ºE, Blanes 41.7ºN 2.8ºE, Montgó 42.1ºN 3.2ºE and Llançà 42.4ºN 3.1ºE in the Catalan coast and Costa Paradiso 41.0ºN 9.0ºE, Torre Porticciolo 40.6ºN 8.2ºE, Santa Caterina 40.1ºN 8.5ºE and Su Palosu 40.0ºN 8.4ºE in Sardinia Island), characterised by high abundances of sea urchins. At each locality, we visually sampled the subtidal benthic substrate (1-7m depth) using 50x50cm quadrats at different sea urchin densities (n = 237 for the high nutrient region and n = 185 for the low nutrient region). Within these quadrats we estimated total algal cover (percentage capped at 100% of turf and canopy forming species) as a measure of community state. To assess sea urchin grazing pressure, we counted the number of sea urchins (*P. lividus* and *A. lixula*) within each quadrat, classifying them into age classes based on size (Post-settlers, <1cm Test Diameter, TD; Juveniles, 1-3cm TD; Young adults, 3-5cm TD and Adults, >5cm TD). Size class information was used to calculate sea urchin biomass (wet weight, $g{\cdot}m^2$) based on standard volumetric conversions for these species (Ballesteros 1981). However, only young adults and adults were used to asses thresholds as sea urchins smaller than 3cm are known to make negligible grazing effect. We used total sea urchin biomass as a simple comparative metric of grazing pressure between the two regions since it integrates sea urchin size and accounts for herbivory by both species. However, the principal species across all the localities was *P. lividus*, accounting for 60 – 80 % of the total biomass. We identified system thresholds by analysing the differential levels of variance (abrupt changes in algal community state) for a particular range of sea urchin biomass for both the forward and backward transitions (Dakos et al. 2015). Additionally, we computed a more accurate analysis to identify significant thresholds, Chow Test statistic, in both nutrient regions. Unfortunately, it was impossible to use this method to identify the backward process (macroalgal community recovery) and was only used to assess the forward threshold (barren precipitation). Since this statistic requires data sets with one cover value per sea urchin biomass we used mean values of percentage cover. The same analysis was also performed using maximum values of percentage cover to further confirm the results. The package strucchange (Zeileis et al. 2013) was used to determine the position of the forward threshold using the statistical software (R Development Core Team 2013), as used in (Gera et al. 2014).

2) Mechanisms underlying the thresholds

We used controlled experiments to explore potential mechanisms (compensatory feeding behaviour and enhanced growth) that could help explain how nutrient conditions mediate grazing thresholds by sea urchins.

i) Compensatory feeding. To test the effect of nutrients on grazing activity we used laboratory-based experiments to measure if macroalgae nutrient content affected the per capita herbivory rates of sea urchins. We used the Mediterranean endemic seaweed *Cystoseria mediterranea* to feed *Paracentrotus lividus* sea urchins. This macroalgal species is one preferred by *P. lividus* and a typical structural Fucales species characteristic of well-developed Mediterranean algal forests (Ballesteros 1988). Sea urchins and macroalgae were both collected in the same area (Sant Francesc cove, 41.7ºN 2.8ºE). Half of the macroalgae was fertilised (F) in aquaria (~10 L) with running seawater for 3 days using 6g of fertiliser (12% N, 8% P and 16% K) while the other non-fertilised half (NF) was kept in natural sea water aquaria. To assess food (algal) quality, we measured leaf nitrogen (%N) from algal fronds from each treatment (F and NF). We used 5 replicates for each treatment. Fronds were first ground with a mill and then the resulting powder encapsulated and sent to Unidade de Técnicas Instrumentais de Analise (Universidade de Coruña) where nitrogen concentration was measured using an Elemental Analyser EA1108 (Carlo Erba Instruments). Fertilisation was successful according to the elemental analysis performed (see Figure S2, p-value < 0.01). All collected sea urchins were transported to the ZAE-ICM (Institut de Ciències del Mar – CSIC) in aerated tanks (1-2 hours) and then starved for 3 days in a big holding aquarium (~1000 L). Afterwards, sea urchins were transferred to 6 independent experimental aquaria for testing. Each aquarium was divided in 6 compartments. Five out of six compartments fit one single sea urchin, and the 6th compartment was left without sea urchin (total number of sea urchins tested 5x6, n = 30). We fed half of the sea urchins with 4g (wet weight) of the control non-fertilized (NF) *C. mediterranea* (n = 15 sea urchins) while the other half were fed with 4g of the fertilized (F) *C. mediterranea* (n = 15 sea urchins). For each aquaria, the compartment left without sea urchins, served as a control to determine the amount of *C. mediterranea* (F and NF) lost due to factors other than feeding rates. The experiment lasted 6 days after which we weighted the *C. mediterranea* (wet weight) that remained in each of the compartments from each aquarium. We estimated algal consumption by sea urchins, subtracting the final from the initial algal weight and dividing by the number of days elapsed (6 days). No significant change in fertilised or unfertilised algal wet weight was detected in control compartments (without sea urchins). One-way ANOVA was used to test differences in consumption rates using the R software (R Development Core Team 2013). Prior to the analyses ANOVA assumptions of normality and homogeneity of variances were checked both visually and statistically (i.e. Shapiro test and Bartlett test respectively).

ii) *Algal growth.* To measure how nutrients influenced algal growth in field conditions, we identified two completely bare rock areas, one in the high nutrient region and the other in the low nutrient region. In each area, three herbivore exclusion cages of 50x50cm (2cm mesh size) were placed on the bare rock (completely overgrazed barren areas, 0% algal cover) in each region. We measured changes in total macroalgal cover (of both erect and turf algae) after one month inside each plot in both high and low nutrient conditions. This was used as an indirect field measure of algal growth in the absence of sea urchins. We measured algal cover using the same methods employed in our field surveys (see above). One-way ANOVA was used to test differences in macroalgal growth using the R software (R Development Core Team 2013). Prior to the analyses ANOVA assumptions of normality and homogeneity of variances were checked both visually and statistically (i.e. Shapiro test and Bartlett test respectively).

RESULTS

DIFFERENT NUTRIENT CONDITIONS RESULT IN DIFFERENT THRESHOLDS

Clear evidence of alternative states was found in both high and low nutrient regions (Fig. 3. 2). A sudden change in the community state (percent cover) occurred when the stressor (sea urchin biomass) crossed critical values (tipping point). In high nutrient conditions, our results show that this tipping point was reached at sea urchin biomasses more than twice (Fig. 3.2a) that of low-nutrient conditions could sustain (Fig. 3.2b). The threshold analysis (Chow test) confirmed the existence of regime shifts in both systems; according to nutrient conditions, thresholds were found at significantly different levels (confidence intervals did not overlap) of the stressor (sea urchin biomass, Fig. 3.2c and 2d). Macroalgal rocky communities in low nutrient conditions shifted abruptly to urchin barrens when stressor values went beyond 736g/m^2 sea urchin biomass (~20 sea urchins/m^2 of 5 cm TD, 380 – 1250g/m^2, 95% confidence intervals, Fig. 3.2d and 2f). In contrast, in high nutrient conditions canopy-forming algae were still present at stressor values of around 1832g/m^2 sea urchin biomass (~40 sea urchins/m^2, 1484 – 2494g/m^2, 95% confidence intervals, Fig. 3.2c and 2e); beyond this level however, these systems also showed dramatic shifts to urchin-dominated barrens. We identified three peaks in the variance of the community state depending on the stressor levels in both regions indicating the proximities of thresholds (Fig. S3.1). Two of them were found in preceding the forward thresholds determined by the more precise Chow test (see above), and the other, precede the backward process, related to the recovery of the macroalgal community at low urchin densities. Unfortunately, it was not possible to assess the backward process using the more trustable Chow Test statistics because the recovery of the macroalgal state occurs at very low sea urchin biomass (close to the complete removal of urchins). According to the analysis of the variance, this backward process was almost identical independently of nutrient condition, and indicated a switch back to erect macroalgae when urchins are nearly absent (275 g/m^2 see Fig. S3.2).

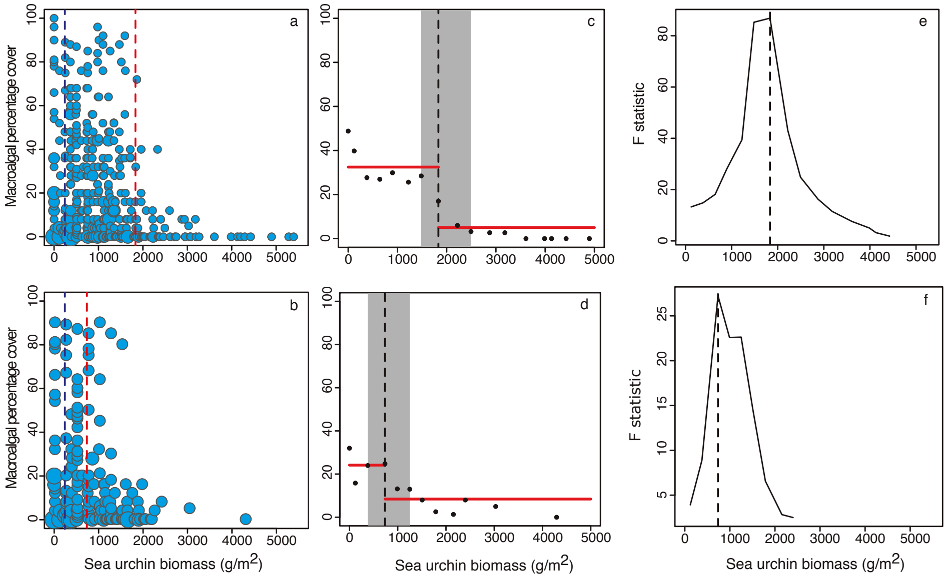


FIGURE 3.2. Raw data in a bubble plot, mean values for combinations o levels of stressor (sea urchin biomass in g/m^2 of wet weight to macroalgal percentage cover) and results for threshold analyses (Chow test) are presented for the two regions a) Catalan coast and b) Sardinia Island by using mean of cover (%) data for each sea urchin density. The size of the bubbles is proportionally related to the number of times a specific combination of sea urchin biomass to macroalgal cover was recorded. The dashed lines indicate the position of the forward (red line) and backward (blue line) thresholds.

MECHANISMS UNDERLYING THE THRESHOLDS

Our controlled experiments showed that sea urchins displayed compensatory feeding when offered non-fertilised algae (algae with low nutrient levels) and lower algal growth rates were found in the low nutrient region. Both these mechanisms likely work together to make macroalgal systems in low-nutrient states shift to urchin barrens at much lower levels of the stressor (herbivore biomass) compared with communities in high nutrient conditions (Fig. 3. 3.). In the consumption experiment we found that sea urchin grazing rates were 25% higher when offered non-fertilized *C. mediterranea* than fertilized algae (Fig. 3. 3a, p-value < 0.04). These results confirm that sea urchins resort to compensatory

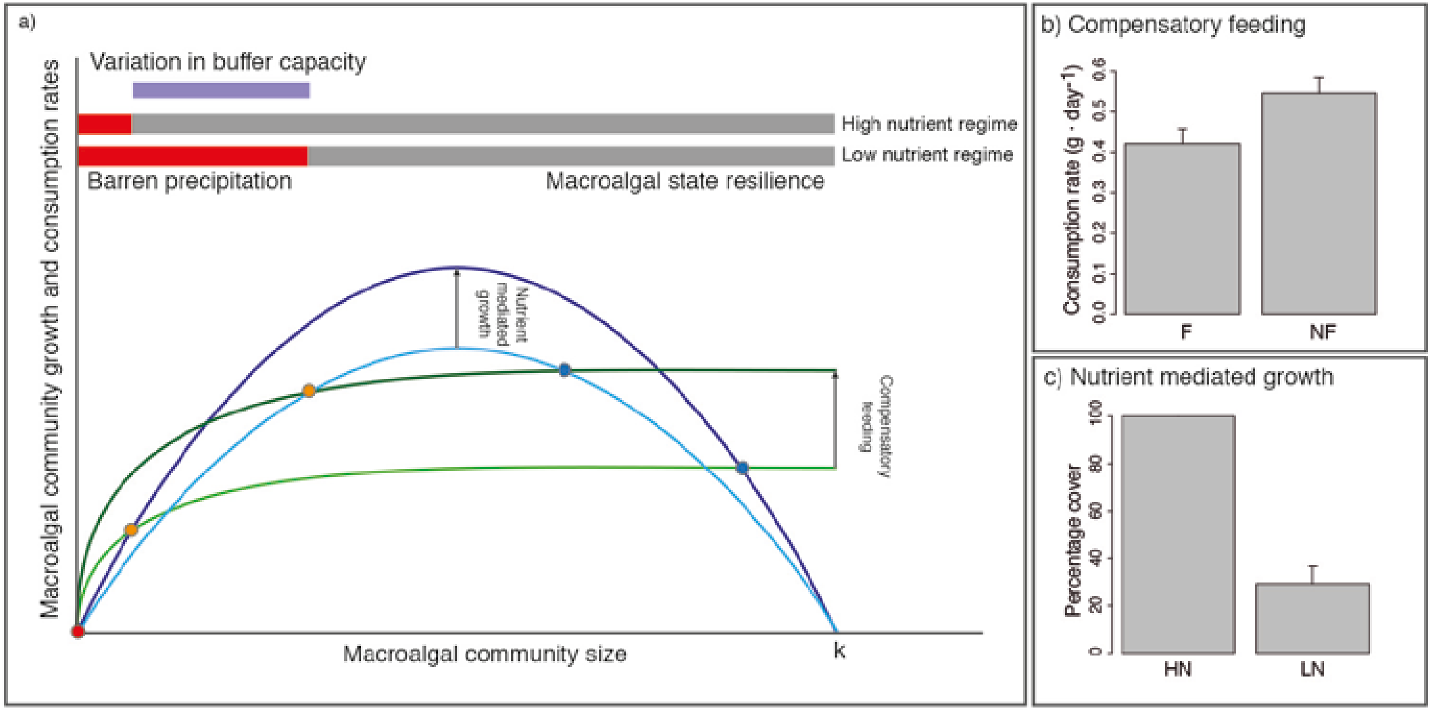


Figure 3.3. Conceptual diagram describing the two evaluated mechanisms; the compensatory feeding and nutrient mediated growth of the macroalgal community and sea urchin population described in Figure 1. Under low nutrient regimes sea urchins compensate their feeding increasing consumption rates a) (mean consumption + SE on fertilized, F and non-fertilized, NF Cystoseira mediterranea in laboratory experiments after 6 days). At the same time under low nutrient regimes the macroalgal community presents retarded growth rates b) (mean percentage cover + SE) of erect and turf algae in the high nutrient and low nutrient regions after one month of herbivore exclusion).

feeding when nutrient availability is low. In addition, in high nutrient conditions, algal cover completely recovered after one month of herbivore exclusion (100% cover) while in low nutrient conditions only ca. 30% of the substrate was recolonized the same period (Fig. 3.3b, Table 3.1, p-value < 0.01).

DISCUSSION

While the non-linear dynamics of temperate macrophtye communities have long been recognized, identifying where these thresholds lie has resisted prediction. Our results indicate that nutrient regimes can be strong drivers of threshold effects, mediating the buffer capacity of macroalgal communities to sea urchin grazing pressure. Specifically, we observed that regions characterised by oligotrophic conditions are much less resilient to grazing compared to regions richer in nutrients (Fig. 3.4). We documented a clear shift to algal barrens at around half the sea urchin biomass in oligotrophic systems (relative to less nutrient-depleted areas), indicating that they may be intrinsically less able to cope with grazing. However, the barren states in both nutrient regimes were remarkably stable, and our analyses indicate that it would require an almost-complete disappearance of sea urchins before these system recover their macroalgal state. Our experiments help unpack the mechanisms underlying these shifts, and indicate that both compensatory sea urchin grazing and reduced algal growth rates act together in oligotrophic systems, endowing them with roughly half the buffer capacity of more nutrient-rich macroalgal systems (Fig. 3.3 and Fig. 3.4). Understanding and quantifying how context-specific conditions can influence threshold dynamics will take us one step closer towards reducing the inevitable surprise of non-linear ecological systems.

Table 3.1. Analyses of variance (one way ANOVA) for the studied mechanisms of compensatory feeding and nutrient enhanced growth of algae between regions.

Mechanism	Df	F value	p-value
Consumption rate	1	90.61	**0.036**
Algal growth	1	4.84	**< 0.01**

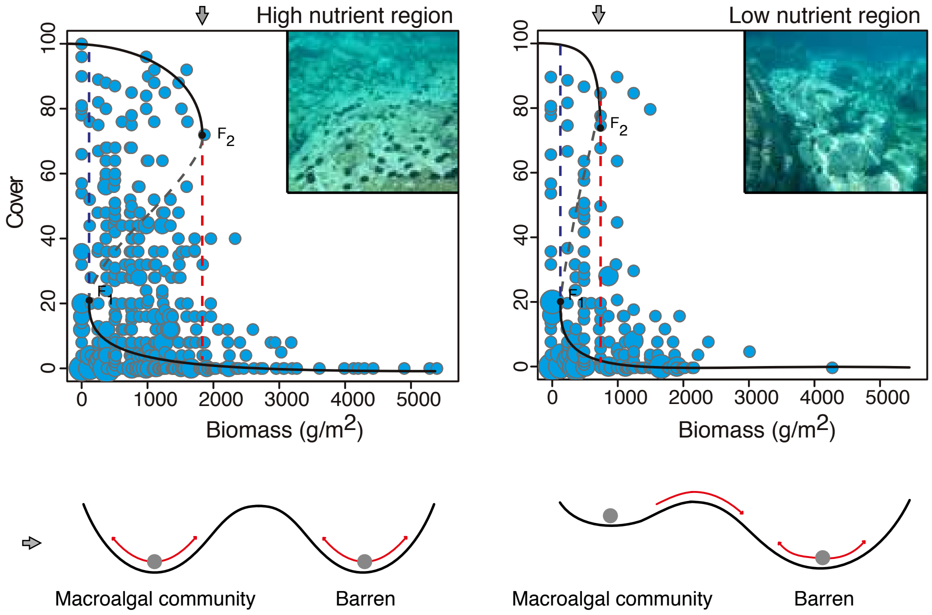


Figure 3.4. Schematic description of the buffer capacity of both the macroalgal community and the barren in each nutrient regime. The bubble graph shows the obtained raw data on macroalgal percentage cover related to sea urchin biomass in g/m^2 of wet weight in the two regions a) Catalan Coast (NE Spain)-high nutrient region and b) Sardinia Island-the low nutrient region (n = 221 and n = 258 respectively). The size of bubbles is in proportion to the number of times the same combination of macroalgal cover to sea urchin biomass was counted. The dashed red line represents the forward threshold after a tipping point F2 is reached and the dashed blue line represents the backward recovery of the macroalgal state when the tipping point F1 is reached (both are set in the position determined by the threshold analyses, see methods). The supporting photos show differences in sea urchin numbers in barren formations between the two studied regions. The conceptual schematic draw below the graph shows the resilience of each stable state in both nutrient regimes. The valleys represent the alternative stable states and the depth of the valley represents the resilience of that particular state.

Temperate macrophyte communities appear particularly prone to catastrophic shifts (Fig. 3.4, Pinnegar et al. 2000; Filbee-Dexter & Scheibling 2014), with sea urchin overgrazing (linked to population outbreaks) often being the primary trigger of these events. While it is uncertain from our work what drives differences in urchin populations themselves, a range of studies have highlighted the importance of both supply-side processes linked to recruitment and settlement (Prado et al. 2012) as well as subsequent top-down control by fish predators (Sala 1997; Pinnegar et al. 2000; Guidetti 2004; 2006) in driving urchin population dynamics. Temperate macroagal systems show catastrophic state-shifting behaviour independently of local and regional conditions. We documented dramatic shifts to algal barrens in both nutrient regimes, linked clearly to increases in sea urchin abundance. The difference between these regions was in where these thresholds lay, with relatively nutrient rich systems being more than twice as resilient to urchin grazing compared to nutrient-poor systems. Given their apparent susceptibility to discontinuities, determining these boundary conditions is all the more important if we have to manage temperate macrophyte communities as functionally healthy systems. This becomes particularly important because our results suggest that there is strong evidence of hysteretic behaviour, a key indicator of the resilience of the community states (Filbee-Dexter & Scheibling 2014; Ling et al. 2015). While low nutrient conditions make macrophyte systems much more prone to barrens, the recovery from the barren state appears not to be dependent on nutrient regime (Fig. S3.2 and Fig. 3.4). While the backward process needs to be interpreted with some caution, it indicates that erect algae are likely to recover only when sea urchin abundances reach close to zero, regardless of nutrient conditions. Once the barrens are created, the urchin population generally does not collapse although individual growth rates may decrease as urchins are forced to switch their feeding to less nutritious encrusting algae (Ling et al. 2015). In macroalgal-barren systems, sea urchins continue scraping the substrate, maintaining areas free of algae extensions. In the Mediterranean, barren maintenance is apparently more efficient when *A. lixula* is abundant (Bulleri 2013). Other barren-associated biota, particularly limpets, also help maintaining the barren state and it has been recently reported that they play significant roles in enhancing the stability of rocky barrens (Piazzi *et al.* 2016). Such positive feedbacks make barren systems particularly difficult to recover and emphasize the need to prevent collapses from occurring, since recovering a macroalgal state from these barrens may require an almost complete elimination of sea urchins from the area (Pinnegar et al. 2000; Ling et al. 2015). Accurately being able to predict the forward threshold is essential if preventative action has to be effective.

Whatever drives variations in urchin populations, our work indicates that the inherent nutrient regimes of each region can strongly influence the functional consequences their populations can have on macroalgal habitats. Nutrient regimes appear to be a critical driver of buffer capacity at least for Mediterranean macrophytes. Under nutrient-poor conditions, habitats were much more prone to ecosystem shifts; very oligotrophic systems may thus be much more vulnerable to population outbreaks of herbivores precipitated either by overfishing of their predators or by stochastic recruitment pulses. In fact, Cardona and others in (2013) described how, after a pulse in productivity in a low nutrient region, the abundance of sea urchins increased, producing a dramatic reduction of the macroalgal community and an increase in barren-associated coralline biota. Similarly, in the eastern Mediterranean, with significantly lower nutrient conditions compared to the western basin, the arrival of the voracious fish herbivores *Siganus* spp through the Suez Canal has led to an extreme depletion of canopy-forming algae species and, where well-developed macrophyte communities were once dominant, now bare rock systems are prevalent (Sala et al. 2012; Vergés et al. 2014). Acknowledging this differential vulnerability may require designing context-specific strategies for managing these systems based on measurable differences in their inherent buffer capacity.

Our work also explores the potential mechanisms that can explain the differential resilience of these ecosystems. Under relatively nutrient-poor conditions, macroalgae showed clearly reduced rates of growth and urchins offset the low quality of plant tissue by increasing their feeding rates to meet their nutritional requirements. Both these mechanisms act synergistically enhancing their combined effects in maintaining the barren states even stronger (Pedersen & Borum 1996; Valentine & Heck 2001). The underlying nutrient regime determines the degree to which macroalgal growth can support urchin consumption before the system collapses completely. As we demonstrate, low nutrient regimes increased rates of consumption by herbivores (compensatory feeding, Fig. 3.3a) while simultaneously reducing the growth capacity of macroalgae (Fig. 3.3b). This maximized grazing:primary production ratio caused a faster shift to a new macroalgae-free urchin barren (Fig. 3.3) and increased the stability of the subsequent new state (Fig. 3. 4). These overshoots are much more likely to occur in the characteristic nutrient-poor conditions of islands making them much less resilient to urchin herbivory compared to other nutrient-rich systems. While our work explored how nutrient regimes mediate the location of these thresholds, under extremes of high nutrient conditions the situation may change completely. Under these scenarios, frequent in most seas but relatively rare in the Mediterranean, macroalgal community composition may revert to fast-growing species making the system much more vulnerable to collapse through a completely different suite of mechanisms including shading and overgrowth. Based on our current results however, we suspect that oligotrophic systems may follow inherently different trajectories than non-oligotrophic systems, and need to be addressed separately.

A suite of recent studies has focused on determining potential signals of impending collapse in non-linear systems (Scheffer & Carpenter 2003; Scheffer et al. 2009; Dakos et al. 2015). These studies have identified potentially useful proxies (critical slowing down, increasing variance and skewness, etc.) that may herald an approaching ecosystem threshold. Identifying these signals depends heavily on reliable long-term monitoring, as well as an adequate demonstration that these signals correlate with hysteretic change. More importantly, there have been few (if any) real-world examples where these leading indicators have been able to adequately predict imminent collapse in time for ameliorative action, making them of little practical utility. One of the difficulties with these approaches is that, being essentially phenomenological, they are not based on clearly understood mechanisms of stability and collapse. Our work indicates that, where it is possible to unpack these underlying drivers, it can help substantially in identifying where and why state-changing thresholds occur. In addition, clarifying the mechanisms that govern these dynamics allows us to determine the critical role that inherent conditions can play in mediating these thresholds. Taken together, it provides us a way forward to make regime-specific predictions on the buffer capacity of systems at local to regional scales. These relationships are admittedly much more difficult to establish in more complex systems where multiple mechanisms may act in several synergistic and antagonistic ways. However, our contention is that, even in these more complex ecosystems, inherent conditions may predispose the system to very different behaviours, implying very different ecosystem responses. It is critical to shift attention to a more mechanistic understanding of the ecological feedbacks that govern these non-linear systems. Determining how these feedbacks interact with context-specific conditions will help considerably improve the predictive power of resilience models, reducing the surprise in identifying thresholds and improving our ability to manage systems characterised by intrinsically non-linear behaviours.

ACKNOWLEDGMENTS

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4

EVALUATING POTENTIAL ARTIFACTS OF TETHERING TECHNIQUES TO ESTIMATE PREDATION ON SEA URCHINS

ABSTRACT

Measuring the strength of trophic interactions in marine systems has been central to our understanding of community structuring. Sea urchin tethering has been the method of choice to evaluate rates of predation in marine benthic ecosystems. As standardly practiced, this method involves piercing the urchin test, potentially introducing significant methodological artifacts that may influence survival or detection by predators. Here we assess possible artifacts of tethering comparing invasive (pierced) and non-invasive tethering techniques using the sea urchin *Paracentrotus lividus*. Specifically we looked at how degree of confinement and high water temperature (first order artifacts), and predator guild and size of the prey (second order artifacts) affect the survival and/or detectability of pierced urchins. Our results show that first order artifacts only arise when pierced sea urchins are placed in sheltered bays with confined waters, especially when water temperature reaches extremely high levels. Prey detectability did not increase in pierced sea urchins for the most common predators. Also, test piercing did not alter the preferences of predators for given prey sizes. We conclude that the standard tethering technique is a robust method to test relative rates of sea urchin predation. However, local conditions could increase mortality of the tethered urchin in sheltered bays or in very high temperature regimes. Under these conditions adequate pierced controls (within predator exclusions) need to be included in assays to evaluate artifactual sources of mortality.

INTRODUCTION

Measuring the strength of trophic interactions has been central to our understanding of community structure (Paine 1966; Estes & Palmisano 1974). Estimating predation and its effects is critical to understand the ability of predators to control prey populations (Estes *et al.* 2011). This is especially important in marine systems, where such control often trigger cascading effects. While directly measuring rates of predation in real world ecosystems is generally unfeasible, researchers have developed assay techniques to obtain relative estimates that can integrate longer periods of time and avoid observer artifacts (Hairston 1989). This has been done with the assumption that these techniques can serve, at the very least, as relative indices of actual predation rates that can still give valuable ways to compare ecosystems or track changes through time. In marine systems, measures of predation have relied heavily on tethering techniques, often using sea urchins as a model prey (McClanahan & Muthiga 1989). In addition, sea urchins are often themselves keystone herbivores in rocky reefs, coral reefs, seagrass meadows and kelp forests. When sea urchin outbreaks take place, these communities can shift to a less productive and diverse state –termed "urchin barrens" (Pinnegar *et al.* 2000). In this context, estimating the ability of predators to control urchin numbers is critical to understand ecosystem functioning (Heck & Wilson 1987; McClanahan & Muthiga 1989; Heck & Valentine 1995; McClanahan 1999; Shears & Babcock 2002; Pederson & Johnson 2006; Clemente *et al.* 2007; Farina *et al.* 2014). Tethering experiments can provide insight on the degree to which differences in predation rates between different localities contribute to barren formation through cascading effects (Clemente *et al.* 2008). Nevertheless, these assays are artificial by design and invasive in their manipulation. It has, thus far, been difficult to assess how prone they are to methodological artifacts, precluding thus the evaluation of their reliability

Tethering techniques have been extensively used in experimental ecology as a tagging and constraining technique to assess predation for different species in various ecosystems and conditions (Watanabe 1984; Shulman 1985; Witman 1985; Herrnkind & Butler 1986; Aronson 1987; Wilson *et al.* 1990). This method consists of marking and restraining target prey for a known period of time in natural conditions and documenting mortality. While it is commonly used in invertebrates, it has some disadvantages (Peterson & Black 1994; Aronson & Heck 1995). Individuals can be tagged by using different tethering techniques depending on the targeted prey and some methods that clearly restrain the movement of the tethered individual can substantially increase the encounter rate by certain predators (Barbeau and Scheibling 1994). The most effective and commonly used tethering methods involve piercing the target organism with a hypodermic needle. For instance, with sea urchins this involves piercing the test from the oral to the aboral region, and passing a monofilament line through the skeleton, which is then used as a tether (Ebert 1965). Although sufficient care is taken not to affect the gonads inside the carcass, this procedure is still invasive, and has a number of potential associated artifacts, which Peterson and Black (1994) have classified as first and second order artifacts. First order artifacts can arise if the wound caused by piercing increases the probability of infections under different environmental conditions; increased temperatures, pollution or nutrient levels, wave flushing and other local factors could interact strongly to influence the disease susceptibility and survival of sea urchins (Lafferty *et al.* 2004; Girard *et al.* 2012), and likely also that of pierced organisms. In addition, second order artifacts could result from the leaking of coelomic fluids into to the water column. These fluids could potentially act as chemical clues for certain benthic predators (Valentinčič 1973; Sloan & Northway 1982) increasing prey detectability, but not for others that base their predation on a more visual search. These biases can clearly affect the comparative estimates of predation when predator guild differs between sites. Despite these limitations, pierced tethering continues to be the most commonly used method to estimate comparative predation rates or predation risk in marine systems (Aronson & Heck 1995). To reduce possible artifacts some authors held tethered urchins in the laboratory for a period of time to allow urchins to heal as monitoring mortality revealed that field survival rates of tethered urchins were higher if they were maintained some days under laboratory conditions prior to using them in field experiments (Shears & Babcock 2002; Fagerli *et al.* 2014), but often this is unfeasible when using this field assay far from laboratories. Still, there have been a few attempts, although incomplete, to evaluate the possibility, magnitude, and sources of biases appearing as a result of first and second order artifacts due to this experimental manipulation (McClanahan & Muthiga 1989; Shears & Babcock 2002).

In this study we investigate possible artifacts of tethering techniques, using the sea urchin *Paracentrotus lividus* (Lamarck, 1816), a keystone herbivore in Mediterranean ecosystems. Pierced tethering has been employed extensively in this species, and has been used to examine the importance of predation on *P. lividus* (Sala & Zabala 1996; Guidetti & Sala 2007), the importance of habitat-engineering species in providing refuge from predation (Farina *et al.* 2009), and the existence of indirect interactions between herbivores and predators in seagrass systems (Pagès *et al.* 2012), among others. In this work we analyze: first, whether test piercing affects prey survival under different environmental conditions (first order artifacts), and second, whether this tagging technique enhances prey detectability under different sizes of the prey or for the most common predators (second order artifacts).

MATERIALS AND METHODS

SAMPLING DESIGN

We designed a series of four separate experiments to test if the pierced tethering method applied to the sea urchin *Paracentrotus lividus* modify mortality rates and prey detectability. For first order artifacts, we conducted two experiments using predator exclusion cages to test the effect of a) degree of confinement (Fig. 4.1, A) and b) water temperature as factors increasing sea urchin mortality after piercing (Fig. 4.1, B). For the second order artifacts we conducted two experiments, c) one to test the effect of pierced tethering on observed predation success for different prey sizes (Fig. 4.1, C) and the second to test d) the effect of pierced tethering in modifying prey detectability as a function of the predator guild (fish, gastropods and sea stars, Fig.4.1, D).

For all experiments, pierced urchins (P) where threaded according to the common methodology described for the target sea urchin species (Sala & Zabala 1996). Unpierced urchins (UP) were used for the first order artifacts as controls. For the second order artifacts, unpierced urchins (UP) were restrained with a line directly wrapped around the sea urchin body twice and then tied to a weight or to experimental cages. This tagging method is useful to tether sea urchins for short periods of time and keep them within the experimental area, but is not useful for longer experiments as they manage to escape. All the experiments were conducted under field conditions rather than in the laboratory, since tethering methods are only relevant for in situ experiments and measures of predation rates.

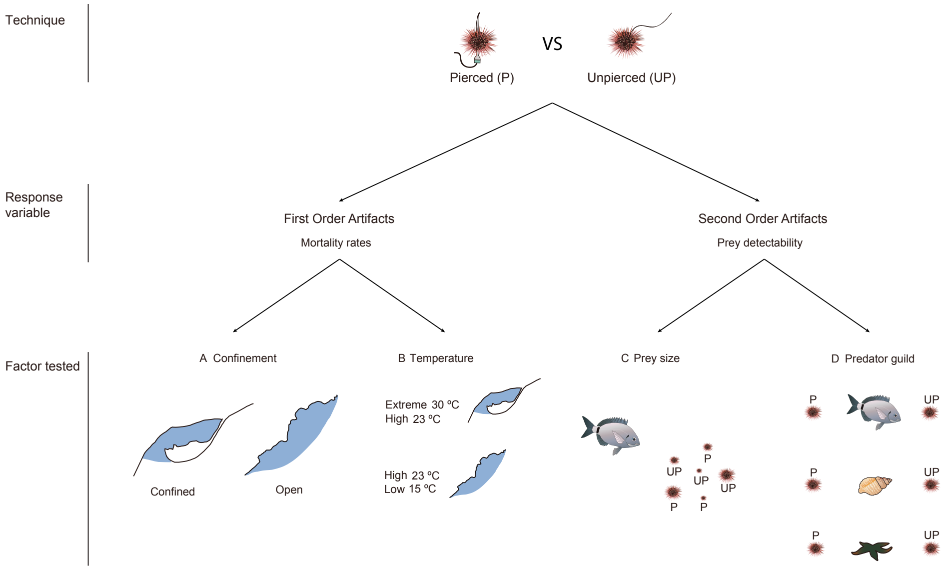

Figure 4.1. Schematic description of the methodology used and the experiments developed to test tethering artifacts. Experiments are classified according to the explored variable A) confinement degree (under fixed temperature conditions; 23ºC), B) seawater temperature (for confined and opened conditions) for which we analyzed the prey mortality and C) prey size and d) predator guild type for which we analyzed prey detectability. In section A) and B) we present a drawing of the study sites; coastline (black line) and the water (shadow area) to show differences in the confinement degree of each site.

First Order Artifacts/direct sea urchin mortality: confinement and water temperature

Confinement effect

We chose a site representative of an open Mediterranean coast (Fenals, 41° 41′ 23″ N, 02° 49′ 42″ E, total surface ca. 92ha, aperture distance ca. 2500m, maximum summer temperature ~23ºC) and an area with limited exchange with the open sea (Alfacs Bay, 40° 36′ 38″ N, 00° 39′ 37″ E, total surface ca. 3000ha, aperture distance ca. 2500m, maximum summer temperature ~30ºC) to assess the confinement effect (Fig. 4.1, A). Mortality was measured for 48 pierced urchins, 32 of them were placed in Alfacs Bay and the other 16 in Fenals under the same temperature conditions (23º C). Thirty-two unpierced urchins were used as controls, 16 in each site. All pierced and unpierced sea urchins were placed in groups of 4 in 1.5 cm mesh exclusion cages (50cm x 20cm x 20cm) at 1m depth in Alfacs Bay and at 8m depth in Fenals and tracked for 12 days. We test for the significance of differences in sea urchin mortality between treatments (pierced and unpierced) in the two sites using one-way ANOVA in the statistical software R (R Development Core Team, 2013).

Water temperature effect

For the temperature experiment (Fig. 4.1, B) we compared pierced sea urchins with unpierced ones (controls) during high temperature conditions in summer (23ºC) and during low temperature conditions in spring (15ºC) in Fenals. We also checked the effect of extreme high conditions of temperature that occur only in very confined areas in the Mediterranean. To do this we compared mortality of pierced and unpierced urchins in extreme high temperatures in summer (30ºC) and high temperatures in spring (23ºC) in Alfacs Bay. A set of 16 sea urchins was pierced using a 0.8mm needle and 16 unpierced sea urchins were used as controls in each site and temperature condition. Urchins were placed in 1.5 cm mesh exclusion cages in groups of 4 (50cm x 20cm x 20cm) at 1 m depths in Alfacs Bay and at 8 m depths in Fenals and tracked for 12 days. We used one-way ANOVA to test for differences in mortality between treatments (pierced and unpierced) in the two sites and temperature conditions.

Second Order Artifacts/prey detectability: prey size and predatory guild

Prey size effect

To test if the treatment (pierced vs unpierced) influenced predation success by fish (Sala et al. 1996) depending on prey size (small; 1-3 cm diameter without spines, TD, medium; 3-5 cm TD and large individuals; >5 cm TD) (Fig. 4.1) we performed an experiment in Medes Islands MPA (42° 02′ 47″ N, 03° 13′ 11″ E) where predation impacts on sea urchin populations is known to be very high (Hereu *et al.* 2005). The experiment was conducted during daylight hours because nocturnally active urchin-feeding fishes are uncommon (Savy 1987; Sala 1997). A total of 90 sea urchins were used for this experiment, 30 small, 30 medium and 30 large. Test diameters were measured with a caliper to determine size classes. Pierced and unpierced urchins were tethered to a 1kg weight and randomly distributed on a macroalgal habitat (5m depth) within a total rocky area of around 330 m^2 to avoid transmission of the chemical clues due to currents or waves action. The principal predator of this urchin is *Diplodus sargus* (Sala *et al.* 1996); large individuals of this species can consume the entire range of *P. lividus* sizes while smaller fishes are potential consumers of only juvenile sea urchins (Sala 1997). The experiment was repeated on 3 different days; on each day a total of 5 individuals of each size class were pierced (P) and other 5 unpierced (UP) were used as controls. Three experienced divers were responsible for visual observations to track the experiment from a certain distance to avoid biasing the information. Each experiment was terminated when 50% of the total initial urchins (P + UP) had been eaten by fish (average time around 45 minutes), and the percentage of both P and UP eaten in each size class were recorded. Generalized Linear Models (GLMs) with binomial distributions were used to evaluate predation impact. The state of the urchin (Dead / Alive) was analyzed as the response variable. Explanatory variables selected were 'Size' (S, M, L) and 'Treatment' (P / UP). These analyses were developed using the R software (R Development Core Team 2013).

PREDATORY GUILD EFFECT

Prey detectability of pierced (P) and unpierced (UP) sea urchins was assessed for the principal urchin predators (fish, gastropods and starfish, Fig.4.1, d) (Boudouresque & Verlaque 2001). We used guild-specific methods to assess prey detectability by each one of these organisms according to response times and behaviors (see below). We used Wilcoxon matched-pairs tests using R software (R Development Core Team 2013) in order to determine prey detectability for fish and benthic predators (see below).

Prey detectability by fish: To test if pierced tethering enhance prey detectability by fish, predation was monitored on 24 sea urchins (3 to 5 cm of test diameter, TD), of which 12 were pierced and 12 were unpierced, using underwater video cameras. We used medium size urchins as this is the main targeted size by fish predators (Sala 1997). The experiment was done in Medes Islands MPA where the density of predatory fish is very high. GoPro Hero 2 (10MP, FullHD) cameras were placed in front of pierced and unpierced urchins randomly distributed in a rocky macroalgal habitat. The experiment was done on three different days (8 cameras were placed each sampling day). For each video, predator species were identified, and the time of first attack was measured as a proxy of detectability, with shorter attack times indicating faster detection.

Prey detectability by gastropods and starfish: To test if pierced tethering affects prey detectability by benthic invertebrate predators, experiments were conducted at locations where gastropods (*Hexaplex trunculus*) and starfish (*Marthasterias glacialis*) were abundant (Alfacs Bay and Fenals, respectively). Predation rates of these predators are very low, so rather than depending on random, low-probability encounter rates, we placed one predator and two sea urchins (one pierced and one unpierced) into a 1.5 cm mesh cages and evaluated the detectability of each predator for each type of urchin. We did prior assessments to analyze mobility of the benthic predators to determine the size of the cages and the variable to be measured. We observed that *M. glacialis* followed a less directional path compared with *H. trunculus* that presented a more ballistic movement to the prey. According to the predator behavior we used different cage sizes and different variables to test preferences for pierced and unpierced sea urchins. For *H. trunculus*, we deployed 20 cages measuring 50 x 20cm in Alfacs Bay, while for M. glacialis, we used 6 cages of 100 x 30cm, deployed in Fenals. One pierced and one unpierced sea urchins (3-5cm TD) were placed at each side of the cage while the benthic predator was placed in the center. The side for the pierced and the unpierced urchins was randomly selected for each trial to avoid biases to a particular direction due to currents or waves. For the *H. trunculus* experiments, prey preference was estimated as the number of times the predator was found at each of the sides of the cage that had either a pierced or an unpierced urchin after 30 minutes of visual observation. We expressed the variable as a percentage of the total number of observations. If the gastropod remained at the center of the cage (10cm wide), it was recorded as no preference and not included in the analyses. For the *M. glacialis* experiments we estimated prey preference by video recording the time the predator spent in the cage area near the pierced or the unpierced urchin, expressed as percentage of total time in the cage. The time that sea stars spent at the center of the cage (20cm wide) was recorded as no preference.

RESULTS

FIRST ORDER ARTIFACTS: INCREASED MORTALITY DUE TO MANIPULATION

Mortality of pierced urchins was significantly affected by the degree of Tab confinement (Table 4.1, p = 0.024). Mortalities (around 20 %) were found exclusively for pierced urchins in confined waters from the sheltered Alfacs Bay compared with no mortality (0%) in the exposed location Fenals (Table 4.1). Unpierced sea urchins (UP, control) did not show any mortality (0%), even inside the bay, indicating that the mortality observed was a direct result of the manipulation (piercing). Extreme high temperature also significantly increased mortality in pierced urchins in the confined site (Table 4.2, p = 0.001). Around 60% of pierced sea urchins died in extreme conditions of high temperatures (30ºC). Once again this mortality was attributable to the combination of piercing and temperature, since controls (unpierced sea urchins) did not show any mortality. Meanwhile, both pierced and unpierced sea urchin controls under the high (23ºC) and low (15ºC) temperatures in open coast (Fenals) did not show any mortality.

Table 4.1. Confinement effect (first order artifacts). Mortality found at 23ºC, expressed in percentage for the whole experimental period in the confined site (Alfacs Bay) and in the open site (Fenals). SE: Standard error. P values of one-way ANOVA test for differences between pierced and unpierced urchins in each confinement condition.

Confined					**Open**				
Pierced		**Control**		**ANOVA**	**Pierced**		**Control**		**ANOVA**
mean	**SE**	**mean**	**SE**	**p-value**	**mean**	**SE**	**mean**	**SE**	**p-value**
22%	6%	0%	0%	**0.024**	0%	0%	0%	0%	**-**

Table 4.2. Temperature effect (first order artifacts). Mortality found under each temperature condition in confined and open waters, expressed in percentage for the whole experimental period. SE: Standard error. P values of one-way ANOVA tests for differences between pierced and unpierced urchins in each experimental condition.

	Extreme (30°C)					**High (23°C)**				
	Pierced		Control		ANOVA	Pierced		Control		ANOVA
Confined	mean	SE	mean	SE	p-value	mean	SE	mean	SE	p-value
	59%	9%	0%	0%	0.001	22%	6%	0%	0%	0.024
	High (23°C)					**Low (15°C)**				
	Pierced		Control		ANOVA	Pierced		Control		ANOVA
Open	mean	SE	mean	SE	p-value	mean	SE	mean	SE	p-value
	0%	0%	0%	0%	-	0%	0%	0%	0%	-

SECOND ORDER ARTIFACTS: INCREASED MORTALITY DUE TO A HIGHER DETECTION RATE BY PREDATORS

Prey size significantly influenced predation rate by fishes (Table 4.3; p = 0.001). Smaller sea urchin sizes were more vulnerable to predation (Fig. 4.2). The highest predation rates were found for small (~ 80%), closely followed by medium (~ 75%) while large urchins attracted the lowest overall predation (~ 30%) (Fig. 4.2). Nonetheless, the influence of treatments (pierced versus unpierced) on predation rates was not significant regardless of the size class considered (Table 4. 3). Of the three experiments designed to detect guild-specific differences in detection rate (or mortality) of predators between pierced or unpierced sea urchins we recorded only a slight preference for unpierced urchins by gastropods while no trend was observed for fish and starfish (Table 4.3). Fish chose equally pierced and unpierced urchins (Fig. 4.3). Only half the *Hexaplex trunculus* showed a preference either for pierced or unpierced urchins. Of the individuals that did display preference, unpierced urchins were slightly preferred (Fig. 4.3; p = 0.04). Of the total experimental time in which individuals occupied the areas with the urchins, *Marthasterias glacialis* spent the same amount of time in pierced than in the unpierced sea urchins areas (Fig. 4.3; p = 0.81).

TABLE 4.3. Second order artifacts analyses. Generalized Linear Models (GLM) analysis for predation according to treatment (pierced and unpierced) and size (juveniles, young adults and adults). We present the initial complete model and the selected one after a stepwise process (AIC criterion). Wilcoxon matched paired test for preferences of each predator guild (fish, gastropods and sea stars). Significant p-values are presented in bold for each of the analyses.

Analyses	Type of artifact	Model	Selected	Effects	Chisq	p
GLM	**Predation**	Status ~ Treatment * Size (binomial)	Status ~Size (binomial)	Size	25.814	**0.001**
				Treatment	0.370	0.543
Wilcoxon	**Preferences**	Fish	–	–	–	0.962
		Hexaplex trunculus	–	–	–	**0.037**
		Marthasterias glacialis	–	–	–	0.809

Discussion

Although pierced tethering has been criticized as a tagging method because of the possibility of introducing artifacts by altering either prey mortality or predator behavior, our study shows that first order artifacts, linked directly to prey mortality due to experimental manipulation, only arise under very specific conditions of extreme high temperature or very low water turnover rate, both of which occur in confined waters, such as closed bays. In contrast, along exposed coastlines and under normal high (summer) temperature conditions, piercing and tethering the sea urchins does not induce any mortality. Interestingly, second order artifacts, arising because of increased prey detectability by predators, also do not represent an important confounding effect when applying this technique. Taken together we demonstrate that tethering sea urchins with piercing is a reliable and robust method for assessing comparative predation rates in *Paracentrotus lividus* for most common conditions in the Mediterranean. The present study is, to the best of our knowledge, the first to explicitly examine the possible artifacts that could bias results when using such a method, and provides support for earlier and future studies using these techniques for field-based estimates of predation.

Our results indicate that only the effects of water confinement and unusual extreme high water temperature appear to significantly increase prey mortality after piercing, while in most common conditions high temperature seems not to affect mortality rates. In large areas of the North Western Mediterranean, summer temperatures do not surpass 23ºC (+/- 1ºC) (data since 1969 from Josep Pascual; www.meteoestartit.cat) except in shallow bays with high degree of confinement where seawater temperature can register values up to 30ºC. However, when working in other areas, where temperatures can cross this limit even under exposed conditions, these second-order artifacts are likely to be important. For instance, exposed waters in the Eastern Mediterranean, or areas in the South Western Mediterranean, may standardly experience higher summer temperatures, and experiments done here should consider the possible by-side effects of piercing. We believe that under extreme temperatures and in high confined areas, keeping sea urchins under control after piercing and using the ones that resist these effects to test predation will improve the method. Mortality of sea urchins due to piercing was accompanied by an evident deterioration in the body’s external tissues and a loosening of the spines. Recent studies have demonstrated the negative effects of an increase in sea water temperature on the ability of urchins to resist pathogens, and a positive effect of waves and water renewal that diminish their susceptibility to disease (Girard *et al.* 2012; Clemente *et al.* 2014). It is also possible that the mortality found in confined waters would also be associated with pathogens, even at normal temperatures with increased abundance of pathogens. There is a high diversity of pathogens that can cause urchin diseases, making it difficult to identify the exact cause of elevated mortalities in such conditions.

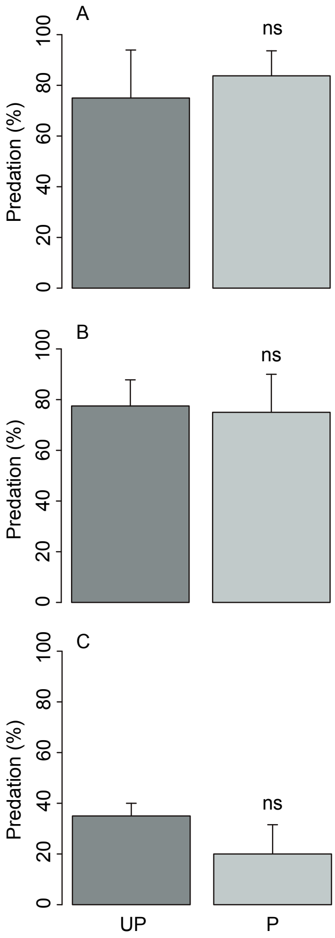

FIGURE 4.2. Predation rates (%) for pierced (P) and unpierced (UP) sea urchins (mean values +/- SE) according to prey size classes A) small (1-3cm test diameter; TD), B) medium (3-5cm TD) and C) large (>5cm TD). Differences between pierced and unpierced sea urchins were non significant in all cases (Table 4,2).

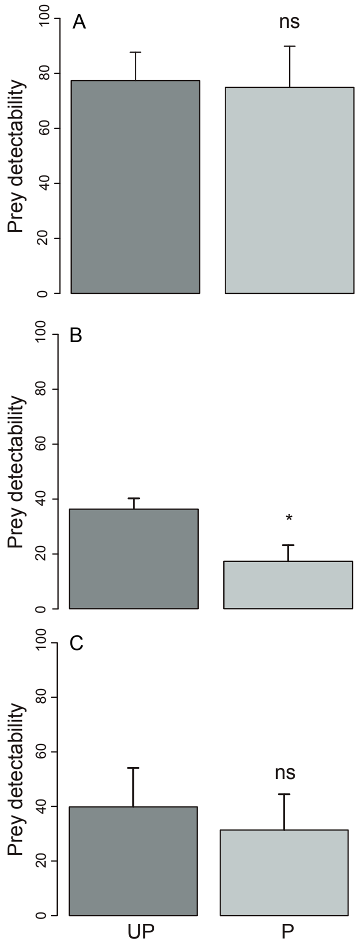

FIGURE 4.3. Prey detectability (mean values +/- SE) of unpierced (UP) and pierced (P) urchins by each predator guild; A) predation impact by *Diplodus sargus*, B) percentage of detection times for *Hexaplex trunculus* and C) percentage of time preference for *Marthasterias glacialis*.

Interestingly, in our work none of the studied predator species preferred pierced urchins, which, a priori, were more likely to emit chemical cues. Only the *H. trunculus* showed preferences for unpierced urchins (10% more than pierced urchins) but low significance was found in statistical analysis. This does not however imply that these predators were unable to detect and respond to chemical cues but that differences between the emitted signals by a pierced and unpierced sea urchin were undistinguishable for the predator under natural field conditions. In fact, it is known that benthic predators, such as gastropods and starfish are cryptic chemotactic organisms, relying heavily on chemical cues for their predation success in highly structured habitats such as seagrass meadows (Farina *et al.* 2014). However, the lack of preference for pierced urchins indicates that second order artifacts are minimal for fish and benthic predators. This was also true for the main fish predator observed in video trials; the prey detection time of the sparid *Diplodus sargus* was not influenced by piercing. Once the urchin was detected and preyed on, several other fish species were attracted to the kill, and scavenged of it (i.e. *S. aurata*, *D. vulgaris* and *L. merula*). This has been previously described in other studies in the Mediterranean (Sala & Zabala 1996; Guidetti 2004).

Fish did not display a preference for pierced urchins in any of the size classes. This suggests that fish predation success under field conditions is primarily driven by visual cues and they are not sensitive to any potential enhanced chemical cues derived from puncturing the urchin. Our video analysis shows that there was no difference in prey detection time between pierced and unpierced urchins, confirming this pattern. It has been previously shown that predator-prey interactions between sea urchins and its fish predators are size dependent (Sala & Zabala 1996). Smaller sizes have a larger predator guild (Guidetti 2004) and they typically rely on finding adequate refuge to escape predation (Sala & Zabala 1996). While larger urchins have fewer available refuges, their size itself serves as a refuge, making them invulnerable to all but the largest extant fish predators.

To summarize, our results show that, at least for the tethering method most commonly used to estimate rates of urchin predation, artifacts are negligible in most standard environmental conditions in the Mediterranean (open coast with low to moderate water residence times and normal high temperatures). Thus, except for uncommon conditions (extreme temperatures, high water confinement) we can confirm that pierced tethering is a very useful tool to mark individuals of this sea urchin species in the Mediterranean when assessing predation rate for comparative purposes (e.g. between regions and treatments, between different predators or to evaluate the effectiveness of MPAs). This is encouraging given the critical importance of assessing predation in marine benthic ecosystems, many of which are strongly controlled by top-down processes, often showing non-linear responses to changes in predation rates. Whether this method is a useful test of absolute (natural) rates of predation will depend on the ability of urchins to properly find refuges as they would if they were not tethered, on non-altering their attachment strength and on maintaining the relative sea urchin abundances at the study sites. In fact, this technique can potentially alter encounter rates between certain predators and the tagged prey if its movement is strongly limited (Barbeau and Scheibling 1994). Nevertheless, in this study we observed that even for comparative studies it is important to account for the environmental conditions at sites in which the experiment will be deployed to effectively apply this technique, since high temperatures and water residence times could potentially bias the results. In conclusion, we consider that the tethering method used to evaluate comparative predation impacts on the sea urchin *P. lividus* in the Mediterranean is a robust technique useful to provide accurate results and that ecological biases in measuring predatory-prey interactions are negligible at least in the most common conditions. Our work confirms the robustness of pierced tethering as a valuable technique in the marine field ecologist's toolbox to measure essential functional rates that shape communities and ecosystems.

ACKNOWLEDGMENTS

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5

HERBIVORE CONTROL IN CONNECTED SEASCAPES: HABITAT DETERMINES WHEN IN THE LIFE HISTORY OF A KEY HERBIVORE POPULATION REGULATION OCCURS

Abstract

Herbivore outbreaks can often trigger catastrophic overgrazing events in marine ecosystems dominated by macrophytes. In the Mediterranean, macroalgal communities (on rocky substrates) and *Posidonia oceanica* seagrass meadows (mostly on sandy substrates) occupy the shallow coastal waters, often as a patchy mosaic that dominates the seascape. The sea urchin *Paracentrotus lividus* frequents both habitats as a key herbivore, and has been known to precipitate overgrazing barrens in macroalgal communities. In this study we sought to identify habitat-specific regulating agents of this herbivore's populations by comparing the strength of supply-side, migration and top-down control processes in seascapes with both habitats present. We measured each of these processes in 8 locations across a wide stretch of the Mediterranean coast (ca. 600km). Our results show that habitat-specific regulation is critical in understanding sea urchin population dynamics, with each habitat limiting urchin populations at different life stages. Early post-settlement mortality was a clear bottleneck for urchins in seagrass meadows. In both habitats, predation (top-down control) was the dominant regulatory mechanism, being the sole significant factor in macroalgal communities. In contrast, in seagrass meadows, settlement and adult migration (from adjacent habitats) were additionally important as critical control agents. The fact that population regulation is linked closely to habitat suggests that sea urchin populations may be significantly buffered from bottlenecks in seascapes with presence of both habitats. By migrating (either actively or passively) or by maintaining reproductive outputs in one of the habitats, sea urchin populations can persist across the seascape despite these regulatory mechanisms. Our results highlight the importance of evaluating the regulatory agents of key structural herbivore populations across complex seascapes to understand and manage these species from reaching outbreak proportions.

INTRODUCTION

Understanding the processes regulating the population of key species is critical in determining the probability of certain ecosystems shifting towards unproductive alternative states (Jackson 2001; Terborgh *et al.* 2001; Denno *et al.* 2003; Baskett & Salomon 2010; Ripple *et al.* 2014). Populations are often strongly mediated by habitat-specific processes as individuals recruit, grow, move, feed and reproduce within the ecosystems they colonize and inhabit. How habitats determine the life history processes of key species can have major population consequences that, in turn, can significantly influence ecosystem function. Habitat choices can strongly influence future life-history stages since each habitat may differ considerably in its availability of food and refugia, and in the nature and strength of species interactions (e.g. competition) individuals are exposed to. At every life history stage, a range of habitat-contingent factors can serve to regulate the fate of individuals in a population. This can have major flow-on effects when the species of concern is also an important ecosystem modifier. In many nearshore marine environments, population outbreaks of key herbivores can often trigger major overgrazing events, leading, in the extreme, to undesirable stable state shifts (Wolf *et al.* 2007; Ling *et al.* 2015). Understanding how habitat choices at any time in the life history can modify the population dynamics of these herbivore species can have profound implications for managing the functional consequences of population trends across the seascape. This is particularly critical in marine systems that range widely in their type of habitats, from large open pelagic systems to patchy, dense benthic environments, and species often occur across these habitats in the course of their complex life cycles. For instance, larvae of many benthic species can show clear early-settlement choices of suitable substrates to recruit based on habitat-specific cues. However, marine habitats are seldom isolated, as seascapes are often a mosaic of more or less connected patches, each with their own suite of habitat-specific population constraints. Understanding how these constraints vary among habitats becomes particularly critical when managing populations of species that can move across the seascape within their life cycle.

Released from control, marine herbivores can transform high-structured and diverse macrophyte ecosystems into completely denuded, almost unproductive states (Estes & Palmisano 1974; Eklöf *et al.* 2008b; Ling *et al.* 2009b; Ripple *et al.* 2014). There is increasing evidence of overgrazed areas expanding across the world oceans, particularly in temperate seas in the Northern hemisphere associated with herbivore population outbreaks (Filbee-Dexter & Scheibling 2014; Conversi *et al.*

2015; Ling *et al.* 2015). Understanding how numbers of key herbivores fluctuate in a population becomes critical to predict and potentially prevent these ecosystems from tipping over to functionally poorer states. However, our ability to accurately predict population fluctuations requires detailed empirical studies to understand how habitat-specific drivers modify the arrival (recruitment and immigration) and loss (mortality and emigration) of individuals in a population (Ripple & Larsen 2000; Ballard *et al.* 2001; Ling *et al.* 2009a; Petraitis & Dudgeon 2015), and how these drivers vary from one habitat to another. While several studies have sought to determine key herbivore regulation, they are often based on the study of a single habitat and a single major process such as larval supply (Cardona *et al.* 2013; Petraitis & Dudgeon 2015), predation (Sala & Zabala 1996; Estes *et al.* 2011; Ripple *et al.* 2014), or migration (Kayal et al. 2012), (but see Ling *et al.* 2009a). Few studies accounted for multiple regulation processes acting at a seascape level where more than one habitat exist; difficult though they are, these approaches provide a more complete knowledge of population control through the life cycle of the species, and allow for a holistic understanding of how these processes could influence ecosystem functioning (Doropoulos *et al.* 2015; Marzloff *et al.* 2015).

Shallow seascapes in the Mediterranean are largely dominated by a mosaic of macroalgal communities (on rocky substrates) and *Posidonia oceanica* seagrass meadows (mostly on sandy bottoms). Both habitats are prone to overgrazing events, often precipitated by outbreaks of the sea urchin *Paracentrotus lividus* (Pinnegar *et al.* 2000; Planes *et al.* 2011). Macroalgal communities are particularly susceptible, and urchin outbreaks can trigger sudden, often unpredictable community shifts to stable barrens (Pinnegar *et al.* 2000; Ling *et al.* 2015). *P. oceanica* meadows seem less prone to sea urchin outbreaks but few cases have also been observed. Outbreak events in Mediterranean macrophyte ecosystems have been related to both unexpected increases in settlement rates (Cardona et al. 2013) and predator release (Sala 1997; Pinnegar *et al.* 2000). Differences in settlement are likely highly dependent on inherent local or regional characteristics like nutrient availability, currents or seawater temperature (Cardona *et al.* 2013; García *et al.* 2015). Once sea urchins settle, their populations experience a significant bottleneck of mass mortality (López et al. 1998). Individuals that survive to older size classes are subject to fish and invertebrate size-specific predators which can be important controllers of urchin populations (Sala 1997; Bonaviri *et al.* 2012). Additionally, there is some evidence of potential urchin migration between macroalgal and seagrass habitats in connected seascapes serving as a potential mechanism of individuals fluctuation within populations (Ceccherelli *et al.* 2009; Prado *et al.* 2012). However, the relative importance of supply-side, top-down and migration processes in regulating herbivore populations in Mediterranean seascapes is still unknown. In this study, we attempt to identify the principal habitat-specific agent of population control in the life history of the key herbivore *P. lividus* in a connected seascape mosaic of rocky macroalgal habitats and *P. oceanica* seagrass meadows. We explore the influence of settlement, post-settlement survival, predation and potential migration on *P. lividus* populations in both habitats to determine the strength of these processes and its consequences for the shallow Mediterranean seascape.

MATERIALS AND METHODS STUDY SITE AND SAMPLING DESIGN.

To assess the effect of settlement, post-settlement survival, predation and migration on *Paracentrotus lividus* abundance in macroalgal and seagrass *Posidonia oceanica* habitats we measured each of these processes in the field along a wide stretch of the Western Mediterranean coast (8 locations, ca. 600km) where both habitats were present (Fig. 5.1). One of the eight localities was inside a marine protected area (Medes Islands marine reserve). Different techniques (see below) were applied to measure settlement, post-settlement survival and predation of *Paracentrotus lividus* at the habitat level at each locality. Additionally, we indirectly estimated the potential migration from macroalgal habitats to *P. oceanica* beds by using the adult sea urchin densities on adjacent macroalgal habitats as a potential explanatory variable for adult populations in seagrass habitats. Sampling was done in two different seasons to remove potential variation or season-related behavioural effects and at a fixed depth of 5 to 8 meters.

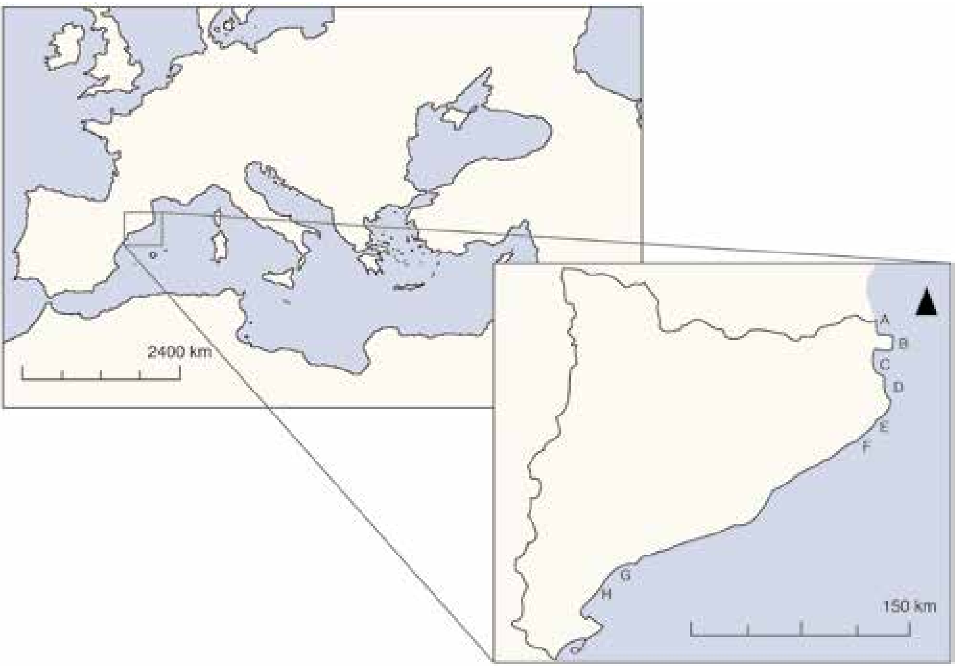


FIGURE 5.1. Map of the Mediterranean Sea showing the study locations within the Catalan coast (ca. 600km). Llançà (A), Portlligat (B), Montgó (C), Illes Medes (D), Giverola (E), Fenals (F), Hospitalet de l'Infant (G) and Ametlla de mar (H).

SETTLEMENT RATES.

Artificial collectors (i.e. 18x10cm wood brushes) were used to estimate *P. lividus* settlement rates (Hereu *et al.* 2004; Prado *et al.* 2012). Three collectors were randomly placed in each location and habitat during the highest settling peak season (May-June), (Tomas et al. 2004). Collectors were placed floating in the water column, 20cm from the bottom and attached to a line. A buoy was fixed to one end of the line to maintain the brush in a vertical position. A weight was attached to the other end to fix the brush to the bottom. Collectors were recovered after 15 days (collectors T1) and replaced with new ones placed on the same lines. These were recovered after an additional 15 days (collectors T2). This was done in order to maximize the probability of capturing the peak settlement period, which is related to seasonal increases in seawater temperature (Tomas et al. 2004). Once in the laboratory, collectors were washed under flowing water and the water filtered through a 250µm mesh net. The filtered samples were then fixed in 70% ethanol for further analysis (see Prado et al. 2012). Samples were examined under a binocular lens and the number of urchin settlers in each sample was counted.

Recruitment - post-settlement survival.

The abundance of sea urchins less than 3cm test diameter (TD) was measured in the same 8 localities sampled above to estimate post-settlement survival. Three different areas were selected within each habitat and site to measure the abundance of *P. lividus* individuals. Sea urchins were counted in five 50x50 cm quadrats, located haphazardly in each of areas. We sampled post-settlement abundances in two different seasons (summer and winter). Sea urchin densities (individuals · m^{-2}) at a location level were then calculated from abundances in quadrats.

Top down – Predation impact.

Tethering techniques were used to measure predation impact on the adult sea urchin *P. lividus* (Boada *et al.* 2015b). This technique is the most extensively used method to assess predation in marine ecosystems of temperate and tropical seas and we have discussed its principal methodological benefits and constraints elsewhere (Boada et al. 2015a). Each individual sea urchin was pierced through the test using a hypodermic needle and threaded with a nylon line. The line was then knotted to create a harness for the urchin, which was attached to a brick or metal stake. Twenty tethered sea urchins were randomly distributed in each habitat in groups of 5 at each locality. Predation impact was calculated after 15 days from the start of the experiment, based on the percentage of urchins preyed on in each group. This experiment was repeated in summer and winter to account for potential seasonal variations in predation impact.

Adult population densities.

Adult sea urchin (>3cm TD) abundances were also measured in the same 8 localities to account for the adult population densities. Three different areas were selected within each habitat and site to measure the abundance of *P. lividus* individuals. Sea urchin numbers were counted in five randomly located 50x50 cm quadrats cm in each of the selected areas in two different seasons (summer and winter). Sea urchin densities (individuals · m^{-2}) for each size class were then calculated from abundances in quadrats.

Statistical analyses.

We explored how processes varied between habitats for different life stages using a series of two-way mixed ANOVA with 'Habitat' as a fixed factor (2 levels, macroalgal habitats and *Posidonia oceanica* meadows) and 'Site' as a random factor (8 levels, localities). With this design we tested for habitat-level differences in settlement (pooled data from collectors T1 and T2), post-settlement survival (pooled summer and winter) and predation impact (pooled summer and winter). The same analysis was used to test habitat preferences of the adult classes as well (pooled summer and winter). Prior to the analyses we tested for normality (Shapiro-Wilk test) and homogeneity of the variance (Bartlett's test). When assumptions of normality were not met, the significance level was set to $p < 0.01$ since the F statistic is robust despite violation of these assumptions when the sampling size is large enough (Underwood 1981a). Generalized Linear Models (GLMs) were used to study the relative contribution of each process (see below) to the adult classes for each habitat separately, with the response variable being the adult sea urchin densities (mean values), and n=8 (the 8 sites). Analyses were done for each habitat separately because different explanatory variables were tested in each one. Settlement rates (mean values, T1 and T2), post-settlement survival (mean values for summer and winter) and predation impact were tested as explanatory variables in both cases. Additionally, the number of adult sea urchins in the adjacent macroalgal habitat (mean values for summer and winter) was used as a potential explanatory variable for the GLM of the seagrass habitat as a proxy for migration. A negative binomial distribution was fixed as the best method to deal with overdispersion (Zuur et al. 2009). We use the Akaike's Information Criterion (AIC) to select the best model when necessary. All statistical analyses were performed using 'lme4' package (Bates et al. 2014) in the open source software (R Development Core Team 2013).

RESULTS

HABITAT-SPECIFIC PARACENTROTUS LIVIDUS SETTLEMENT.

Settlement varied greatly between the different studied localities with average values ranging from less than 5 settlers brush^{-1} in some locations to a maximum of 232 settlers brush^{-1} in other sites (per sampling interval). Interestingly, the average settlement in *P. oceanica* seagrass meadows (35 settlers brush^{-1} ± 1) and the average settlement in macroalgal habitats (38 settlers brush^{-1} ± 2) was very similar. Settlement in both habitats, across sites and sampling periods, were highly correlated with a slope close to 1, indicating that neither of the two habitats had a major settlement attraction (Fig. 5.2a; see also non-significant ANOVA results, Table 5.1).

HABITAT-SPECIFIC PARACENTROTUS LIVIDUS POST-SETTLEMENT SURVIVAL.

Post-settlement survival (as densities of individuals < 3 cm) differed significantly between habitats ($p < 0.01$, Table 5.1, Fig 5.2b). While in *P. oceanica* meadows no juveniles were found in any of the 8 localities, an average of 19 ± 3 sea urchin recruits per 10 square meters was recorded in macroalgal habitats. Nevertheless, strong differences in recruits were found between sites, ranging from 6 to close to 80 recruits per 10 square meters.

HABITAT-SPECIFIC YOUNG ADULT PREDATION.

Predation was substantially different between localities and habitats with the lowest values in *P. oceanica* meadows being around 5% individuals eaten in the 15-days experimental period and 35% in macroalgal habitats. The maximum predation we recorded in meadows was, on average, around 60% observed in Medes Islands marine reserve. In macroalgal habitats the maximum predation impact observed was around 90%. This maximum predation impact values where again found in the Medes Island marine reserve while similar predation values were registered in outside reserve locations as well. Mean predation impact was substantially lower in *P. oceanica* meadows, with values close to 22.5% (± 5%) relative to the mean predation impact in macroalgal habitats 55.5% (± 3%) (Table 5.1, $p < 0.01$). No correlation was observed between the predation impact in both habitats (across seasons and localities), confirming that habitat specific factors determine this parameter (Fig. 5. 2c)

HABITAT-SPECIFIC ADULT POPULATIONS.

Adult population also differed significantly between locations with some sites in which no urchins were found (i.e. Medes Islands marine reserve) and some sites had values of up to 8 urchins/square meter (average of both habitats). The average number of sea urchins larger than 3 cm TD was 4.2 ind-m^{-2} (± 0.6) in *P.* meadows and 3.9 ind-m^{-2} (± 0.4) in macroalgal habitats. Differences between habitats were non-significant (Table 5.1). Interestingly, adult sea urchin populations were similar in both habitats although no correlation was observed between habitats at the level of the location, confirming that habitat specific factors determine adult populations (Figure 5. 2d).

TABLE 5.1. Habitat-specific regulation processes; settlement, post-settlement survival and predation and habitat adult sea urchin densities. Two-way ANOVA tests for differences between habitats. Significant p-values are given in bold.

Process	Df	F-value	P-value
Settlement	1	0.007	0.936
Post-settlement survival	1	7.919	**0.009**
Adult predation	1	10.33	**0.006**
Adult densities	1	0.036	0.853

Habitat-specific adult sea urchin regulation.

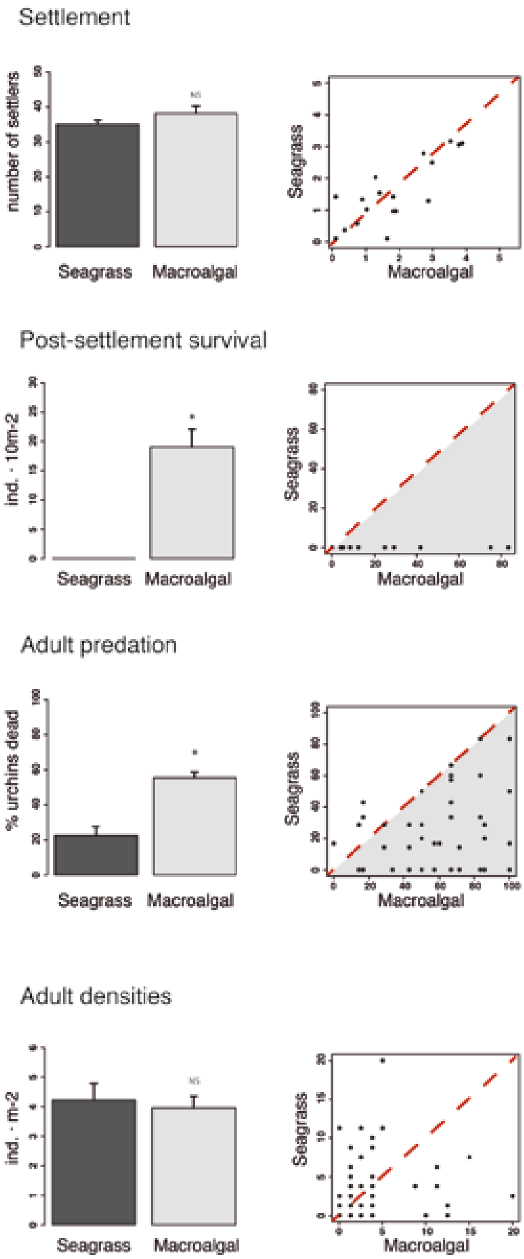
For adult sea urchin populations living in macroalgal habitats, predation emerged as the sole significant factor contributing to its numbers (Table 5.2). In clear contrast, a combination of settlement, predation and sea urchin numbers in the adjacent macroalgal habitats help predict sea urchin numbers in *P. oceanica* meadows. Top-down control through predator effects was the most important process in both habitats explaining the greatest variation in sea urchin numbers compared with the other ecological drivers we studied. After predation, the number of sea urchins in the adjacent macroalgal habitat determined the abundance of sea urchins in seagrass meadows indicating that potential migration from rocky substrates to seagrass ecosystems was an important agent of adult urchin numbers. Settlement of larvae had the lowest impact explaining the abundance of sea urchins in seagrass meadows but still played a secondary role in this habitat (Table 5.2).

Table 5.2. Habitat-specific model fitting for adult sea urchin densities as response variable dependent on settlement, post-settlement survival, predation and potential migration (only from macroalgal rocky substrates to *Posidonia oceanica* meadows). Significant p-values are given in bold.

Habitat	Full model	Selected model	Effects	Df	p-value
Macroalgae	Density ~ Settlement + Post-settlement survival + Predation + (Site)	Density ~ Settlement + Predation + (Site)	Settlement	1	0.25
			Predation	1	**0.04**
Seagrass	Density ~ Settlement + Post-settlement survival + Predation + Macroalgal density (Site)	Density ~ Settlement + Predation + Macroalgal density (Site)	Settlement	1	**0.03**
			Predation	1	**< 0.01**
			Macroalgal density	1	**0.03**

DISCUSSION

Habitat-specific processes contribute to determining when in the life history of sea urchins its abundance is significantly regulated (Fig. 5.3). As shown by previous studies for this urchin (López *et al.* 1998; Tomas *et al.* 2004; Prado *et al.* 2012) and other benthic species (Hunt & Scheibling 1997), we identified a clear bottleneck during the post-settlement stage with no recruits found in *Posidonia oceanica* seagrass meadows and only a few (relative to the abundance of settlers, several orders of magnitude higher) in macroalgal habitats. However, despite such high early life-history mortality rates, adult urchins were abundant in both habitats, presenting a potential paradox, particularly in seagrass meadows, of how these populations continue to be maintained. The high adult abundances in seagrass meadows despite heavy post-settlement mortality indicates that either the few that survive each year (below our sampling resolution) or individuals that migrate from adjacent macroalgal habitats serve to supply the adult population. In macroalgal habitats predation was the sole significant regulating agent of adult sea urchin populations and other factors such as post-settlement survival appear to be of minor importance. The fact that these demographic controls occur at different times the life-cycle of the urchin in each habitat has profound implications in connected habitat matrices since populations can potentially find refuge in adjacent ecosystems. In addition, our work highlights the importance of habitat-specific top-down processes in controlling key herbivore populations – both directly and indirectly.


Settlement

Post-settlement survival

Adult predation

Adult densities

FIGURE 5.2. Bar plots showing habitat-specific values for settlement, post-settlement survival, predation on adult sea urchins, and adult densities on the left (mean values +/- SE). Scatter plot of pair raw data found for each process in each habitat and site. Dashed red lines show trend for no preference between habitats and grey shadows highlight the preferred habitat.

Although the predator release hypothesis has been tested in several studies and described as the main reason for population explosions of many herbivore species (Sala & Zabala 1996; Pinnegar *et al.* 2000; Wolf *et al.* 2007), much of the existing literature evaluates cascading effects of predator decline at rather narrow spatial scales (Sala & Zabala 1996), typically comparing marine reserves with unprotected areas. Our study expands this to a much wider regional scale (hundreds of kilometres) and demonstrates that predation is still the major habitat-specific factor controlling population numbers of the key herbivore *P. lividus* particularly in macroalgal habitats across the Western Mediterranean. Interestingly, sea urchins in seagrass habitats also appear to be indirectly affected by predation. The need to find refuge from this intense predation pressure within a habitat (Hereu et al. 2005) or in more complex adjacent habitats within a landscape (Farina et al. 2009) may increase the probability that urchins migrate between habitats. Our results indicate that predation pressure could both influence the numbers of sea urchins in seagrass meadows by direct predation and indirectly by controlling the number of available migrants of the adjacent subpopulation in the macroalgal habitats. Additionally, migration to segrass meadows can also be indirectly precipitated by the higher predator abundance observed in macroalgal habitats and the lower structural refuges compared to seagrass meadows (Farina et al. 2009). While in seagrass habitats factors other than predation may also control urchins, predator release is the strongest agent of population regulation in macroalgal habitats. Settlement in macroalgal habitats could not adequately predict adult population numbers probably because settlement rates were not limiting in the studied area. When, in contrast, settlement rates are normally limiting (Prado et al. 2012), Cardona et al (2013) showed significant cascading effects in a macroalgal community associated with a peak of recruitment indicating that under certain scenarios, where predation is very low, the functional effect of supply-side processes across the trophic chain can be sufficiently important. However, this study confirms that, more globally, predation control is the main process regulating benthic herbivore populations in complex marine landscapes in the Western Mediterranean. For macroalgal communities this takes on a special importance, given its susceptibility to overgrazing pressure and its propensity to shift to unproductive barren states (Pinnegar et al. 2000).

Seagrass meadows present a more complex picture for populations of sea urchins. Here, supply-side processes and predation are not the only factors controlling adult populations and migration from adjacent habitats apparently plays a very important role in determining adult abundances. Although we did not track urchin migration directly, given the absence of young individuals in the meadow found in this and other studies (Prado et al. 2012), the adult populations we encountered could be explained by active or passive migration from adjacent habitats, as in our models, adults in nearby macroalgae are a strong predictor of urchin adults in the meadow. Earlier studies suggest similar migratory behaviours (Prado et al. 2012) that have been linked to a potential escape from predation (Farina et al. 2009). This ability to move between habitats could be an important strategy to avoid habitat-specific survival risks as urchins seek food or refuge in more complex nearby habitats (Farina et al. 2009). In fact, in terrestrial systems, the ability of species to migrate between habitats has been shown to be an important factor meditating population numbers (Sinclair & Arcese 1995; Lima 1998). It is possible that mobile organisms may select switch between habitats to maximize foraging intake (Fretwell & Lucas 1969). This habitat selection hypothesis assumes that individuals are potentially able to identify the intrinsic quality or availability of different resources and the capacity to move between these habitats. Although no evidence exists to suggest that urchins prefer feeding in seagrass habitat over macroalgal habitat, recent novel findings describe massive sea urchin migration events to seagrass meadows from recently overgrazed rocky habitats (unpublished results S. D. Ling). This indicates that urchins may retreat to suboptimal feeding grounds when adjacent habitats are depleted. Responses other than foraging (such as predation risk) could also trigger movement between habitats. This assumes that individuals are able to perceive, evaluate and respond to differential predation risk between habitats (Abramsky *et al.* 1996; Heithaus & Dill 2002), an interesting area for further investigation in sea urchins. In addition to the active migration, passive migration cannot be fully discarded. During storm conditions, it has been observed that sea urchins are easily displaced from rocky habitats compared to seagrass meadows, probably as a result of the lower structural refuge (Pagès *et al.* 2013). This passive movement, storm-linked, can contribute to a global net gain of sea urchins in seagrass meadows within mixed seascapes.

In seagrass meadows supply-side processes were also found to play a significant though minor role in influencing sea urchin numbers. This was surprising given the large post-settlement mortality we recorded in this habitat. Although we cannot identify the processes causing this mortality they are most likely related to early post-settlement predation (Hunt & Scheibling 1997; Bonaviri *et al.* 2012), the mechanical action of the sandy sediment itself (Prado et al. 2009) or other environmental factors. Recruitment rates may not necessarily be zero, but are most likely below the ability of our methods to detect them. The few settlers that survive this early bottleneck each year are likely sufficient to cumulatively influence the overall abundance of the adult population. Successful sea urchin recruitment in seagrasses is known to occur in meadows with unburied seagrass matte, a mixture of live and dead rhizome and roots that can be critical for the survival of newly settled individuals (Prado et al. 2009). This can be especially important in completely isolated seagrass patches, were migration between habitats is practically absent and a cumulative year-by-year effect of settlement together with a low predation pressure could be the only factor contributing to the adult population, particularly in sediment-deficient seagrass meadows with characteristically high-unburied rhizome layers (Prado et al. 2009).

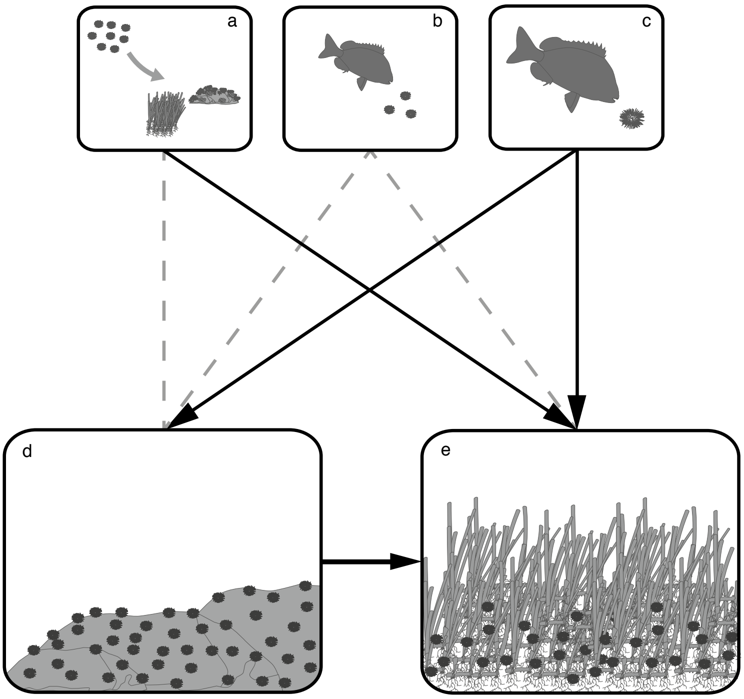


FIGURE 5.3. Schematic description of main results found. Soft grey lines indicate the studied habitat-specific regulation processes of a) settlement, b) post-settlement survival, c) predation and d) numbers of potential migrants from the macroalgal rocky substrate while black lines indicate the significant regulation processes in each habitat.

Taken together, these findings highlight the necessity of evaluating ecological processes that regulate the demographic fate of populations that inhabit connected seascapes. Emigration and immigration to or from neighbouring habitats are generally neglected in the study of populations of species with limited mobility (such as sea urchins) but should be seriously taken into consideration in complex landscapes that include well-connected habitats with suitable alternative resources and differential refuges. As we have shown, population regulation could be strongly mediated by habitat and species can retreat to neighbouring environments within the matrix to search for potential refuges For example, by migrating (either actively or passively) or by maintaining reproductive outputs in one of the habitats, sea urchin populations can persist across the seascape despite regulatory mechanisms occur in one of the habitats. This buffering capacity of connected landscapes could have critical consequences for the functional (and dysfunctional) roles key herbivores often play in macrophyte systems. Recognizing that these key populations range within their lifespan between habitats that may differ considerably in their regulatory processes is a critical learning for managing their populations. Preventing their numbers from reaching outbreak proportions, potentially triggering ecosystem collapse requires a more holistic approach to managing entire seascapes rather than individual ecosystems. Maintaining top-down functioning to control herbivore populations in both habitats is a critical first step given the demonstrated importance of predatory control of urchins across the matrix.

ACKNOWLEDGMENTS

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6

HOTSPOTS OF PREDATION PERSIST OUTSIDE MARINE RESERVES IN THE HISTORICALLY FISHED MEDITERRANEAN SEA

Abstract

The Mediterranean Sea has sustained historically high levels of fishing since pre-Roman times. This once-abundant sea has witnessed major declines in apex predators, now largely restricted to isolated pockets within marine reserves. This depletion could critically impact macrophyte communities that are strongly structured by top-down processes. We evaluated rates of predation on the sea urchin *Paracentrotus lividus*, a key herbivore of macroalgal and *Posidonia oceanica* seagrass seascapes, across a large stretch of the Western Mediterranean coastline. Fish predation was generally higher inside reserves, but was equally high at several locations outside these boundaries. Although critically low at some locations compared to reserves, predation was functionally ubiquitous in most habitats, seasons and sites. Fish were still primarily responsible for this predation with no clear evidence of meso-predator release. Macroalgal habitats were consistently subject to higher predation than in seagrass meadows, functionally critical given the vulnerability of macroalgal systems to overgrazing. Predation hotspots were clearly associated with high fish predator numbers and low refuge availability. Taken together, these results suggest that long-term overfishing may not necessarily reflect a complete loss of trophic function. Pockets of fish predation may still persist, linked to habitat complexity, predator behavioural adaptations and landscape-level features. Given the essential role top-down control plays in macroalgal communities, regulating fishing at these predation hotspots is vital to effectively conserving habitats from future hysteretic shifts. Even historically fished seas may retain areas where trophic function persists; identifying these areas is critical to preserving the remaining ecological integrity of these coastlines.

INTRODUCTION

One of the clearest signatures of the increasing human imprint on the biosphere is the gradual weakening of trophic processes as top predators decline from natural ecosystems under the combined onslaught of direct extraction and habitat loss (Ripple *et al.* 2014). Predation is a critical agent of community structuring (Hairston *et al.* 1960); the depletion of key predators leave both terrestrial and marine ecosystems increasingly prone to catastrophic and often hysteretic collapses from which recovery can be protracted. Marine macrophyte communities are particularly susceptible; uncontrolled by predation, marine herbivores can undergo major population explosions, overgrazing macrophyte-dominated ecosystems (Kempf 1962). In a classic example, otters have been identified as principal structuring agents of kelp communities in the Eastern Pacific by regulating urchin populations (Tegner & Dayton 2000). Similarly, the structuring of Western Mediterranean macrophytes appears to be strongly mediated by top-down control of urchins by fish predators (Pinnegar *et al.* 2000).

Marine ecosystem managers have long recognized the importance of conserving higher trophic functions, and regulating fishing of top predators has been the instrument of choice in managing nearshore ecosystems (Estes *et al.* 2011). There has been a growing call to expand networks of marine reserves and impose fishing restrictions to protect key predators and enhance the natural resilience of the ecosystems they structure (Pinnegar *et al.* 2000). This is predicated on the assumption that fish predator numbers link well with rates of predation, and that healthy predator populations will ensure their functional roles within the ecosystem (Clemente *et al.* 2010). There is growing evidence demonstrating that marine reserves have been largely effective in reversing the direct and indirect effects of trophic decline (Shears & Babcock 2002), and they clearly enhance ecosystem functioning. However, it is becoming increasingly clear that predation is an inherently dynamic process, and predator-prey interactions can vary considerably across the seascape. The distribution and densities of predators and prey within the mosaic may be influenced by recruit supply, which may, in turn, be mediated by habitat differences (Hereu *et al.* 2004). Independent of numbers, predator-prey interactions may be strongly driven by how both predators and their prey use these habitats (Farina *et al.* 2014). These habitat-specific factors may also interact in complex ways making predator-prey interactions often difficult to predict. Both fish predators and their prey may modify their behaviours in relation to each other's presence, the abundance of conspecifics, the availability of refugia and the configuration of the habitat within the larger seascape. For instance, habitat structural complexity, by modifying the presence of prey refugia is fundamental in determining predation rates and, in turn, prey population structures (Hereu *et al.* 2005; Farina *et al.* 2009). Moreover, predators may also be implicated in complex indirect interactions in macrophyte communities; fish herbivores, by reducing the leaf canopy of macrophyte communities, can enhance fish predation on urchin herbivores by reducing refuge availability (Pagès *et al.* 2012). Further, a reduction of top predators can sometimes lead to the competitive release of benthic meso-predators that may potentially compensate rates of functional predation experienced by the system (Levi & Wilmers 2012). This can also be highly habitat dependent since every system could be host to a very different suite of predators. Finally, both predators and prey may move between habitats in the mosaic, and predation may be strongly influenced by patterns of habitat connectivity or isolation within the larger seascape (Hitt *et al.* 2011).

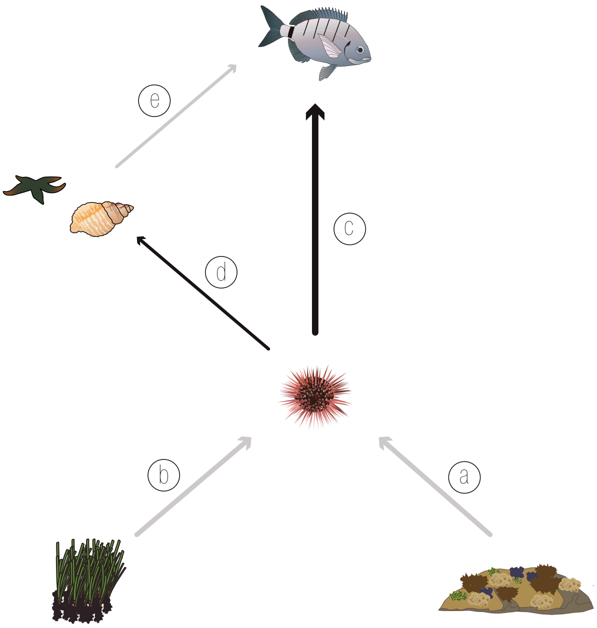

Figure 6.1. Principal interactions in Mediterranean macrophyte communities. The sea urchin *Paracentrotus lividus* lives in both seagrass meadows of *Posidonia oceanica* and macroalgal dominated rocky habitats in the Mediterranean. Letters represent trophic interactions; herbivory (a) and (b), and predation (c), (d) and (e). Black arrows show the predator-prey interactions studied in the present work.

Two macrophyte habitats dominate the North Western Mediterranean: *Posidonia oceanica* seagrass meadows and shallow macroalgae-dominated rocky habitats, both potentially structured by top-down control of the herbivorous sea urchin *Paracentrotus lividus* (Fig. 6.1.) (Verlaque 1987). The Mediterranean has been seriously overfished for millennia (Sala *et al.* 2012), and determining if predation still plays a functional role is essential to planning conservation actions across the region (e.g. creations of marine reserves, management of coastal development, etc.). While it is well established that predation intensity is relatively high inside existing protected areas (Sala & Zabala 1996) it is unclear to what extent this function is conserved beyond their boundaries, although it is generally assumed to be low because of this historically sustained fishing pressure (Guidetti et al., 2010). However, there is little information available on the factors that influence predation in different macrophyte habitats. The decline of fish predators could have triggered a functional substitution by other benthic predators. In addition, given that reserves are principally established to enhance predator numbers, understanding how predation activity is linked to fish predator abundance is critical. To answer these questions, we measured relative rates of sea urchin predation by fish and benthic predators at eight representative locations across a large stretch of the NW Mediterranean coast in both algal communities and seagrass meadows in different seasons. In addition, we attempted to identify if predator habitat use or habitat-specific factors (presence of refuges) can drive functional rates of predation in these dominant macrophyte habitats.

MATERIALS AND METHODS

STUDY SYSTEM

The shallow seascape of the Western Mediterranean is dominated by rocky macroalgal communities and *P. oceanica* seagrass meadows. Although the sea urchin *P. lividus* is a key herbivore in both habitats, they may differ considerably in their susceptibility to urchin herbivory (Boudouresque & Verlaque 2001). In macroalgal systems, urchin overgrazing can cause ecosystem barrens from which recovery is often protracted (Pinnegar *et al.* 2000). Predators likely play a vital role in regulating sea urchin populations, preventing these ecosystem shifts (Sala 1997; Guidetti 2004). While *P. oceanica* meadows may experience very similar rates of urchin herbivory, they may cope better with this offtake because of their inherent evolutionary adaptations (Vergés *et al.* 2008). However, heavy eutrophication could make meadows susceptible to overgrazing (Ruiz *et al.* 2009). Several fish species prey on *P. lividus*, and many of these are important commercial and recreational fishery targets (Guidetti 2006). Additionally, benthic predators including starfish and some gastropods may also be important contributors to sea urchin predation (Boudouresque & Verlaque 2001).

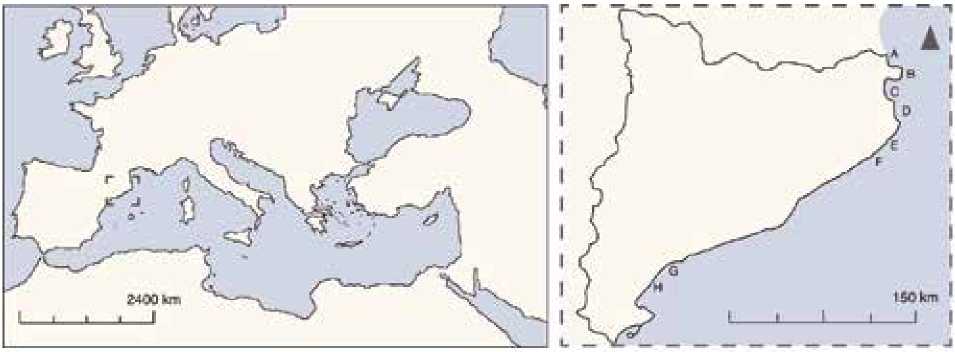

Figure 6.2. Map of the Mediterranean Sea showing the study locations within the Catalan Coast. Llançà (A), Portlligat (B), Montgó (C), Medes Islands (D), Giverola (E), Fenals (F), Hospitalet (G) and Ametlla de Mar (H).

STUDY SITE AND SAMPLING DESIGN

The study was conducted along the NW Mediterranean (~600km). Eight sites were selected along the coast, characterized by shallow seagrass *P. oceanica* habitats and photophi lic macroalgae on rocky substrates (Fig. 6.2). Sites were not randomly selected since all sites required both habitats to be present and at least one unfished reserve was required for the study objectives. Fishing is permitted at all sites except the Medes Island Marine Protected Area, which has been a marine reserve since 1990, and partially controlled in Portlligat since 2006 as part of the Cap de Creus Natural Park but with low fishing regulation. The reserve is characterized by a high abundance and biomass of predatory fish (Garcia-Rubies *et al.* 2013). In each habitat we assessed predation on the sea urchin *P. lividus*, the most important key herbivore in NW Mediterranean macrophyte habitats (Harmelin *et al.* 1980). We evaluated predation impact by fish and benthic predators (see below) in each of the selected sites in summer and winter. In addition, we estimated the habitat use by the most important urchin predators, and evaluated habitat characteristics that could constitute an effective predation refuge for the urchin (i.e. canopy height in both habitats, crevices in rocky substrates and bare root-rhizome layer in seagrass meadows), (Orth *et al.* 1984). All measurements were recorded within a depth range of 3 to 8 meters for both habitats.

PREDATION IMPACT

Predation impact was measured using tethering techniques (Aronson & Heck 1995) on adult urchins. This comparative method has been used extensively to assess predation in Mediterranean and other temperate ecosystems (Guidetti & Sala 2007; Farina *et al.* 2009) and coral reefs (McClanahan & Muthiga 1989; Pederson & Johnson 2006). The urchin was harnessed by threading a thin nylon line through its test. The harness was then attached to a brick or metal stake, which were deployed in rocky reefs or meadows respectively. The harness left the urchin a 50 cm radius space to move from its point of attachment. Within this radius, the urchin could actively seek available shelters as it would in natural conditions. To assess tethering-related mortality before the experiment we tracked the survival of caged urchins (to exclude predators); none of the 14 tethered urchins died in the 12 days of this assessment. Twenty urchins were placed in groups of 5 at each site and habitat (total of 4 replicated groups) in two different seasons (i.e. summer and winter). Predation impact was measured 15 days after the start of the experiment. Predation impact was calculated for each group of urchins using the percentage of dead urchins with respect to the initial number (0, 20, 40, 60, 80 and 100% predation). Fish or benthic predators leave clearly distinguishable bites/marks on sea urchin carcasses (Shears & Babcock 2002) and we carefully examined dead urchin tests to assign predation impact to their respective consumers; fish predator impact (FPI) and benthic predator impact (BPI).

PREDATOR ABUNDANCES AND HABITAT USE

We measured the habitat use of the most common identified benthic predators of *P. lividus* within the two selected habitats, *Hexaplex trunculus* and *Marthasterias glacialis* (Boudouresque & Verlaque 2001). We frequently found these benthic predators still attached to the prey. In addition we recorded a few predation events by the starfish *Coscinasterias tenuispina* (S. Farina pers. obs.) and added this species to our sampling. Benthic predator habitat use was estimated through accounting the abundance of benthic predators at each site and habitat along 4 underwater visual belt transects (10m x 2m). These were used to calculate benthic predator densities per square meter for each site.

We also investigated the habitat use by fish predators during daylight hours, when predators are most active (Savy 1987). We used underwater video cameras in each habitat and season (i.e. GoPro Hero 2, 10Mp) (Harasti *et al.* 2014) to record 4 fixed videos of 20 minutes within each site. We used underwater buoys to mark a 5 x 5 m area in front of the camera and counted the number of individuals of the principal predators and scavengers of medium and large sea urchins (*Diplodus sargus, D. vulgaris, Sparus aurata* and *Labrus merula*) (Sala 1997; Guidetti 2004) and two more potential predator or scavenger species of the genus *Diplodus* (*D. puntazzo* and *D. cervinus*) seen traveling through the marked area. This was then multiplied by the total time each species spent within the zone and divided by the total observed area. Habitat use (U) was calculated as follows:

- $U = \frac{\Sigma(Ti)}{T * A}$

where i is the number of predators observed during the sampling interval, Ti is the total time each predator species spent in the area, A is the total area observed in each video and T is the video recording time (approx. 20 minutes each).

Habitat structural parameters

To test the influence of habitat structural parameters on predation rates, we measured habitat complexity (canopy height and number of refuges) in both habitats. We measured canopy height with a measuring tape at 20 random locations within the macroalgal and *P. oceanica* canopy at each site and for each season. In addition, we measured the depth of the total unburied rhizome layer (Prado *et al.* 2009) in *P. oceanica* meadows since this is often used as an important refuge by sea urchins in seagrass meadows (Orth *et al.* 1984). This was done at 20 random points at each meadow with a measuring stick inserted into the unburied matrix. In rocky macroalgal systems we counted the number of potential shelters (crevices and niches that were estimated to harbor an urchin of at least 4 cm diameter) within a 50 cm diameter range at 20 random points per site and season.

Statistical analyses

3-way ANOVA's tests were performed to establish the effect of habitat, season and site on the following dependent variables: total predation impact, fish predator impact (FPI), bottom predator impact (BPI), sea urchin predator fish habitat use (U) and canopy height. The factors considered were 'site' (8 levels, fixed factor), 'habitat' (2 levels; *P. oceanica* meadows and macroalgal habitats, fixed factor) and 'season' (2 levels; summer and winter, fixed factor). Prior to the analyses we tested for normality (Shapiro-Wilk test) and homogeneity of the variance (Bartlett's test). When assumptions were not met, we set the significance level to $p < 0.01$ as the F statistic is robust despite violation of these assumptions when the sampling size is large enough (Underwood 1981b). Significant differences between sites were further explored with Tukey HSD *post hoc* tests.

A continuous approach (GLM) was used to test the significance of the explanatory variables related to fish habitat use (for the three main predators) and habitat structural parameters (presence of habitat-specific refuges, see below) to explain the observed patterns of fish predation (FPI) within each habitat. We could not test this model for bottom predation impact (BPI) as the number of predation events observed was too low to reliably establish any causal link. A General Linear Model (GLM) with a Binomial distribution (and a logarithmic link function) was fitted to test significance. To describe the response of FPI within each habitat a specific analysis was performed according to habitat-specific explanatory variables. For the *P. oceanica* habitats the specific variables included in the model were canopy height (canopy), depth of the dead matte rhizome layer (matte) and the habitat use of fish of the *Diplodus* genus as the main *P. lividus* predators. For the macroalgal dominated rocky habitats the variables included were canopy height (canopy), number of refuges (shelters) and the habitat use of fish of the *Diplodus* genus as the main *P. lividus* predators. We used mean values of habitat use from the 8 replicates (summer and winter together) to better investigate the use in each location and habitat. We started with a full model considering all predator and habitat-associated variables for model selection. We then chose the best model by dropping each effect sequentially and using Akaike's Information Criterion (AIC) and likelihood ratio tests (Zuur *et al.* 2009). All the statistical analyses were performed using 'lme4' package (Bates et al. 2014) in the open source software R (R Development Core Team 2013).

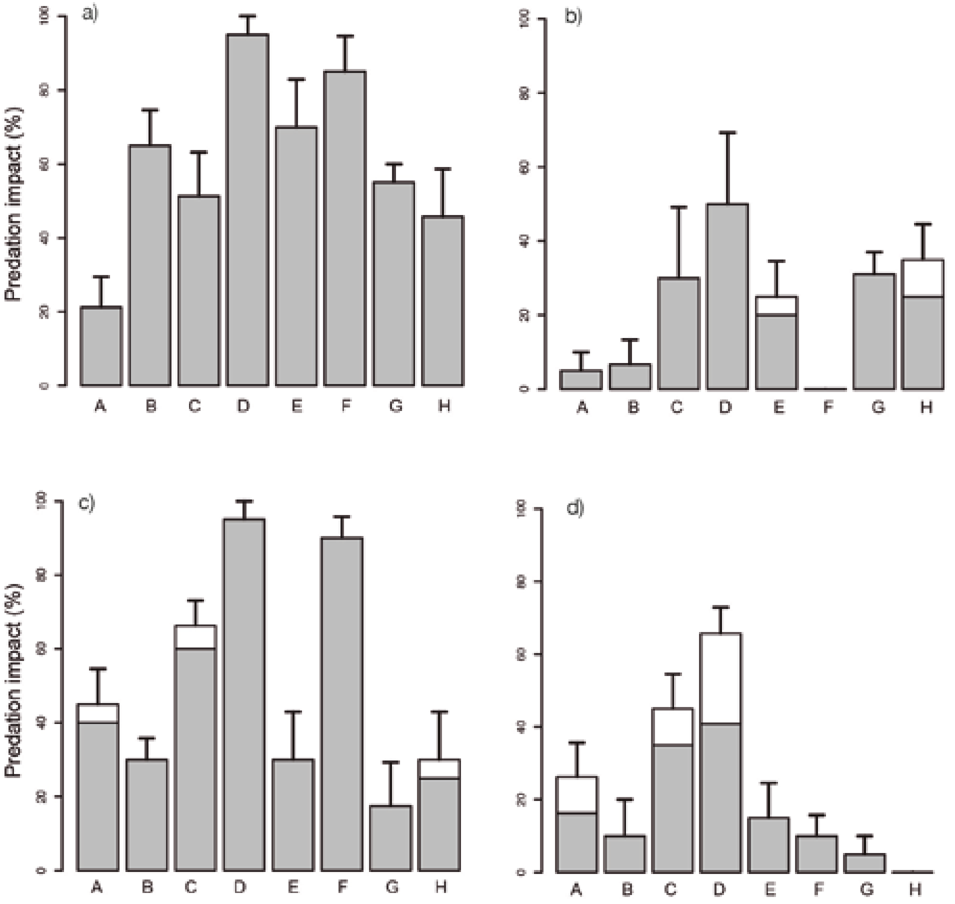

Figure 6.3. Mean predation impact on *P. lividus* (%) + SE in a) rocky habitats in summer, b) *P. oceanica* meadows in summer, c) rocky habitats in winter and d) *P. oceanica* meadows in winter. Filled bars represent fish predator impact (FPI) and clear bars represent benthic predator impact (BPI). Total absence of predation (0%) in sites F and H in b) and a) respectively.

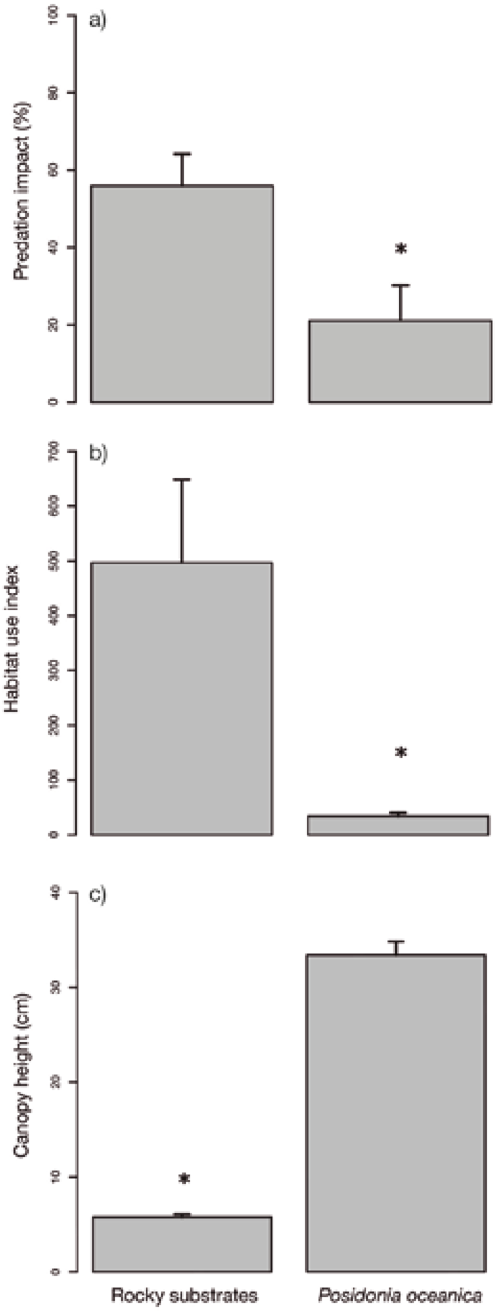

Figure 6.4. Percentage of urchins eaten (a), index of habitat use by sea urchin fish predator guild (b) and length of the canopy height (c) in both habitats. Mean values of two seasons ± SE, n=64. Asterisk indicates significant differences based on ANOVA test.

RESULTS

SPATIO-TEMPORAL VARIATION IN BENTHIC AND FISH PREDATION RATES: HABITAT, SEASON AND SITE.

Predation impact varied substantially between habitats, sites and seasons (Table 6.1, Fig. 6.3). Predation impact in rocky habitats was at least double of that measured in *P. oceanica* habitats, while at some sites this difference was even more marked (Table 6.1, Fig. 6.3). On average, the predation impact in rocky reef communities was 54.9% (± 9.2%), compared with a predation impact of 17.8% (± 8.6%) in *P. oceanica* meadows (Fig. 6.4). The highest predation impact for both habitats was found inside the Medes Islands Marine Reserve in which fishing had been restricted for more than 2 decades. Interestingly though, other locations along the coast, outside any NTA, experienced similar predation impact both in macroalgal communities (*post hoc* Site A = B = C < D = F > E = G = H) and in *P. oceanica* meadows (*post hoc* Site A = B = E = F = G = H < C = D) (Fig. 6.3). In contrast, predation impact in other sites was very low in both habitats especially in a particular season (e.g. Site A or Site B, Fig. 6.3). While predation was generally higher in summer than in winter, this was only true for some sites (Table 6.1, Fig. 6.3). At sites where predation was very high (i.e. Site D or Site F), the difference between seasons was almost absent; where predation was low, these differences were considerably more marked (Fig. 6.3). Even outside the marine reserves, fish predators continued to be responsible for the bulk of predation (Fig. 6.3). In macroalgal habitats the few observed cases of benthic predation occurred outside the reserve. In contrast, in *P. oceanica* habitats predation by benthic predators was more prevalent, although still considerably lower than fish predation. A high peak of benthic predation was observed in the seagrass habitat in winter in Medes Islands as a result of an observed increase in the abundance of the predator starfish *C. tenuispina* (a few tethered sea urchins still had the star attached to the carcass). An interesting pattern is that season was significant when both fish and benthic predation were considered separately, but not together (Table 6.1, Table 6.2) since both predators seem to prefer distinct seasons particularly in determinate sites. Predation by fish was significantly higher in summer (41.5% ± 9.7%, Table 6.2), than in winter (32.3% ± 8.1%) while benthic predation was significantly lower in summer (1.0% ± 0.7%) than in winter (3.5% ± 2.2%).

Table 6.1. Three-way ANOVAs for total and specific predation impact. p-values correspond to F-test results. d.f. = degrees of freedom.

Variable	Source of variation	d.f.	p-value
Total Predation	Site	7	**< 0.001**
	Habitat	1	**< 0.001**
	Season	1	0.062
	Site x Habitat	7	**< 0.001**
	Site x Season	7	**< 0.001**
	Habitat x Season	1	0.117
	Site x Habitat x Season	7	0.523
Predation Fish	Site	7	**< 0.001**
	Habitat	1	**< 0.001**
	Season	1	**0.009**
	Site x Habitat	7	**< 0.001**
	Site x Season	7	**0.005**
	Habitat x Season	1	0.202
	Site x Habitat x Season	7	0.494
Predation Benthic	Site	7	**0.033**
	Habitat	1	**0.016**
	Season	1	**0.017**
	Site x Habitat	7	0.092
	Site x Season	7	**0.003**
	Habitat x Season	1	0.601
	Site x Habitat x Season	7	**0.003**

VARIATION IN PREDATOR HABITAT USE AND CANOPY HEIGHT BETWEEN HABITATS, SITES AND SEASON.

Predator habitat use also showed clear differences between habitats (Fig. 6.4) and sites for certain seasons (Table 6.2, Fig. S6.1). The index of predator habitat use was more than 10 times higher in rocky habitats than in seagrass habitats, a difference not reflected in the magnitude of predation impact (Fig. 6.4). This suggests that although predators may use the habitat much less, predation rates continue to be relatively high in seagrass meadows.

As expected, canopy height varied considerably between macrophyte habitats; *P. oceanica* canopies were almost an order of magnitude taller (~ 35 cm long) than macroalgal dominated habitats (~ 6 cm height, Fig. 6.4, Table 6.2). Canopy height also varied between sites and seasons, although in the same direction for both habitats (Table 6.2, interaction between site, habitat and season, Fig. S6.2). Within each habitat, canopy height varied considerably between seasons, with summer canopies consistently taller than winter canopies (average values summer *P. oceanica* > average winter *P. oceanica* > average macroalgal summer and winter), a trend that was more pronounced at some sites (Table 6.2, interaction between site and season).

Habitat-specific refugia also varied considerably between sites (Fig. S6.1). In macroalgal habitats the number of refugia differed considerably between sites with a mean maximum value of 9.2 refugia per sampling and a mean minimum of 2 (ANOVA p-value < 0.001). In seagrass habitats as well, the depth of the dead matte rhizome layer could differ by an order of magnitude between sites (mean max. 14.4 cm and min. 0.8 cm; ANOVA p-value < 0.001).

Table 6.2. ANOVA analyses for predator habitat use U (see text) and the canopy height. p-values correspond to those provided by an F-test. d.f., degrees of freedom.

Variable	Source of variation	d.f.	p-value
Predators	Site	7	**< 0.001**
	Habitat	1	**< 0.001**
	Season	1	**0.018**
	Site x Habitat	7	**< 0.001**
	Site x Season	7	**0.005**
	Habitat x Season	1	0.236
	Site x Habitat x Season	7	0.493
Canopy	Site	7	**< 0.001**
	Habitat	1	**< 0.001**
	Season	1	**< 0.001**
	Site x Habitat	7	**< 0.001**
	Site x Season	7	**< 0.001**
	Habitat x Season	1	**< 0.001**
	Site x Habitat x Season	6	**< 0.001**

DETERMINANTS OF PREDATION RATES IN ROCKY AND SEAGRASS HABITATS

The GLM results showed that in rocky habitats, predation rates were best predicted by the number of available shelters ($p < 0.05$) together with the abundance of fish predator-species of the genus *Diplodus* ($p < 0.05$, Table 6.3). The same pattern was found in *P. oceanica* meadows where the depth of the dead matte rhizome layer ($p < 0.05$) together with habitat use by fish species in the genus *Diplodus* were key determinants of predation impact ($p < 0.05$, Table 6.3).

TABLE 6.3. Model fitting using GLM for fish predator impact (FPI) inside rocky habitats and *Posidonia oceanica* meadows as a response variable dependent on predator species use of habitat (U) and specific structural parameters of each habitat.

Habitat	Response variable	Full model	Selected model	Effects	df	p-value
Rocky habitat	Predation Fish	~Canopy + Shelters + *Diplodus spp* + *S. aurata* + *L. merula*	~Shelters + *Diplodus spp*	Shelters	1	0.009
				Diplodus spp	1	0.008
	Predation Benthic	Insufficient data				
Posidonia	Predation Fish	~Canopy + Matte + *Diplodus spp* + *S. aurata* + *L. merula*	~Matte + *Diplodus spp*	Matte	1	0.012
				Diplodus spp	1	0.001
	Predation Benthic	Insufficient data				

DISCUSSION

Despite a long history of commercial, artisanal and recreational fishing, fish predation continues to be a ubiquitous process along the North Western Mediterranean coast, albeit with considerable site-level variation in intensity. While the Medes Islands marine reserve (with over two decades of fishing prohibition) unsurprisingly received the highest level of predation in both habitats, locations with no such restrictions also received comparable levels of functional predation. Fish predators continue to be the principal agent of predation in these waters with benthic predators apparently playing a relatively minor role. Perhaps most strikingly, there were strong differences in the intensity of predation between macrophyte habitats, even when separated by just a few meters. Predation impact in macroalgal habitats were at least twice as high as in *P. oceanica* seagrass meadows, even when these habitats were very closely connected, a difference that appears clearly linked to the greater abundance of predators in rocky systems. These predator-prey interactions appeared to be controlled by the same agents in both habitats, the number of available refugia as well as predator habitat use.

The now well-documented specter of trophic downgrading across the world's oceans has raised serious questions of the continued functional resilience of important coastal ecosystems (Estes et al., 2011). On coastlines that have been dominated by heavy human extractive use for as long as the Mediterranean has, it is difficult to conceive of fish predatory functions still being ubiquitous and relevant outside the most strictly protected reserves. However, this assumption has rarely been tested at regional scales; our results are an encouraging indication that, despite the considerable trophic downgrading the Mediterranean has experienced with centuries of human use (Pauly *et al.* 1998), predation continues to be a relevant trophic process in its macrophyte communities. To be sure, predation was highest in the only marine reserve we studied (Medes Islands), where predatory fish guilds have increased notably in the last decades (Garcia-Rubies *et al.* 2013). However, a few sites along the coast without the benefit of this careful management (i.e. site F, site C) showed rates very similar to Medes, indicating that locations across the NW Mediterranean were still able to maintain intact higher trophic functions. These sites were characterised by subtidal rocky extensions and outcrops that may attract predators (personal observation); these contingent factors may help determine how predator-prey processes vary across the coastline. Each of these processes is likely governed by a complex suite of factors acting together to determine the relative importance of predatory functions, supply side processes and bottom-up drivers in structuring macrophyte communities. For instance, while predation may be an important agent of population control, urchin populations can themselves be highly dependent on recruitment (Prado *et al.* 2012) and bottom-up processes controlling algal growth (Menge 2000); these factors likely interact in complex ways to determine the capacity of predators to control the system.

What is clear, however, is that benthic predators do not substitute fish as the top trophic agents along this coast, even in sites where fish predation is very low (Site A, Fig. 6.2). The only observation that could potentially indicate a certain level of competitive release is seen at a seasonal level. The fish predatory guild is less active during winter, and bottom predators become more active during these months, especially in seagrass meadows. This increased winter activity may represent a competitive exclusion between predatory groups. Chemotactic benthic predators may take much longer to locate their prey than visual fish predators, and since winter temperatures constrain fish metabolic activity, benthic invertebrates may derive seasonal benefits from this reduced movement (Bonaviri *et al.* 2009) but see also (Farina *et al.* 2014). This competitive exclusion was not observed in sites where fish predation was high throughout the year and the impact of benthic predators was low (i.e. in rocky habitats from Site D and Site F). Clearly though, these appear to represent merely seasonal changes in behavior rather than any real change in community composition, and the overall predominance of fish predators indicates that these systems are not witnessing either a release of benthic meso-predators due to overfishing or competitive exclusion. This contrasts with macrophyte habitats in other fished regions (like Australia) where benthic predators dominate top trophic roles, with fish predators virtually absent (Farina *et al.* 2014). In other coastal ecosystems, notably Caribbean coral reefs, the removal of large predatory fish triggers a distinct meso-predator release (Burkepile & Hay 2007). The apparently low functional replaceability of the predator guild in the Mediterranean highlights the centrality of fish to predation pathways in these systems. This further emphasizes the importance of managing predatory fish against fishing overexploitation if this crucial process is to be preserved.

The intensity of predation we recorded was clearly habitat dependent, even when the seascape was a patchy mosaic dominated by both habitats. Macroalgal habitats received rates of predation at least twice as high as seagrass meadows. This can, in part, be explained by higher habitat use of predators in rocky systems and the taller canopies characteristic of *P. oceanica* meadows. Surprisingly, predation impact in seagrass meadows were disproportionately high compared to predator use of these habitats. This uncoupling between predator numbers and predation impact between habitats indicates that predator-prey interactions may be highly dependent on habitat-specific traits in relation to refuge availability, predator efficiency, prey abundance, and other factors. It is well known that predatory fish abundance and habitat attributes plays a critical and often complex role in determining predation impact in marine ecosystems (Hereu *et al.* 2005; Canion & Heck 2009; Farina *et al.* 2009). These habitat-specific differences in predation have important implications given what we know of the functioning of these ecosystems. Rocky macroalgal communities along the Mediterranean (as in several regions) appear inherently vulnerable to urchin overgrazing and may be subject to functional discontinuities once they switch to urchin barrens (Ling *et al.* 2015). A series of feedbacks – continued scraping by urchins preventing recolonisation by algae, urchin bioerosion of rocks creating their own refugia, etc – may make recovery very difficult past this threshold (Guidetti *et al.* 2003). The generally high levels of predation experienced here may be critical in ensuring that these points of discontinuity are not breached. In contrast, *P. oceanica* meadows may be less "dependent" on top-down control as a process in maintaining ecosystem function (Vergés *et al.* 2008; Prado *et al.* 2011). This may be due to the inherent ability of this seagrass to resist herbivory with its suite of coping mechanisms (Ruiz *et al.* 2009) unless urchin density is particularly high.

Perhaps most interestingly, predation was clearly predictable in both seagrass systems and macroalgal dominated rocky systems. Predation impact was a clear function of refuge availability and fish predators. *D. sargus* has been previously described as the main sea urchin consumer inside marine reserves (Sala 1997; Guidetti 2004); our results confirm that it may be a key agent of top-down control in Mediterranean macroalgal habitats even outside these protected areas. Unfortunately it also underscores the low functional redundancy these systems have, since no species appears to substitute *D. sargus* when its numbers decline. *D. sargus* may be one of few extant species in the Mediterranean capable of breaking the urchin carapace after they reach adulthood although several other fish predators can be important secondary consumers (Sala 1997; Guidetti 2004). This coupling of number and function validates the effectiveness of fishing restrictions in ensuring high predation in areas that require conservation. It is important however, to account for the structural complexity of these sites since, as this study and others indicate, refuge availability can critically mediate predatory-prey interactions in rocky macroalgal systems (Hereu *et al.* 2005; Clemente *et al.* 2012) and in seagrass meadows.

Our results show that fish interactions with their sea urchin prey are still prevalent across the Mediterranean coast despite centuries of human use and are highly dependent on site specificities and habitat characteristics. Given that the release from predatory functions can have cascading effects (e.g. creation of urchin barrens in rocky bottoms), it is critical to maintain and protect these higher trophic functions, particularly where they continue to be important. Identifying these hotspots of functional predation may be an essential first step when establishing new marine reserves to conserve macrophyte communities in temperate seas vulnerable to overgrazing events. This is particularly important given that meso-predators do not appear able to replace fish, even when their numbers decline, as the principal predator in coastal habitats. Our results indicate that, despite a long history of fishing, conserving functional predation may still be achievable outside marine reserves and is critical to ensure the resilience of ecosystems where top down processes still control the structuring of ecological communities.

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7

MAIN CONCLUSIONS

MAIN CONCLUSIONS

Chapter 3. *Immanent conditions determine imminent collapses: nutrient regimes define the grazing resilience of macroalgal communities.* The resilience of macroalgal communities in the Mediterranean is strongly influenced by inherent, region-specific nutrient conditions. Although macroalgal communities in the Mediterranean are universally prone to critical transitions in the wake of increasing sea urchin numbers, communities in nutrient-poor regions (i.e. Mediterranean islands) are less tolerant to increases in herbivore numbers than continental coastal waters, which are richer in nutrients. Two synergistic mechanisms underlie this differential tolerance – compensatory feeding by herbivores and nutrient-mediated algal growth. Determining the location of critical thresholds has important implications for ecosystem management to prevent ecosystem collapse. Understanding the role of nutrients in mediating where these thresholds lie is vital for a more regionally-relevant approach to ecosystem management.

Chapter 4. *Evaluating potential artefacts of tethering techniques to estimate predation on sea urchins.* Piercing sea urchins with the standard tethering technique neither increases mortality nor facilitates prey detectability in most environmental conditions found in the Mediterranean. If the needle employed in the procedure is small, the piercing of the test does not cause mortality under standard conditions of temperature (below 30 ºC) and water turnover rate (in open coastal waters). Moreover, this technique does not increase the prey detectability by the predator guild, independently of predator identity (e.g. benthic or fish). These results validate the use of sea urchin tethering techniques using piercing as a simple, powerful and reliable assay for comparative studies of predation impact.

Chapter 5. *Top-down processes govern sea urchin outbreaks in Mediterranean macrophyte ecosystems.* Sea urchins in shallow macrophyte ecosystems (macroalgal habitats and seagrass meadows) show habitat-specific population regulation, with each habitat limiting urchin populations at different life stages. The principal control is through predation acting directly in both habitats and indirectly in seagrass meadows (probably mediated by migration). The fact that population regulation is clearly habitat-specific suggests that sea urchin populations may be significantly buffered from demographic bottlenecks in connected seascapes. Our results highlight the importance of evaluating the regulatory agents of key structural herbivore populations across complex seascapes to understand and manage these species from reaching outbreak proportions.

Chapter 6. *Hotspots of predation persist outside marine reserves in the historically fished Mediterranean Sea.* Despite a long history of overfishing, fish predation is still a ubiquitous process along the Western Mediterranean. Predation rates remain very high at certain hotspots along the Catalan Coast and are driven primarily by fish abundance and the presence of available refuges. Identifying locations where functional predation still serves to prevent key species outbreaks should be a high priority in guiding conservation actions to guarantee the long-term persistence of benthic vegetation and its associated ecological services even outside the network of marine protected areas.

8

GENERAL DISCUSSION

8. General Discussion

This thesis seeks to improve our understanding of regime shifts in Mediterranean macrophyte ecosystems by i) unveiling mechanisms precipitating these shifts and ii) determining the main population control agents of the sea urchin *Paracentrotus lividus*, a species identified as a key structuring agent of the two shallow macrophyte habitats I studied – rocky macroalgal habitats and *Posidonia oceanica* seagrass meadows. The results of this thesis provide clear insights on how to reduce the associated uncertainty of sudden state shifts and helps identify areas of conservation priority to manage these systems against catastrophic transitions. In the following sections I discuss the factors regulating sea urchin population in macrophyte ecosystems in the Mediterranean and highlight the importance of striving towards a mechanistic understanding of state shifts in order to improve our ability to predict them well before they occur. Additionally, I explore how the data presented in this thesis can be effectively used to build mathematical models capable of predicting the time to barren-formation in macroalgal habitats under different scenarios. While these studies apply specifically to nearshore Mediterranean macrophyte communities, the conceptual approaches I have used have a more general applicability, and the ecosystem responses I document are likely valid for a range of marine temperate ecosystems worldwide.

8.1. Developing sound methods to test hypotheses in the field

Measuring ecological processes and, more specifically, estimating the strength of species interactions is central to understanding community structure and functioning (Paine 1966). Observing and isolating these processes in natural ecosystems is a non-trivial problem and ecologists have developed a suite of experimental techniques to accurately estimate them in field and lab conditions. While using these techniques however, it is critical to be aware of their inherent limitations and potential artefacts to be confident that the results obtained are an adequate reflection of the processes being estimated. The central objectives of this thesis involved the estimation of predation impact on sea urchin populations, using assays developed both for the field and the laboratory. In Chapter 4 I critically examined the effectiveness of one of the most common marking techniques used to assess predation rates in sea urchins worldwide. My results showed that urchin tethering with piercing is reliable and has few procedural artefacts. The technique is remarkably robust and significant procedural mortality (compared with wild, unmanipulated individuals) occurs only in extreme conditions of high temperature and low water renewal. These results are particularly relevant given how important sea urchins are as key structuring agents of macrophyte habitats: marking and tethering methods are standardly used in estimating functional predation on these species without an explicit assessment of the possible associated artefacts of the technique. While tethering techniques are still essentially comparative, with sufficient care, they may serve as very close proxies of absolute (natural) predation rates. For one, tethering should not interfere with the urchin´s natural ability to seek shelter – something that can be addressed merely by ensuring that the tether is sufficiently long. In addition, tethering can artificially modify natural densities. However, in most natural conditions, the assay inflates natural densities by an insignificant fraction, and it is highly unlikely that this could produce noticeable artefacts except when natural urchin densities are extremely low. If these factors are kept in mind and their assumptions made clear, tethering could well serve as an excellent measure of natural predation rates, not merely as a relative index. These procedural tests serve to identify and assess the potential artefacts and assumptions of the techniques we standardly use in our experiments and considerably increase our confidence in the results we get from these methods. Often, these indirect measures are the best we can do in understanding complex processes in even more complex ecosystems, and building faith in their reliability is critical when interpreting them. These simple procedural assessments need to increasingly become part of our experimental toolbox as we strive to understand the underlying processes that structure ecosystems.

8.2. Understanding regime shifts: a priority in a fast-changing world

While ecosystems are naturally dynamic, human actions have historically triggered them to change structurally and functionally at much faster rates than this dynamism would allow. The overharvesting of natural resources and habitat destruction for human use are among the major causes that, together with pollution and climate change, are rapidly modifying ecosystems across the world. There is growing evidence that we are in the midst of the sixth mass extinction, with actual rates of species loss much higher than those in the past (Ceballos et al. 2015). Unlike previous events, this extinction carries an unmistakably human imprint. Marine environments face a set of anthropogenic impacts including overfishing, particularly of top-trophic groups (Pauly et al. 1998), increasing eutrophication affecting temperate and tropical seas (Smith 2003), direct destruction of habitats as our coastlines get increasingly modified, particularly important in the Mediterranean, and several other effects climate change-related effects, including the so-called tropicalisation of temperate waters (Vergés et al. 2014). All these human alterations can have consequences beyond the most directly observable effects, particularly when ecosystems are prone to changes of state. Understanding the mechanisms by which the structure of a given ecosystem changes suddenly and discontinuous due to human alterations becomes critical to maintain and improve the health of marine systems. In this thesis I have described how two of the most prevalent anthropogenic impacts in Mediterranean waters (i.e. overfishing and eutrophication) influence shifts in macrophyte ecosystems. Overfishing has been described as one of the principal drivers of state shifts in macroalgal habitats in shallow Mediterranean areas. Eutrophication is also recognised as the most pervasive human impact in European waters and address this has been the central focus of the European Water Framework Directive over the last decade. Identifying what precipitates shifts in Mediterranean macrophyte communities will help marine managers make science–based decisions in systems much less understood than their terrestrial counterparts.

At present, it is widely accepted that ecosystems might change as a function of the ineraction between several intrinsic and extrinsic factors, both biological and physical (Conversi et al. 2015). I have shown in **Chapter 3** that this clearly true in shallow Mediterranean macroalgal communities. While sea urchin outbreaks are the major factor driving heavy structural changes in these ecosystems, inherent nutrient regimes can determine the resilience of macroalgal states. Macroalgal communities in the Mediterranean are comparable with kelp beds and both are characterised by distinct and well-documented alternative stable states (Filbee-Dexter & Scheibling 2014; Ling *et al.* 2015). However, observations of extreme shifts are not sufficient to demonstrate alternative states as ecosystems may also respond to disturbances by following more continuous non-linear paths (**Chapter 1**). In contrast, alternate states are characterised by discontinuous changes in ecosystem state and hysteretic responses – where ecosystem takes very different trajectories during decline and recovery. While it generally accepted that there are typical difficult to conclusively demonstrate without long-term monitoring, in **Chapter 3**, I show that it is possible to convincingly measure discontinuities and hysteresis with extensive field surveys.

Identifying that our systems are characterised by alternative states with critical thresholds is a necessary first step. Beyond this, it becomes essential to determine where along a gradient of disturbance, these critical thresholds lie. Researchers have been reasonably successful in identifying early warning signals of approaching tipping points, but these approaches have two major shortcomings: on the one hand, they can only warn of an approaching threshold when it is very close – often too close for remedial action; in addition they are only useful in areas where long-term monitoring data is available. In **Chapter 3** I take a completely different approach, attempting to identify the specific mechanisms that trigger these phase shifts. I identified nutrients as a critical factor that modifies the resilience of different stable states, because of compensatory feeding by sea urchins and nutrient-dependent growth of macroalgae. Understanding these processes is very useful since they give us a clear mechanistic framework to predict discontinuous behaviours in macrophyte communities facing herbivory.

Although the resilience of macrophyte ecosystems relies on inherent nutrient regimes, it is clear that the ecosystem stability is ultimately determined by sea urchin grazing pressure. Understanding sea urchin population control was my third objective with the overall aim of reducing the uncertainty underlying macrophyte transitions in the Mediterranean. I first identified a set of potential population controls occurring at different life-stages of the sea urchin including: recruitment, early post-settlement survival, predation on adult urchins and potential migration. I explored these regulatory processes in landscapes that were connected matrices of seagrass and rocky macroalgae - the two principal benthic habitats where sea urchins occur. As I describe in **Chapter 5**, I found a strong variability in settlement, post-settlement survival and sea urchin predation at a regional scale. Perhaps more interestingly, I found that sea urchin populations have habitat-specific regulating agents limiting urchin populations at different life-stages. While predation (top-down control) was the main process directly controlling populations in both habitats, in *P. oceanica* seagrass meadows, other habitat-specific factors (i.e. settlement and migration) were also important in determining adult herbivore populations. Although earlier stages are strongly controlled in seagrass meadows, adult populations of sea urchins were still relatively abundant in meadows, suggesting that migration from neighbouring habitats plays an important role in determining population numbers. This, combined with the fact that population regulation is linked closely to habitat, suggests that sea urchin populations may be significantly buffered from bottlenecks in connected seascapes, which makes this species more resistant to controls. Overall, these results highlight the importance of evaluating the regulatory agents of key structural herbivore populations across seascapes to understand and manage these species from reaching outbreak proportions. It is important to integrate this seascape-level population dynamics of key species (**Chapter 5**) together with an understanding of the mechanisms influencing the resilience of alternative stable states (**Chapter 3**) to improve our understanding of ecosystem behaviour.

8.3. Mapping functional attributes to evaluate macrophyte resilience

Historically fished seas around the world like the Mediterranean are experiencing an extreme trophic downgrading (Estes et al. 2011) as they are increasingly subject to a progressive 'fishing down the food web' (Pauly et al. 1998). This raises serious questions of the continued functioning of critical ecosystems, such as those dominated by macroalgae and seagrasses. However, while most top predators are already practically extinct across much of the coast, this does not necessarily mean that top predatory functions have disappeared with them. In **Chapter 5** and **Chapter 6** I show that predation continues to play an important part in regulating adult sea urchin populations in Mediterranean macrophyte systems. These findings add to several others that highlight how critical top-down control is in both marine and terrestrial systems (Sala & Zabala 1996; Menge 2000; Wolf *et al.* 2007). However, my results show that, apart from predation, other factors (i.e. settlement and migration) are significant in determining adult herbivore populations in specific habitats (i.e. seagrass meadows), highlighting the importance of incorporating seascape approaches to management actions. This is of particular importance in places where predation is low; here, habitat-specific factors like settlement and migration can have major cascading effects on the ecosystem (Cardona et al. 2013).

Acknowledging the importance of top-down processes in structuring ecosystems, marine reserves have increasingly become the principal instrument of management, established to ensure the recovery of biodiversity and biomass of fish predators (Garcia-Rubies et al. 2013). MPAs have proven remarkably effective in conserving and enhancing top-down control even if this does not necessarily spill over outside its boundaries (Pinnegar et al. 2000). However, as my large-scale assessment of urchin predation across the Catalan coast shows (in **Chapter 6**) that predation may still be functionally relevant even outside marine reserves, albeit patchily distributed across the region. I mapped these hotspots of predation along the coast, and showed that predation is highly dependent on fish predator numbers and the availability of refuges for urchin prey. Given that predation links clearly with ecosystem resilience, this provides a framework to map resilience across the coastline, even outside reserves – which represents the vast majority of the coastline. Together with compensatory feeding and nutrient-mediated growth found for regions with relatively high nutrient availability in **Chapter 3**, macrophyte communities in some areas may still be resilient despite centuries of heavy fishing. An important cautionary note however is that the size of predatory fish outside reserves was considerably smaller than inside them (personal observations), indicating that despite the surprisingly positive results, ecosystems may still be vulnerable to a collapse in their predatory function. Further research is urgently required to determine if these declining sizes of predators can continue to sustain the thresholds of functional predation (Sala 1997) required to properly control herbivores in vegetated habitats. In **Chapter 6** I showed how heavily structured habitats like seagrass meadows (i.e. *P. oceanica*) offer protection to benthic herbivore populations like sea urchins that, most probably, actively choose this habitat when possible (**Chapter 5**). I additionally found that benthic predators would not be able to substitute fish predators (mesopredator release hypotheses) in preventing sea urchin outbreaks. Fish continue to be critical in maintaining top-down processes, and there are few alternatives to reducing overfishing in the Mediterranean if we are to properly conserve its ecosystems.

Taken together, these results highlight how important it is to conserve the last remaining pockets where predation by fish still maintains the functionality and services macrophyte systems provide. While marine reserves are undoubtedly successful in protecting and recovering depleted macrophyte states, they represent a mere 1-2% of the global ocean, leaving the rest completely open to fishing and other extractive pressures. Human societies depend heavily on the natural resources and services that nearshore coastal systems provide, and finding innovate means to ensure these resources are sustainably managed is vital. MPAs will always be a critical tool in this endeavour but it is essential to evolve a much larger toolbox of management measures to mitigate ecosystem depletion, based on a better understanding of how our nearshore ecosystems function. Identifying hotspots of active predation may be the first step in preventing ecosystem degradation at regional scales. For one, it could serve to determine locations where conservation actions are more likely to succeed (high predator impact areas). For another, it may help identify places in where collapse might be imminent, allowing managers to initiate remedial action before these systems tip over. We are still in the early stages of understanding our ecosystems in a holistic way, but striving towards this is critical to increase the probabilities of success of marine management actions in order to preserve our natural ecological heritage and the goods and services it provides. This thesis is a modest attempt to contribute towards that goal.

8.4. Modelling ecosystems to improve management actions

Ecosystems shifts are infrequently monitored and, more often than not, their occurrence is examined retrospectively. While academically interesting, these post-hoc analyses rarely translate into anything that managers can use to prevent catastrophic shifts. In my thesis I attempt to show that it is possible to develop field and laboratory experiments to understand the key processes underlying phase shifts. Using a mechanistic approach to determining catastrophic behaviour is particularly helpful since it lends itself to building predictive models of ecosystem response. If it were possible to construct models based on realistic field-obtained data that can help predict phase shifts in Mediterranean macrophyte ecosystems, it would considerably advance the practical management of these systems. Qualitative models are particularly effective in testing and describing ecosystem function because of their high mathematical rigor, testability and realism (Dambacher et al. 2009). This thesis identifies several critical processes governing macrophyte ecosystems in the Mediterranean and could well serve to construct qualitative models to further explore and predict state shifts affecting them (for instance, see Marzloff et al. 2011). However, the results of this thesis go beyond a qualitative description of species interactions, and provide quantitative estimates of some key processes. Although a complete understanding of ecosystem dynamics is rarely possible, the results obtained in this thesis, together with other available information, could be used to build very realistic models to explore phase shifts in macrophyte systems in the Mediterranean (Box 6). Recent studies by Marzloff et al. (2013; 2015) have successfully employed empirical information obtained from kelp bed on the East coast of Tasmania, where climate-related expansion of sea urchins has caused major overgrazing events, to predict the probability of barren occurrence and recovery. I believe that implementing the proposed model (Box. 6) and testing this against observational data (sea urchin population structures) under different scenarios of nutrient regimes and predation impact could serve as a powerful predictive tool for managers. One advantage of this approach is that it allows for realistic predictions of the lead-up time to catastrophic shifts under different scenarios.

8.5. FUTURE PERSPECTIVES

HOW LONG DOES IT TAKE FOR URCHINS TO CREATE BARRENS? AND HOW LONG DOES IT TAKE TO RECOVER?

In this thesis I have been able to disentangle the main processes that regulate sea urchin population dynamics in connected habitats, and determine the main mechanisms driving macroalgal community transitions under different nutrient regimes. This information can help build models, as proposed above, aimed at warning of the proximity of critical thresholds under potential scenarios of predation impact and nutrient regimes. These models require much more refinement before they can be employed. For one, apart from the described effects of nutrients on compensatory feeding and algal growth, the known relationship between nutrient availability and sea urchin settlement also needs to be formally included in the model, to explain why oligotrophic areas have, in general, less recruits that eutrophic ones. For another, the inclusion of size-specific predation (the relationship between prey size and predator size) will allow for a much more realistic approach to describing of the interaction between carnivorous fishes and sea urchins through size-structured models. Developing size structured mathematical models incorporating realistic data for each sea urchin life-stage will help us predict the time to barren formation under different scenarios of predation and nutrient conditions. This can be very useful to predict when ecosystems are approaching critical thresholds and prevent them from tipping over. Additionally, while the hysteresis of the system has been partially addressed in this thesis, more experimental effort is needed to determine the effects of inherent conditions on the recovery pathway.

CAN OUR RESULTS BE APPLIED TO OTHER MACROPHYTE TEMPERATE HABITATS GLOBALLY?

Although more productive and structured, kelp beds in temperate seas are very similar to macroalgal communities in the Mediterranean. Both are vulnerable to barren formation when sea urchin outbreaks occur. In **Chapter 3** I described how inherent nutrient regimes could modify where critical thresholds lay. Temperate seas around the world are in general more productive than the Mediterranean, and thus investigating if results in **Chapter 3** can be extrapolated to kelp beds around the world would be of general interest. This would be particularly important in regions where tropicalisation of ecosystems occurs as a result of global warming. Under these scenarios of tropical herbivores expanding to new areas highly susceptible to grazing pressure, inherently high nutrient regimes may or may not increase the buffer capacity of the newly colonized ecosystems. Considering that herbivore expansions are fast, knowing if nutrient regimes do (or do not) provide this buffer capacity would significantly inform and improve management reaction times.

Does the regional species pool determine recovery capacity?

The diversity-stability hypothesis claims that the stability of an ecosystem is directly to its diversity. In the Mediterranean, macroalgal habitats are multispecific assemblages, with variable degrees of dominance or evenness among species. The susceptibility of these species to grazing varies greatly, and how this "defence diversity" in macroalgae contributes to the stability of the system will be the natural next question to ask. In addition, macroalgal species diversity can also be critical during periods of recovery. The ability of macroalgal species to colonize barrens from the regional species pool will strongly mediate the effectiveness of management interventions aimed at reducing urchin populations and increasing predator numbers. In addition, this will provide insights on the natural probability of shifting between alternative states depending on the regional species pool.

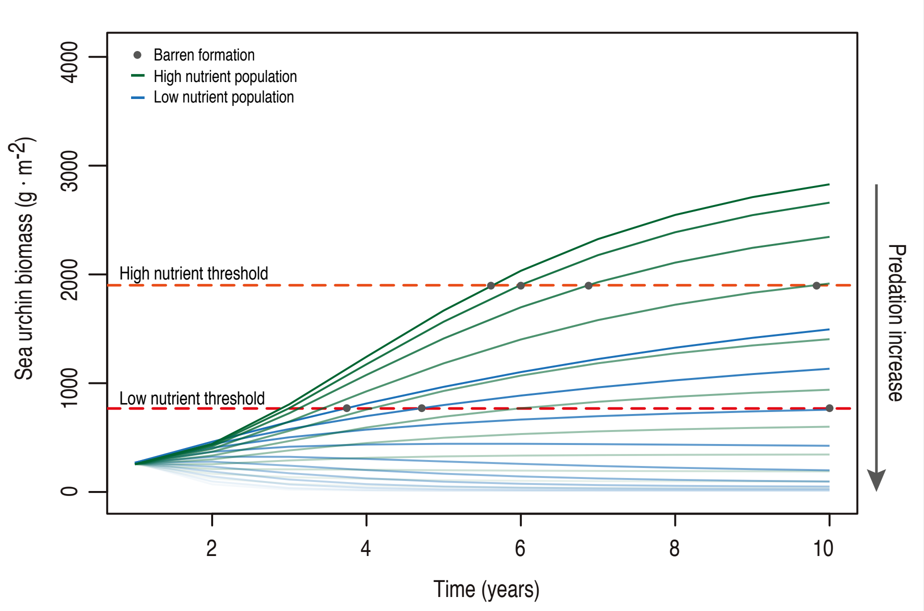
Evaluating ecosystem goods and services

Catastrophic shifts in ecosystems generally occur from a productive and biodiverse state to a degraded low-productivity, low-diversity state. Well-structured communities like macrophyte habitats in the Mediterranean provide a suite of ecosystem goods and services essential not merely for environmental quality and health of the overall marine coastal waters, but also for human wellbeing. It must be remembered that these communities are important nursery habitats guaranteeing the sustainability of many fish and invertebrate marine populations that support local (and international) human demand and the livelihoods of fishing communities. Quantifying the overall goods and services that these critical ecosystems provide will help i) to evaluate ecosystem functions, goods and services threatened by sea urchin outbreaks, ii) help convince governments, policy makers and the general public why it is essential to protect these systems from catastrophically tipping over to unproductive states.

Modeling state shifts

BOX 6

Building up mathematical models based on reliable empirical data could serve as powerful tools to predict the probability of crossing catastrophic thresholds. Here I present some preliminar outputs of the Mediterranean Sea Urchin Population size structured matrix model for *Paracentrotus lividus* (MEDSUP). Using this model based on the ecological data compiled within this thesis I predict the time to barren-formation under a set of different scenarios of high nutrient regimes (green projections) and low nutrient regimes (blue projections) with a determined predation impact. These resutls are an example to illustrate the objectives of the model parametrization and not definitive.



The time predicted and here presented could vary from the final outputs of the MEDSUP model being the first ones more conservative. This is part of undergoing research in collaboration with S. D. Ling and R. L. Selden.

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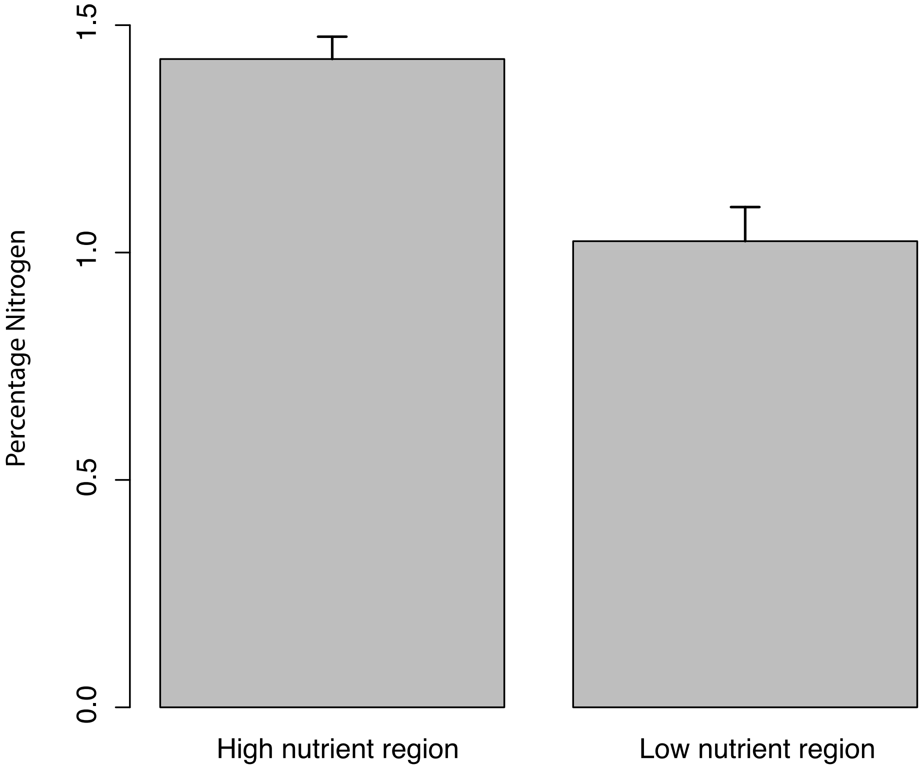
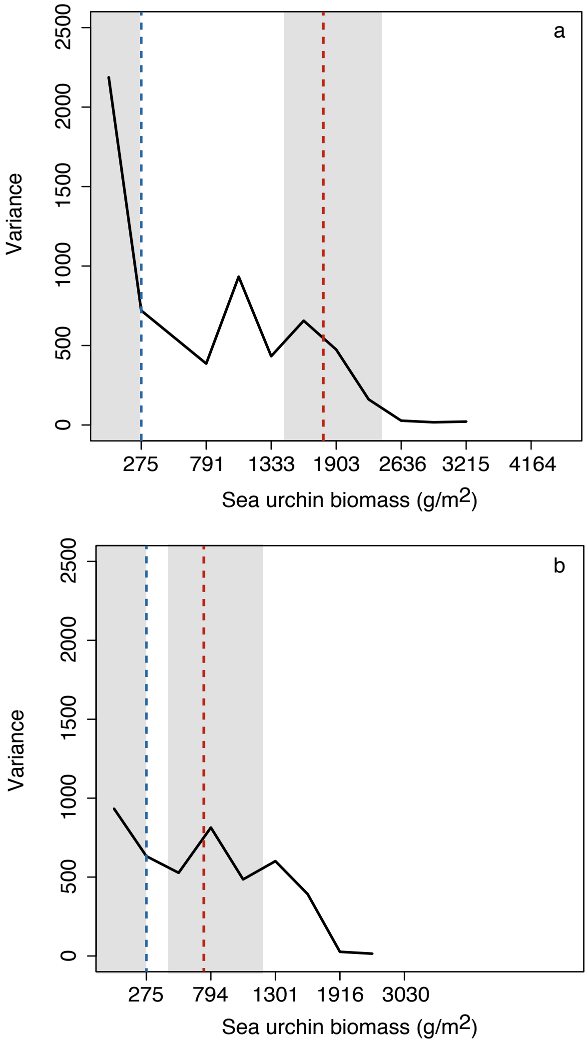
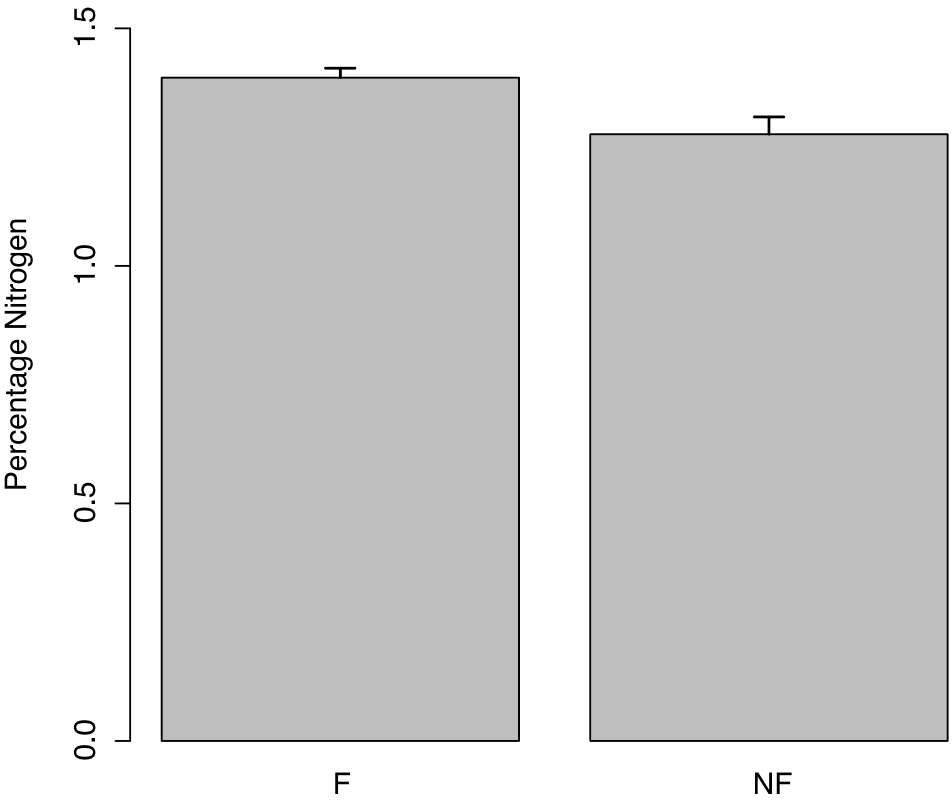
APPENDIX



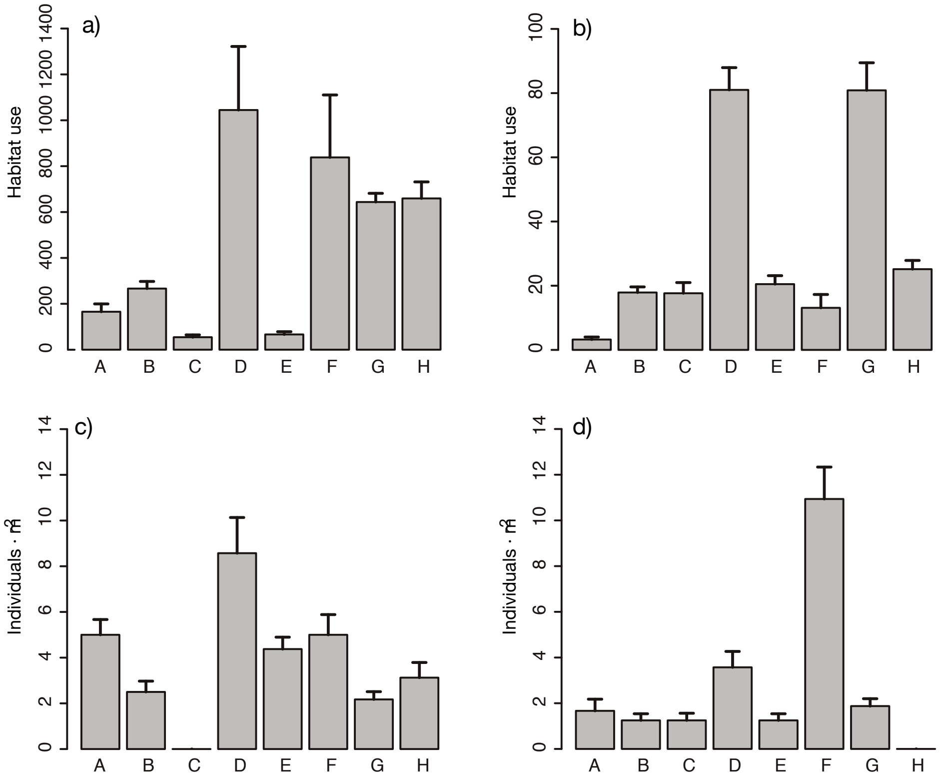
SUPPLEMENTARY 3.1. *Posidonia oceanica* tissues nutrient content (mean + SE) was analysed in each region as an indicator of nutrient availability to confirm differences in nutrient regimes (Roca *et al.* 2015). Significant differences (ANOVA, p-value < 0.03) confirmed that the Catalan coast is more eutrophic than Sardinia (Bosc *et al.* 2004).



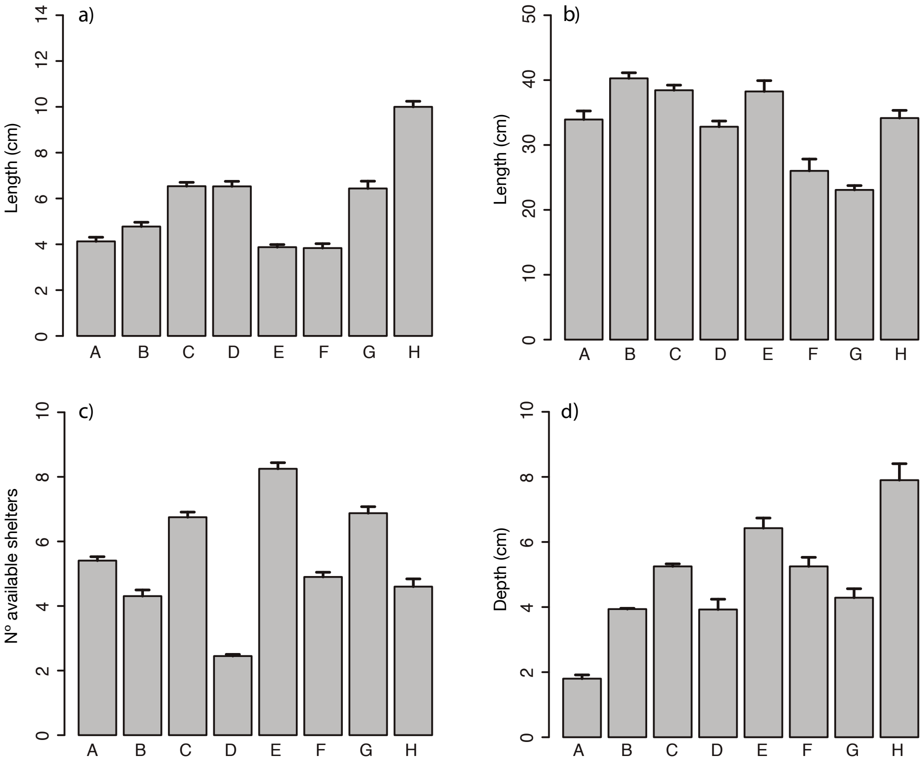
Supplementary 3.2. Analysis of the variance of data on percentage of macroalgal cover and sea urchin biomass in both high nutrient region a) the Catalan coast and low nutrient region b) Sardinia Island. The dashed blue line represents the backward threshold from which the recovery of the macroalgal community is possible (according to a previous peak in variance). The dashed red line represents the forward threshold from which the barren is created (according to Chow test, see methods). The grey shadow around the backward threshold represents the area in which the recovery of the macroalgal community may occur while the grey shadow around the forward process shows the confidence intervals (95%) obtained in the threshold analyses.



Supplementary 3.3. *C. mediterranea* tissue nutrient content (mean + SE, n=15) in fertilized (F) and non-fertilized (NF) conditions. Significant differences indicate fertilizer effectiveness (ANOVA, p-value < 0.01).



SUPPLEMENTARY 6.2. Mean values ± SE (summer and winter) habitat use index U (see text) of the fish predatory guild within a) the rocky habitats and b) *P. oceanica* meadows, and densities of benthic predators within c) rocky habitats and d) *P. oceanica* meadows. We recorded no benthic predators in sites C and H for c) and d) respectively.



Supplementary 6.3. Mean values ± SE (summer and winter) for habitat structural parameters; a) Algal canopy height within the rocky habitats, b) Canopy height of *P. oceanica* leaves, c) number of available shelters and d) depth of the dead matte rhizome layer. Each structural parameter was estimated from 20 random samples per site and season.

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Evaluating potential artifacts of tethering techniques to estimate predation on sea urchins

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ABSTRACT

Measuring the strength of trophic interactions in marine systems has been central to our understanding of community structuring. Sea urchin tethering has been the method of choice to evaluate rates of predation in marine benthic ecosystems. As standardly practiced, this method involves piercing the urchin test, potentially introducing significant methodological artifacts that may influence survival or detection by predators. Here we assess possible artifacts of tethering comparing invasive (pierced) and non-invasive tethering techniques using the sea urchin *Paracentrotus lividus*. Specifically, we looked at how degree of confinement and high water temperature (first order artifacts) and predator guild and size of the prey (second order artifacts) affect the survival and/or detectability of pierced urchins. Our results show that first order artifacts only arise when pierced sea urchins are placed in sheltered bays with confined waters, especially when water temperature reaches extremely high levels. Prey detectability did not increase in pierced sea urchins for the most common predators. Also, test piercing did not alter the preferences of predators for given prey sizes. We conclude that the standard tethering technique is a robust method to test relative rates of sea urchin predation. However, local conditions could increase mortality of the tethered urchin in sheltered bays or in very high temperature regimes. Under these conditions, adequate pierced controls (within predator exclusions) need to be included in assays to evaluate artifactual sources of mortality.

1. Introduction

Measuring the strength of trophic interactions has been central to our understanding of community structure (Estes and Palmisano, 1974; Paine, 1966). Estimating predation and its effects is critical to understand the ability of predators to control prey populations (Estes et al., 2011). This is especially important in marine systems, where such control often trigger cascading effects. While directly measuring rates of predation in real world ecosystems is generally unfeasible, researchers have developed assay techniques to obtain relative estimates that can integrate longer periods of time and avoid observer artifacts (Hairston, 1989). This has been done with the assumption that these techniques can serve, at the very least, as relative indices of actual predation rates that can still give valuable ways to compare ecosystems or track changes through time. In marine systems, measures of predation have relied heavily on tethering techniques, often using sea urchins as a model prey (McClanahan and Muthiga, 1989). In addition, sea urchins are often themselves keystone herbivores in rocky reefs, coral reefs, seagrass meadows, and kelp forests. When sea urchin outbreaks take place, these communities can shift to a less productive and diverse state—termed “urchin barrens” (Pinnegar et al., 2000). In this context, estimating the ability of predators to control urchin numbers is critical to understand ecosystem functioning (Clemente et al., 2007; Farina et al., 2014; Heck and Valentine, 1995; Heck and Wilson, 1987; McClanahan, 1999; McClanahan and Muthiga, 1989; Pederson and Johnson, 2006; Shears and Babcock, 2002). Tethering experiments can provide insight on the degree to which differences in predation rates between different localities contribute to barren formation through cascading effects (Clemente et al., 2008). Nevertheless, these assays are artificial by design and invasive in their manipulation. It has, thus far, been difficult to assess how prone they are to methodological artifacts, precluding thus the evaluation of their reliability.

Tethering techniques have been extensively used in experimental ecology as a tagging and constraining technique to assess predation for different species in various ecosystems and conditions (Aronson, 1987; Herrnkind and Butler, 1986; Shulman, 1985; Watanabe, 1984; Wilson et al., 1990; Witman, 1985). This method consists of marking

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and restraining target prey for a known period of time in natural conditions and documenting mortality. While it is commonly used in invertebrates, it has some disadvantages (Aronson and Heck, 1995; Peterson and Black, 1994). Individuals can be tagged by using different tethering techniques depending on the targeted prey, and some methods that clearly restrain the movement of the tethered individual can substantially increase the encounter rate by certain predators (Barbeau and Scheibling, 1994). The most effective and commonly used tethering methods involve piercing the target organism with a hypodermic needle. For instance, with sea urchins, this involves piercing the test from the oral to the aboral region and passing a monofilament line through the skeleton, which is then used as a tether (Ebert, 1965). Although sufficient care is taken not to affect the gonads inside the carcass, this procedure is still invasive and has a number of potential associated artifacts, which Peterson and Black (1994) have classified as first and second order artifacts. First order artifacts can arise if the wound caused by piercing increases the probability of infections under different environmental conditions; increased temperatures, pollution or nutrient levels, wave flushing, and other local factors could interact strongly to influence the disease susceptibility and survival of sea urchins (Girard et al., 2012; Lafferty et al., 2004), and likely also that of pierced organisms. In addition, second order artifacts could result from the leaking of coelomic fluids into the water column. These fluids could potentially act as chemical clues for certain benthic predators (Sloan and Northway, 1982; Valentinčič, 1973) increasing prey detectability, but not for others that base their predation on a more visual search. These biases can clearly affect the comparative estimates of predation when predator guild differs between sites. Despite these limitations, pierced tethering continues to be the most commonly used method to estimate comparative predation rates or predation risk in marine systems (Aronson and Heck, 1995). To reduce possible artifacts, some authors held tethered urchins in the laboratory for a period of time to allow urchins to heal as monitoring mortality revealed that field survival rates of tethered urchins were higher if they were maintained some days under laboratory conditions prior to using them in field experiments (Fagerli et al., 2014; Shears and Babcock, 2002), but often, this is unfeasible when using this field assay far from laboratories. Still, there have been a few attempts, although incomplete, to evaluate the possibility, magnitude, and sources of biases appearing as a result of first and second order artifacts due to this experimental manipulation (McClanahan and Muthiga, 1989; Shears and Babcock, 2002).

In this study, we investigate possible artifacts of tethering techniques, using the sea urchin *Paracentrotus lividus* (Lamarck), a keystone herbivore in Mediterranean ecosystems. Pierced tethering has been employed extensively in this species and has been used to examine the importance of predation on *P. lividus* (Guidetti and Sala, 2007; Sala and Zabala, 1996), the importance of habitat-engineering species in providing refuge from predation (Farina et al., 2009), and the existence of indirect interactions between herbivores and predators in seagrass systems (Pagès et al., 2012), among others. In this work, we analyze, first, whether test piercing affects prey survival under different environmental conditions (first order artifacts), and second, whether this tagging technique enhances prey detectability under different sizes of the prey or for the most common predators (second order artifacts).

2. Materials and methods

2.1. Sampling design

We designed a series of four separate experiments to test if the pierced tethering method applied to the sea urchin *Paracentrotus lividus* modify mortality rates and prey detectability. For first order artifacts, we conducted two experiments using predator exclusion cages to test the effect of a) degree of confinement (Fig. 1, A) and B) water temperature as factors increasing sea urchin mortality after piercing (Fig. 1, B). For the second order artifacts, we conducted two experiments: c) one to test the effect of pierced tethering on observed predation success for different prey sizes (Fig. 1, C) and the second to test d) the effect of pierced tethering in modifying prey detectability as a function of the predator guild (fish, gastropods, and sea stars, Fig. 1, D).

For all experiments, pierced urchins (P) where threaded according to the common methodology described for the target sea urchin species (Sala and Zabala, 1996). Unpierced urchins (UP) were used for the first order artifacts as controls. For the second order artifacts, unpierced urchins (UP) were restrained with a line directly wrapped around the sea urchin body twice and then tied to a weight or to experimental cages. This tagging method is useful to tether sea urchins for short

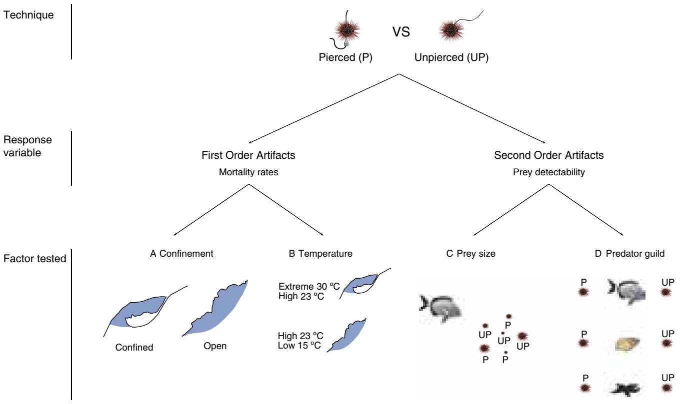


Fig. 1. Schematic description of the methodology used and the experiments developed to test tethering artifacts. Experiments are classified according to the explored variable A) confinement degree (under fixed temperature conditions; 23 °C), B) seawater temperature (for confined and opened conditions) for which we analyzed the prey mortality, and C) prey size, and D) predator guild type for which we analyzed prey detectability. In section A) and B), we present a drawing of the study sites; coastline (black line) and the water (shadow area) to show differences in the confinement degree of each site.

periods of time and keep them within the experimental area but is not useful for longer experiments as they manage to escape. All the experiments were conducted under field conditions rather than in the laboratory, since tethering methods are only relevant for *in situ* experiments and measures of predation rates.

2.2. First order artifacts/direct sea urchin mortality: confinement and water temperature

2.2.1. Confinement effect

We chose a site representative of an open Mediterranean coast (Fenals, 41° 41' 23" N, 02° 49' 42" E, total surface ca. 92 ha, aperture distance ca. 2500 m, maximum summer temperature ~23 °C) and an area with limited exchange with the open sea (Alfacs Bay, 40° 36' 38" N, 00° 39' 37" E, total surface ca. 3000 ha, aperture distance ca. 2500 m, maximum summer temperature ~30 °C) to assess the confinement effect (Fig. 1, A). Mortality was measured for 48 pierced urchins, 32 of them were placed in Alfacs Bay and the other 16 in Fenals under the same temperature conditions (23 ° C). Thirty-two unpierced urchins were used as controls, 16 in each site. All pierced and unpierced sea urchins were placed in groups of 4 in 1.5 cm mesh exclusion cages (50 cm × 20 cm × 20 cm) at 1 m depth in Alfacs Bay and at 8 m depth in Fenals and tracked for 12 days. We test for the significance of differences in sea urchin mortality between treatments (pierced and unpierced) in the two sites using one-way ANOVA in the statistical software R (R Development Core Team, 2013).

2.2.2. Water temperature effect

For the temperature experiment (Fig. 1, B), we compared pierced sea urchins with unpierced ones (controls) during high temperature conditions in summer (23 °C) and during low temperature conditions in spring (15 °C) in Fenals. We also checked the effect of extreme high conditions of temperature that occur only in very confined areas in the Mediterranean. To do this, we compared mortality of pierced and unpierced urchins in extreme high temperatures in summer (30 °C) and high temperatures in spring (23 °C) in Alfacs Bay. A set of 16 sea urchins was pierced using a 0.8 mm needle and 16 unpierced sea urchins were used as controls in each site and temperature condition. Urchins were placed in 1.5 cm mesh exclusion cages in groups of 4 (50 cm × 20 cm × 20 cm) at 1 m depths in Alfacs Bay and at 8 m depths in Fenals and tracked for 12 days. We used one-way ANOVA to test for differences in mortality between treatments (pierced and unpierced) in the two sites and temperature conditions.

2.3. Second order artifacts/prey detectability: prey size and predatory guild

2.3.1. Prey size effect

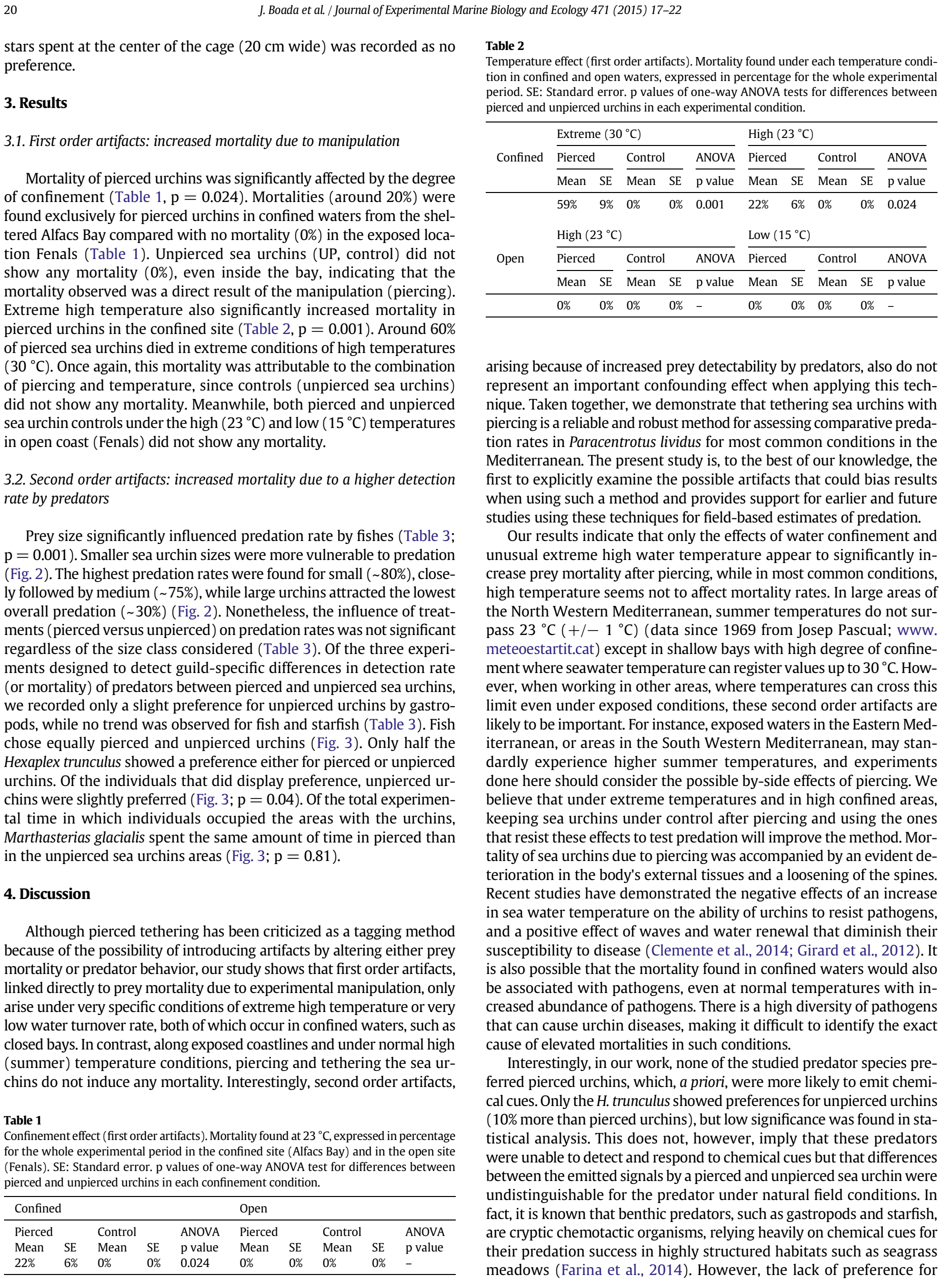
To test if the treatment (pierced versus unpierced) influenced predation success by fish (Sala, 1997) depending on prey size (small; 1–3 cm diameter without spines, TD, medium; 3–5 cm TD, and large individuals >5 cm TD) (Fig. 1), we performed an experiment in Medes Islands MPA (42° 02' 47" N, 03° 13' 11" E) where predation impacts on sea urchin populations is known to be very high (Hereu et al., 2005). The experiment was conducted during daylight hours because nocturnally active urchin-feeding fishes are uncommon (Sala, 1997; Savy, 1987). A total of 90 sea urchins were used for this experiment: 30 small, 30 medium, and 30 large. Test diameters were measured with a caliper to determine size classes. Pierced and unpierced urchins were tethered to a 1 kg weight and randomly distributed on a macroalgal habitat (5 m depth) within a total rocky area of around 330 m^2 to avoid transmission of the chemical clues due to currents or waves action. The principal predator of this urchin is *Diplodus sargus* (Sala, 1997); large individuals of this species can consume the entire range of *P. lividus* sizes, while smaller fishes are potential consumers of only juvenile sea urchins (Sala, 1997). The experiment was repeated on 3 different days; on each day a total of 5 individuals of each size class were pierced (P) and other 5 unpierced (UP) were used as controls. Three experienced divers were responsible for visual observations to track the experiment from a certain distance to avoid biasing the information. Each experiment was terminated when 50% of the total initial urchins (P + UP) had been eaten by fish (average time around 45 minutes), and the percentage of both P and UP eaten in each size class were recorded. Generalized Linear Models (GLMs) with binomial distributions were used to evaluate predation impact. The state of the urchin (dead/alive) was analyzed as the response variable. Explanatory variables selected were 'size' (S, M, L) and 'treatment' (P/UP). These analyses were developed using the R software (R Development Core Team, 2013)

2.3.2. Predatory guild effect

Prey detectability of pierced (P) and unpierced (UP) sea urchins was assessed for the principal urchin predators (fish, gastropods, and starfish, Fig. 1, D) (Boudouresque and Verlaque, 2001). We used guild-specific methods to assess prey detectability by each one of these organisms according to response times and behaviors (see below). We used Wilcoxon matched paired tests using R software (R Development Team, 2013) in order to determine prey detectability for fish and benthic predators (see below).

Prey detectability by fish: To test if pierced tethering enhances prey detectability by fish, predation was monitored on 24 sea urchins (3–5 cm of test diameter, TD), of which 12 were pierced and 12 were unpierced, using underwater video cameras. We used medium size urchins as this is the main targeted size by fish predators (Sala, 1997). The experiment was done in Medes Islands MPA, where the density of predatory fish is very high. GoPro Hero 2 (10MP, FullHD) cameras were placed in front of pierced and unpierced urchins randomly distributed in a rocky macroalgal habitat. The experiment was done on three different days (8 cameras were placed each sampling day). For each video, predator species were identified, and the time of first attack was measured as a proxy of detectability, with shorter attack times indicating faster detection.

Prey detectability by gastropods and starfish: To test if pierced tethering affects prey detectability by benthic invertebrate predators, experiments were conducted at locations where gastropods (*Hexaplex trunculus*) and starfish (*Marthasterias glacialis*) were abundant (Alfacs Bay and Fenals, respectively). Predation rates of these predators are very low, so rather than depending on random, low-probability encounter rates, we placed one predator and two sea urchins (one pierced and one unpierced) into 1.5 cm mesh cages and evaluated the detectability of each predator for each type of urchin. We did prior assessments to analyze mobility of the benthic predators to determine the size of the cages and the variable to be measured. We observed that *M. glacialis* followed a less directional path compared with *H. trunculus* that presented a more ballistic movement to the prey. According to the predator behavior, we used different cage sizes and different variables to test preferences for pierced and unpierced sea urchins. For *H. trunculus*, we deployed 20 cages measuring 50 × 20 cm in Alfacs Bay, while for *M. glacialis*, we used 6 cages of 100 × 30 cm, deployed in Fenals. One pierced and one unpierced sea urchins (3–5 cm TD) were placed at each side of the cage while the benthic predator was placed in the center. The side for the pierced and the unpierced urchins was randomly selected for each trial to avoid biases to a particular direction due to currents or waves. For the *H. trunculus* experiments, prey preference was estimated as the number of times the predator was found at each of the sides of the cage that had either a pierced or an unpierced urchin after 30 minutes of visual observation. We expressed the variable as a percentage of the total number of observations. If the gastropod remained at the center of the cage (10 cm wide), it was recorded as no preference and not included in the analyses. For the *M. glacialis* experiments, we estimated prey preference by video recording the time the predator spent in the cage area near the pierced or the unpierced urchin, expressed as percentage of total time in the cage. The time that sea



stars spent at the center of the cage (20 cm wide) was recorded as no preference.

3. Results

3.1. First order artifacts: increased mortality due to manipulation

Mortality of pierced urchins was significantly affected by the degree of confinement (Table 1, p = 0.024). Mortalities (around 20%) were found exclusively for pierced urchins in confined waters from the sheltered Alfacs Bay compared with no mortality (0%) in the exposed location Fenals (Table 1). Unpierced sea urchins (UP, control) did not show any mortality (0%), even inside the bay, indicating that the mortality observed was a direct result of the manipulation (piercing). Extreme high temperature also significantly increased mortality in pierced urchins in the confined site (Table 2, p = 0.001). Around 60% of pierced sea urchins died in extreme conditions of high temperatures (30 °C). Once again, this mortality was attributable to the combination of piercing and temperature, since controls (unpierced sea urchins) did not show any mortality. Meanwhile, both pierced and unpierced sea urchin controls under the high (23 °C) and low (15 °C) temperatures in open coast (Fenals) did not show any mortality.

3.2. Second order artifacts: increased mortality due to a higher detection rate by predators

Prey size significantly influenced predation rate by fishes (Table 3; p = 0.001). Smaller sea urchin sizes were more vulnerable to predation (Fig. 2). The highest predation rates were found for small (~80%), closely followed by medium (~75%), while large urchins attracted the lowest overall predation (~30%) (Fig. 2). Nonetheless, the influence of treatments (pierced versus unpierced) on predation rates was not significant regardless of the size class considered (Table 3). Of the three experiments designed to detect guild-specific differences in detection rate (or mortality) of predators between pierced and unpierced sea urchins, we recorded only a slight preference for unpierced urchins by gastropods, while no trend was observed for fish and starfish (Table 3). Fish chose equally pierced and unpierced urchins (Fig. 3). Only half the *Hexaplex trunculus* showed a preference either for pierced or unpierced urchins. Of the individuals that did display preference, unpierced urchins were slightly preferred (Fig. 3; p = 0.04). Of the total experimental time in which individuals occupied the areas with the urchins, *Marthasterias glacialis* spent the same amount of time in pierced than in the unpierced sea urchins areas (Fig. 3; p = 0.81).

4. Discussion

Although pierced tethering has been criticized as a tagging method because of the possibility of introducing artifacts by altering either prey mortality or predator behavior, our study shows that first order artifacts, linked directly to prey mortality due to experimental manipulation, only arise under very specific conditions of extreme high temperature or very low water turnover rate, both of which occur in confined waters, such as closed bays. In contrast, along exposed coastlines and under normal high (summer) temperature conditions, piercing and tethering the sea urchins do not induce any mortality. Interestingly, second order artifacts, arising because of increased prey detectability by predators, also do not represent an important confounding effect when applying this technique. Taken together, we demonstrate that tethering sea urchins with piercing is a reliable and robust method for assessing comparative predation rates in *Paracentrotus lividus* for most common conditions in the Mediterranean. The present study is, to the best of our knowledge, the first to explicitly examine the possible artifacts that could bias results when using such a method and provides support for earlier and future studies using these techniques for field-based estimates of predation.

Table 1
Confinement effect (first order artifacts). Mortality found at 23 °C, expressed in percentage for the whole experimental period in the confined site (Alfacs Bay) and in the open site (Fenals). SE: Standard error. p values of one-way ANOVA test for differences between pierced and unpierced urchins in each confinement condition.

Confined					Open				
Pierced		Control		ANOVA	Pierced		Control		ANOVA
Mean	SE	Mean	SE	p value	Mean	SE	Mean	SE	p value
22%	6%	0%	0%	0.024	0%	0%	0%	0%	–

Table 2
Temperature effect (first order artifacts). Mortality found under each temperature condition in confined and open waters, expressed in percentage for the whole experimental period. SE: Standard error. p values of one-way ANOVA tests for differences between pierced and unpierced urchins in each experimental condition.

	Extreme (30 °C)					High (23 °C)				
Confined	Pierced		Control		ANOVA	Pierced		Control		ANOVA
	Mean	SE	Mean	SE	p value	Mean	SE	Mean	SE	p value
	59%	9%	0%	0%	0.001	22%	6%	0%	0%	0.024
	High (23 °C)					Low (15 °C)				
Open	Pierced		Control		ANOVA	Pierced		Control		ANOVA
	Mean	SE	Mean	SE	p value	Mean	SE	Mean	SE	p value
	0%	0%	0%	0%	–	0%	0%	0%	0%	–

Our results indicate that only the effects of water confinement and unusual extreme high water temperature appear to significantly increase prey mortality after piercing, while in most common conditions, high temperature seems not to affect mortality rates. In large areas of the North Western Mediterranean, summer temperatures do not surpass 23 °C (+/− 1 °C) (data since 1969 from Josep Pascual; www.meteoestartit.cat) except in shallow bays with high degree of confinement where seawater temperature can register values up to 30 °C. However, when working in other areas, where temperatures can cross this limit even under exposed conditions, these second order artifacts are likely to be important. For instance, exposed waters in the Eastern Mediterranean, or areas in the South Western Mediterranean, may standardly experience higher summer temperatures, and experiments done here should consider the possible by-side effects of piercing. We believe that under extreme temperatures and in high confined areas, keeping sea urchins under control after piercing and using the ones that resist these effects to test predation will improve the method. Mortality of sea urchins due to piercing was accompanied by an evident deterioration in the body's external tissues and a loosening of the spines. Recent studies have demonstrated the negative effects of an increase in sea water temperature on the ability of urchins to resist pathogens, and a positive effect of waves and water renewal that diminish their susceptibility to disease (Clemente et al., 2014; Girard et al., 2012). It is also possible that the mortality found in confined waters would also be associated with pathogens, even at normal temperatures with increased abundance of pathogens. There is a high diversity of pathogens that can cause urchin diseases, making it difficult to identify the exact cause of elevated mortalities in such conditions.

Interestingly, in our work, none of the studied predator species preferred pierced urchins, which, *a priori*, were more likely to emit chemical cues. Only the *H. trunculus* showed preferences for unpierced urchins (10% more than pierced urchins), but low significance was found in statistical analysis. This does not, however, imply that these predators were unable to detect and respond to chemical cues but that differences between the emitted signals by a pierced and unpierced sea urchin were undistinguishable for the predator under natural field conditions. In fact, it is known that benthic predators, such as gastropods and starfish, are cryptic chemotactic organisms, relying heavily on chemical cues for their predation success in highly structured habitats such as seagrass meadows (Farina et al., 2014). However, the lack of preference for

Table 3
Second order artifacts analyses. Generalized Linear Models (GLM) analysis for predation according to treatment (pierced and unpierced) and size (juveniles, young adults and adults). We present the initial complete model and the selected one after a stepwise process (AIC criterion). Wilcoxon matched paired test for preferences of each predator guild (fish, gastropods, and sea stars). Significant p values are presented in bold for each of the analyses.

Analyses	Type of artifact	Model	Selected	Effects	Chisq	p
GLM	Predation	Status ~ Treatment * Size (binomial)	Status ~ Size (binomial)	Size	25.814	**0.001**
				Treatment	0.370	0.543
Wilcoxon	Preferences	Fish	–	–	–	0.962
		Hexaplex trunculus	–	–	–	**0.037**
		Marthasterias glacialis	–	–	–	0.809

pierced urchins indicates that second order artifacts are minimal for fish and benthic predators. This was also true for the main fish predator observed in video trials; the prey detection time of the sparid *Diplodus sargus* was not influenced by piercing. Once the urchin was detected and preyed on, several other fish species were attracted to the kill, and scavenged of it (i.e. *S. aurata*, *D. vulgaris*, and *L. merula*). This has been previously described in other studies in the Mediterranean (Guidetti, 2004; Sala and Zabala, 1996).

Fish did not display a preference for pierced urchins in any of the size classes. This suggests that fish predation success under field conditions is primarily driven by visual cues and they are not sensitive to any potential enhanced chemical cues derived from puncturing the urchin. Our video analysis shows that there was no difference in prey detection time between pierced and unpierced urchins, confirming this pattern. It has been previously shown that predator–prey interactions between sea urchins and its fish predators are size dependent (Sala and Zabala, 1996). Smaller sizes have a larger predator guild (Guidetti, 2004), and they typically rely on finding adequate refuge to escape predation (Sala and Zabala, 1996). While larger urchins have fewer available refuges, their size itself serves as a refuge, making them invulnerable to all but the largest extant fish predators.

To summarize, our results show that, at least for the tethering method most commonly used to estimate rates of urchin predation, artifacts are negligible in most standard environmental conditions in the

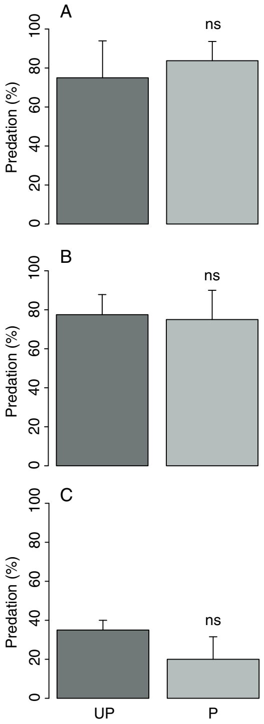


Fig. 2. Predation rates (%) for pierced (P) and unpierced (UP) sea urchins (mean values +/− SE) according to prey size classes A) small (1–3 cm test diameter; TD), B) medium (3–5 cm TD), and C) large (>5 cm TD). Differences between pierced and unpierced sea urchins were non-significant in all cases (Table 2).

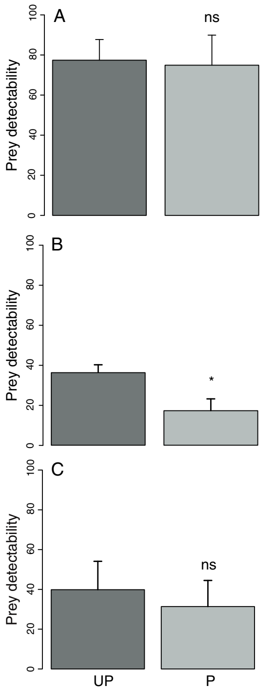


Fig. 3. Prey detectability (mean values +/− SE) of unpierced (UP) and pierced (P) urchins by each predator guild; A) predation impact by *Diplodus sargus*, B) percentage of detection times for *Hexaplex trunculus*, and C) percentage of time preference for *Marthasterias glacialis*.

Mediterranean (open coast with low to moderate water residence times and normal high temperatures). Thus, except for uncommon conditions (extreme temperatures, high water confinement), we can confirm that pierced tethering is a very useful tool to mark individuals of this sea urchin species in the Mediterranean when assessing predation rate for comparative purposes (e.g. between regions and treatments, between different predators or to evaluate the effectiveness of MPAs). This is encouraging given the critical importance of assessing predation in marine benthic ecosystems, many of which are strongly controlled by top-down processes, often showing non-linear responses to changes in predation rates. Whether this method is a useful test of absolute (natural) rates of predation will depend on the ability of urchins to properly find refuges as they would if they were not tethered, on non-altering their attachment strength and on maintaining the relative sea urchin abundances at the study sites. In fact, this technique can potentially alter encounter rates between certain predators and the tagged prey if its movement is strongly limited (Barbeau and Scheibling, 1994). Nevertheless, in this study, we observed that even for comparative studies, it is important to account for the environmental conditions at sites in which the experiment will be deployed to effectively apply this technique, since high temperatures and water residence times could potentially bias the results. In conclusion, we consider that the tethering method used to evaluate comparative predation impacts on the sea urchin *P. lividus* in the Mediterranean is a robust technique useful to provide accurate results and that ecological biases in measuring predator–prey interactions are negligible at least in the most common conditions. Our work confirms the robustness of pierced tethering as a valuable technique in the marine field ecologist's toolbox to measure essential functional rates that shape communities and ecosystems.

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Hotspots of predation persist outside marine reserves in the historically fished Mediterranean Sea

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ABSTRACT

The Mediterranean Sea has sustained historically high levels of fishing since pre-Roman times. This once-abundant sea has witnessed major declines in apex predators, now largely restricted to isolated pockets within marine reserves. This depletion could critically impact macrophyte communities that are strongly structured by top-down processes. We evaluated rates of predation on the sea urchin *Paracentrotus lividus*, a key herbivore of macroalgal and *Posidonia oceanica* seagrass seascapes, across a large stretch of the Western Mediterranean coastline. Fish predation was generally higher inside reserves, but was equally high at several locations outside these boundaries. Although critically low at some locations compared to reserves, predation was functionally ubiquitous in most habitats, seasons and sites. Fish were still primarily responsible for this predation with no clear evidence of meso-predator release. Macroalgal habitats were consistently subject to higher predation than in seagrass meadows, functionally critical given the vulnerability of macroalgal systems to overgrazing. Predation hotspots were clearly associated with high fish predator numbers and low refuge availability. Taken together, these results suggest that long-term overfishing may not necessarily reflect a complete loss of trophic function. Pockets of fish predation may still persist, linked to habitat complexity, predator behavioral adaptations and landscape-level features. Given the essential role top-down control plays in macroalgal communities, regulating fishing at these predation hotspots is vital to effectively conserve habitats from future hysteretic shifts. Even historically fished seas may retain areas where trophic function persists; identifying these areas is critical to preserving the remaining ecological integrity of these coastlines.

1. Introduction

One of the clearest signatures of the increasing human imprint on the biosphere is the gradual weakening of trophic processes as top predators decline from natural ecosystems under the combined onslaught of direct extraction and habitat loss (Ripple et al., 2014). Predation is a critical agent of community structuring (Hairston, Smith, & Slobodkin, 1960); the depletion of key predators leaves both terrestrial and marine ecosystems increasingly prone to catastrophic and often hysteretic collapses from which recovery can be protracted. Marine macrophyte communities are particularly susceptible; uncontrolled by predation, marine herbivores can undergo major population explosions, overgrazing macrophyte-dominated ecosystems (Kempf, 1962). In a classic example, otters have been identified as principal structuring agents of kelp communities in the Eastern Pacific by regulating urchin populations (Tegner & Dayton, 2000). Similarly, the structuring of Western Mediterranean macrophytes appears to be strongly mediated by top-down control of urchins by fish predators (Pinnegar et al., 2000).

Marine ecosystem managers have long recognized the importance of conserving higher trophic functions, and regulating fishing of top predators has been the instrument of choice in managing nearshore ecosystems (Estes et al., 2011). There has been a growing call to expand networks of marine reserves and impose fishing restrictions to protect key predators and enhance the natural resilience of the ecosystems they structure (Pinnegar et al., 2000). This is predicated on the assumption that fish predator numbers link well with rates of predation, and that healthy predator populations will ensure their functional roles within the ecosystem (Clemente, Hernandez, Rodríguez, & Brito, 2010). There is growing evidence demonstrating that marine reserves have been largely effective in reversing the direct and indirect effects of trophic decline (Shears & Babcock, 2002), and they clearly enhance ecosystem functioning. However, it is becoming increasingly clear that predation is an inherently dynamic process, and predator–prey

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interactions can vary considerably across the seascape. The distribution and densities of predators and prey within the mosaic may be influenced by recruit supply, which may, in turn, be mediated by habitat differences (Hereu, Zabala, Linares, & Sala, 2004). Independent of numbers, predator–prey interactions may be strongly driven by how both predators and their prey use these habitats (Farina et al., 2014). These habitat-specific factors may also interact in complex ways making predator–prey interactions often difficult to predict. Both fish predators and their prey may modify their behaviors in relation to each other's presence, the abundance of conspecifics, the availability of refugia and the configuration of the habitat within the larger seascape. For instance, habitat structural complexity, by modifying the presence of prey refugia is fundamental in determining predation rates and, in turn, prey population structures (Farina, Tomas, Prado, Romero, & Alcoverro, 2009; Hereu, Zabala, Linares, & Sala, 2005). Moreover, predators may also be implicated in complex indirect interactions in macrophyte communities; fish herbivores, by reducing the leaf canopy of macrophyte communities, can enhance fish predation on urchin herbivores by reducing refuge availability (Pagès et al., 2012). Further, a reduction of top predators can sometimes lead to the competitive release of benthic meso-predators that may potentially compensate rates of functional predation experienced by the system (Levi & Wilmers, 2012). This can also be highly habitat dependent since every system could be host to a very different suite of predators. Finally, both predators and prey may move between habitats in the mosaic, and predation may be strongly influenced by patterns of habitat connectivity or isolation within the larger seascape (Hitt, Pittman, & Nemeth, 2011).

Two macrophyte habitats dominate the North Western Mediterranean: *Posidonia oceanica* seagrass meadows and shallow macroalgae-dominated rocky habitats, both potentially structured by top-down control of the herbivorous sea urchin *Paracentrotus lividus* (Fig. 1) (Verlaque, 1987). The Mediterranean has been seriously overfished for millennia (Sala et al., 2012), and determining if predation still plays a functional role is essential to planning conservation actions across the region (e.g. creations of marine reserves, management of coastal development). While it is well established that predation intensity is relatively high inside existing protected areas (Sala & Zabala, 1996) it is unclear to what extent this function is conserved beyond their boundaries, although it is generally assumed to be low because of this historically sustained fishing pressure (Guidetti et al., 2010). However, there is little information available on the factors that influence predation in different macrophyte habitats. The decline of fish predators could have triggered a functional substitution by other benthic predators. In addition, given that reserves are principally established to enhance predator numbers, understanding how predation activity is linked to fish predator abundance is critical. To answer these questions, we measured relative rates of sea urchin predation by fish and benthic predators at eight representative locations across a large stretch of the NW Mediterranean coast in both algal communities and seagrass meadows in different seasons. In addition, we attempted to identify if predator habitat use or habitat-specific factors (presence of refuges) can drive functional rates of predation in these dominant macrophyte habitats.

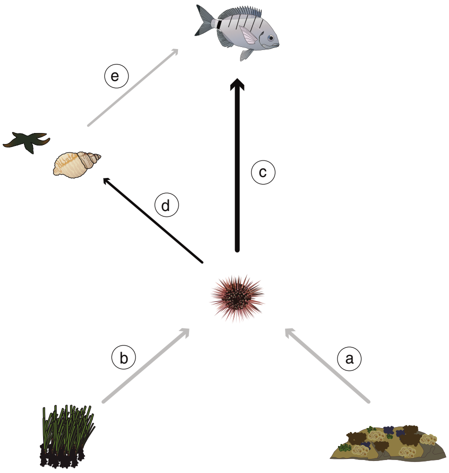

Fig. 1. Principal interactions in Mediterranean macrophyte communities. The sea urchin *Paracentrotus lividus* lives in both seagrass meadows of *Posidonia oceanica* and macroalgal dominated rocky habitats in the Mediterranean. Letters represent trophic interactions; herbivory (a) and (b), and predation (c), (d) and (e). Black arrows show the predator–prey interactions studied in the present work.

2. Materials and methods

2.1. Study system

The shallow seascape of the Western Mediterranean is dominated by rocky macroalgal communities and *P. oceanica* seagrass meadows. Although the sea urchin *P. lividus* is a key herbivore in both habitats, they may differ considerably in their susceptibility to urchin herbivory (Boudouresque & Verlaque, 2001). In macroalgal systems, urchin overgrazing can cause ecosystem barrens from which recovery is often protracted (Pinnegar et al., 2000). Predators likely play a vital role in regulating sea urchin populations (Supplementary, A1), preventing these ecosystem shifts (Guidetti, 2004; Sala, 1997). While *P. oceanica* meadows may experience very similar rates of urchin herbivory, they may cope better with this offtake because of their inherent evolutionary adaptations (Vergés, Pérez, Alcoverro, & Romero, 2008). However, heavy eutrophication could make meadows susceptible to overgrazing (Ruiz, Pérez, Romero, & Tomas, 2009). Several fish species prey on *P. lividus*, and many of these are important commercial and recreational fishery targets (Guidetti, 2006). Additionally, benthic predators including starfish and some gastropods may also be important contributors to sea urchin predation (Boudouresque & Verlaque, 2001).

2.2. Study site and sampling design

The study was conducted along the NW Mediterranean (~600 km). Eight sites were selected along the coast, characterized by shallow seagrass *P. oceanica* habitats and photophilic macroalgae on rocky substrates (Fig. 2). Sites were not randomly selected since all sites required both habitats to be present and at least one unfished reserve was required for the study objectives. Fishing is permitted at all sites except the Medes Island Marine Protected Area, which has been a marine reserve since 1990, and partially controlled in Portlligat since 2006 as part of the Cap de Creus Natural Park but with low fishing regulation. The reserve is characterized by high abundance and biomass of predatory fish (Garcia-Rubies, Hereu, & Zabala, 2013). In each habitat we assessed predation on the sea urchin *P. lividus*, the most important key herbivore in NW Mediterranean macrophyte habitats (Harmelin, Bouchon, Duval, & Hong, 1980). We evaluated predation impact by fish and benthic predators (see below) in each of the selected sites in summer and winter. In addition, we estimated the habitat use by the most important urchin predators, and evaluated habitat characteristics that could constitute an effective predation refuge for the urchin (i.e. canopy height in both habitats, crevices in rocky substrates and bare root-rhizome layer in seagrass meadows) (Orth, Kenneth, Heck, & van Montfrans, 1984). All measurements were recorded within a depth range of 3 to 8 m for both habitats.

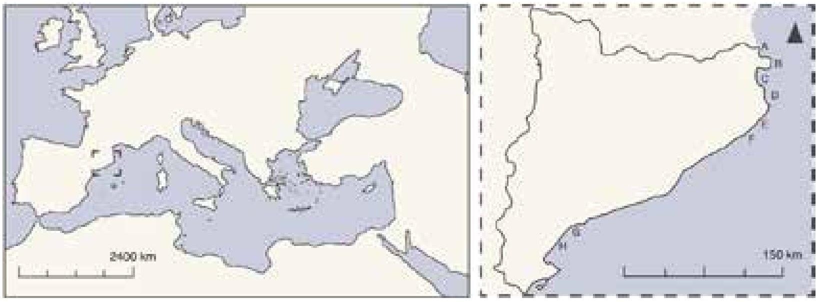

Fig. 2. Map of the Mediterranean Sea showing the study locations within the Catalan Coast. Llançà (A), Portlligat (B), Montgó (C), Medes Islands (D), Giverola (E), Fenals (F), Hospitalet (G) and Ametlla de Mar (H).

2.3. Predation impact

Predation impact was measured using tethering techniques (Aronson & Heck, 1995; Boada et al., 2015) on adult urchins. This comparative method has been used extensively to assess predation in Mediterranean and other temperate ecosystems (Farina et al., 2009; Guidetti & Sala, 2007) and coral reefs (McClanahan & Muthiga, 1989; Pederson & Johnson, 2006). The urchin was harnessed by threading a thin nylon line through its test. The harness was then attached to a brick or metal stake, which was deployed in rocky reefs or meadows. The harness left the urchin a 50 cm radius space to move from its point of attachment. Within this radius, the urchin could actively seek available shelters as it would in natural conditions. To assess tethering-related mortality before the experiment we tracked the survival of caged urchins (to exclude predators); none of the 14 tethered urchins died in the 12 days of this assessment. Twenty urchins were placed in groups of 5 at each site and habitat (total of 4 replicated groups) in two different seasons (i.e. summer and winter). Predation impact was measured 15 days after the start of the experiment. Predation impact was calculated for each group of urchins using the percentage of dead urchins with respect to the initial number (0, 20, 40, 60, 80 and 100% predation). Fish or benthic predators leave clearly distinguishable bites/marks on sea urchin carcasses (Shears & Babcock, 2002) and we carefully examined dead urchin tests to assign predation impact to their respective consumers; fish predator impact (FPI) and benthic predator impact (BPI).

2.4. Predator abundances and habitat use

We measured the habitat use of the most common identified benthic predators of *P. lividus* within the two selected habitats, *Hexaplex trunculus* and *Marthasterias glacialis* (Boudouresque & Verlaque, 2001). We frequently found these benthic predators still attached to the prey. In addition we recorded a few predation events by the starfish *Coscinasterias tenuispina* (S. Farina pers. obs.) and added this species to our sampling. Benthic predator habitat use was estimated through accounting the abundance of benthic predators at each site and habitat along 4 underwater visual belt transects (10 m × 2 m). These were used to calculate benthic predator densities per square meter for each site.

We also investigated the habitat use by fish predators during daylight hours, when predators are most active (Savy, 1987). We used underwater video cameras in each habitat and season (i.e. GoPro Hero 2, 10 Mp) (Harasti, Gallen, Malcolm, Tegart, & Hughes, 2014) to record 4 fixed videos of 20 min within each site. We used underwater buoys to mark a 5 × 5 m area in front of the camera and counted the number of individuals of the principal predators and scavengers of medium and large sea urchins (*Diplodus sargus*, *Diplodus vulgaris*, *Sparus aurata* and *Labrus merula*) (Guidetti, 2004; Sala, 1997) and two more potential predators or scavenger species of the genus *Diplodus* (*Diplodus puntazzo* and *Diplodus cervinus*) were seen traveling through the marked area. This was then multiplied by the total time each species spent within the zone and divided by the total observed area. Habitat use (U) was calculated as follows:

$$\mathrm{U} = \frac{\Sigma(Ti)}{T * A}$$

where *i* is the number of predators observed during the sampling interval, *Ti* is the total time each predator species spent in the area, *A* is the total area observed in each video and *T* is the video recording time (approx. 20 min each).

2.5. Habitat structural parameters

To test the influence of habitat structural parameters on predation rates, we measured habitat complexity (canopy height and number of refuges) in both habitats. We measured canopy height with a measuring tape at 20 random locations within the macroalgal and *P. oceanica* canopy at each site and for each season. In addition, we measured the depth of the total unburied rhizome layer (Prado, Romero, & Alcoverro, 2009) in *P. oceanica* meadows since this is often used as an important refuge by sea urchins in seagrass meadows (Orth et al., 1984). This was done at 20 random points at each meadow with a measuring stick inserted into the unburied matrix. In rocky macroalgal systems we counted the number of potential shelters (crevices and niches that were estimated to harbor an urchin of at least 4 cm diameter) within a 50 cm diameter range at 20 random points per site and season.

2.6. Statistical analyses

3-way ANOVA's tests were performed to establish the effect of habitat, season and site on the following dependent variables: total predation impact, fish predator impact (FPI), bottom predator impact (BPI), sea urchin predator fish habitat use (U) and canopy height. The factors considered were 'site' (8 levels, fixed factor), 'habitat' (2 levels; *P. oceanica* meadows and macroalgal habitats, fixed factor) and 'season' (2 levels; summer and winter, fixed factor). Prior to the analyses we tested for normality (Shapiro–Wilk test) and homogeneity of the variance (Bartlett's test). When assumptions were not met, we set the significance level to $p < 0.01$ as the F statistic is robust despite violation of these assumptions when the sampling size is large enough

(Underwood, 1981). Significant differences between sites were further explored with Tukey HSD post hoc tests.

A continuous approach (GLM) was used to test the significance of the explanatory variables related to fish habitat use (for the three main predators) and habitat structural parameters (presence of habitat-specific refuges, see below) to explain the observed patterns of fish predation (FPI) within each habitat. We could not test this model for bottom predation impact (BPI) as the number of predation events observed was too low to reliably establish any causal link. A General Linear Model (GLM) with a Binomial distribution (and a logarithmic link function) was fitted to test significance. To describe the response of FPI within each habitat a specific analysis was performed according to habitat-specific explanatory variables. For the *P. oceanica* habitats the specific variables included in the model were canopy height (canopy), depth of the dead matte rhizome layer (matte) and the habitat use of fish of the *Diplodus* genus as the main *P. lividus* predators. For the macroalgal dominated rocky habitats the variables included were canopy height (canopy), number of refuges (shelters) and the habitat use of fish of the *Diplodus* genus as the main *P. lividus* predators. We used mean values of habitat use from the 8 replicates (summer and winter together) to better investigate the use in each location and habitat. We started with a full model considering all predator and habitat-associated variables for model selection. We then chose the best model by dropping each effect sequentially and using Akaike's Information Criterion (AIC) and likelihood ratio tests (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). All the statistical analyses were performed using 'lme4' package (Bates, Maechler, Bolker, & Walker, 2014) in the open source software R (Bates et al., 2014; R Development Core Team, 2013).

3. Results

3.1. Spatio-temporal variation in benthic and fish predation rates: habitat, season and site

Predation impact varied substantially between habitats, sites and seasons (Table 1, Fig. 3). Predation impact in rocky habitats was at least double of that measured in *P. oceanica* habitats, while at some sites this difference was even more marked (Table 1, Fig. 3). On average, the predation impact in rocky reef communities was 54.9% (±9.2%), compared with a predation impact of 17.8% (±8.6%) in *P. oceanica* meadows (Fig. 4). The highest predation impact for both habitats was found inside the Medes Islands Marine Reserve in which fishing had been restricted for more than 2 decades. Interestingly though, other locations along the coast, outside any NTA, experienced similar predation impact both in macroalgal communities (post hoc Site $A = B = C < D = F > E = G = H$) and in *P. oceanica* meadows (post hoc Site $A = B = E = F = G = H < C = D$) (Fig. 3). In contrast, predation impact in other sites was very low in both habitats especially in a particular season (e.g. Site A or Site B, Fig. 3). While predation was generally higher in summer than in winter, this was only true for some sites (Table 1, Fig. 3). At sites where predation was very high (i.e. Site D or Site F), the difference between seasons was almost absent; where predation was low, these differences were considerably more marked (Fig. 3). Even outside the marine reserves, fish predators continued to be responsible for the bulk of predation (Fig. 3). In macroalgal habitats the few observed cases of benthic predation occurred outside the reserve. In contrast, in *P. oceanica* habitats predation by benthic predators was more prevalent, although still considerably lower than fish predation. A high peak of benthic predation was observed in the seagrass habitat in winter in Medes Islands as a result of an observed increase in the abundance of the predator starfish *C. tenuispina* (a few tethered sea urchins still had the star attached to the carcass). An interesting pattern is that season was significant when both fish and benthic predation were considered separately, but not together (Table 1, Table 2) since both predators seem to prefer distinct seasons particularly in determinate sites. Predation by fish was significantly higher in summer (41.5% ± 9.7%, Table 2), than in winter (32.3% ± 8.1%) while benthic predation was significantly lower in summer (1.0% ± 0.7%) than in winter (3.5% ± 2.2%).

Table 1
Three-way ANOVAs for total and specific predation impact. p-Values correspond to F-test results. d.f. = degrees of freedom.

Variable	Source of variation	d.f.	p-Value
Total predation	Site	7	<0.001
	Habitat	1	<0.001
	Season	1	0.062
	Site × habitat	7	<0.001
	Site × season	7	<0.001
	Habitat × season	1	0.117
	Site × habitat × season	7	0.523
Predation fish	Site	7	<0.001
	Habitat	1	<0.001
	Season	1	0.009
	Site × habitat	7	<0.001
	Site × season	7	0.005
	Habitat × season	1	0.202
	Site × habitat × season	7	0.494
Predation benthic	Site	7	0.033
	Habitat	1	0.016
	Season	1	0.017
	Site × habitat	7	0.092
	Site × season	7	0.003
	Habitat × season	1	0.601
	Site × habitat × season	7	0.003

3.2. Variation in predator habitat use and canopy height between habitats, sites and season

Predator habitat use also showed clear differences between habitats (Fig. 4) and sites for certain seasons (Table 2, Fig. A2). The index of predator habitat use was more than 10 times higher in rocky habitats than in seagrass habitats, a difference not reflected in the magnitude of predation impact (Fig. 4). This suggests that although predators may use the habitat much less, predation rates continue to be relatively high in seagrass meadows.

As expected, canopy height varied considerably between macrophyte habitats; *P. oceanica* canopies were almost an order of magnitude taller (~35 cm long) than macroalgal dominated habitats (~6 cm height, Fig. 4, Table 2). Canopy height also varied between sites and seasons, although in the same direction for both habitats (Table 2, interaction between site, habitat and season, Fig. A3). Within each habitat, canopy height varied considerably between seasons, with summer canopies consistently taller than winter canopies (average values summer *P. oceanica* > average winter *P. oceanica* > average macroalgal summer and winter), a trend that was more pronounced at some sites (Table 2, interaction between site and season).

Habitat-specific refugia also varied considerably between sites (Fig. A2). In macroalgal habitats the number of refugia differed considerably between sites with a mean maximum value of 9.2 refugia per sampling and a mean minimum of 2 (ANOVA p-value < 0.001). In seagrass habitats as well, the depth of the dead matte rhizome layer could differ by an order of magnitude between sites (mean max. 14.4 cm and min. 0.8 cm; ANOVA p-value < 0.001).

3.3. Determinants of predation rates in rocky and seagrass habitats

The GLM results showed that in rocky habitats, predation rates were best predicted by the number of available shelters ($p < 0.05$) together with the abundance of fish predator-species of the genus *Diplodus* ($p < 0.05$, Table 3). The same pattern was found in *P. oceanica* meadows where the depth of the dead matte rhizome layer ($p < 0.05$) together with habitat use by fish species in the genus *Diplodus* were key determinants of predation impact ($p < 0.05$, Table 3).

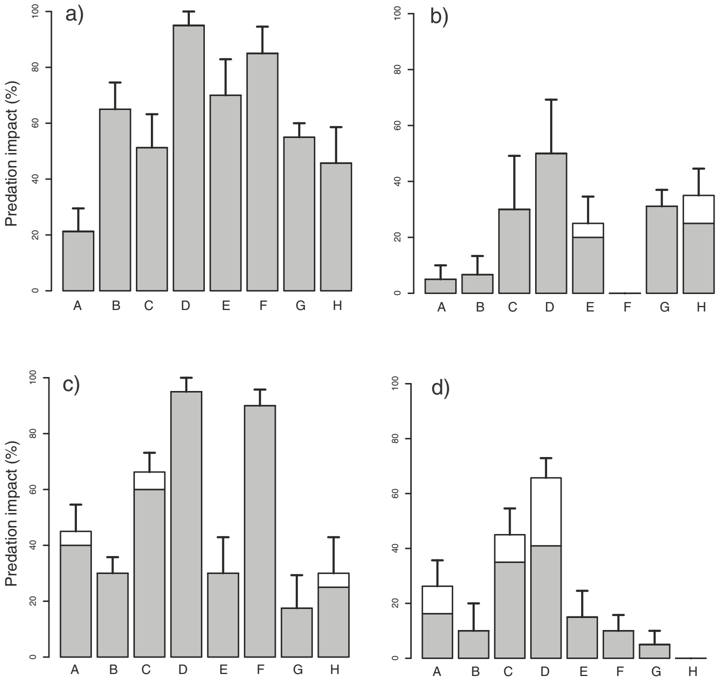

Fig. 3. Mean predation impact on *P. lividus* (%) + SE in a) rocky habitats in summer, b) *P. oceanica* meadows in summer, c) rocky habitats in winter and d) *P. oceanica* meadows in winter. Filled bars represent fish predator impact (FPI) and clear bars represent benthic predator impact (BPI). Total absence of predation (0%) in sites F and H in b) and a) respectively.

4. Discussion

Despite a long history of commercial, artisanal and recreational fishing, fish predation continues to be a ubiquitous process along the North Western Mediterranean coast, albeit with considerable site-level variation in intensity. While the Medes Islands marine reserve (with over two decades of fishing prohibition) unsurprisingly received the highest level of predation in both habitats, locations with no such restrictions also received comparable levels of functional predation. Fish predators continue to be the principal agent of predation in these waters with benthic predators apparently playing a relatively minor role. Perhaps most strikingly, there were strong differences in the intensity of predation between macrophyte habitats, even when separated by just a few meters. Predation impact in macroalgal habitats were at least twice as high as in *P. oceanica* seagrass meadows, even when these habitats were very closely connected, a difference that appears clearly linked to the greater abundance of predators in rocky systems. These predator–prey interactions appeared to be controlled by the same agents in both habitats, the number of available refugia as well as predator habitat use.

The now well-documented specter of trophic downgrading across the world's oceans has raised serious questions of the continued functional resilience of important coastal ecosystems (Estes et al., 2011). In coastlines that have been dominated by heavy human extractive use for as long as the Mediterranean has, it is difficult to conceive of fish predatory functions still being ubiquitous and relevant outside the most strictly protected reserves. However, this assumption has rarely been tested at regional scales; our results are an encouraging indication that, despite the considerable trophic downgrading the Mediterranean has experienced with centuries of human use (Pauly, Christensen, Dalsgaard, Froese, & Torres, 1998), predation continues to be a relevant trophic process in its macrophyte communities. To be sure, predation was highest in the only marine reserve we studied (Medes Islands), where predatory fish guilds have increased notably in the last decades (Garcia-Rubies et al., 2013). However, a few sites along the coast without the benefit of this careful management (i.e. site F, site C) showed rates very similar to Medes, indicating that locations across the NW Mediterranean were still able to maintain intact higher trophic functions. These sites were characterized by subtidal rocky extensions and outcrops that may attract predators (personal observation); these contingent factors may help determine how predator–prey processes vary across the coastline. Each of these processes is likely governed by a complex suite of factors acting together to determine the relative importance of predatory functions, supply side processes and bottom-up drivers in structuring macrophyte communities. For instance, while predation may be an important agent of population control, urchin populations can themselves be highly dependent on recruitment (Prado et al., 2012) and bottom-up processes controlling algal growth (Menge, 2000); these factors likely interact in complex ways to determine the capacity of predators to control the system.

What is clear, however, is that benthic predators do not substitute fish as the top trophic agents along this coast, even in sites where fish

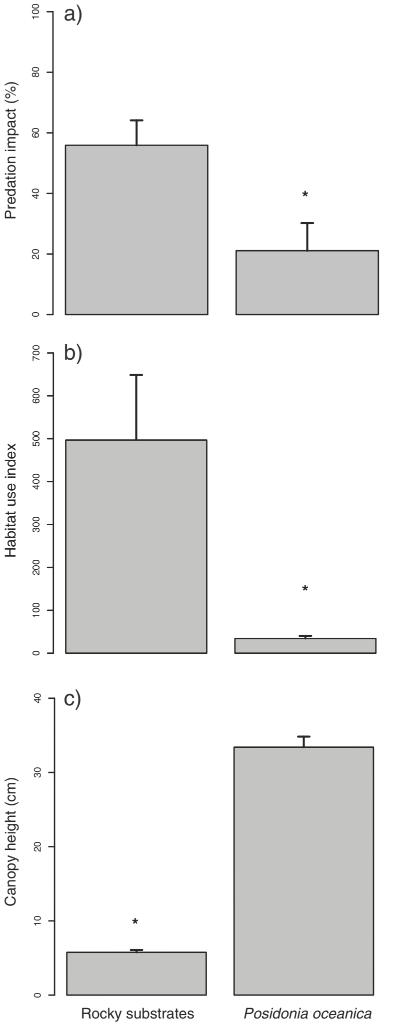

Fig. 4. Percentage of urchins eaten (a), index of habitat use by sea urchin fish predator guild (b) and length of the canopy height (c) in both habitats. Mean values of two seasons ± SE, n = 64. Asterisk indicates significant differences based on ANOVA test.

Table 2
ANOVA analyses for predator habitat use U (see text) and the canopy height. p-Values correspond to those provided by an F-test. d.f., degrees of freedom.

Variable	Source of variation	d.f.	p-Value
Predators	Site	7	<0.001
	Habitat	1	<0.001
	Season	1	0.018
	Site × habitat	7	<0.001
	Site × season	7	0.005
	Habitat × season	1	0.236
	Site × habitat × season	7	0.493
Canopy	Site	7	<0.001
	Habitat	1	<0.001
	Season	1	<0.001
	Site × habitat	7	<0.001
	Site × season	7	<0.001
	Habitat × season	1	<0.001
	Site × habitat × season	6	<0.001

predation is very low (Site A, Fig. 2). The only observation that could potentially indicate a certain level of competitive release is seen at a seasonal level. The fish predatory guild is less active during winter, and bottom predators become more active during these months, especially in seagrass meadows. This increased winter activity may represent a competitive exclusion between predatory groups. Chemotactic benthic predators may take much longer to locate their prey than visual fish predators, and since winter temperatures constrain fish metabolic activity, benthic invertebrates may derive seasonal benefits from this reduced movement (Bonaviri et al., 2009) but see also (Farina et al., 2014). This competitive exclusion was not observed in sites where fish predation was high throughout the year and the impact of benthic predators was low (i.e. in rocky habitats from Site D and Site F). Clearly though, these appear to represent merely seasonal changes in behavior rather than any real change in community composition, and the overall predominance of fish predators indicates that these systems are not witnessing either a release of benthic meso-predators due to overfishing or competitive exclusion. This contrasts with macrophyte habitats in other fished regions (like Australia) where benthic predators dominate top trophic roles, with fish predators virtually absent (Farina et al., 2014). In other coastal ecosystems, notably Caribbean coral reefs, the removal of large predatory fish triggers a distinct meso-predator release (Burkepile & Hay, 2007). The apparently low functional replaceability of the predator guild in the Mediterranean highlights the centrality of fish to predation pathways in these systems. This further emphasizes the importance of managing predatory fish against fishing overexploitation if this crucial process is to be preserved.

The intensity of predation we recorded was clearly habitat dependent, even when the seascape was a patchy mosaic dominated by both habitats. Macroalgal habitats received rates of predation at least twice as high as seagrass meadows. This can, in part, be explained by higher habitat use of predators in rocky systems and the taller canopies characteristic of *P. oceanica* meadows. Surprisingly, predation impact in seagrass meadows was disproportionately high compared to predator use of these habitats. This uncoupling between predator numbers and predation impact between habitats indicates that predator–prey interactions may be highly dependent on habitat-specific traits in relation to refuge availability, predator efficiency, prey abundance, and other factors. It is well known that predatory fish abundance and habitat attributes play a critical and often complex role in determining predation impact in marine ecosystems (Canion & Heck, 2009; Farina et al., 2009; Hereu et al., 2005). These habitat-specific differences in predation have important implications given what we know of the functioning of these ecosystems. Rocky macroalgal communities along the Mediterranean (as in several regions) appear inherently vulnerable to urchin overgrazing and may be subject to functional discontinuities once they switch to urchin barrens (Ling et al., 2014). A series of feedbacks – continued scraping by urchins preventing recolonization by algae, urchin bioerosion of rocks creating their own refugia, etc – may make recovery very difficult past this threshold (Guidetti, Fraschetti, Terlizzi, & Boero, 2003). The generally high levels of predation experienced here may be critical in ensuring that these points of discontinuity are not breached. In contrast, *P. oceanica* meadows may be less “dependent” on top-down control as a process in maintaining ecosystem function (Prado, Collier, Romero, & Alcoverro, 2011; Vergés et al., 2008). This may be

Table 3
Model fitting using GLM for fish predator impact (FPI) inside rocky habitats and *Posidonia oceanica* meadows as a response variable dependent on predator species use of habitat (U) and specific structural parameters of each habitat.

Habitat	Response variable	Full model	Selected model	Effects	df	p-Value
Rocky habitat	Predation fish	~Canopy + shelters + *Diplodus* spp. + *S. aurata* + *L. merula*	~Shelters + *Diplodus* spp.	Shelters	1	0.009
				Diplodus spp.	1	0.008
	Predation benthic	Insufficient data				
Posidonia	Predation fish	~Canopy + matte + *Diplodus* spp. + *S. aurata* + *L. merula*	~Matte + *Diplodus* spp.	Matte	1	0.012
				Diplodus spp.	1	0.001
	Predation benthic	Insufficient data				

due to the inherent ability of this seagrass to resist herbivory with its suite of coping mechanisms (Ruiz et al., 2009) unless urchin density is particularly high.

Perhaps most interestingly, predation was clearly predictable in both seagrass systems and macroalgal dominated rocky systems. Predation impact was a clear function of refuge availability and fish predators. *D. sargus* has been previously described as the main sea urchin consumer inside marine reserves (Guidetti, 2004; Sala, 1997); our results confirm that it may be a key agent of top-down control in Mediterranean macroalgal habitats even outside these protected areas. Unfortunately it also underscores the low functional redundancy these systems have, since no species appears to substitute *D. sargus* when its numbers decline. *D. sargus* may be one of the few extant species in the Mediterranean capable of breaking the urchin carapace after they reach adulthood although several other fish predators can be important secondary consumers (Guidetti, 2004; Sala, 1997). This coupling of number and function validates the effectiveness of fishing restrictions in ensuring high predation in areas that require conservation. It is important however, to account for the structural complexity of these sites since, as this study and others indicate, refuge availability can critically mediate predatory–prey interactions in rocky macroalgal systems (Clemente, Hernandez, Montaño-Moctezuma, Russell, & Ebert, 2012; Hereu et al., 2005) and in seagrass meadows.

Our results show that fish interactions with their sea urchin prey are still prevalent across the Mediterranean coast despite centuries of human use and are highly dependent on site specificities and habitat characteristics. Given that the release from predatory functions can have cascading effects (e.g. creation of urchin barrens in rocky bottoms), it is critical to maintain and protect these higher trophic functions, particularly where they continue to be important. Identifying these hotspots of functional predation may be an essential first step when establishing new marine reserves to conserve macrophyte communities in temperate seas vulnerable to overgrazing events. This is particularly important given that meso-predators do not appear able to replace fish, even when their numbers decline, as the principal predator in coastal habitats. Our results indicate that, despite a long history of fishing, conserving functional predation may still be achievable outside marine reserves and is critical to ensure the resilience of ecosystems where top down processes still control the structuring of ecological communities.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.biocon.2015.06.017.

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