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Conservation and ecological restoration of Mediterranean marine forests

Fabrizio Gianni

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Ecole Doctorale de Sciences Fondamentales et Appliquées

T H E S E

pour obtenir le titre de
Docteur en Sciences
de l'Université Nice Sophia Antipolis
Discipline : Sciences de l'Environnement

présentée et soutenue par

Fabrizio GIANNI

**Conservation et restauration écologique des forêts marines
Méditerranéennes**

Conservation and ecological restoration of Mediterranean marine forests

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*"Se è vero che siamo fatti della stessa materia che si trova sparsa nel cosmo,
si potrebbe dire che questa materia dopo un lungo viaggio e infinite vicissitudini
si è aggregata in una struttura straordinaria, il cervello umano, che ora si gira indietro,
guarda l'universo e, in un certo senso, contempla se stesso."*

Piero Angela

“We are what we eat”

Ludwig Feuerbach

Abstract

Several important marine habitats exist worldwide, both in tropical and in temperate waters, and many of them are already suffering the effects of multiple human impacts. A widespread loss of marine forests of large brown seaweeds has been observed in the recent decades. Their loss leads to an ecosystem-shift towards less complex turf beds or sea urchin barren grounds, devoid of any erect vegetation. A wide arrays of human activities are causing this regression: eutrophication, coastal urbanisation, high sedimentation rates, destructive fishing and overfishing of sea urchins predators.

In the framework of the MMMPA project, this PhD work aimed to address some important topics related to the conservation and restoration of algal forests, with a particular attention to the role of Marine Protected Areas (MPAs) and fish herbivory. Multiple complementary approaches were used: macroalgae surveys, literature reviews, manipulative experiments in the field, tank-based experiments and GIS habitat mapping.

Results from field experiments showed that native herbivorous fish, likely *Sarpa salpa* (salemas), can be the most effective herbivore of intertidal *Cystoseira* belts both on natural and artificial substrates. Indeed, salemas strongly affected *Cystoseira stricta* fitness, being able to decrease the growth, biomass and reproductive output of natural forests and limit restoration success on artificial substrates. Likely, the role of the herbivorous fish in structuring macroalgal communities has been overlooked in the Mediterranean Sea so far.

A review of the existing literature showed that knowledge on marine forests forming species has improved in recent decades. However, most of the research is not in relation to MPAs, likely due to the fact that marine forests are not always included in MPAs planning and management plans. Studies on marine forests are not homogeneously distributed in the world, being concentrated in the developed countries where marine forests sustain industrial activities or where their importance is recognised. Interestingly, an increase of the awareness of marine forests importance and of the scientific interest (published papers) was observed.

Nowadays, marine forests are under continuous threats and especially sensitive to multiple impacts. Hence, conservation measures and recovery strategies should be urgently set up. Degraded/lost forests should be restored according to the guidelines and suggestions discussed in this PhD work, keeping in mind that the conservation of the existing forests in MPAs has always to be considered as a priority.

Keywords

Marine forests, marine protected areas, herbivorous fishes, conservation, restoration, seaweeds, macroalgae, Mediterranean Sea, artificial structures, herbivory, monitoring, management, temperate seas

Résumé

Plusieurs importants habitats marins existent de par le monde, à la fois dans les régions tropicales comme tempérées, et beaucoup d'entre eux souffrent déjà des effets des impacts humains cumulés. Une perte généralisée des forêts marines de grandes algues brunes a été observée dans les récentes décennies. Leur perte cause un changement des écosystèmes vers des gazons moins complexes, ou des déserts des oursins dépourvus de la végétation dressée. De nombreuses activités humaines sont à l'origine de cette régression: l'eutrophisation, l'urbanisation côtière, les taux élevés de sédimentation, la pêche destructive et la surpêche des prédateurs des oursins.

Dans le cadre du projet MMMPA, ce travail de thèse vise à répondre à certaines importantes questions liés à la conservation et à la restauration des forêts d'algues, avec une attention particulière à le rôle des Aires Marines Protégées (AMP) et les poissons herbivores. Différents approches ont été utilisées: suivis de macroalgues, revues de la littérature, expériences de manipulation en mer et en aquarium et la cartographie avec le logiciel SIG.

Les résultats des expériences sur le terrain ont montré que les poissons herbivores indigènes, probablement *Salpa Sarpa* (saupes), peuvent être les herbivores les plus efficace des ceintures intertidales de *Cystoseira* à la fois sur substrats naturels et artificiels. En effet, les saupes affectent fortement la fitness de *Cystoseira stricta*, en étant capable de diminuer la croissance, la biomasse et la reproduction des forêts naturelles et de limiter le succès de la restauration sur des substrats artificiels. Le rôle des poissons herbivores a probablement été négligé dans la régulation des communautés macroalgales Méditerranéennes, jusqu'à au présent.

Une revue de la littérature existante a montré que les connaissances sur les espèces formant les forêts marines se sont améliorées au cours des dernières décennies. Cependant, la plupart de la recherche scientifique n'est pas en relation avec les AMPs, probablement en raison du fait que les forêts marines ne sont pas toujours considérées pendant la création des AMPs et dans les plans de gestion. Les études sur les forêts marines étant concentrée dans les pays développés où les forêts marines soutiennent les activités industrielles ou lorsque leur importance est reconnue, elle ne sont pas répartis de manière homogène dans le monde. De façon intéressante, on a observé une amélioration de la prise de conscience de l'importance des forêts marines et de l'intérêt scientifique (articles publiés).

De nos jours, les forêts marines, particulièrement sensibles aux impacts cumulés, sont sous menaces continues. Par conséquent, les mesures de conservation et les stratégies de rétablissement devraient être mises en place de toute urgence. Les forêts dégradées / perdues

devraient être restaurées selon les lignes directrices et les suggestions discuté dans ce travail de thèse, en gardant à l'esprit que la conservation des forêts dans AMPs doit toujours être considérée comme une priorité.

Mot clés

Forêts marines, aires marines protégées, poissons herbivores, conservation, restauration, algues, macro-algues, Mer Méditerranée, structures artificielles, herbivorie, suivi, gestion, mers tempérées

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Chapter 1 – General introduction

1.1 - Why focus on marine forests of large brown seaweeds?

Many important marine habitats exist worldwide, both in tropical and in temperate waters. Some of them are suffering the effects of multiple human impacts¹ and may no longer be able to maintain and support the associated marine biodiversity, the resulting ecological processes and the ecosystem functions. While in tropical areas, the major role of primary producers and habitat formers is played by corals; in temperate areas canopy-forming seaweeds build the major three-dimensional, high productive habitats on rocky substrates². Many species of large brown seaweeds are able to create dense forests, extending from the surface to several meters in depth. Their canopies can range from few dozen centimetres (some fucoids) to 50 meters in height (giant kelps), and they are all considered ecosystem engineers and main components of the marine environment³. Indeed, as a transposition of the land forests into the sea, algal forests produce oxygen, export organic matter to other systems, are reproductive nurseries for fishes and substrate for sessile organisms, and they can be used as food by humans⁴. For instance, most of the biomass they produce is transported onshore or in deep waters, the rest is quickly consumed by grazers or decomposed in situ. This source of carbon is transferred to the highest levels of the food-webs, up to top-predators. Marine forests thus provide a wide series of advantages in temperate waters that cannot be ignored. However, large brown seaweeds have been chronically understudied in the last decades, especially in European waters⁵ and only recently the awareness on their importance is rising and more efforts are done in research.

¹ Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F et al 2008. A global map of human impact on marine ecosystems. *Science*, 319(5865), 948-952.

² Dayton PK 1985. Ecology of kelp communities. *Annu. Rev. Ecol. Syst.* 16, 215–245.

³ Jones CG, Lawton JH, Shachak M 1994. Organisms as ecosystem engineers. *Oikos*. 69, 373-86.

⁴ Mann KH 1973. Seaweeds: their productivity and strategy for growth. *Science* 182, 975–981.

⁵ Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ 2013. Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. *Ecology and Evolution*, 3(11), 4016-4038.

1.2 - Algal forests at risk: direct and indirect effects of human activities

Coastal zones host more than 60% of human population and recent estimations suggest the possibility of a further dramatic increase for the next decades⁶. Urbanization, extractive activities and agriculture inputs into the sea represent growing pressures for coastal ecosystems and potential drivers of loss. Marine forests are not exempt, and their regression or, in some cases, a widespread loss has been described along many areas⁷.

Among the most direct stressors, marine forests harvesting is a common activity that is responsible to remove thousands of tonnes of seaweeds each year, for food or for extracting substances. Urbanisation of the coastline is also one of the major threats to marine forests, due to the consequent decrease in water quality, increase in sedimentation and habitat destruction. Habitat modification can reduce connectivity among populations and contribute to the loss of marine forests and to ecosystem-shifts⁸. Marine forests regression may be also due to the outbreak of herbivores caused by natural or human-induced drivers. For instance, in many coastal regions of the Mediterranean Sea, the proliferation of sea urchins, caused by overfishing of their natural predators or by destructive date-mussel fishing, have created extensive barren grounds, devoid of erect vegetation (Fig. 1)⁹. Due to the rise of sea water temperatures, tropical herbivorous fishes are expanding their range in temperate waters, creating similar barrens in different basins¹⁰. Climate change can also affect marine forests by the increase of high intensity storms frequency, able to eradicate large brown algae at a higher rate than the natural recovery. Finally, the regression of marine forests affects marine ecosystems, inducing a decrease in biodiversity and complexity of benthic communities, shifts in species composition and loss in ecosystem functions¹¹. More effort should be put in research, to increase our knowledge on algal forests, their ecology, distribution and evolution. In addition, a higher interest should be given to the measures and tools that may be applied to reduce the loss and enhance the resilience of these systems.

⁶ Cohen JE 2003. Human population: the next half century. *Science* 302:5648, 1172-1175.

⁷ Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM et al 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental conservation* 29(04), 436-459.

⁸ Airoidi L, Beck MW 2007. Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology: An Annual Review* 45, 347-407.

⁹ Micheli F, Benedetti-Cecchi I, Gambaccini s, Bertocci I, Borsini C et al 2005. Cascading human impacts, marine protected areas, and the structure of Mediterranean reef assemblages. *Ecological Monographs* 75:81-102.

¹⁰ Vergés A, Steinberg PD, Hay ME, Poore AG, Campbell AH et al 2014. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. In *Proceedings of the Royal Society B* Vol. 281, No. 1789, p. 20140846. The Royal Society.

¹¹ Estes JA, Duggins DO, Rathbun GB 1989. The ecology of extinctions in kelp forest communities. *Conservation Biology* 3.3, 252-264.



Figure 1. Trophic cascades in the Mediterranean rocky-sublittoral: one of the causes of marine forests regression in temperate areas. Drawings from a short movie realized for outreach purposes (Annex VI). © Celine Barrier.

1.3 - Marine protected areas and ecological restoration to halt algal forests loss?

In the global scenario of marine landscapes, threatened by human activities, the conservation of algal forests emerges as a priority. One of the most common and efficient management tools in marine systems are Marine Protected Areas (MPAs). With the help of some regulations, MPAs are a guarantee of preserving healthy ecosystems from various human pressures such as, fishing activities, anchoring, coastal modification and waste-water discharge. A multitude of studies proved that where MPAs are well-managed, fish abundance recovers and trophic food-webs can be restored¹². As a consequence, in MPAs, the restored/preserved high-level predators may control the abundance of herbivores, limiting their grazing pressure and macroalgae are expected to recover. In fact, the recovery process is not always immediate and depends on several factors, among others the presence of close source of propagules and the suitability of the substrate for recruitment. Events of algal forests natural recovery mostly occur in the long-established MPAs. The re-establishment of trophic cascades in the Pacific Ocean is one of the most emblematic examples: the return of sea otters (and/or other apical predators after fishing regulation) controlled sea urchins abundance, allowing the natural recovery of kelp forests¹³. Therefore, MPAs may represent important sanctuaries where the conservation or the natural recovery of marine forests is expected to occur. However, at present, information on marine forests in MPAs is limited and more research is needed to assess the actual and potential role of MPAs in the conservation and management of marine forests.

When the natural recovery of marine forests is unlikely or require time, a valuable tool is the human-guided restoration. Ecological restoration is an activity that starts or accelerates the natural recovery of degraded ecosystems with respect to its health, integrity and sustainability¹⁴. In the simplest cases, restoration only remove or modify disturbances, allowing ecological processes to follow an independent and often slow recovery. In more complex circumstances, restoration requires the deliberate reintroduction of species or habitats that have been lost, even if not necessarily until the point to restore their historical conditions (often unknown). It can be useful especially when species are not supposed to

¹² Russ GR, Alcala AC 1996. Marine reserves: rates and patterns of recovery and decline of large predatory fish. *Ecological applications*, 947-961.

¹³ Estes JA, Duggins DO 1995. Sea otters and kelp forests in Alaska: generality and variation in a community ecological paradigm. *Ecological Monographs*, 65(1), 75-100.

¹⁴ Jackson LL, Lopoukhine N, Hillyard D 1995. Ecological restoration: a definition and comments. *Restoration Ecology*, 3(2), 71-75.

recover alone in a short time, being severely degraded or characterized by low-dispersal ability/slow-growing rates. Such actions may require integrated measures, such as the protection of the reintroduced species from herbivores. When the desired trajectory is reached, the restored habitat may no longer require any other assistance, becoming self-sustaining and the restoration can be considered successful.

While ecological restoration has been applied since long-time to restore terrestrial or transitional habitats, such as forests, saltmarshes and mangroves, it is a quite new science in the marine environment, and particularly applied to corals and seagrasses. Ecological restoration is potentially an efficient tool for re-establishing marine forests, as they have efficient reproductive strategies, relatively fast growth-rates and they are usually easy to manipulate. Nowadays, experimental studies on large brown seaweeds restoration are increasing, especially in Asia, North and South America and, to some extents, in the Mediterranean Sea. Experiments were not only focused on restoring marine forests in natural habitats, but also on gardening artificial substrates¹⁵, such as coastal defence structures, widely diffused along urbanised coastal areas. Since they generally host low complexity macroalgal assemblages, gardening such structures with key-species would increase their ecological value and re-establish some of the associated ecological processes. Although in many cases algal forests restoration proved to be feasible, the success of such actions remains controversial and extremely variable due to several factors, such as water quality and herbivory pressure, particularly high on artificial habitats.

1.4 - Thesis objectives and approaches

The present PhD thesis was performed in the framework of the European MMMPA project (Training Network for Monitoring Mediterranean Marine Protected Areas, Call FP7-PEOPLE-2011-ITN)¹⁶, and in particular in the work package “Biodiversity assessment and ecosystem functioning”. The research I carried out was focused on the conservation and restoration of Mediterranean marine forests, with a particular interest on the potential role of MPAs.

¹⁵ Firth LB, Thompson RC, Bohn K, Abbiati M, Airolidi L et al 2014. Between a rock and a hard place: environmental and engineering considerations when designing coastal defence structures. *Coastal Engineering*, 87, 122-135.

¹⁶ www.mmmmpa.eu

The first step consisted in reviewing the state of art at the global scale of:

- Marine forests knowledge in MPAs and their inclusion in monitoring programs
- Ecological restoration of marine forests and the potential role of MPAs.

The review of the existing literature on marine forests in global MPAs is addressed in **chapter 2**. This synthesis research allowed to quantify the level of information on algal forests that is available in MPAs, reflecting the historical interest on marine forests and their actual consideration as key-habitats in the MPA management. A smaller-scale review focused only on Mediterranean marine forests and MPAs, was published in the proceedings of the Mediterranean Marine Vegetation Symposium (**annex I**)¹⁷.

A specific literature review was also done on the ecological restoration of marine forests and published on an international journal (**chapter 3**)¹⁸. This paper summarises the experiments performed around the world for restoring canopy-forming species, with a particular focus on the techniques used and their outcomes. The role of MPAs in restoration initiatives was also discussed, as much as the eventuality of gardening artificial structures for increasing their ecological value. A flow-chart was proposed as a conceptual tool, suggesting clues for a reasoned conservation and restoration of marine forests.

Thanks to this work, I had the chance of being included in an international group of researchers that presented a small contribution on the same topic to the Mediterranean Marine Vegetation Symposium and published in the proceedings of this conference (**annex II**)¹⁹. In particular, it synthetized the documented regression of algal forests in the Mediterranean Sea and the possibility of restoring them with adequate methods.

These reviews allowed to highlight the main gaps on marine forests conservation and restoration, such as the scarce knowledge on their distribution and the causes of restoration failure. The experimental work of my PhD was thus devoted to increase knowledge on marine forests distribution in three Mediterranean MPAs partners of the project and to study the role of plant-herbivore interactions for Mediterranean marine forests conservation and restoration.

¹⁷ Gianni F, Mangialajo L 2014. Are Mediterranean MPAs protecting marine forests? Proceedings of the 5th Mediterranean Symposium on Marine Vegetation, Portoroz, Slovenia, 27-28 October 2014, pp. 74-79.

¹⁸ Gianni F, Bartolini F, Airoidi L, Ballesteros E, Francour P et al 2013. Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. *Advances in oceanography and limnology* 4,83-101.

¹⁹ Airoidi L, Ballesteros E, Buonuomo R, Van Belzen J, Bouma TJ et al 2014. Marine forests at risk: solutions to halt the loss and promote the recovery of Mediterranean canopy-forming seaweeds. Proceedings of the 5th Mediterranean Symposium on Marine Vegetation, Portoroz, Slovenia, 27-28 October 2014, pp. 28-33.

Indeed, if herbivory pressure is too high, existing forests could be lost²⁰ and ecological restoration actions may not be successful¹⁸.

Detailed cartographies of algal forests and the evaluation of species diversity in three partners MPAs was done by visual census and geo-referenced in GIS maps in order to create a baseline for future management plans (**annex III**). Since most *Cystoseira* and *Sargassum* species are not easily identified in the field, some specimens were collected during the surveys and preserved with an innovative method that allows the conservation of the original structure and the consistency of algae. A reference collection of algal specimens, adequately stored and catalogued, is a useful tool for researchers and for MPA staff to validate future scientific observations (**annex IV**).

In order to better understand the plant-herbivore relationships, a manipulative experiment on an artificial structure, associated to tank experiments, was performed. The potential effect of different herbivores on transplanted *Cystoseira* individuals was tested (**chapter 4**). This study allowed to highlight that, unexpectedly, native herbivorous fish are the main herbivores able to reduce restoration success in the infralittoral fringe of man-made structures.

Successively, in order to better understand their role in natural habitats and quantify their potential pressure on very shallow marine forests, another field experiment was carried out on natural rocky shores (**chapter 5**), using a new conceived device that efficiently reduced fish grazing, without limiting light penetration (**annex V**).

Therefore, in the present PhD work, multiple and diversified approaches were used to improve the conservation of marine forests in the Mediterranean Sea: from literature summary to macroalgae survey, from application of a geographic information system (GIS) to manipulative experiments in the field and in tanks.

The main results of the research I carried out during my PhD are discussed in **chapter 6**, together with some perspectives for future research priorities.

In agreement with the MMMPA project communication objectives, several actions and outreach products were made, including a set of guidelines to support the development of management plans and a short movie (**annex VI**).

²⁰ Sala E, Kizilkaya Z, Yildirim D, Ballesteros E 2011. Alien marine fishes deplete algal biomass in the eastern Mediterranean. PloS one, 6(2), e17356.

Chapitre 1 – Introduction générale (en français)

1.1 – Pourquoi étudier les forêts marines des grandes algues brunes?

De nombreux habitats marins importants existent dans le monde entier, à la fois dans les régions tropicales comme dans les eaux tempérées. Certains d'entre eux souffrent des effets des impacts humains multiples¹ et ne peut plus être en mesure de maintenir et de soutenir la biodiversité marine associée, les processus écologiques résultant et les fonctions des écosystèmes. Alors que dans les régions tropicales, le rôle majeur de producteurs primaires et de constructeurs d'habitats est joué par les coraux; dans les zones tempérées les forêts marines de macro-algues forment les habitats les plus productifs sur les substrats rocheux². De nombreuses espèces de grandes algues brunes sont en mesure de créer des forêts denses, allant de la surface à plusieurs mètres de profondeur. Leurs auvents peuvent aller de quelques dizaines de centimètres (certains fucoides) à 50 mètres de hauteur (laminaires géants), et ils sont tous considérés comme des ingénieurs de l'écosystème et les principaux composants de l'environnement marin³. En effet, comme une transposition des forêts terrestres dans la mer, les forêts d'algues produisent de l'oxygène, exportent de la matière organique à d'autres systèmes, sont des nurseries de reproduction pour les poissons et substrat pour les organismes sessiles, et elles peuvent être utilisés comme nourriture par les humains⁴. Par exemple, la majeure partie de la biomasse qu'ils produisent est transportée près des côtes ou dans les eaux profondes, le reste est rapidement consommé par les herbivores ou décomposé in situ. Cette source de carbone est transférée aux plus hauts niveaux de la chaîne alimentaire, jusqu'à les grands prédateurs. Les forêts marines fournissent ainsi une grande série d'avantages dans les eaux tempérées qui ne peuvent pas être ignorés. Cependant, les grandes algues brunes ont été chroniquement sous-étudiées au cours des dernières décennies, en particulier dans les eaux européennes⁵ et, récemment, la prise de conscience de leur importance est augmentée et des efforts sont faits dans la recherche.

¹ Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F et al 2008. A global map of human impact on marine ecosystems. *Science*, 319(5865), 948-952.

² Dayton PK 1985. Ecology of kelp communities. *Annu. Rev. Ecol. Syst.* 16, 215–245.

³ Jones CG, Lawton JH, Shachak M 1994. Organisms as ecosystem engineers. *Oikos*. 69, 373-86.

⁴ Mann KH 1973. Seaweeds: their productivity and strategy for growth. *Science* 182, 975–981.

⁵ Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ 2013. Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. *Ecology and Evolution*, 3(11), 4016-4038.

1.2 – Les forêts d’algues brunes en danger: les effets directs et indirects des activités humaines

Les zones côtières accueillent plus de 60% de la population humaine et des estimations récentes suggèrent la possibilité d'une nouvelle hausse spectaculaire pour les prochaines décennies⁶. L'urbanisation, les activités extractives et les rejets de l'agriculture entrant dans la mer, représentent des pressions croissantes pour les écosystèmes côtiers et les facteurs causant la perte des habitats. Les forêts marines ne sont pas exemptées, et leur régression ou, dans certains cas, une perte généralisée a été décrite dans de nombreux domaines⁷.

Parmi les facteurs de stress les plus directs, le ramassage des algues est une activité commune qui est responsable de supprimer des milliers de tonnes d'algues chaque année, pour faire de la nourriture ou des substances. L'urbanisation du littoral est également une des principales menaces pour les forêts marines, en raison de la diminution de la qualité de l'eau, de l'augmentation de la sédimentation et de la destruction des habitats. La modification des habitats peut réduire la connectivité entre les populations et contribuer à la disparition des forêts et aux changements des écosystèmes marins⁸. La régression des forêts marines peut être aussi due à la prolifération des herbivores causée par des facteurs naturels ou induits par l'homme. Par exemple, dans de nombreuses zones côtières de la mer Méditerranée, la prolifération des oursins, causée par la surpêche de leurs prédateurs ou par la pêche destructive aux dattes de mer, a produit la formation de vaste fonds désertiques, dépourvu de végétation dressée (Fig. 1)⁹. En raison de la hausse des températures de l'eau de mer, les poissons herbivores tropicaux ont élargi leur distribution spatiale dans les eaux tempérées, créant des zones désertifiées similaires dans différents bassins¹⁰. Le changement climatique peut également affecter les forêts marines par l'augmentation de la fréquence des tempêtes d'intensité plus grande, capables d'éradiquer les grandes algues brunes à un taux plus élevé

⁶ Cohen JE 2003. Human population: the next half century. *Science* 302:5648, 1172-1175.

⁷ Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM et al 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental conservation* 29(04), 436-459.

⁸ Airoldi L, Beck MW 2007. Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology: An Annual Review* 45, 347-407.

⁹ Micheli F, Benedetti-Cecchi I, Gambaccini s, Bertocci I, Borsini C et al 2005. Cascading human impacts, marine protected areas, and the structure of Mediterranean reef assemblages. *Ecological Monographs* 75:81-102.

¹⁰ Vergés A, Steinberg PD, Hay ME, Poore AG, Campbell AH et al 2014. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. In *Proceedings of the Royal Society B Vol. 281, No. 1789, p. 20140846. The Royal Society.*



Figure 1. Cascades trophiques sur le fonds rocheux subtidal dans la Mer Méditerranée : l'une des causes de régression des forêts marines dans les zones tempérées. Dessins dans un court-métrage réalisé à des fins de sensibilisation (annexe VI). © Celine Barrier.

que le rétablissement naturel. Enfin, la régression des forêts marines affecte les écosystèmes marins, causant une diminution de la biodiversité et de la complexité des communautés benthiques et la perte sur des fonctions des écosystèmes¹¹. Plus d'efforts devraient être déployés dans la recherche, afin d'accroître nos connaissances sur les forêts d'algues, leur écologie, leur distribution et leur évolution. Un intérêt plus élevé devrait être donné aux mesures et aux outils qui peuvent être appliquées pour réduire les pertes et améliorer la résilience de ces systèmes.

1.3 – Les aires marines protégées et la restauration écologique comme outil pour arrêter la régression des forêts marines?

Dans le scénario global de paysages marins, menacés par les activités humaines, la conservation des forêts d'algues apparaît comme une priorité. L'un des outils de gestion les plus courantes et les plus efficaces sont les Aires Marines Protégées (AMPs). Avec la mise en place de certaines régulations, les AMPs constituent une garantie pour la préservation des écosystèmes sains des diverses pressions humaines telles que : les activités de pêche, l'ancrage, les aménagements côtiers et la décharge des eaux usées. Une multitude d'études a prouvé que lorsque les AMPs sont bien gérées, les écosystèmes récupèrent, les réseaux trophiques peuvent être restaurés et l'abondance des poissons augmente. En effet, dans les AMPs, les prédateurs de hauts niveaux trophiques peuvent contrôler l'abondance des herbivores. Ce qui peut diminuer la pression de pâturage. Les espèces formant les forêts marines pourraient alors récupérer et proliférer¹². Cependant, les processus de récupération ne sont pas toujours immédiats et sont dépendant de plusieurs facteurs, entre autres la présence de sources proches de propagules et la disponibilité du substrat pour le recrutement. Les processus de rétablissements naturels des forêts d'algues se produisent principalement dans les anciennes AMPs. L'un des exemples les plus emblématiques est le rétablissement des cascades trophiques dans l'océan Pacifique. Le retour de la loutre de mer (et / ou d'autres prédateurs apicaux après la réglementation de la pêche) a contrôlé l'abondance des oursins et favorisé la récupération naturelle des forêts de kelp¹³. Par conséquent, les AMPs peuvent

¹¹ Estes JA, Duggins DO, Rathbun GB 1989. The ecology of extinctions in kelp forest communities. *Conservation Biology* 3.3, 252-264.

¹² Russ GR, Alcala AC 1996. Marine reserves: rates and patterns of recovery and decline of large predatory fish. *Ecological applications*, 947-961.

¹³ Estes JA, Duggins DO 1995. Sea otters and kelp forests in Alaska: generality and variation in a community ecological paradigm. *Ecological Monographs*, 65(1), 75-100.

représenter des sanctuaires importants où la conservation et la régénération naturelle des forêts marines est possible. Cependant, actuellement, l'information sur les forêts marines dans les AMPs est limitée. Pour évaluer le rôle actuel et potentiel des AMPs dans la conservation et la gestion des forêts marines, plusieurs travaux de recherche sont encore nécessaires.

Dans le cas où la récupération naturelle des forêts marines est peu probable ou lente, la restauration guidée par l'homme est un outil important. La restauration écologique est une activité qui a pour objectif de démarrer ou accélérer la régénération naturelle des écosystèmes dégradés par rapport à son état, son intégrité et sa durabilité¹⁴. Dans les cas les plus simples, la restauration supprime ou modifie les perturbations, ce qui permet aux processus écologiques d'avoir une reprise indépendante et souvent lente. Dans des circonstances plus complexes, la restauration exige la réintroduction délibérée d'espèces ou d'habitats qui ont été perdus pas nécessairement jusqu'au point de rétablir leurs conditions historiques (souvent inconnues). Ce qui peut être utile en particulier lorsque les espèces ne sont pas capable de récupérer naturellement dans un court laps de temps, parce qu'elles sont sévèrement dégradées ou se caractérisent par une faible dispersion / taux de croissance lente. Ces actions peuvent nécessiter des mesures intégrées, telles que la protection des espèces réintroduites des herbivores. Lorsque l'objectif est atteint, l'habitat restauré peut devenir autonome (ne nécessite pas d'assistance) et la restauration peut être considérée comme un succès.

Bien que la restauration écologique dans les habitats terrestres ou de transition (les marais salants et les mangroves) a été utilisée depuis longtemps, dans le milieu marin, ses applications sont encore récentes, appliquées principalement aux coraux et aux herbiers de phanérogames marines. La restauration écologique peut être un outil efficace pour rétablir les forêts marines. En effet, ces derniers ont des stratégies de reproduction efficaces, des taux de croissance relativement rapides et sont généralement faciles à manipuler. Aujourd'hui, les études expérimentales pour la restauration des algues brunes sont en augmentation, en particulier en Asie, Amérique du Nord et du Sud et, pour certains cas, en mer Méditerranée. Les expériences ont été non seulement axés sur la restauration des forêts marines dans les habitats naturels, mais aussi sur des substrats artificiels¹⁵, tels que les structures de défense côtière, largement diffusées dans les zones côtières urbanisées. Comme ils accueillent généralement des communautés de macro-algues à faible complexité, leur recouvrement avec des espèces clés, augmenterait leur valeur écologique et rétablirait certains processus

¹⁴ Jackson LL, Lopoukhine N, Hilyard D 1995. Ecological restoration: a definition and comments. *Restoration Ecology*, 3(2), 71-75.

¹⁵ Firth LB, Thompson RC, Bohn K, Abbiati M, Airoidi L et al 2014. Between a rock and a hard place: environmental and engineering considerations when designing coastal defence structures. *Coastal Engineering*, 87, 122-135.

écologiques associés. Bien que dans plusieurs cas, il a été démontré que la restauration des forêts d'algues est possible, le succès de ces actions reste controversé et extrêmement variable et dépendant de plusieurs facteurs, tels que la qualité de l'eau et la pression de broutage particulièrement élevée sur les habitats artificiels.

1.4 - Objectifs de la thèse et approches utilisées

Cette thèse de doctorat a été réalisée dans le cadre du projet Européen MMMPA (Training Network for Monitoring Mediterranean Marine Protected Areas, Call FP7-PEOPLE-2011-ITN)¹⁶, et en particulier dans le *work package* “*Biodiversity assessment and ecosystem functioning*”. La recherche que j’ai effectué était axée sur la conservation et la restauration des forêts marines méditerranéennes, avec un intérêt particulier sur le rôle potentiel des AMPs.

La première étape consistait à examiner l'état de l'art à l'échelle mondiale de:

- la connaissance sur les forêts marines dans les AMPs et leur inclusion dans les programmes de surveillance
- la restauration écologique des forêts marines et le rôle potentiel des AMPs.

Une revue de la littérature existante sur les forêts marines dans les AMPs mondiales est abordée au **chapitre 2**. Cette recherche de synthèse a permis de quantifier le niveau d'information sur les forêts d'algues dans les AMPs et leur considération dans la gestion des AMPs. Une revue de la littérature à plus petite échelle, axée uniquement sur les forêts marines et les AMPs méditerranéennes, a été publiée dans les actes du *Mediterranean Marine Vegetation Symposium (annexe I)*¹⁷.

Une revue de la littérature a également été effectuée sur la restauration écologique des forêts marines et a fait le sujet d’une publication dans un journal international (**chapitre 3**)¹⁸. Cet article résume les expériences réalisées dans le monde entier pour la restauration des forêts marines, avec un accent particulier sur les techniques utilisées et leurs résultats. Le rôle des

¹⁶ www.mmmmpa.eu

¹⁷ Gianni F, Mangialajo L 2014. Are Mediterranean MPAs protecting marine forests? Proceedings of the 5th Mediterranean Symposium on Marine Vegetation, Portoroz, Slovenia, 27-28 October 2014, pp. 74-79.

¹⁸ Gianni F, Bartolini F, Airoidi L, Ballesteros E, Francour P et al 2013. Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. *Advances in oceanography and limnology* 4,83-101.

AMPs dans les initiatives de restauration a également été discuté, autant que l'éventualité de boiser les structures artificielles pour augmenter leur valeur écologique. Un organigramme a été proposé comme outil conceptuel, suggérant des pistes pour une conservation et une restauration raisonnée des forêts marines.

Grâce à ce travail, j'ai eu la chance d'être inclus dans un groupe international de chercheurs qui ont présenté leur recherche sur le même sujet au *Mediterranean Marine Vegetation Symposium* et publié dans les actes de cette conférence (**annexe II**)¹⁹. En particulier, cette recherche synthétise l'ensemble des études qui ont documenté la régression des forêts d'algues en mer Méditerranée et la possibilité de les restaurer avec des méthodes appropriées.

Ces revues ont permis de mettre en évidence les lacunes principales de la conservation et la restauration des forêts marines, tels que les connaissances limitées sur leur distribution et les causes de l'échec de leur restauration. Le travail expérimental de ma thèse a donc été consacré à accroître les connaissances sur la distribution des forêts marines au niveau de trois AMPs Méditerranéennes partenaires du projet et d'étudier les interactions plantes-herbivores. En effet, si la pression de l'herbivorie est trop élevée, les forêts existantes pourraient être perdues²⁰ et les actions de restauration écologique échoueraient¹⁸.

Une cartographie détaillée des forêts marines ont été faite dans les trois AMPs partenaires du projet par recensement visuel et géo-référencée dans des cartes à l'aide du logiciel SIG. Elles seront une base de référence pour les plans de gestion futurs (**annexe III**). Comme la plupart des espèces de *Cystoseira* et *Sargassum* ne sont pas faciles à identifier sur le terrain, certains spécimens ont été recueillis au cours des suivis et conservées avec une méthode innovante permettant la conservation de la structure d'origine et la consistance des algues. Une collection de référence d'espèces d'algues, stocké de manière adéquate et cataloguée, est un outil utile pour les chercheurs et pour le personnel des AMPs pour valider les observations scientifiques futurs (**annexe IV**).

Afin de mieux comprendre les relations plantes-herbivores, une expérience sur une structure artificielle, associée à des expériences en aquarium, a été réalisée. J'ai testé l'effet potentiel de

¹⁹ Airoidi L, Ballesteros E, Buonomo R, Van Belzen J, Bouma TJ et al 2014. Marine forests at risk: solutions to halt the loss and promote the recovery of Mediterranean canopy-forming seaweeds. *Proceedings of the 5th Mediterranean Symposium on Marine Vegetation*, Portoroz, Slovenia, 27-28 October 2014, pp. 28-33.

²⁰ Sala E, Kizilkaya Z, Yildirim D, Ballesteros E 2011. Alien marine fishes deplete algal biomass in the eastern Mediterranean. *PloS one*, 6(2), e17356.

différents herbivores sur des individus de *Cystoseira* transplantées (**chapitre 4**). Cette étude a permis de mettre en évidence que, de façon inattendue, les poissons herbivores indigènes sont les principaux herbivores capables de réduire le succès de la restauration dans la frange infralittoral des structures artificielles.

Ultérieurement, afin de mieux comprendre le rôle des poissons herbivores indigènes dans les habitats naturels et de quantifier leur pression potentielle sur les forêts marines, une autre expérience sur le terrain a été réalisée au niveau de côtes rocheuses naturelles (**chapitre 5**). Pour réaliser cette expérience, j'ai utilisé un nouveau dispositif qui a efficacement réduit le broutage des poissons, sans limiter la pénétration de la lumière (**annexe V**).

En conclusion, dans ce travail de thèse, différentes approches ont été utilisées pour améliorer la conservation des forêts marines dans la mer Méditerranée: synthèses de la littérature, suivis de macro-algues, application d'un système d'information géographique (SIG) et expérimentation sur le terrain et en aquarium.

Les principaux résultats de cette recherche sont examinés dans le **chapitre 6**, suivi de quelques perspectives et les priorités pour la recherche future.

En accord avec les objectifs de communication du projet MMMPA, plusieurs actions et produits de sensibilisation ont été faits, comprenant un ensemble de lignes directrices afin de faciliter l'élaboration de plans de gestion et un court-métrage (**annexe VI**).



Chapter 2 – Marine forests of large brown seaweeds in MPAs: how much do we know?*

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Underwater census of marine forests of large brown seaweeds in the subtidal zone. Photo: Mangialajo L.

* This chapter is a draft that will be submitted to several potential co-authors, preferably marine forests experts working in the MPAs relevant for our study. The collaboration with an international team will allow the validation and eventual integration of results. A further bibliometric analysis will be conducted for classifying papers on the base of the research topics (i.e. physiology, ecology, etc.). This classification will potentially explain some regional differences observed in the study.

2.1 - Introduction

In the last century and in particular in the last decades, human activities have altered the oceans through direct and indirect impacts, more often cumulated (Halpern et al. 2008). This caused the loss of several ecosystem functions, biodiversity, regression of species distribution and alteration of food-webs (Jackson et al. 2001, Cheevaporn and Menasveta 2003, Jones et al. 2004, Harley et al. 2006, Fabry et al. 2008). A rising awareness about the necessity of managing the oceans has led to the development of a wide arrange of strategies and conservation programs, the first of which are Marine Protected Areas (MPAs) (Agardy 1994). They guarantee protection from different human impacts and, if well-managed, represents one of the most successful tools to prevent biodiversity loss (Lubchenco et al. 2003). Around the world, 5000 MPAs, parks, sanctuaries, natural monuments, ecological reserves have been established, covering 2.8% of the oceans, and many others are going to be created (www.protectplanetoocean.org). Their principal aims are managing fisheries, protecting highly harvested or charismatic species (Zacharias and Roff 2001, Hooker and Gerber 2004) and conserving high-diversity ecosystems.

In temperate waters, marine forests, mainly formed by kelps and fucoids, are key-habitats, comparable to land forests for the goods and services provided (Mann 1973, Jones et al. 1994). Their canopies offer refuge and subsistence to many organisms and sustain complex food-webs (Foster and Schiel 1985, Ballesteros 1990, Bologna and Steneck 1993, Bustamante et al. 1995). In addition, many species are economically valuables and are exploited by maritime people since thousands of years (Erlandson 2001, Jackson et al. 2001). Nowadays, marine forests are threatened by human activities and several studies have described their loss or regression worldwide (Graham 2004, Ling et al. 2009, Scheibling and Gagnon 2009, Connell and Russell 2010, Nicastro et al. 2013, Thibaut et al. 2014). In this context, MPAs may represent a good tool for marine forests conservation, excluding or regulating some activities that directly (i.e. coastal development and destructive fishing) or indirectly (i.e. cascade effects of overfishing) have an impact on their conservation (Guidetti et al. 2003, Airoidi and Beck 2007, Foster and Schiel 2010, Gianni et al. 2013). Indeed, events of marine forests recovery have been especially reported in MPAs of the Southwest (Babcock et al. 1999, Edgar and Barrett 1999, Shears and Babcock 2003) and Northeast Pacific (Behrens and Lafferty 2004, Hamilton et al. 2014), of the Northwest Atlantic (Steneck et al. 2013) and in rare cases also in the Mediterranean Sea (Hereu Fina and Quintana Pou 2012, Galasso et al. 2015).

However, contrarily to land forests, whose protection represents the primary condition to save the associated terrestrial biodiversity (Myers et al. 2000), marine forests usually have a marginal role compared to other charismatic species. Thus, MPAs are often established on the base of highly protected and known species, such as mammals, fishes assemblages, corals and seagrasses meadows, listed in important environment legislations (e.g. the European Habitat Directive). As well as, the evaluation of MPAs efficacy is mostly based on fishes or seagrasses than on algal forests, with the exception of some regions, such as the Pacific Northeast, where their importance is recognised. As a consequence, knowledge on the status and distribution of marine forests can be locally incomplete (Raybaud et al. 2013), also in MPAs. Therefore, the objective of this paper was to quantify the contribution of scientific research to the study of large brown algae forests in MPAs, globally, and estimate the degree of knowledge of this habitat compared to other important habitats or species.

2.2 - Materials and methods

To quantify the number of international scientific papers on marine forests of large brown seaweeds and other habitats/species carried out in MPAs worldwide, we searched ISI Web of Science and Aquatic Sciences and Fisheries Abstracts (ASFA) databases, allowing the inclusion of a large amount of grey literature. The search was performed in February 2016 and only papers in English were considered.

Firstly, we searched for studies that addressed any topic related to marine forests inside or outside MPAs, in order to assess the trend in the number of papers published in the last decades. Successively, we also evaluated the number of studies performed on seagrasses and fishes in MPAs for a comparison with the number of studies on marine forests. The keywords used in the two databases are reported in Tab 1. After excluding not relevant and duplicated papers, the articles performed in MPAs were organized by geographic area. Papers on marine forests were also divided by MPA, in order to class MPAs according to the relevance they have in the research on marine forests. MPAs with more than five studies on marine forests were considered as relevant for our study and further comparisons with the other key habitats/assemblages (seagrasses and fishes) were done. In order to perform these comparisons, the name of the MPA was used as a filter in the query, together with the same keywords used for the marine forests search (Tab. 1).

Finally, in order to highlight if research on marine forests is increasing, for each selected MPA, we calculated the rate of papers published on marine forests per year, as the ratio between the number of papers and the number of years of protection (since their establishment). In this analysis, studies performed before the creation of the MPAs were excluded.

Tab. 1. Keywords used in ISI Web of Science and ASFA databases. First search: papers on algal forests inside or outside MPAs. Second search: papers on seagrasses or fishes in MPAs.

First search
<p><u>Algal forests outside MPAs:</u></p> <p>(Fucales OR Fucoi* OR laminariales OR kelp* OR "marine forest*" OR "brown alga*" OR "brown seaweed*" OR Ascoseira OR Akkesiphycus OR Aureophycus OR Alaria OR Agarum OR Anthophycus OR Acrocarpia OR Ascophyllum OR Axillariella OR Costaria OR Cymathere OR Cystosphaera OR Cystoseira OR Sargassum OR Caulocystis OR Cystophora OR Cystophyllum OR Cladophyllum OR Carpophyllum OR Carpoglossum OR Coccophora OR Brassicophycus OR Bifurcaria OR Fucus OR Desmarestia OR Durvillaea OR Dictyoneurum OR Eदारachne OR Eualaria OR Ecklonia OR Eckloniopsis OR Egregia OR Eisenia OR Halidrys OR Hormophysa OR Himantothallus OR Haligenia OR Hesperophycus OR Himanthalia OR Lessoniopsis OR Lessonia OR Laminaria OR Myriodesma OR Nizamuddinia OR Oerstedtia OR Myagropsis OR Pelvetia OR Pelvetiopsis OR Phyllaria OR Phyllariopsis OR Petalonia OR Pelagophycus OR Postelsia OR Punctaria OR Pleurophycus OR Pterygophora OR Undaria OR Macrocystis OR Nereocystis OR Phyllospora OR Saccharina OR Saccorhiza OR Seirococcus OR Scytothalia OR Stephanocystis OR Silvetia OR Sirophysalis OR Scaberia OR Sargassopsis OR Phyllotricha OR Platythalia OR Palaeohalidrys OR Xiphophora OR Marginariella OR Turbinaria OR Landsburgia)</p>
<p><u>Algal forest inside MPAs:</u></p> <p>(keywords like above)</p> <p>AND</p> <p>("marine protected area*" OR "nat* protected area*" OR "marine reserve*" OR "nat* reserve*" OR "marine park*" OR "nat* park*" OR "special* reserve*" OR "special* protected area*" OR sanctuary OR monument)</p>

Second search

("marine protected area*" OR "nat* protected area*" OR "marine reserve*" OR "nat* reserve*" OR "marine park*" OR "nat* park*" OR "special* reserve*" OR "special* protected area*" OR sanctuary OR monument)

AND

fish or seagrasses with the following keywords:

for seagrasses: (Eelgrass* OR seagrass* OR "marine plant*" OR "marine phanerogam*" OR Posidonia OR Cymodocea OR Zostera OR Phyllospadix OR Halophila OR Amphibolis OR Halodule OR Syringodium OR Thalassodendron OR Thalassia)

For fish: (fish)

2.3 - Results

The research on marine forests forming species started being relevant in the 80ies and it has regularly increased since then, reaching a pick of 1696 papers in 2015 (Fig. 1). It is worth noting that most of research on large brown seaweeds is mainly not related to protection or MPAs.

Our synthesis highlighted 518 papers on marine forests forming species in 244 MPAs (Fig. 2). Studies on marine forests are particularly abundant in the Pacific Northeast (40% of the global amount of research on marine forests), even if relevant percentages of papers were also found in the Mediterranean Sea (16%) and in the Pacific Southwest (15%).

When comparing, globally, with seagrasses and fish, our research found respectively 739 and 2.723 papers. Fish are consistently the more studied subject, with 68.4% of published papers, followed by seagrasses with 18.6% and marine forests with 13.0%. A similar trend was also observed when papers were divided by geographical area (Fig. 3). However, the percentage of studies on marine forests and seagrasses change according to the region. Studies on marine forests prevail mainly in the Pacific Ocean, Atlantic Ocean and Black Sea, while studies on seagrasses prevail in the Indian Ocean, Caribbean and Mediterranean Sea.

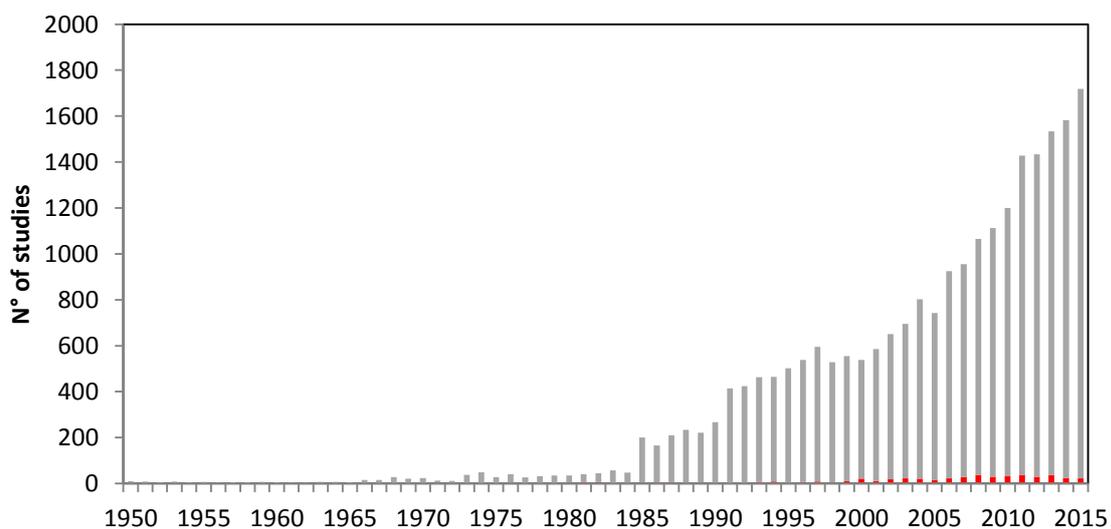


Figure 1. Number of papers carried out per year in the world on algal forests, inside (in red) and outside MPAs (in grey).

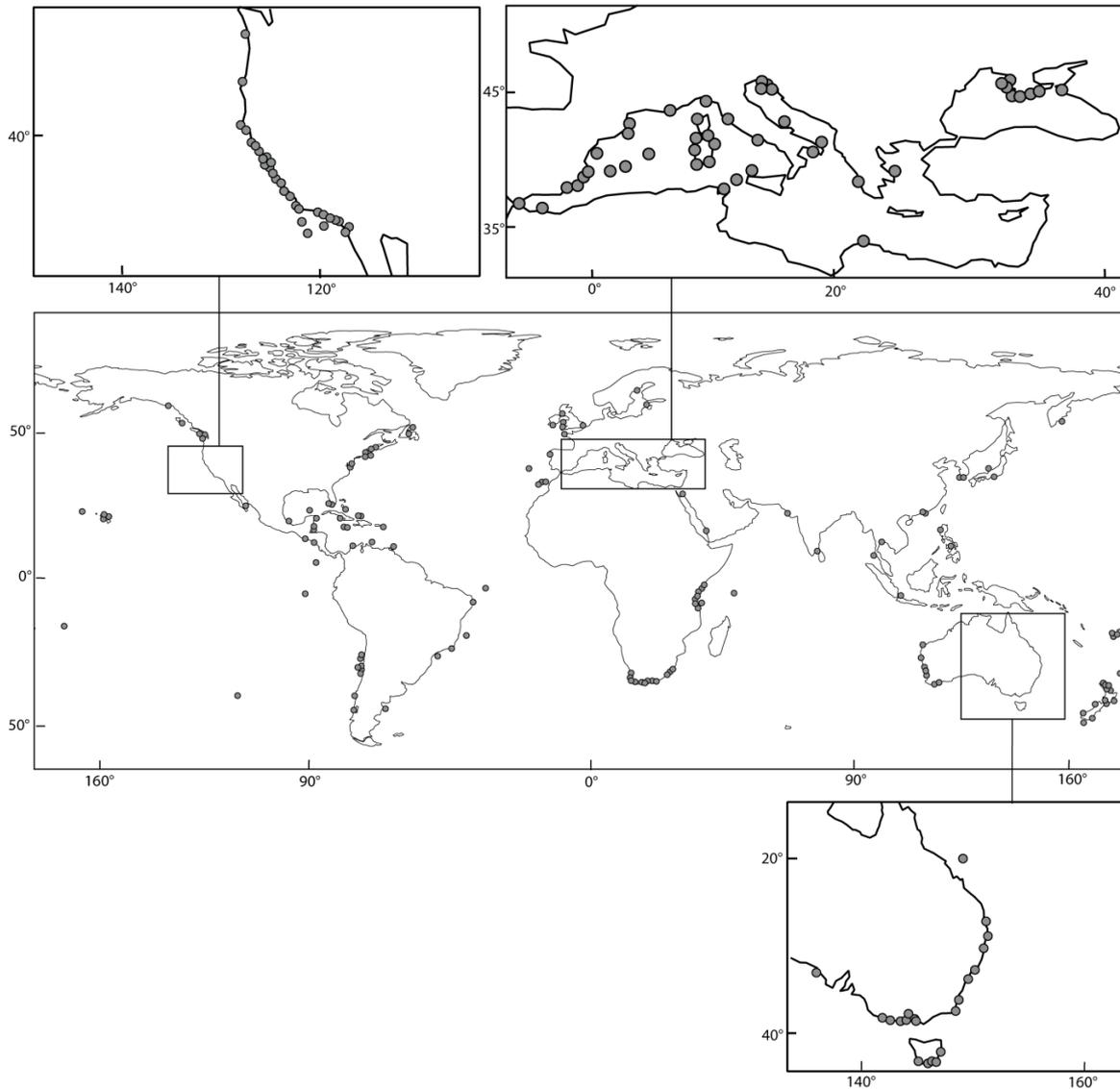


Figure 2. Geographical distribution of the studies on algal forests in all MPAs.

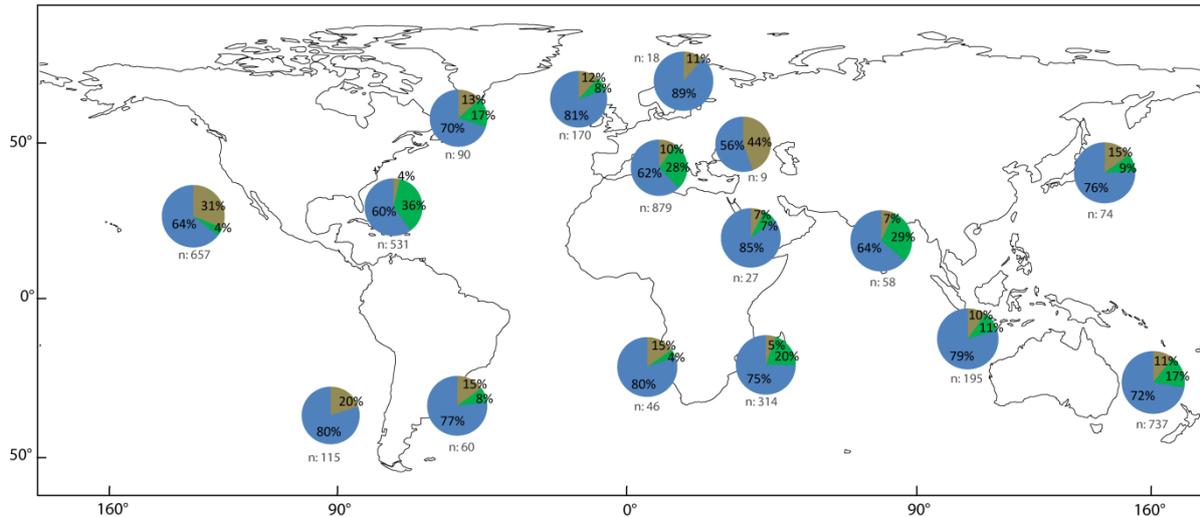


Figure 3. Percentage of studies carried out in MPAs on marine forests (in brown), seagrasses (in green) and fishes (in blue) divided by geographic zone: NW Atlantic, NE Atlantic, SE Atlantic, SW Atlantic, Mex-Caribbean, Mediterranean, Black Sea, Baltic Sea, Red Sea, W Indian, N Indian, E Indian, SW Pacific, NW Pacific, NE Pacific, SE Pacific. In grey the total number of studies found in each region on marine forests, fish and seagrasses.

Overall, 20 MPAs with more than 5 scientific papers on marine forests were selected. They are located in the Pacific Ocean, Mediterranean Sea and Indian Ocean. The highest number of papers was recorded at Channel Islands National Marine Sanctuary ($n=70$, California), followed by Catalina Island MPAs ($n=50$, California), La Jolla MPA ($n=31$, California) and Cape Rodney – Okakari Point MPA (or Leigh; $n=31$, New Zealand). Less than 15 papers on marine forests were published in the other selected MPAs (Fig. 4).

The age of the MPA does not seem to be a driver of the number of papers on marine forests published per year (Fig. 5). In most MPAs, independently if recently established or not, the research effort is one paper every two years. The highest rate of papers per year was recorded in Channel Islands MPAs established in 1980 (almost two papers per year). A particular case is Catalina Island MPAs network, where a very high number of studies ($n=46$) was carried out before the creation of the MPAs on this island (in 2010). Even if these studies were not considered in the present analysis, Catalina Island MPAs have a great research effort on marine forests, even higher than some long-established MPAs.

The proportion of papers on marine forests compared to the other selected biotic components in the 20 relevant MPAs is reported in figure 6. Marine forests are the dominant research topic

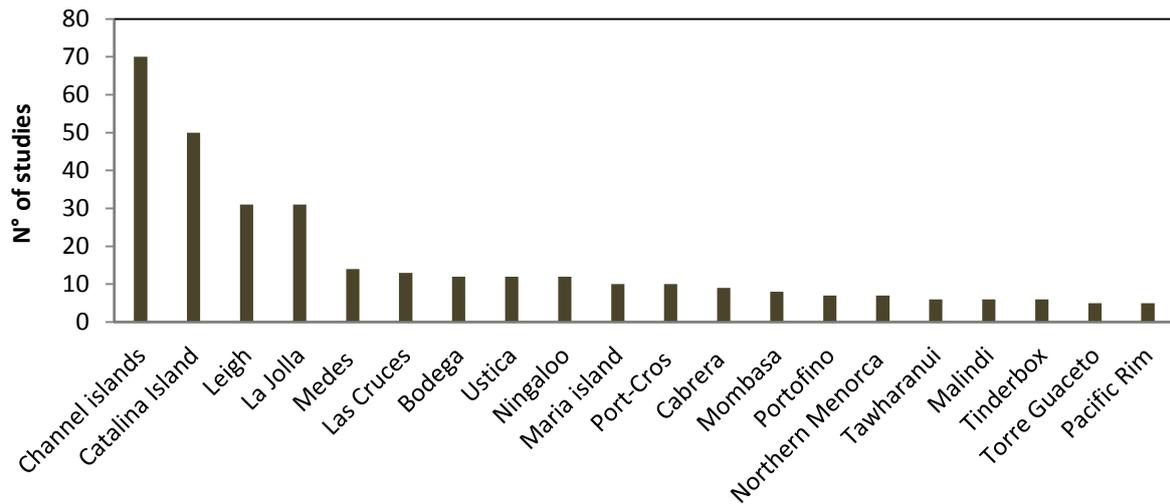


Figure 4. Number of studies on marine forests in the 20 selected MPAs.

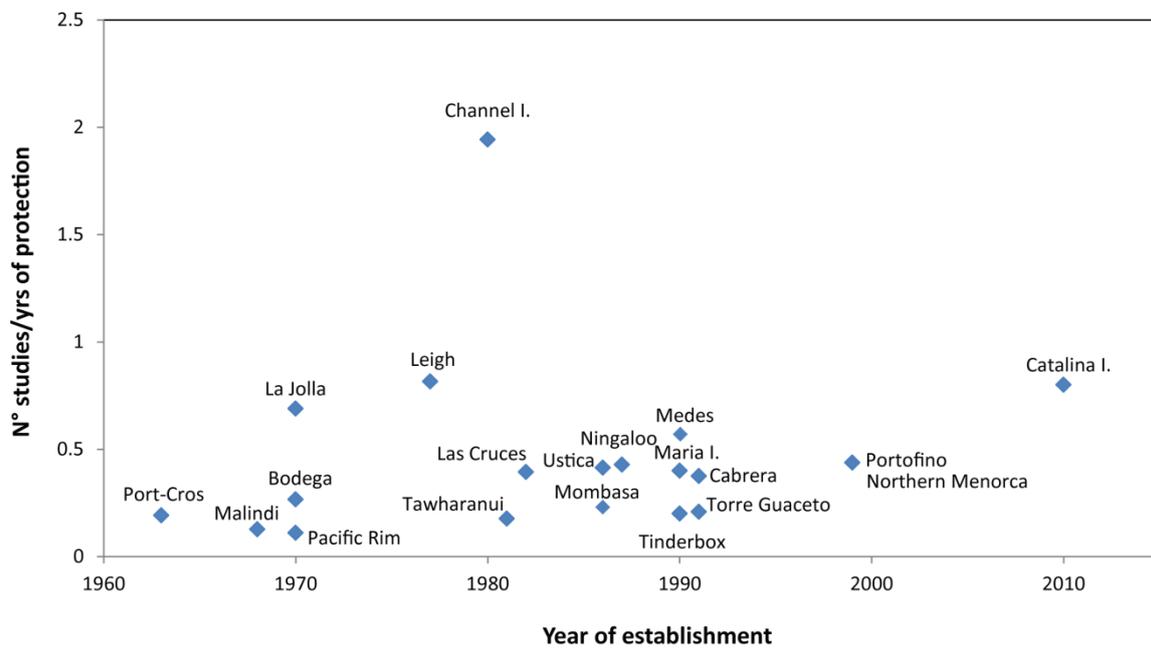


Figure 5. Number of studies on marine forests per year for the selected MPAs.

in Tinderbox (67% of the selected papers, Tasmania), Maria Island MPAs (63%, Tasmania), Northern Menorca MPA (54%, Spain), Las Cruces MPA (52%, Chile) and Channel Islands MPA (51%). A high number of papers on marine forests was also found in other MPAs of the Pacific Ocean: La Jolla MPA (49% of the papers), Tawharanui MPA (46%, New Zealand), Cape Rodney – Okakari Point MPA (40%) and Bodega MPA (34%, California). On the contrary, in Mediterranean MPAs, with the exception of Balearic Islands MPAs (Northern Menorca and Cabrera), papers on marine forests are less than 30%, with a minimum value in Port-Cros MPA (7%). However, interestingly, the percentage of studies on marine forests is, in many cases, comparable to the research effort performed on seagrasses. In the Indian Ocean MPAs, the proportion of studies on marine forests forming species is lower, since they are located in tropical ecosystems, where canopy forming species are not highly represented.

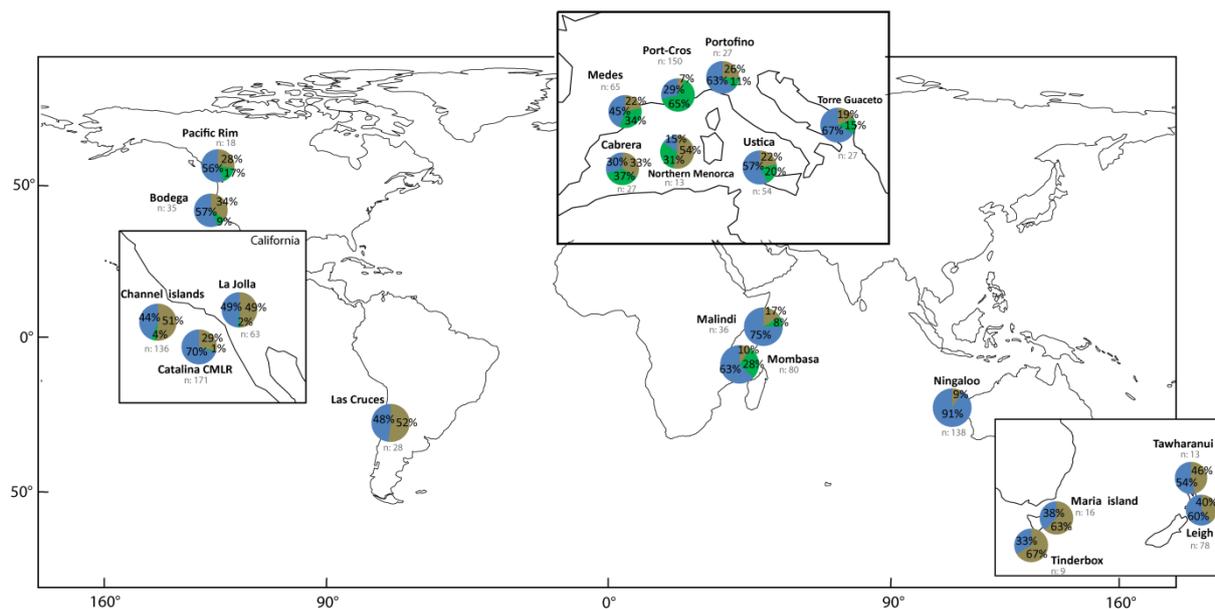


Figure 6. Percentage of papers on marine forests (in brown), seagrasses (in green) and fishes (in blue) in the 20 MPAs with the highest number of studies on marine forests.

2.4 - Discussions and perspectives

Aichi biodiversity targets require that more than 10 per cent of the ocean be protected by 2020 and nearly 200 countries committed on such goal in 2010 (www.cbd.int). This is valuable being MPAs one of the best tools for protecting marine environment: they are anchors for the long-term and large-scale conservation of the oceans, as well as they secure future for developing sustainable local economies (Allison et al. 1998, Lubchenco et al. 2003, Russ et al. 2004). In order to adequately reach such targets, protected areas should be effectively planned, with a good representativeness of habitats, and well-managed, by evaluating regularly the effects of protection (Ward et al. 1999, Agardy et al. 2003, Thomas et al. 2014, Duarte et al. 2016).

In this study, we showed that even if the number of papers on marine forests forming species has increased in the last years, only a small proportion of this research is globally conducted in relation to MPAs: 244 MPAs have performed some kind of research on marine forests forming species, but only 4 of them have more than 20 papers. In the 20 MPAs considered relevant for this study (with more than 5 papers on marine forests), the amount of research is highly variable according to the MPAs and does not seem to depend on the age of the protected area. Most MPAs produce less than one study every two years; the highest rate (2 papers per year) was recorded in Channel Islands MPAs. In fact, information about marine forests distribution in MPAs is not always available, due to the fact that often, such as in most Mediterranean MPAs, marine forests are not included in MPAs planning and monitoring programs. Without information on their presence and distribution, it is difficult to assess if: i) marine forests are well represented in MPAs worldwide and ii) an adequate percentage of marine forests is protected.

Knowledge on marine forests is not evenly distributed around the world. Most of the information available on kelp or furoid forests is restricted to few geographic areas: the Pacific Southwest and Northeast and the Western Mediterranean Sea (including one MPA in the Adriatic Sea). This is frequently correlated to the presence of historical academic institutions, specialised research teams and funding availability. Obviously, MPAs with the highest number of papers on marine forests forming species are located in temperate areas: Tinderbox, Maria Island, Channel Islands, Las Cruces, Northern Menorca, and to a lesser extent, Cape Rodney – Okakari Point, La Jolla, Tawharanui and Bodega. It is worth noting that in tropical MPAs, most of the papers focused on marine forests forming species are

describing the degradation of coral reefs and the regime shifts towards macroalgal-dominated assemblages (Hughes et al. 2007, Roff et al. 2015).

The geographical distribution of studies on marine forests is also related to the presence of emblematic species, such as the giant kelps. As an example, in Channel Islands Marine Sanctuary, where *Macrocystis pyrifera* is abundant, a monitoring program has been established since long time (Davis 1988, Davis et al. 1997). This program originated extensive research on kelp recovery processes, food-web interactions and ecology of commercially important species, associated to kelp forests (North and Pearse 1970, Leighton 1971, Bodkin 1988, Graham 2004, Blamey et al. 2010, Rogers-Bennett et al. 2011). Similarly, in MPAs of Australian and New Zealand temperate waters, the complex dynamics of kelp forests and the regime shifts from kelp beds to sea urchin barren grounds have been the focus of recent research studies (Edgar and Barrett 1999, Shears and Babcock 2002, 2003, Ling and Johnson 2012). Remarkably, other ecologically and commercially important kelp and fucoid forests thriving along the North and South Atlantic and Northwest Pacific Oceans (Raffaelli and Hawkins 1996) produced less ecological and conservation studies (but see Valero et al. (2011), Couceiro et al. (2013)). For instance, in Northern Brittany (Atlantic French coast), where traditionally scientific research is focused on kelp forests, papers on their conservation in MPAs are scarce, since only recently an MPA (Iroise Marine Park) was established. The Mediterranean Sea represents a particular case, where endemic, but “miniaturised”, marine forests forming species (mostly *Cystoseira* spp.) (Feldmann 1937) are abundant (Sales and Ballesteros 2009, Capdevila et al. 2015, Thibaut et al. 2016), even if less known, likely due to the complexity of taxonomic identification. In this Basin, numerous studies focused on marine forests, but they mostly described the regime shifts from vegetation to barren state, caused by the overfishing of sea urchin predators. For example, it is worth noting that extensive barren grounds appeared as an unexpected consequence of Ustica MPA establishment, due to the regulation of sea-urchin harvesting (Gianguzza et al. 2006). In the same MPA, interesting biotic interaction among sea urchin predators, fostered the recovery of *Cystoseira* forests (Galasso et al. 2015): one of the rare events of *Cystoseira* natural recovery (see also Hereu Fina and Quintana Pou (2012)).

When comparing marine forests research to other important biotic components, it appears evident that fishes are largely the most common focus of MPAs papers. Fish assemblages, in fact, are very often the principal target of MPAs establishment and the protection measures are generally limited to fishery regulations. In addition, fishes usually recover quickly after MPAs establishment, allowing the assessment of the protection effect (Russ and Alcala 1996,

Halpern and Warner 2002). Concerning marine vegetation, globally the percentage of studies on seagrasses (18.6%) is higher than the percentage of studies on marine forests (13%). When focusing at the regional scale or on the 20 selected MPAs, such proportions are more variable, but a potential bias may stem from the fact that in several oceanic coastal systems seagrasses are rare, being principally found in estuaries. In the Mediterranean Sea, *Posidonia oceanica* is widely distributed in open coastal systems and it is often associated to forests of large brown seaweeds. Its ecological importance is widely recognised and it represents the only species of marine vegetation listed in the annex IV of the European Habitat Directive. Interestingly, in the Mediterranean MPAs, the amount of research performed on large brown seaweeds of the order Fucales and Laminariales, that are not included in the Habitat Directive, is comparable or higher than the research on seagrasses. This likely reflects a growing scientific interest and recognition of their ecological role. However, this positive perception of marine forests in the scientific community, does not seem to be always reflected in the management measures at local (MPAs) and international (Directives) levels.

On the light of these results, we can argue that, even if the research effort on marine forest is still lower than the one performed on other charismatic biotic components, the scientific interest for this habitat is increasing, also in MPAs. A growing number of studies is being carried out globally to understand marine forests ecology, plant-herbivore relationships and the consequent ecosystem-shifts (Folke et al. 2004, Sala et al. 2011, Filbee-Dexter and Scheibling 2014, Ling et al. 2015).

In this perspective, more research effort is needed on the ecological and conservation aspects related to marine forests, particularly in areas where extensive healthy forests still exist. Marine forests should be included in the existing protection schemes (Fraschetti et al. 2005), as MPAs may represent refugia for marine forests in a mosaic of cumulative impacts (Micheli et al. 2013, Strain et al. 2014, Mineur et al. 2015, Guarnieri et al. 2016). The detailed distribution of marine forests is required for the implementation of effective protection measures (Fraschetti et al. 2008) and for the assessment of MPAs effectiveness in marine forests conservation (Lester et al. 2009, Sala et al. 2012).

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Chapter 3 – Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas

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A mixed *Cystoseira* forest (*C. stricta* and *C. compressa*) in the infralittoral fringe of Saint Jean Cap Ferrat, French Riviera (NW Mediterranean Sea). Photo: Gianni F.

Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas

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Cystoseira species are some of the most important marine ecosystem-engineers, forming extended canopies comparable to land forests. Such forests are sensitive to human disturbances, like the decrease in water quality, the coastal development and the outbreak of herbivores. Conspicuous historical declines have been reported in many regions and several *Cystoseira* species are presently protected by European Union (EU) environmental policies. The aim of this work was to synthesize the conservation perspectives of *Cystoseira* forests in the Mediterranean Sea, focusing on the opportunities offered by artificial restoration and highlighting the potential role of Marine Protected Areas (MPAs). MPAs give a better protection to healthy forests than non-managed sites and may be a source of propagules for natural recovery and/or for non-destructive artificial restoration of nearby damaged forests. MPAs lacking *Cystoseira* forests may also represent preferential sites for reforestation. We proposed a flow-chart for the conservation and a reasoned restoration of *Cystoseira* in the Mediterranean Sea. The successful conservation of *Cystoseira* forests is still possible, via raising public awareness on the role of *Cystoseira* and reducing human impacts on coastal ecosystems. Such actions have to be coupled with more specific large-scale management plans, encompassing restoration actions and enforcement of protection within MPAs.

Keywords: *Cystoseira*; Fucales; forests; conservation; restoration; recovery; human impacts; Marine Protected Areas

1. Introduction

The genus *Cystoseira* is represented by 42 species, mostly distributed in the Mediterranean Sea, but also in the Atlantic Ocean [1], from the surface to the upper circalittoral zone [2,3]. Several species are endemic to the Mediterranean Basin, that is considered the hot-spot for *Cystoseira* species [4], some of the most important marine ecosystem-engineers, forming extended canopies comparable to land forests [5]. They increase three-dimensional complexity and spatial heterogeneity of rocky bottoms, providing refuge and food for many invertebrates and fishes at different life history stages [6–13]. *Cystoseira* forests, hence, play an important functional role in Mediterranean coastal ecosystems, sustaining complex food webs and maintaining a high biodiversity.

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Similar to other large brown seaweeds, *Cystoseira* species are highly sensitive to several human disturbances, so that conspicuous historical declines, for at least a century and especially of species thriving in rock-pools and in the infralittoral zone, have been reported in many regions of the Mediterranean Sea [14–19]. Among human impacts responsible for such regression, the increasing coastline urbanization [17] causes habitat destruction and modification of environmental characteristics (such as hydrodynamics, loads of sediments [20], nutrients [21,22] and chemical pollutants [23,24]). Proliferation of urban structures is common along the Mediterranean coasts: as an example, more than 17 km of coast close to Genoa Harbour (North West of Italy), 11.1% and 88.96% of the French whole Mediterranean and Monaco coastlines are entirely artificial (www.medam.org, last access 6 September 2013). Loss of *Cystoseira* has also been attributed to the outbreak of herbivores [15,25,26], which is a common phenomenon in many regions of the world and is sometimes caused by overfishing of their predators [27–37]. In the Mediterranean Sea, sea-urchins are considered the most important herbivores, being able to graze the macroalgal communities and to create barren grounds (i.e. rocky reef, bare or covered by encrusting coralline algae) [26,38]. High densities of sea-urchins are usually associated with over-fishing of their predators (sea-breams of the genus *Diplodus*) [38–40] and/or date mussels (*Lithophaga lithophaga*) harvest [41–45]. Although *L. lithophaga* is a protected species and its harvest banned (included in Annex IV of the Habitats Directive, in Annex II of the Bern Convention and Barcelona Convention), such fishing was very common in the past in some parts of the Mediterranean Sea and is still illegally carried on in several regions [46–50]. Another important herbivore in Mediterranean rocky bottoms is salema (*Sarpa salpa*), known to selectively graze on some *Cystoseira* species [51]. Its contribution to the general loss of *Cystoseira* in the Mediterranean Sea cannot be quantified with the present knowledge, but we cannot exclude an increase of salema abundances due to the over-fishing of their predators [52–54]. Other potential impacts causing *Cystoseira* regression that are not considered in this study, as less known and/or spread, may be agriculture, bivalve farming and scientific research in the past.

For these reasons, *Cystoseira* forests are locally considered under threat. Several Mediterranean species (*C. amentacea* var. *stricta*, *C. mediterranea*, *C. sedoides*, *C. spinosa*, *C. zosteroides*) are listed in the Annex I of the Bern Convention (Council of Europe 1979). The Mediterranean Action Plan, adopted within the framework of the Barcelona Convention (1976), identifies, in an amendment of 2009 (Annex IV, SPA/BD Protocol – United Nations Environment Programme [UNEP]), the conservation of all but one Mediterranean *Cystoseira* species (*C. compressa*) as a priority. Nevertheless, the overall benefits of these protection measures have been low so far, urging for effective conservation actions.

Here we discuss how the establishment of Marine Protected Areas (MPAs) where dense *Cystoseira* forests are still present, could favour the conservation of these habitats and their recovery. In this synthesis, we only consider MPAs that are effectively enforced [55], so that illegal fishing is not carried on and predators can control densities of sea-urchins, preventing the formation of barren grounds [38].

Cystoseira species are characterized by high reproductive potential, with the production of abundant large and easily sinking eggs and zygotes [56–58]. This reproductive strategy favours the formation of dense monospecific assemblages, but limits the dispersal ability [58]. The low dispersal reduces the potential for natural recovery of wide lost/degraded areas [59,60], such that artificial restoration has been suggested to be an effective way to favour the recovery of low-dispersal/long-lived species [61]. This is the case for *Cystoseira* species, whose effective reproductive strategy would allow the use of non-destructive restoration methods such as propagules or reproductive structures [62]. On

the contrary, the benefits of the restoration undertaken to restore, for instance, degraded *Posidonia oceanica* meadows have been contentious [63] because *P. oceanica* has a very low sexual reproduction potential [64] that imposes the use of techniques that may damage the source meadows [65–67]. More studies are needed to find a non-destructive technique for *P. oceanica* restoration. Finally, when *Cystoseira* forests are lost over wide areas, it may be envisaged to couple artificial restoration [19,62,68,69] with conservation and management in MPAs.

The aim of this work is to synthesize the conservation perspectives of *Cystoseira* forests in the Mediterranean Sea. We focus on the opportunities offered by artificial restoration of *Cystoseira* species, taking into account previous experiences with other large brown seaweeds worldwide, and highlighting the potential role of MPAs.

2. Restoration of marine forests

2.1. Large brown seaweeds forestation

Restoration ecology in estuarine and marine systems is a relatively recent science [61] compared to the historical restoration actions extensively carried out in terrestrial environments [70,71]. Nevertheless restoration actions have been experimented within estuarine habitats [72–75], coastal urbanized areas [76–78], wetlands [79], coral reefs [80], seagrass and eelgrass beds [81,82]. Restoration of kelp and furoid forests has also been explored in Asia, especially China, Japan and Korea and in North and South America [83–90].

Marine forests restoration has been generally performed through three methods: transplanting juvenile or adult individuals [69,83,87,89,91], enhancing recruitment potential (by releasing a suspension of gametes/zygotes or installing fertile receptacles in the target area) [62,83,92,93] or artificially supplementing recruitment (culture of embryos/juveniles in laboratory) [83,90,94].

Transplanting juvenile or adult individuals has been the most frequently tested restoration technique. Kelps and fucoids mainly thrive on rocky exposed shores, so that an efficient fixing of individuals to the rocks has been a major challenge in these forestation attempts. In Chile individuals of *Lessonia nigrescens* were transplanted using plastic nets, rubber bands or epoxy [83,91]. In Southern California *Silvetia compressa* was transplanted by attaching small pieces of rock bearing adults or juveniles to the shore [89]. In North-western Washington (USA), juveniles of *Nereocystis luetkeana* were embedded in a propylene rope that was successively inserted in a hard plastic clip attached to the rock with epoxy putty [87]. In Southern California, Hernández-Carmona *et al.* [85] tested, in different years, two techniques to restore *Macrocystis pyrifera*: transplantation of juveniles, by tying them to the base of previously cut *Eisenia arborea*, and the enhancement of recruitment potential, by putting reproductive blades in cage-like lobster traps. The effort required by each of the two methods was comparable, and the results suggested that combining transplantation of juveniles and seeding during spring would increase the probability of a successful restoration. The enhancement of recruitment potential was also tested for *Sargassum thunbergii* in China using a concentrated suspension of germlings [93]. In another study, such a technique was applied for increasing the recruitment potential of *Lessonia nigrescens* in Northern Chile and then compared to the use of bundles of reproductive fronds fixed to the rock [83]. The same authors also tested an artificially supplemented recruitment, by seeding spores in the laboratory on different substrata that were, afterward, fixed to the rock. Another study artificially supplemented the recruitment of *N. luetkeana* by using Petri dishes as a support, but the rate of loss was very high [87].

All of the studies discussed above considered the restoration successful when a high survival and/or density of recruits was observed at the end of the experiment. However, for restoration to be successful over time, some maintenance actions also have to be planned. For example, a suspension of germlings of *Sargassum thunbergii* was released in artificial rock-pools in China made to control water motion and nutrients and favour the settling of embryos [93]. Grazing is another factor that may need to be controlled, depending on local conditions and method applied (especially when using embryos or juveniles). Many studies on the restoration of kelps and fucoids included methods to exclude herbivores: cages [95], nets [96], antifouling paint [89] or removal by hand [88,97]. The effects of grazing may vary with the density of germlings [97] and may increase with other stressors [97–99].

2.2. *Cystoseira* forestation

The restoration of *Cystoseira* forests in the Mediterranean Sea has been less well studied compared to that of kelps and fucoids in other parts of the world. Some studies used several methods for transplanting *Cystoseira* individuals in order to test ecological hypotheses (different from restoration): plants were tethered to other macrophytes [51], entangled in nets screwed into the rock [100], attached to plastic meshes fixed to ceramic plates [101], fastened to bricks with polyurethane foam [102] and fixed with epoxy putty, both detaching pieces of rocks bearing adult individuals [24] and directly in holes drilled into the rocks [58]. A few other studies explored specifically the reforestation potential of Mediterranean rocky shores (reviewed in Table 1) using different techniques depending on *Cystoseira* species. *Cystoseira barbata*, a species thriving in shallow and relatively sheltered waters, was transplanted in the Northern Adriatic Sea fastened to bricks with polyurethane foam [68], or fragments of rocks bearing juveniles were chiselled off, transferred and reattached with epoxy putty to the shore [19,62]. Attempts of *C. barbata* transplantation (together with *C. crinita* and *C. foeniculacea* f. *tenuiramosa*) were performed in the south of France, fixing adult plants with epoxy putty to boulders disposed in rock-pools [103]. *Cystoseira amentacea* var. *stricta*, a species that forms belts in the exposed infralittoral fringe, was transplanted fixing adult plants with epoxy putty in holes drilled into the rock [69]. *Cystoseira compressa*, which thrives in both exposed and sheltered shallow waters, was transplanted, in the infralittoral fringe of exposed shores, using the same technique as for *Cystoseira amentacea* var. *stricta* [58,69] or, in shallow sheltered zones, hooking the base to cubes of cork fitted in the holes of bricks [68]. For this latter species, however, there was a relatively high loss of transplants [68,69], because the morphology of the base of *C. compressa* made the fixing unstable. In most of the cited studies, transplantation success was high: more than 70% survival after six months for *C. compressa* and *C. amentacea* var. *stricta* [69] and about 30% survival for *C. barbata* after eight months [62]. More interestingly, fertile receptacles or new recruits were often observed in the same year in the case of adult transplantation and one year later in the case of juvenile transplantation [24,58,62,68,69]. Capitalising on the reproductive season of the target species of *Cystoseira* could, therefore, help optimising reforestation efforts.

Despite the generally high reproductive potential of *Cystoseira* species [104,105], few studies have used gametes/zygotes for restoration purposes. Perkol-Finkel *et al.* [62] intercepted recruits of *C. barbata* in the field by using a variety of artificial plates that were located in areas with high settlement potential, but low post-settlement survival probability. They compared plates of different materials (limestone, concrete and clay) and different levels of roughness (only for the clay plates), but neither factor significantly

Table 1. Studies reporting a transplantation method used either for restoring *Cystoseira* species or for testing ecological hypotheses.

Transplanted species	Stage of transplant	Topic	References	Location
<i>C. barbata</i> , <i>C. spinosa</i> var. <i>tenuior</i> , <i>C. crinita</i>	Adults	Effect of pollution	[24]	Menorca Island, Spain
<i>C. abies-marina</i>	Adults	Effect of pollution	[100]	São Miguel Island, Azores, Portugal
<i>C. amentacea</i> , <i>C. compressa</i> , <i>C. balearica</i> , <i>C. crinita</i> , <i>C. compressa</i> var. <i>pustulata</i> , <i>C. spinosa</i>	Adults	Grazing	[51]	Menorca Island, Spain
<i>Cystoseira</i> sp.	Adults	Grazing	[101]	Medes Islands, Spain
<i>C. barbata</i>	Adults	Phenology	[102]	Izola, Slovenia
<i>C. compressa</i> , <i>C. amentacea</i>	Adults	Zonation pattern	[58]	Bogliasco, Ligurian Sea, Italy
<i>C. foeniculacea</i> f. <i>tenuiramosa</i> , <i>C. barbata</i> , <i>C. crinita</i>	Adults, zygotes (plates in adult canopy)	Restoration	[103]	PACA Region, France
<i>C. amentacea</i> , <i>C. compressa</i>	Adults	Restoration	[69]	St Jean-Cap Ferrat, South of France
<i>C. barbata</i>	Juveniles	Restoration	[19]	Monte Conero, Adriatic Sea, Italy
<i>C. barbata</i>	Juveniles, zygotes (plates in canopy of adults)	Restoration	[62]	Monte Conero and surrounding urbanized coast of the Adriatic Sea, Italy
<i>C. barbata</i> , <i>C. compressa</i>	Adults, cultures of zygotes	Restoration	[68]	Izola, Slovenia; Miramare Natural Reserve, Italy

affected the settlement: after four months the plates supported variable, but generally high densities of recruits that could be used for subsequent transplantation. In Menorca, Spain, fertile receptacles were directly fixed to the rocky shore, and propagules, seeded on small stones in laboratory, were transferred to the sea (M. Sales *personal communication*). These non-destructive methods allowed obtaining new recruits without damaging *Cystoseira* forests, which is essential given the critical conservation status of these species. Important knowledge on how to obtain propagules of *Cystoseira* in tanks or Petri dishes and preserve them alive for a long time, as well as on optimal culture parameters, can be deduced from laboratory cultivation experiments developed principally for industrial and medical aims [106–111]. Such propagules can be directly released at sea (through a suspension) or maintained in culture until they reach an adequate size to be transplanted, as already done for other large brown seaweeds [83,90,93,112,113].

Similar to kelps, *Cystoseira* forestation may need maintenance actions to control biotic and abiotic factors that may decrease the survival of transplants or the density of recruits. Grazing is one of the major causes of failure in restoration activities of large brown seaweeds [87,114,115]. Negative effects of grazing have been observed in almost all *Cystoseira* transplantation experiments carried out in the Mediterranean Sea ([58,62,68,69,116], Ferrario *et al.* unpublished manuscript), and experimental exclusions of herbivores have significantly increased the survival probability ([62], Ferrario *et al.* unpublished manuscript). Potential herbivores comprise species of crustaceans, molluscs, sea-urchins and fish [51,62,101,117,118] that usually graze more on *Cystoseira* juveniles than on adults [98,119]. Other factors, such as the absence of an adult canopy and the slope of the substratum, do not seem to limit the success of transplantation [19,62]. On the contrary, the zonation pattern (the position occupied by the species on the infralittoral fringe), and in particular for *C. amentacea* var. *stricta* or *C. mediterranea*, can be a determining factor [58], being related to variable abiotic and biotic pressures [51]. Locally critical ecological factors need to be identified and taken into account for a successful restoration of *Cystoseira* forests.

2.3. Forestation on artificial structures

Many artificial reefs, already existing or especially conceived, have been used for the restoration of large brown seaweed forests degraded by human impacts [86,90,92,120–126]. Even if in many cases results have been considered as successful, the installation of new artificial structures, including artificial reefs, has some negative effects on the native habitats and their associated assemblages [127,128]. Therefore we consider a more sustainable alternative, the use of already existing coastal infrastructures deployed for other societal needs (i.e. piers, dikes, breakwaters, jetties, wharfs, seawalls, offshore platforms, etc.), as a scaffold for the forestation of threatened algal forests. Since coastal infrastructures are expected to proliferate alongside human population [128–131] and their current ecological value as habitats is often very poor compared to natural habitats [130,132–136], efforts to garden ecologically valuable species on their surfaces could help to elevate their ecological value without compromising their original function [62]. Despite the increasing interest and focus, little is still known about the factors affecting the success of these interventions.

Marine infrastructures offer atypical substrates for benthic assemblages in terms of orientation, exposure, structure, surface texture, physical and biotic disturbances [128], all of which are known to affect the recruitment, survival and growth of many large brown seaweeds [137–139]. Extensive transplantation experiments of juveniles of *Cystoseira barbata* to a number of breakwaters and natural sites along the Italian North Adriatic Sea ([19,62], Ferrario *et al.* unpublished manuscript) have given encouraging results. Transplantation proved to be technically feasible and not affected by the slope of the substratum. This suggests that coastal infrastructures could provide potentially adequate habitats despite the greater proportion of inclined surfaces compared to natural ones [130,140,141]. Moreover, the survival of transplants was not impaired by lack of surrounding adults, suggesting that this would not be a limiting factor when managing assemblages on new man-made infrastructures that would obviously lack adult canopies. Nevertheless, when structures were located in sandy areas, a typical setting of many coastal defence infrastructures, survival rate was low [132]: scouring of sediment could be an important limiting factor for algae development [20]. Grazing pressure also seems to be higher on artificial than on natural substrates ([62,129,142,143], Ferrario *et al.*

unpublished manuscript), so that grazers exclusion should be considered [68]. Finally, individuals transplanted on the seaward side of breakwaters could be subjected to a large dislodgment by wave action [62]. A broad-scale experiment is in progress on the Marseilles harbour dikes where concrete structures are tested to transplant fertile *Cystoseira amentacea* var. *stricta* (T. Thibaut, *personal communication*).

3. *Cystoseira* forests in Marine Protected Areas

Thanks to a wide array of regulations, MPAs may guarantee protection of coastal ecosystems from several kinds of direct human impacts, especially coastal development and overfishing [144–146]. Generally, in well-enforced MPAs, illegal destructive fisheries, such as date mussel harvest and blast fishing, are not practiced and high fish and macroalgal biomass are expected, as the restored/preserved high-level predators in the food webs can control the abundance of herbivores and therefore limit the grazing pressure [39,40], one of the major causes of *Cystoseira* regression [15,25,26,33,38]. Whenever released from predator control, in fact, herbivore species like sea-urchins (e.g. *Paracentrotus lividus* and *Arbacia lixula*) and fish (i.e. *Sarpa salpa*) may greatly increase in population density and overgraze erected macroalgae.

Although healthy *Cystoseira* forests can be found in MPAs, as for example in Formentera-Espardell, in Spain, and in Scandola and Port-Cros, in France [147], where an efficient fishing regulation is in force, this is not a general rule [148]. Most Mediterranean MPAs are established on rocky coasts and exposed promontories, which should be the ideal habitats for algal forests, but *Cystoseira* is often not well developed (for instance in Cap de Creus MPA, in Spain, and Piperi MPA, in Greece) [148]. Alternate states (e.g. high fish biomass and low macroalgal complexity or low fish biomass and barren grounds) are commonly observed in MPAs, probably due to other factors acting at different scales [148]. At some MPAs *Cystoseira* stands may be lacking due to natural factors, such as local physical conditions and the characteristics of the species that are locally dominant, but in other sites the lack might be related to past direct or indirect anthropogenic impacts [15]. Potentially, the date mussel harvest or the cascading effects of sea-urchins predators' overfishing may have depleted macrophyte assemblages in MPAs before the establishment of the protection regime, but historical data are generally lacking. However, at Ustica Island MPA (Sicily), extensive barren grounds appeared after the MPA establishment, likely due to the regulation of sea-urchin harvesting [149], but also to the fact that in this relatively remote island, the population density of natural fish predators (sea-brems) is low, probably due to limited juveniles' settlement [40].

Healthy dense forests can still be found in non-protected, but naturally isolated and lowly human-impacted sites, such as Bledes and Dragonera in the Balearic Islands, Kimolos in Greece or St Peter's Island and Maratea coastline in Italy (authors' *personal observation*, [148]). Such forests should be the object of priority conservation measures.

Due to the limited dispersal capability of *Cystoseira* species, the natural re-colonization of deforested areas is particularly slow [56,58,105]. To our knowledge, the only documented cases of natural recovery of *Cystoseira* have been recorded in MPAs. In the Medes MPA, *Codium vermilara* beds and some barren grounds were dominating the seascape at the moment of its establishment, and *Cystoseira* recovery started occurring only 20 years later [148,150]. In Ustica MPA, about 10 years after the disappearance of *Cystoseira* forests, a potential increase of abundance of the starfish *Marthasterias glacialis* may have contributed to the regulation of sea-urchin density and the observed natural recovery of *Cystoseira* [151]. In both cases, we suppose that fragmented reproductive populations of

Cystoseira were still present in scattered refuge areas, even if at low densities. Rare dispersal events, such as drifting or dispersal by animals ('zoochory') may be more common than generally assumed for some species [103], but it is generally assumed that *Cystoseira* natural recovery is unlikely, or very slow, and human-guided restoration could be a helpful tool. Healthy forests in well-preserved MPAs can represent the source of propagules useful to support rare dispersal events and non-destructive re-forestation programmes. Restoration of large brown seaweeds has already interested MPAs in different parts of the world (e.g. [35,36,68,83,89]) and we suggest that managers of MPAs, where the extension of such forests was reduced by human activities prior to the establishment of the protected area, should consider *Cystoseira* re-forestation. Indeed, the controlled abundance of herbivores in these sites may represent a better guarantee for a successful restoration. Unfortunately historical distribution of *Cystoseira* forests is largely unknown, also in areas hosting MPAs. Where grey literature, experts or local stakeholders knowledge is not enough to effectively assess the past presence/natural absence of *Cystoseira* forests, the decision may be based on similar neighbour sites or on modelling [152].

In conclusion, we suggest that MPAs have a strong potential for conservation and restoration of marine forests: both as a source of propagules and as priority sites for restoration activities. Nevertheless, they do not provide protection from large-scale impacts, such as global warming, biological invasions and decrease in water quality [153]. A large-scale spatial planning applied to MPAs and adjacent unprotected areas [154–156] with long-term monitoring programmes and restoration actions, where necessary, is probably the best perspective for *Cystoseira* forests preservation in the Mediterranean Sea [157].

4. Conservation and a reasoned forestation of *Cystoseira* species

In synthesis, *Cystoseira* forests have already suffered widespread and apparently irreversible loss, much of which may have gone unnoticed. The Mediterranean Action Plan, adopted within the framework of the Barcelona Convention (1976), identifies the conservation of *Cystoseira* species as a priority and several large brown seaweeds are listed in the Red Books of Mediterranean and Black Seas (IUCN, www.iucn.org), but very few tangible focussed actions have been established (no institutional actions have been undertaken in the Mediterranean Sea to our knowledge). Therefore, the overall benefits of these protection measures have been low so far [17] and we do not have information on *Cystoseira* distribution, even in MPAs. Also little information is available about their recovery potential, and possibly, over a certain deterioration threshold, these systems may not be able to recover at all [19,34,158,159]. A correct conservation of Mediterranean marine forests should therefore rely firstly on the protection and management of existing healthy forests and secondly on the restoration of fragmented/lost ones. Some guidelines for hypothetical conservation/non-destructive restoration actions of *Cystoseira* forests can be summarized by a flow-chart (Figure 1).

The first step would be to collect information on the distribution and status of the existing forests. If forest is present, healthy and already protected (e.g. in a MPA), it would be useful establishing a regular monitoring to detect early signals of regression. If the forest is not protected, setting effective conservation actions should be considered, in addition to a regular monitoring. If instead the forest is unhealthy, management actions (including forestation) should be planned.

When the site is not forested, it is important to search for historical data: if *Cystoseira* was previously present, an artificial restoration plan should be considered, after removing

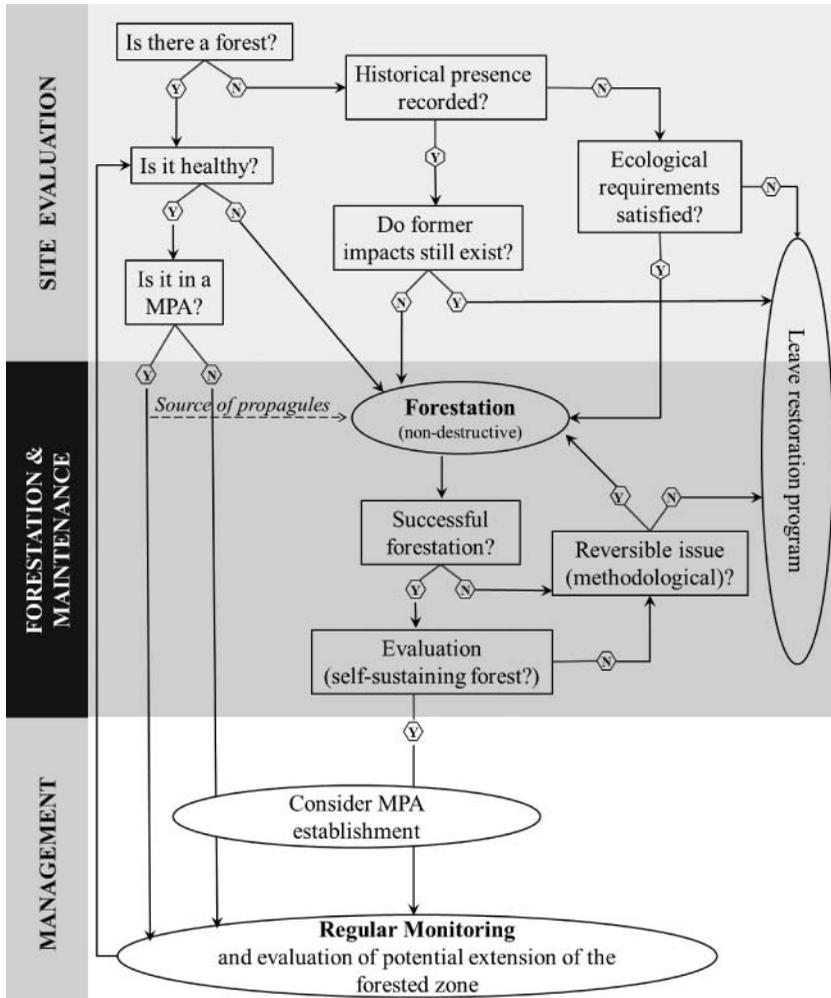


Figure 1. Flow-chart for conservation and reasoned forestation of *Cystoseira* species in the Mediterranean Sea.

the impacts that generated the loss of the forest. If such impacts are still present in the area, no forestation action should be undertaken. If no historical data are available, evaluating the local and regional environmental conditions and anthropogenic pressures could help to understand if *Cystoseira* ecological requirements are satisfied, the likelihood that *Cystoseira* forests might have occurred in the region (e.g. [152]) and if a restoration programme could succeed.

Once restoration action is deemed necessary and likely successful, a forestation method should be chosen. Several approaches have been presented and discussed here and there is not a best technique, as recovery is context-dependent, relying on life-history characteristics of the target species and on the local environmental conditions [159]. Restoration should not involve the transplantation of adults or juveniles collected from healthy forests [68,69]. This approach, although successful, should be avoided and preference should be given to non-destructive techniques based on the enhancement of natural ([62], M. Sales *personal communication*) or artificial supplemented recruitment [68].

After the first forestation phase, the established setup should be maintained (e.g. cages cleaning, nutrients supply and regulation or exclusion of herbivores) and regularly monitored to assess its success. If the forestation is not successful due to high mortality of transplants or absence of recruitment, additional forestation activities could be planned, but only in case the failure is related to reversible issues (e.g. catastrophic events, inadequate choice of the forestation or the maintenance actions). The outcome of restoration should be regularly evaluated by quantifying different variables, in the function of the chosen species/technique: survival of transplants, density and mortality of recruits and/or fertility of second generation individuals. When such variables are comparable to those measured in healthy forests, we may consider the forest as self-sustaining. Afterwards, eventual cages installed for excluding herbivores can be removed, unless they were made from biodegradable materials [84]. Successive monitoring programmes should be undertaken to detect eventual impacts affecting such a forest. If the restored area is not protected, any kind of effective management action devoted to protect the forest may be considered. A successful restoration can be also applied on the adjacent coasts, so to increase the extension of *Cystoseira* stands.

5. Conclusions

Marine forests of large brown seaweeds are locally disappearing in many regions of the world, together with the increase of human activities [17,160]. This trend is also occurring in several areas of the Mediterranean Sea [15], where healthy *Cystoseira* forests are highly threatened and not adequately protected [148]. An important role for forest conservation may be played by MPAs that guarantee protection from various human impacts (e.g. overfishing, urbanization) and that can reduce other ones through an integrated large-scale ecosystem-wide management with adjacent non-protected areas [155,156]. The protection of existing forests should be coupled to regular monitoring programmes in order to promptly highlight potential threats and early signs of regression. Current recovery potential for lost marine forests seems to be limited, even when the proximate drivers of loss are removed [60,161]. An active restoration represents a valuable alternative to assist the conservation of *Cystoseira* forests, but a costs/benefits assessment should be done to evaluate if protection of marine forests would be a better alternative to restore already degraded forests. This should account the economic value of direct, indirect and 'non-use' goods furnished by marine forests, a practice already performed with services provided by MPAs [162].

Several restoration techniques have been presented and discussed here and the choice is species/site dependent. Whenever possible, non-destructive techniques and biodegradable materials should be preferred and, in some cases (e.g. highly variable environments where failure could be higher) the integration of different techniques could enhance success probability [87]. The restoration of *Cystoseira* forests is particularly recommended where historical presence is recorded and the impacts that led to its loss are no longer acting in the area. Nevertheless, forestation could also be considered at sites where the previous distribution cannot be documented, but is likely, based on the local and regional environmental characteristics. Also existing artificial substrata could be considered for forestation, whenever the biotic and abiotic environmental factors are compatible, as this would enhance the ecological value of these artificial substrata without compromising their engineering function. Restoration actions should be preferentially performed in MPAs that can give a better protection than non-managed sites and guarantee the source of propagules for the recovery and/or restoration of close damaged forests.

A successful conservation of *Cystoseira* forests is still possible, as shown by the encouraging results discussed in this synthesis [19,62,68,69]. Reducing cumulative human impacts would still represent one of the most important strategies for the successful conservation and recovery of these systems, but, whenever this alone cannot reverse the loss, well-designed restoration projects can assist. Other important drivers of success would include raising public and political awareness, legal actions and enforcing MPA management plans [159,163].

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Chapter 4 – Ecological amelioration of artificial structures: the role of herbivores

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An herbivore exclusion treatment set up on the breakwater of the Saint Jean Cap Ferrat's harbour, French Riviera. Photo: Gianni F.

Abstract

Coastal areas have been globally transformed by urbanisation, particularly concentrated near the shore, and artificial structures are now already widespread. Even if they physically substitute rocky shores, they do not host structured assemblages as the natural habitats and they do not provide the same ecological functions and services. First attempts to garden artificial structures with habitat-forming species, such as *Cystoseira* spp., were not successful, contrarily to experiments performed in natural areas. Herbivory, and potentially fish grazing, may represent one of the major causes of failure on man-made structures. In this study we set up an herbivory exclusion experiment, coupled with tests in tanks, in order to investigate the potential effect of different herbivores on the forestation success of artificial substrates. Results revealed that fish, namely *Sarpa salpa*, the only strictly herbivorous fish in the Western Mediterranean Sea, can be a very effective grazer of the intertidal *Cystoseira* species. These fish, generally considered as not affecting the very shallow algal belts, were able to cause up to 70-90% of *Cystoseira* loss after few days, in both field and tank experiments. On the contrary, limpets and crabs did not affect significantly the transplanted individuals. Our study proves that fish herbivory may be more important than generally assumed in temperate areas and that it may affect the restoration of large brown seaweeds on artificial substrates. Forestation of artificial habitats is feasible and should be planned whenever possible to ameliorate the ecological value of the already existing man-made substrates. However, the potential effects of grazers, particularly fish, should be previously quantified and adequate exclusion or regulation actions eventually considered.

Keywords: herbivory, *Sarpa salpa*, *Cystoseira*, restoration, intertidal zone, macroalgae

4.1 - Introduction

Coastal areas have been globally transformed by urbanisation, particularly concentrated near the shore (Timmerman and White 1997, EEA 2005). Residential, commercial and tourist activities have required the development of infrastructures such as breakwaters, seawalls, jetties, piers and groynes (Thompson et al. 2002, Bulleri and Chapman 2010). For instance, in Europe about 22,000 km² of the coastline is artificial and in the Mediterranean Sea, infrastructures dominate almost 50% of the shorelines of France, Italy and Spain (Airoidi and Beck (2007) and reference therein). The same scenario and sometimes even worse, occurs worldwide, along the coasts of many industrialized and developing countries (Koike 1996, Chapman and Bulleri 2003, Dugan et al. 2011). Therefore, artificial structures are becoming common features of coastal areas. However, even if they physically substitute rocky shores, they generally do not host structured assemblages as the natural habitats and they do not provide the same ecological functions (Airoidi et al. 2005, Perkol-Finkel et al. 2006). Indeed, assemblages on these substrates are generally characterized by low species and genetic diversity (Chapman 2003, Fauvelot et al. 2009) or dominated by opportunistic and invasive species (Bulleri et al. 2006, Airoidi et al. 2015).

Since coastal defence structures are already widespread and are expected to proliferate with the rise of human population (Firth and Hawkins 2011), more efforts should be made to increase their biodiversity and enhance their ecological value (Perkol-Finkel et al. 2012). Some engineering solutions have already been tested in order to favour the settlement of invertebrates or the recruitment of juveniles of fishes (e.g. Chapman and Blockley (2009), Lapinski et al. (2014)). In addition, despite artificial structures are different to natural systems in terms of substrate features (Chapman and Bulleri 2003, Bulleri et al. 2006), recent studies highlighted that it is possible to increase their biodiversity by fostering the installation of habitat-forming species, such as large brown seaweeds (Dean and Jung 2001, Terawaki et al. 2003, Falace et al. 2006, Perkol-Finkel et al. 2012, Ferrario et al. 2016). Thus, existing defence structures may represent adequate substrates for the installation of valuable species without a supplementary introduction of artificial materials in the marine environment (Gianni et al. 2013).

In the Mediterranean Sea, nursery habitats on rocky shores are mainly represented by species of the genus *Cystoseira* that form dense forests from the surface to several meters depth and support a high biodiversity of organisms (Ballesteros 1990, Ballesteros et al. 2009, Cheminée et al. 2013). However, *Cystoseira* forests are sensitive to most direct and indirect human

impacts, among which coastline urbanisation, considered one of the major causes of loss (Airoldi and Beck 2007, Thibaut et al. 2014). In the last years, the need to restore such paramount habitats has increased (Gianni et al. 2013), not only on natural rocky shores, but also in artificial urbanised landscapes in order to re-establish their important ecosystem functions (Firth et al. 2013). These actions would also increase connectivity among *Cystoseira* populations, characterised by low dispersal ability and high genetic segregation (Robvieux et al. 2012). Despite successful manipulations of *Cystoseira* species for restoration purposes on natural habitats (Susini et al. 2007), few studies experimented *Cystoseira* restoration on artificial habitats (Falace et al. 2006, Perkol-Finkel and Airoldi 2010, Perkol-Finkel et al. 2012, Ferrario et al. 2016) and the success of such interventions remains at present extremely variable.

Among the most important factors that could significantly reduce the success of gardening artificial substrates and inhibit the development of complex algal communities are scouring, poor water quality and herbivory. While the effect of scouring and water quality on *Cystoseira* forests have been investigated in urban areas (Soltan et al. 2001, Airoldi 2003, Arévalo et al. 2007, Mangialajo et al. 2007, Perkol-Finkel and Airoldi 2010, Sales et al. 2011, Devescovi 2015), herbivory associated to artificial habitats has received less attention (Ruitton et al. 2000, Perkol-Finkel et al. 2012, Ferrario et al. 2016).

A greater abundance of grazers is attracted by coastal infrastructures and therefore the herbivorous pressure in these habitats is higher than in natural systems (Bulleri et al. 2000, Rilov and Benayahu 2000, Moschella et al. 2005, Einbinder et al. 2006). This potentially explains the inhibition of complex macroalgal assemblages development on such structures. Indeed, first attempts to forest breakwaters with *Cystoseira amentacea* var. *stricta*, hereafter *C. stricta*, a species forming belts in the very narrow superficial infralittoral fringe, failed in few days (authors' unpublished data). Likely a higher feeding rate on artificial substrates was the cause of such failure, while *C. stricta* restoration performed with the same technique on natural shores gave successful outcomes (Susini et al. 2007). Other studies on deeper *Cystoseira* species (i.e. *Cystoseira barbata* and *Cystoseira compressa*), transplanted on artificial habitats, revealed grazing or non-consumptive behaviours of crabs and fishes (Falace et al. 2006, Perkol-Finkel et al. 2012, Ferrario et al. 2016). However, being the infralittoral fringe a very shallow zone (approximately 0.5 m above and below the mean sea level), *C. stricta* belts are most of time exposed to air and/or wave action. It has been assumed until present that limpets, gastropods and to some extents crabs, are the major herbivores regulating these communities (Benedetti-Cecchi and Cinelli 1992, 1996, Cannicci et al. 2002, Coleman

et al. 2006, Lorenzen 2007) and that such wave exposed zone represents a spatial refuge for *C. stricta* from herbivorous fish (Verges et al. 2009).

Therefore, the aim of this study was to investigate the potential effect of different herbivores, including fish, on the forestation success of intertidal artificial substrates. *C. stricta* individuals were transplanted on an harbour dike and the herbivores access to the experimental plots was regulated by setting up cages of different shapes. The feeding effect of the different herbivores considered in the study was also assessed by tank experiments in order to discern their respective role in affecting *Cystoseira* restoration actions.

4.2 - Materials and Methods

4.2.1 - Study area and species

The caging experiment was carried out in summer 2013 on the breakwater of the Saint Jean Cap Ferrat's harbour (43°41'27" N, 7°20'9" E), in the French Riviera, Western Mediterranean (Fig. 1). In the study area, this is the only artificial structure with close natural *Cystoseira stricta* populations: a guarantee that the area has an adequate water quality for large brown seaweeds development.

Primary branches of *C. stricta* used for the transplantation were sourced at Pointe du Colombier (43°40'58" N, 7°20'32" E), a natural rocky site close to Saint Jean Cap Ferrat, characterized by dense belts of this species. On the contrary, the infralittoral fringe of the breakwater was characterised by less complex macroalgal communities, formed by encrusting and articulates Corallinales and other photophilous algae (mostly *Dictyota* spp. and turf-forming Ceramiales and Sphacelariales).

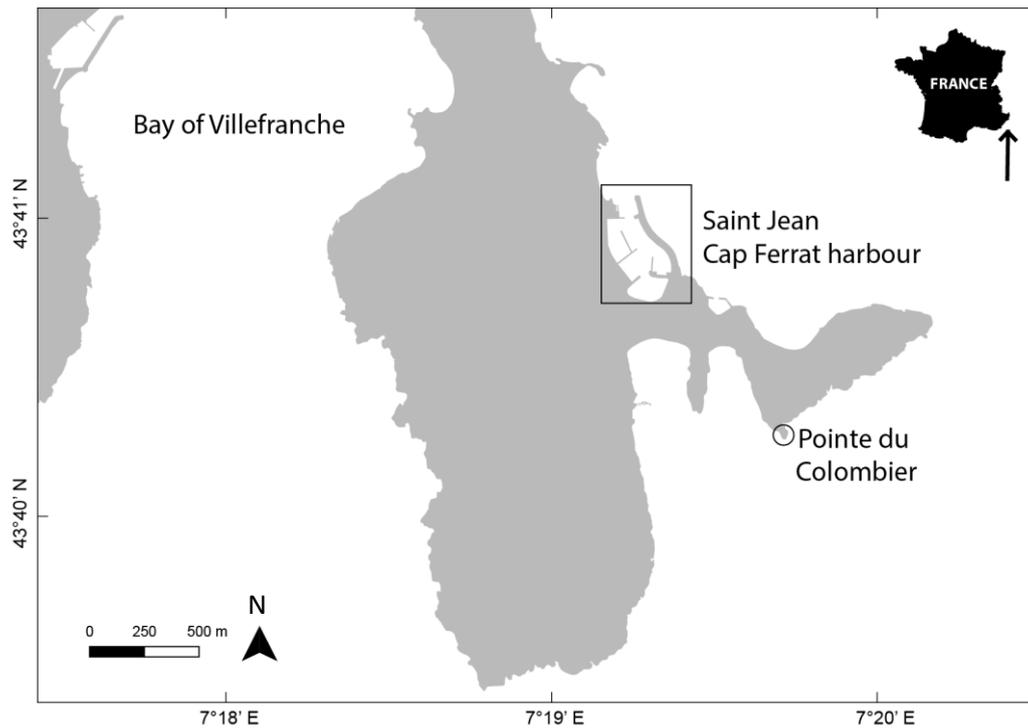


Figure 1. Map of the study area. The experiment was carried out at Saint Jean Cap Ferrat harbour and *Cystoseira stricta* branches were collected at Pointe du Colombier, in the French Riviera (South-East of France).

4.2.2 - Field experiments

The density of all potential herbivores of *Cystoseira stricta* on the breakwater was evaluated by visual census both before starting the experiment, in order to set up appropriate herbivores exclusion treatments, and during the experiment. Sea urchins were absent in the considered zone (only few individuals were found deeper on the breakwater). Density and size (carapace width: CW) of crabs (*Eriphia verrucosa* Forskål, 1775 and *Pachygrapsus marmoratus* Fabricius, 1787) were estimated by six replicated transects (15 × 3 m) (Flores and Paula 2001), paying attention to check each crevice and hole of the breakwater. Density and size of salema, *Sarpa salpa* (L. 1758) were evaluated by ten replicated transects (25 × 5 m) (Harmelin-Vivien et al. 1985). They were performed parallel and perpendicular to the breakwater on a 1200 m² surface characterised by a rocky-sea bottom (maximum depth: 5m). Density and shell maximum diameter (SD) of limpets (*Patella* spp.) were evaluated along six 80 × 20 cm transects, over the mean low water line. Size of limpets was measured by a vernier caliper (precision 0.05 mm).

In order to perform a non-destructive sampling in the source populations at the natural site, we collected only primary branches of *C. stricta*. Branches were preserved in a cool box and immediately transferred to the artificial structure. Twenty-four plots of 30 × 20 cm were previously cleared on the seaward side of the breakwater to remove all the other algae and invertebrates. Four *C. stricta* branches (about 12 cm long) were fixed with epoxy putty in small holes drilled in each plot. Twelve branches were brought back to the natural site and fixed with the same method in three separated plots in order to control for eventual effects of the manipulation on *C. stricta*. Plots were spaced few meters apart to be considered independent.

The grazing effect on the transplants was evaluated by seven treatments. The access of the different herbivores was regulated by setting up, over the plots, plastic net cages (about 30 cm large, 20 cm wide, 10 cm high; 1 cm mesh) with different shapes. Since the breakwater is exposed to high hydrodynamic conditions, cages were fastened with ties to rods (10 cm high) previously fixed by epoxy putty in holes drilled into the rock. The seven treatments ('All', 'Salemas', 'Limpets', 'Crabs', 'Limpets+Crabs', 'Control', 'Artefact control') are described in Table 1. One additional treatment, named 'Control Natural', was set up at the natural site for controlling an eventual effect of the manipulation (Table 1). The experiment lasted one week and it was replicated twice in order to verify consistence of results. The grazing effect in the different treatments was quantified by measuring differences in length (cm) and algal surface (in cm²) of *C. stricta* branches at the beginning and the end of the experiment, by using ImageJ© software (available online at <http://rsb.info.nih.gov/ij/>).

Table 1. Detailed description of the herbivores exclusion treatments.

Treatment name	Herbivores allowed in the plot	Type of treatment	Brief description
All	All	No exclusion	No cage
Artefact control	All	Artefact control (to control for caging artefact)	Plots with a net stripe at one side
Salemas	Only salemas	Selective exclusion treatment	Plots surrounded by a 10 cm height net, folded to avoid crabs access, but open on top
Limpets	Only limpets	Selective exclusion treatment	Plots with a closed cage and four limpets inside (3-4 cm shell length)
Crabs	Only crabs	Selective exclusion treatment	Plots with a closed cage with openings at the base. Limpets were manually removed from the surroundings
Limpets+Crabs	Limpets and crabs	Selective exclusion treatment	Plots with a closed cage with small openings at the base. Presence of limpets in the surroundings.
Control	None	Total exclusion (control)	Plots with a closed cage
Control Natural	None	Total exclusion (control for the manipulation effect)	A closed cage over the plots at the natural site

4.2.3 - Tank experiments

Grazing effect of crabs, limpets and salemas on *Cystoseira stricta* was also evaluated by tank experiments. Some individuals of *Patella* spp., *Pachygrapsus marmoratus* and *Eriphia verrucosa* were collected at the natural site of Pointe du Colombier. In order to avoid harming limpets, we gently induced them to detach spontaneously from the boulders. All animals were transported to the laboratory of the University of Nice in cool boxes filled with seawater and we provided oxygen by air pumps to reduce the stress. In the laboratory we set up two replicated tanks (about 30 L) for each species of crab and for limpets, each with four individuals, except for *E. verrucosa* in order to avoid fighting between conspecifics (Rossi and Parisi 1973). Seawater temperature in the tanks was maintained at 23 °C and we provided a 14L:10D (light:dark cycle) photoperiod by cool-white fluorescent tubes. Concerning *Sarpa salpa*, eight sub-adults individuals (about 8-10 cm) were caught by fishermen at Saint Jean Cap Ferrat and moved to the Marineland Water Park (Antibes, France). Salemas were carried in special bags filled with seawater and pure oxygen (1:3) in order to minimize stress. Two replicated tanks (about 70 L) were set up, each with four *S. salpa* individuals. Fish were acclimatized for some days and fed with lyophilized food before starting the experiment. Tanks hosting fish were provided with continuously renewed seawater (about 20 °C) coming from the sea and exposed to sunlight. All tanks were cleaned every two days. In each tank we placed a concrete tile with four primary branches of *C. stricta* (about 12 cm long) fixed with epoxy putty, as in the field experiment. To evaluate the grazing effect, we measured the length and the surface of *C. stricta* branches at the beginning of the experiment and after seven days. We did not feed the animals during the experiment that lasted one week.

Successively, in another experiment, we also evaluated the feeding choice of *S. salpa* between *C. stricta* and other common macroalgae of the infralittoral fringe: *Dictyota fasciola*, *Corallina elongata* and *Padina pavonica*. We set up two replicated tanks, each with four individuals of salemas. In every tank we put a concrete tile on which we fixed, by epoxy putty, two *C. stricta* branches and two similar quantity of another species of macroalgae. We firstly offered to salemas *C. stricta* and *D. fasciola*. After five days, new *C. stricta* branches were coupled to *C. elongata* and finally, after other five days, to *P. pavonica*. Consumption was measured as the difference between the initial and final algal surface, by using ImageJ© software.

4.2.4 - Statistical analyses

Density of herbivores was calculated as number of individuals/m² and size classes distributions were represented in histogram bars as percentage frequencies.

The loss in length and surface of *Cystoseira stricta* branches was calculated as relative difference in percentage between the beginning and the end of the field and tank experiments. Since results of the analyses performed with these two variables were always consistent, here we will only show the surface-based results, that we consider more representative of the biomass than the length-based measures.

Differences among herbivores exclusion treatments in the field experiment were investigated with permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) based on Euclidian distance matrix. We considered the treatments as a fixed factor with eight levels and the mean value of the surface loss in each plot, as replicates (n = 3) (calculated on the 4 branches, not being independent measures). P-values were obtained by 9999 unrestricted permutations of raw data. Pairwise tests were performed in order to discern eventual differences among treatments. In this case, Monte Carlo test was applied because of the low number of permutations. Analyses were performed using Primer 6 & PERMANOVA+ software package, considering the two experimental sessions separated. We used effect sizes (or magnitude) to show differences between treatments in the field experiment. We calculated effect sizes with log-response ratios (Hedges et al. 1999) for each treatment as:

$$R_t = \ln \left(\frac{E_t}{E_{control}} \right) = \ln \left(\frac{\bar{x} \text{ surface loss}_t}{\bar{x} \text{ surface loss}_{CT}} \right)$$

where R_t is the log-response ratio for the treatment t , and \bar{x} surface loss _{t} and \bar{x} surface loss_{CT} are respectively the mean of surface loss of each treatment and the mean of surface loss of the treatment 'Control'.

Differences among treatments for both tank experiments, were investigated with permutational multivariate analysis of variance (PERMANOVA) based on Euclidian distance matrix. P-values were obtained by 9999 unrestricted permutations of raw data and applying Monte Carlo test, if necessary. Pairwise tests were done to show differences among treatments.

4.3 - Results

4.3.1 - Field experiments

At the artificial reef of Saint Jean Cap Ferrat harbour, the estimated density of crabs was 0.2 ± 0.02 ind./m² (mean \pm SE) for *Pachygrapsus marmoratus* and 0.02 ± 0.006 ind./m² for *Eriphia verrucosa*. Limpets density was estimated at 407 ± 43.1 ind./m² and salemas at 1.5 ± 0.3 ind./m² (13.7 ± 2.7 g/m²). Size classes distributions (Fig. 2) showed that on the breakwater, limpets populations were mainly characterized by small individuals (1-10 and 10-20 mm SD classes, representing about 90% of all individuals). The two species of crabs were represented by different size classes: *P. marmoratus* population was mainly characterized by individuals between 1 and 2 cm CW, while *E. verrucosa* by larger individuals (more than 3 cm CW). In front of the breakwater we observed big schools of *Sarpa salpa* sub-adults individuals (about 6 cm total length) representing more than 60% of all individuals, but large individuals were also observed.

The percentage of surface loss of *Cystoseira stricta* branches on the breakwater was very high (up to 90%) in the plots where all herbivores were allowed ('All', 'Artefact control'), as well as in the plots where only salemas were allowed ('Salemases'), for both experimental sessions (Fig. 3). Such results were confirmed by the analyses of variance (PERMANOVA, $p < 0.01$, Table 2) and by the pairwise tests (see letters above the bars in fig. 3). The analyses showed significant differences among the treatments accessible to salemas ('All', 'Artefact control', 'Salemases') and the ones exclusively accessible to other herbivores ('Crabs', 'Limpets', 'Limpets+Crabs') or completely closed ('Control', 'Control Natural'), in which *C. stricta* was not grazed. In particular, in the treatment where all herbivores were allowed ('All'), primary branches were more grazed compared to the other treatments, but, as expected, no differences were observed compared to the artefact control. The plots accessible to salemas were statistically different from all the other ones except for the artefact control. In the first date in which we performed the experiment the treatment completely closed at the natural site ('Control Natural') did not differ significantly by the artefact control (Fig. 3), probably due to a storm that partially damaged the cages. Effect sizes confirmed these results, highlighting significant grazing effects only for plots where salemas (alone or with other herbivores) were allowed ('All', 'Artefact control', 'Salemases') (Fig. 4).

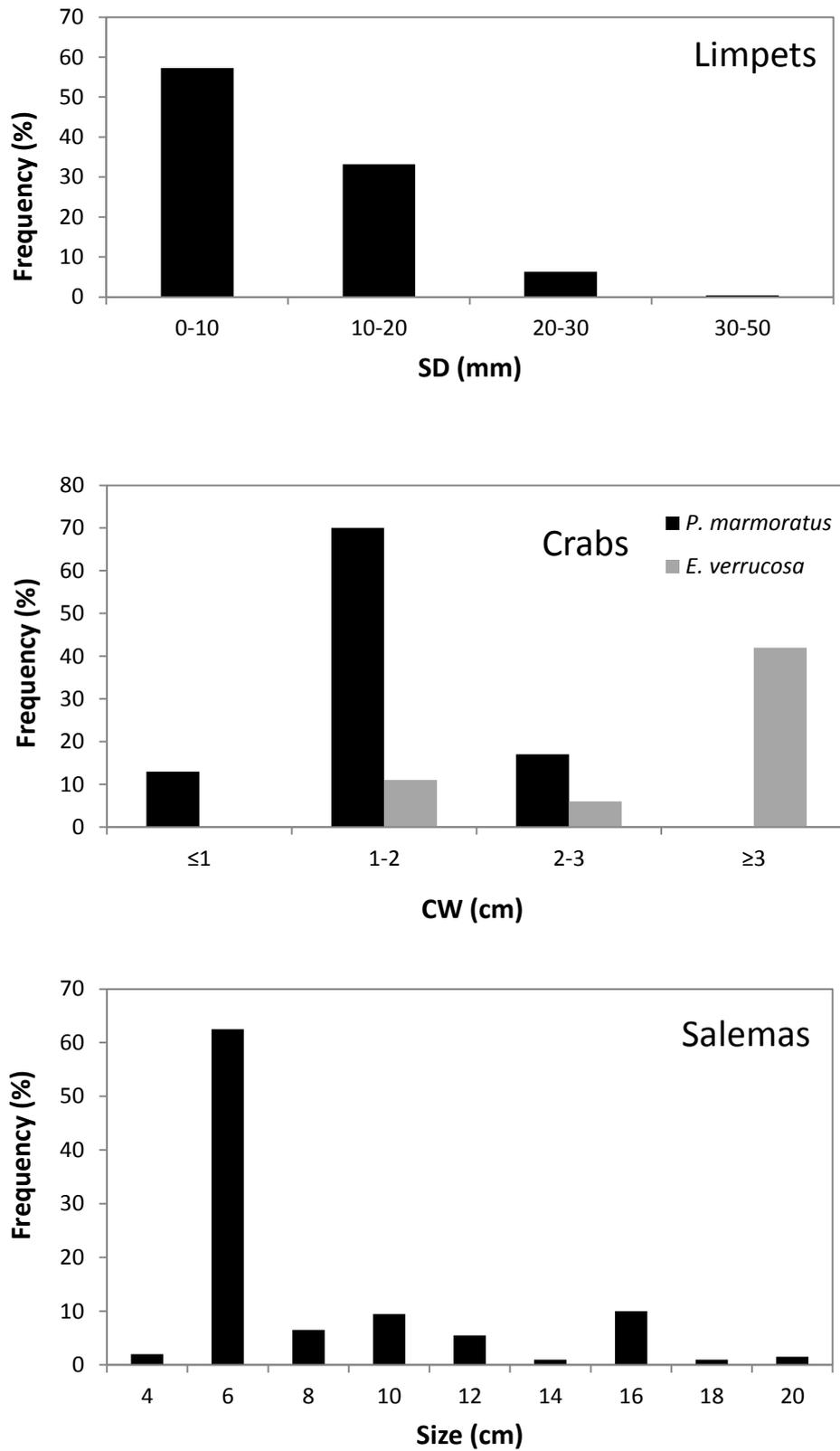


Figure 2. Size classes distribution of the different herbivores at the artificial structure. SD: shell maximum diameter; CW: carapace width.

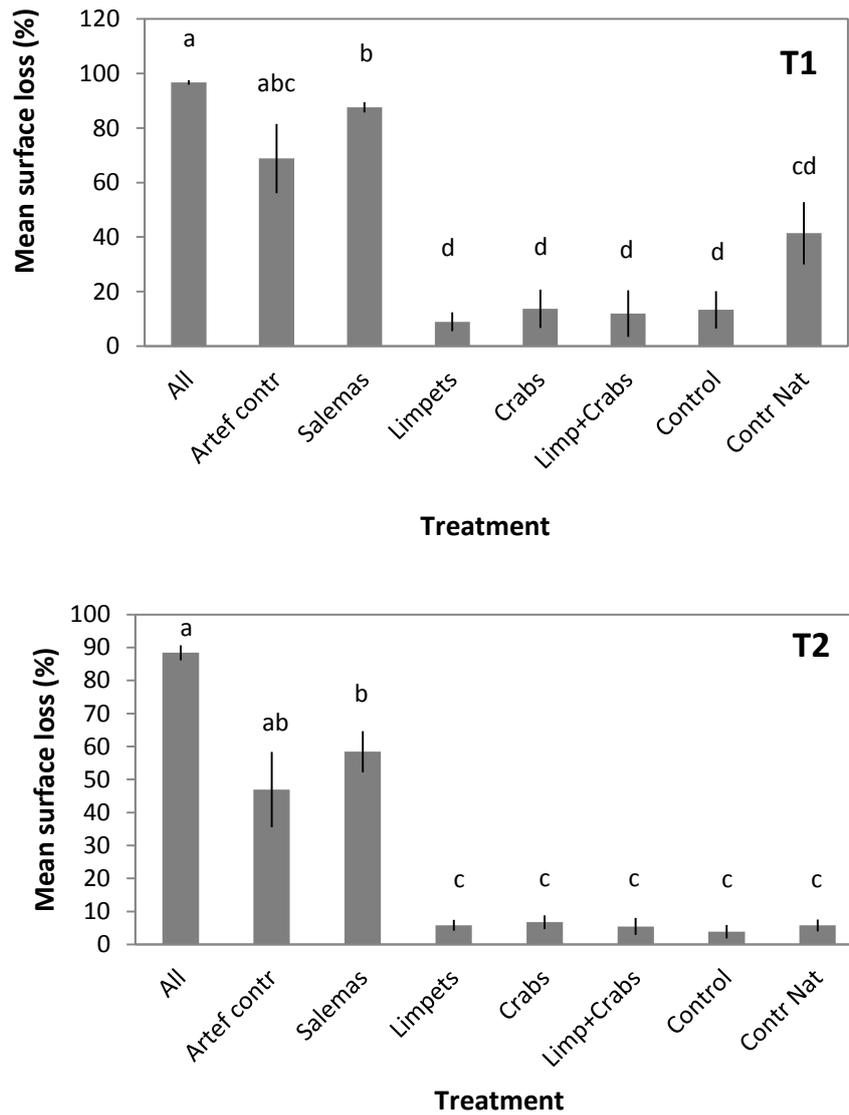


Figure 3. Percentage of surface loss of *Cystoseira stricta* in the field experiments. T1 and T2: field experimental sessions. Letters above the bars indicate significant differences of the pairwise-tests. See Table 1 for treatments description.

Table 2. PERMANOVA tables for the field experiments. Tr: Treatment. Statistically significant values are in bold type. Time 1 and 2: field experimental sessions.

Source	TIME 1				TIME 2		
	df	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Tr	7	4482.7	23.602	0.0001	3725.5	79.026	0.0001
Residual	15	189.93			47.143		

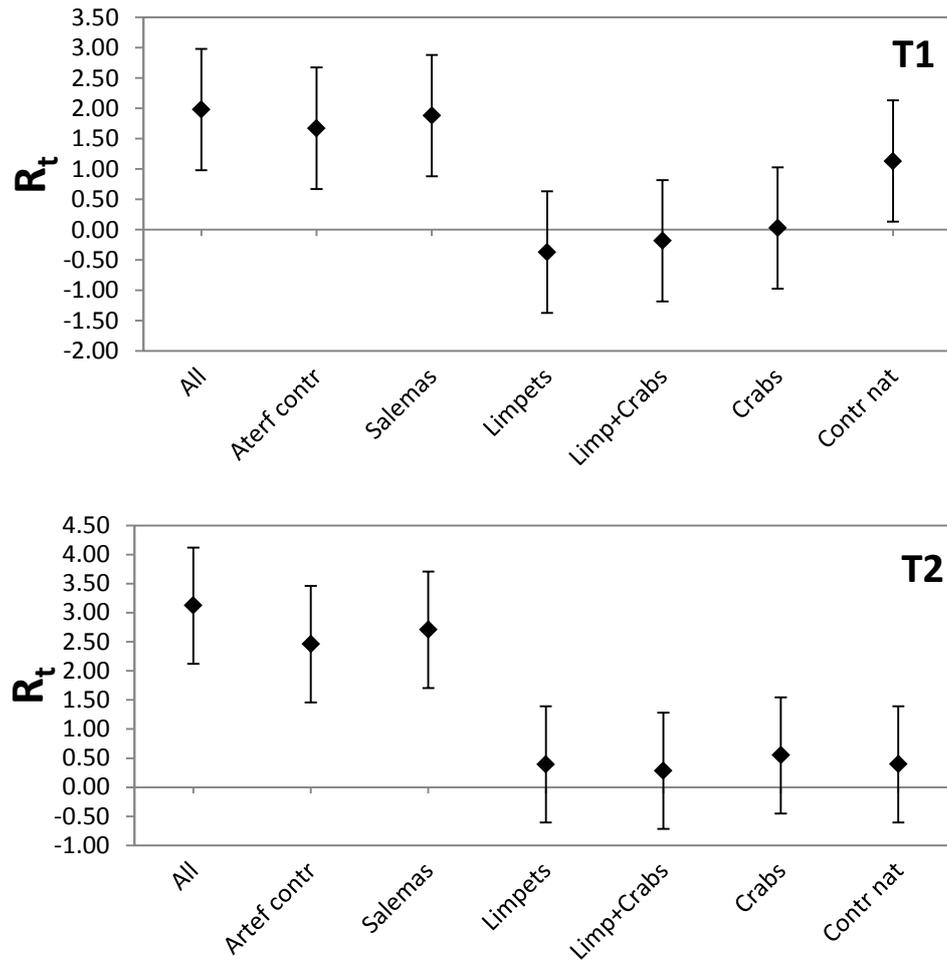


Figure 4. Effect sizes on the surface loss of *Cystoseira stricta* in the field experiment. T1 and T2: field experimental sessions. R_t : the log-response ratio for each treatment. Control treatment is not represented. Effect sizes are significant if confidence intervals do not overlap zero. See Table 1 for treatments description.

4.3.2 - Tank experiments

The first tank experiment, aimed to evaluate the grazing effect of the different herbivores, showed that the main grazer of *Cystoseira stricta* was *Sarpa salpa* (Fig. 5), as also highlighted by the analysis of variance (PERMANOVA, $p < 0.01$, Table 3) and pairwise tests (see Fig. 5). The crab *Pachygrapsus marmoratus*, under food-limitation conditions, showed to be able to cut and, in some cases, to feed on fragments of *C. stricta*. The other herbivores (*Eriphia verrucosa* and limpets) did not graze significantly *C. stricta* primary branches.

In the experiment that investigated the feeding choice of *S. salpa*, salemas always preferred *C. stricta* compared to the other common infralittoral macroalgae (*Dictyota fasciola*, *Padina pavonica*, *Corallina elongata*), consuming more than 60% of *C. stricta* branches in few days (Fig. 6). These results were confirmed by the analyses of variance (PERMANOVA, $p < 0.01$), except for the comparison between *C. stricta* and *D. fasciola* that resulted not statistically significant (PERMANOVA, $p = 0.37$) (Table 4).

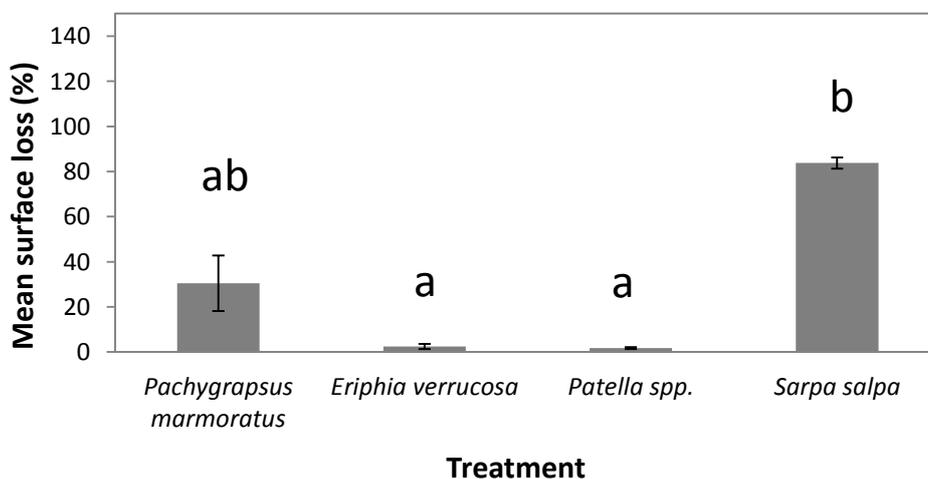


Figure 5. Percentage of surface loss of *Cystoseira stricta* in the tank experiment. Letters above the bars indicate statistically similar groups individuated by pairwise-tests.

Table 3. PERMANOVA table for the herbivory experiment performed in tank. Tr: Treatment. Statistically significant values are in bold type.

Source	df	MS	Pseudo-F	P(perm)
Tr	3	2966.7	27.021	0.0339
Residual	4	109.79		

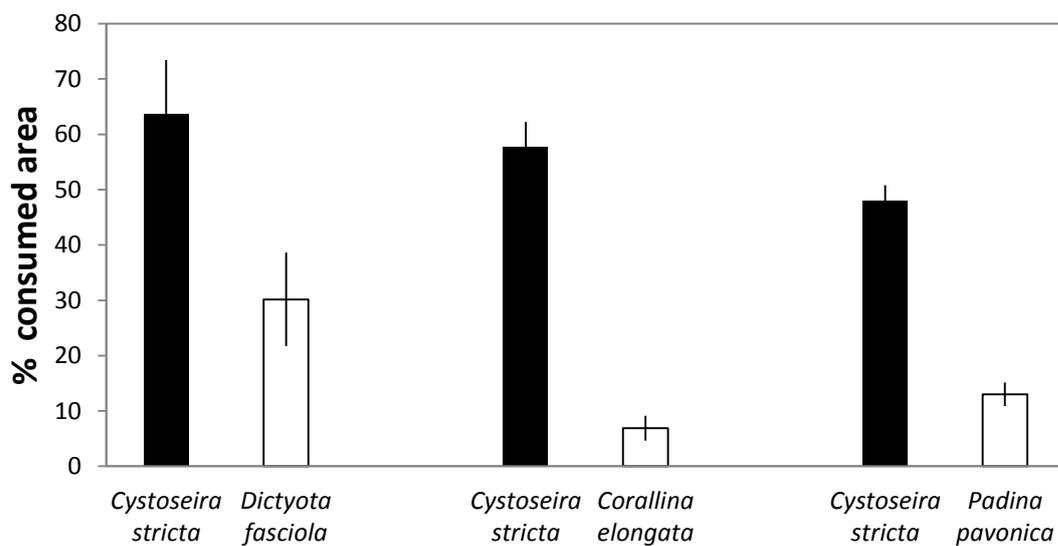


Figure 6. Feeding choice of *Sarpa salpa*. Histogram bars show the preference of salemas between *Cystoseira stricta* and other common macroalgae of the infralittoral fringe. Each couple of algae was offered to fish at different times.

Table 4. PERMANOVA tables for the coupled-choice experiments in tank. Tr: Treatment. Statistically significant values are in bold type.

Source	df	<i>C. stricta</i> - <i>Dictyota fasciola</i>			<i>C. stricta</i> - <i>Corallina elongata</i>			<i>C. stricta</i> - <i>Padina pavonica</i>		
		MS	Pseudo-F	P(MC)	MS	Pseudo-F	P(MC)	MS	Pseudo-F	P(MC)
Tr	1	1398.6	1.2971	0.3767	3032	145.28	0.0072	1250.1	78.052	0.0122
Residual	2	1078.2			20.87			16.016		

4.4 - Discussion

The increase of coastline urbanisation following human development along the shores forces the need to mitigate its impacts and restore natural-like habitats that were lost, fragmented or degraded (Airoldi et al. 2005, Bulleri and Chapman 2010, Firth et al. 2013). This can be done on existing coastal defence structures, that can be used as a scaffold for restoring habitat-forming species (Gianni et al. 2013). Marine forests of large brown seaweeds are among the first species that should be restored because their regression is critically rising (Airoldi et al. 2014) and because if their ecological role is re-established, biodiversity of artificial structure could be quickly augmented. However, our results highlight a paramount role of herbivores, and in particular of the herbivorous fish, in reducing the success of restoration actions.

Density of herbivores in our experimental site was representative of very shallow artificial structures in the Mediterranean Sea. Limpets were very abundant (about 400 ind./m²), in agreement with the densities measured on artificial reefs (10-1100 ind./m²) (Bulleri et al. 2000, Bulleri and Chapman 2004). Crabs densities (0.2 ± 0.02 ind./m² for *Pachygrapsus marmoratus* and 0.02 ± 0.006 ind./m² for *Eriphia verrucosa*) were comparable to the values observed in other studies on natural habitats (*P. marmoratus*: 0.2-2.4 ind./m²; *E. verrucosa*: 0.02-0.05 ind./m²) (Cannicci et al. 1999, Flores and Paula 2001, Cannicci et al. 2002), although data on density in artificial habitats are not available, to our knowledge. Salema density estimations in the Mediterranean Sea are very variable (0.003 – 7.7 ind./m²) (Francour 1997, Ruitton et al. 2000, Verges et al. 2009) and up to 10 g/m² (Sala et al. 2012). Probably this is due to the fact that *Sarpa salpa* is a gregarious species, usually moving in big schools and its density can highly change in space and time (Harmelin-Vivien et al. 1985). Our values (1.5 ± 0.3 ind./m², 13.7 ± 2.7 g/m²), compared to the other estimates at the Mediterranean scale, seem to be representative of high densities.

Our study shows that the success of *Cystoseira stricta* transplantation on artificial structures is mostly affected by herbivorous fish, and in particular salemas. They are able to graze up to the infralittoral fringe, a zone generally considered protected from fish herbivory, being exposed frequently to the air. We cannot exclude that other herbivorous fishes like blennids have contributed to the strong grazing observed. However, the grazing effect of blennids on macroalgal communities is generally considered limited (Verlaque (1990) and references therein). The observed fish grazing pressure was sufficient to cause up to 90% of *C. stricta* surface loss after few days and sometimes even after few hours, especially during bad weather

conditions (low barometric pressure and strong waves action). Such conditions likely make *C. stricta* transplants more accessible to fish and, although discontinuous, grazing may be very effective.

Tank experiments confirmed our field observations: salemas were able to deplete almost completely *C. stricta* branches in few days and, interestingly enough, *C. stricta* was preferred to other three common macroalgal species (*Padina pavonica*, *Corallina elongata* and *Dictyota fasciola*). Evidences proving that *C. stricta* is a preferred food item for *S. salpa* were already obtained in the past: this species can represent up to 60% of the gut content (Verlaque 1990, Tomas et al. 2011) and it is highly consumed when transplanted deeper in the infralittoral (Verges et al. 2009, Tomas et al. 2011). These results, in agreement with our findings in coupled feeding choice experiments, could be explained by the great nutritional value of *Cystoseira* species (Durmaz et al. 2008, Vizetto-Duarte et al. 2014).

Limpets and crabs on the contrary did not affect *C. stricta* transplants both in the field and in tank experiments. Limpets can regulate macroalgal assemblages on intertidal shores being able to remove mature thalli (Lorenzen (2007) and references therein). However, in the Mediterranean Sea they control early patterns of colonisation, grazing on early life stages of algae (i.e. zygotes and juveniles) rather than well-developed individuals (Benedetti-Cecchi and Cinelli 1992, Benedetti-Cecchi et al. 1996). Crabs did not eat or manipulate *C. stricta* on the breakwater, as occurred for individuals transplanted in the upper-subtidal zone (Ferrario et al. 2016), but, interestingly, in our tank experiments a small amount of the branches were eaten. Crabs are generally omnivorous and many species manipulate macroalgae for masking or decoration (Cruz-Rivera 2001). *P. marmoratus* and *E. verrucosa* do not generally mask themselves with algae and in nature they prefer feeding on small invertebrates and filamentous algae than corticated and leathery macrophytes (Cannicci et al. 2002, Cannicci et al. 2007). Since both species are able to regulate their feeding preferences according to food availability (Cannicci et al. 2007) we suppose that they ate *C. stricta* in tanks because of the food limitation condition. It is worth noting that our experiment was planned on a short temporal scale and only using long *C. stricta* branches. Grazing pressure of limpets and crabs would have been greater in a long term experiment, also involving early life stages of *Cystoseira*.

In conclusion, our study proved that fish herbivory is an important factor influencing the success of restoration actions on artificial substrates. In addition, we showed that *S. salpa* is also able to strongly affect macroalgae in a very shallow zone, generally considered protected by these herbivores (Verges et al. (2009) and references therein). It is likely that the role of

herbivorous fish in regulating intertidal macroalgal assemblages is greater than originally thought and has been underestimated so far. Further studies are needed to estimate the effect of salemas on macroalgal communities, in particular on natural *Cystoseira* belts.

Even if few studies tested forestation of artificial habitats by means of endangered and engineer species, such as *Cystoseira*, these actions are feasible and may be planned whenever possible to ameliorate the ecological value of artificial substrates. However, firstly, the potential effects of grazers, and particularly fish, should be quantified and, consequently exclusion or regulation actions considered (Gianni et al. 2013). With this in mind, ecologists and engineers should work together in order to design and build artificial structures with already pre-installed herbivores exclusions devices that would help the restoration of canopy-forming species.

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Chapter 5 – Threats to marine forests in temperate areas: the overlooked role of herbivorous fish

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Cystoseira stricta belts protected from fish grazing by hand-made deterrent devices. Photo: Mangialajo L.

Abstract

Marine forests of large brown seaweeds (i.e. fucoids and kelps) are subjected to multiple impacts, causing local regressions and inducing a general loss along many coastal areas of the world. Fucoids and kelps are also considered the most vulnerable algal functional group to herbivory. In temperate zones the herbivorous pressure of fishes has been generally assumed to be lower if compared to the one of invertebrates (i.e. sea urchins in the subtidal and molluscs in the intertidal zone). Recently, dramatic community phase-shifts from marine forests to barrens, drew scientists attention to the effects of tropical herbivorous fishes expanding their range in temperate areas. Few other evidences allowed to argue that also the role of native herbivorous fishes in shaping macroalgal communities may have been overlooked so far. In this study we evaluated the effect of *Sarpa salpa* (salemas) feeding on the fitness (growth, biomass and reproduction) of *Cystoseira amentacea* var. *stricta* (Fucales). This is a species thriving in very shallow exposed shores, generally considered a refuge from fish herbivory. To test our hypotheses, we set up an experiment using an innovative deterrent device, allowing the decrease of the herbivorous pressure, without affecting the light penetration. The results of this study proved that native herbivorous fish can strongly affect marine forests in temperate areas. The herbivorous pressure in the unprotected blocks caused up to 78% algal growth, 86% biomass and 97% reproductive output loss. Since salemas feed preferentially on the apical reproductive structures, we argue that they may have contributed to the loss of *Cystoseira* forests recorded in the Mediterranean in the last decades. The effect of browser herbivorous fishes should be considered in interaction with invertebrate herbivores (grazers, scrapers), as much as with other stressors threatening marine forests in temperate areas. An increase of herbivorous fish populations is plausible and may be fostered by different causes, such as long-term and large scale fluctuations, changes in fisheries and trophic cascades. The loss of marine forests and the shift to fast-growing, less palatable species (i.e. coralline turfs) results in less complex, productive and diversified benthic assemblages. We suggest that more information on the distribution, abundance and fluctuations of both marine forests and herbivorous fishes would allow a better monitoring and management of coastal ecosystems.

Keywords: fish, herbivory, marine forests, *Cystoseira*, temperate areas, interaction, conservation, restoration, algae

5.1 - Introduction

Marine forests of large brown seaweeds are unique habitats supporting a great variety of organisms worldwide (Dayton 1985, Ballesteros 1990, Jones et al. 1994, Steneck et al. 2002, Schiel and Foster 2006, Cheminée et al. 2013). Several species mostly belonging to the orders Fucales and Laminariales are adapted to different environmental conditions. They can be the dominant species in both very shallow and deep waters (up to the light compensation limit) and in exposed and sheltered zones of macro- and microtidal environments (Schiel and Foster 1986, Leigh et al. 1987, Hereu et al. 2008, Sales and Ballesteros 2009, Nelson et al. 2015).

Marine forests are subjected to multiple impacts, causing local regression and inducing a general loss along many coastal areas of the world (Eriksson et al. 2002, Coleman et al. 2008, Okuda 2008, Perkol-Finkel and Airoidi 2010, Mineur et al. 2015). In particular, species thriving in shallow zones are the most affected, being located in a boundary environment (Cefalì et al. 2016), exposed to both terrestrial and marine sources of impacts. Contaminants (Gledhill et al. 1997, Sales et al. 2011), eutrophication (Berger et al. 2004, Mangialajo et al. 2008, Gorman et al. 2009), suspended sediment in the water (Eriksson et al. 2002, Airoidi 2003), increase of the seawater temperature (Schiel et al. 2004, Raybaud et al. 2013, Pereira et al. 2015) and habitat modification by coastal urbanisation (Airoidi and Beck 2007, Connell et al. 2008, Thibaut et al. 2014), are well-known factors responsible of such loss.

In addition, plant-herbivore relationships are extremely intense in marine environments and leathery macrophytes, as fucoids and kelps, are considered the most vulnerable algal functional group to herbivory (Poore et al. 2012). In this context, outbreaks of herbivores, such as sea urchins, following natural fluctuations or fostered by alteration in trophic webs (i.e. reduction of their predators by overfishing) (Mann and Breen 1972, Lozano et al. 1995, Micheli et al. 2005), are responsible of macroalgal communities depletion and a consequent formation of extensive barren grounds (Mann 1977, Chapman 1981, Agnetta et al. 2015). This phenomenon has been observed in many regions of the world (Hernández et al. 2008, Bonaviri et al. 2011, Flukes et al. 2012, Jeon et al. 2015) and nowadays sea-urchin barren grounds are a common landscape in most temperate bioregions. As a consequence, sea-urchins are considered as the main herbivores of marine forests in the subtidal zone of temperate areas (Choat and Schiel 1982, Byrnes et al. 2013, Ling et al. 2015). In the intertidal zone, on the contrary, gastropods and limpets are considered the major herbivores, because it is generally less accessible to sea urchins (Jenkins et al. 1999, Coleman et al. 2006, Leblanc et

al. 2011). Therefore, in both subtidal and intertidal temperate ecosystems, herbivory pressure is generally associated to invertebrates (Andrew 1993, Vásquez and Buschmann 1997, Estes et al. 1998, Sala et al. 1998, Davies et al. 2007).

The role of herbivorous fishes in regulating macroalgal vegetation is highly variable: in tropical areas, their role in limiting the risk of shifts from coral-dominated to macroalgae-dominated communities is well-known (Lubchenco and Gaines 1981, Mumby et al. 2006, McCauley et al. 2010, Chong-Seng et al. 2014). In temperate zones the herbivorous pressure of fishes has been generally assumed to be lower if compared to the one of invertebrates (Choat 1982, Gaines and Lubchenco 1982, Jones 1988). This may be due to the abundance of herbivorous fishes species that decreases from low to high latitudes, according to different hypotheses (Floeter et al. 2005, Trip et al. 2014).

Recently, the interest of scientists on herbivorous fishes on temperate areas has increased, in particular due to dramatic community phase-shifts from forests to barrens observed, among others, in Japan and in the Eastern Mediterranean Sea (Vergés et al. 2014a). This change has been driven by tropical herbivorous fishes expanding their range in temperate areas with the increase of sea water temperatures. However, recent studies also argue that the role of native herbivorous fishes may have been underestimated so far. Taylor and Schiel (2010) proved that a wide-ranging herbivorous fish, *Odax pullus*, is able to greatly reduce the cover and biomass of the kelp *Durvillaea antarctica*, restricting the alga to wave-exposed conditions (less accessible to fish) in Southern New Zealand. Vergés et al. (2009) observed a similar phenomenon in the Western Mediterranean Sea, where several *Cystoseira* species can be restricted to spatial refuges (in very shallow or deep areas) by the herbivorous pressure of *Sarpa salpa* (salema). The authors of both these studies argue that the impact of this consumer is potentially enormous: they often graze selectively on large brown algae, affecting their spatial distribution.

In this study we evaluated the effect of fish grazing on the fitness (growth, biomass and reproductive output) of *Cystoseira amentacea* var. *stricta* (hereafter *C. stricta*), a canopy-forming seaweed of the Mediterranean Sea. *C. stricta* bears apical reproductive structures (receptacles) and form narrow belts, few dozens of centimetres, in the infralittoral fringe, considered as a refuge from fish herbivory (Vergés et al. 2009). In particular, it is characterized by longer primary branches in the upper part (High zone) of the infralittoral fringe and shorter primary branches in the Low zone, more accessible to fish. In order to assess *S. salpa* herbivory pressure and its effect on *C. stricta* fitness, a manipulating experiment was performed, using an innovative herbivorous fish deterrent device. We tested

the hypotheses that if *C. stricta* belts are protected the length of primary branches, biomass and reproductive output will increase and the differences between the high and low level of the infralittoral fringe will be reduced.

5.2 - Materials and methods

5.2.1 - Study area and species

The experiment was carried out between March and June 2014 in two randomly chosen sites (Pointe du Rubé and Pointe de la Cuisse) located in the Villefranche Bay, French Riviera (Fig. 1). Algal communities of the infralittoral fringe in this geographic area are generally dominated by *Cystoseira stricta* (Fig. 2a). In the Mediterranean Sea, the tide amplitude is few dozens of centimetres and the infralittoral fringe is a very peculiar zone, most of time exposed to air and/or wave action; it is entirely submerged only during spring tides and adverse marine conditions (low barometric pressure).

C. stricta is an habitat-forming species, supporting complex food webs on rocky-bottoms (Ballesteros 1990, Cheminée et al. 2013) and it is protected by the Bern and Barcelona Conventions. It is a long-lived species with the growing period comprised between March and July. The base is sympodial, formed by a creeping axis from which multiple axes arise. Branches can reach 40 cm long and are lost in late summer when the dormant season starts. Receptacles, from few millimetres to 2 cm long, begin to develop in the apical part of all branches at the end of April and are very abundant (Gómez Garreta et al. 2000). Even if this species is characterized by high reproductive potential, zygotes are heavy and tend to sink close to the parental plant (Guern 1962). This strategy favours the formation of monospecific forests, but limit the dispersal ability estimated to be around few centimetres (Mangialajo et al. 2012).

In both sites in which we performed the experiment, *C. stricta* belts were dense and continuous.

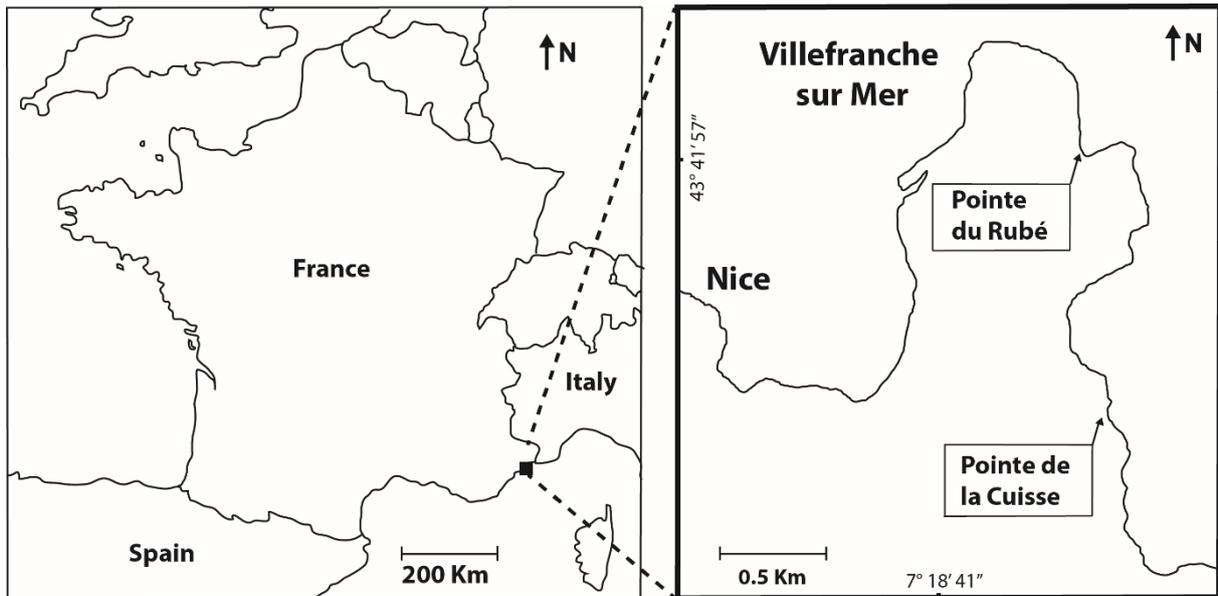


Figure 1. The study area is located in the Villefranche bay, French Riviera, NW Mediterranean Sea.

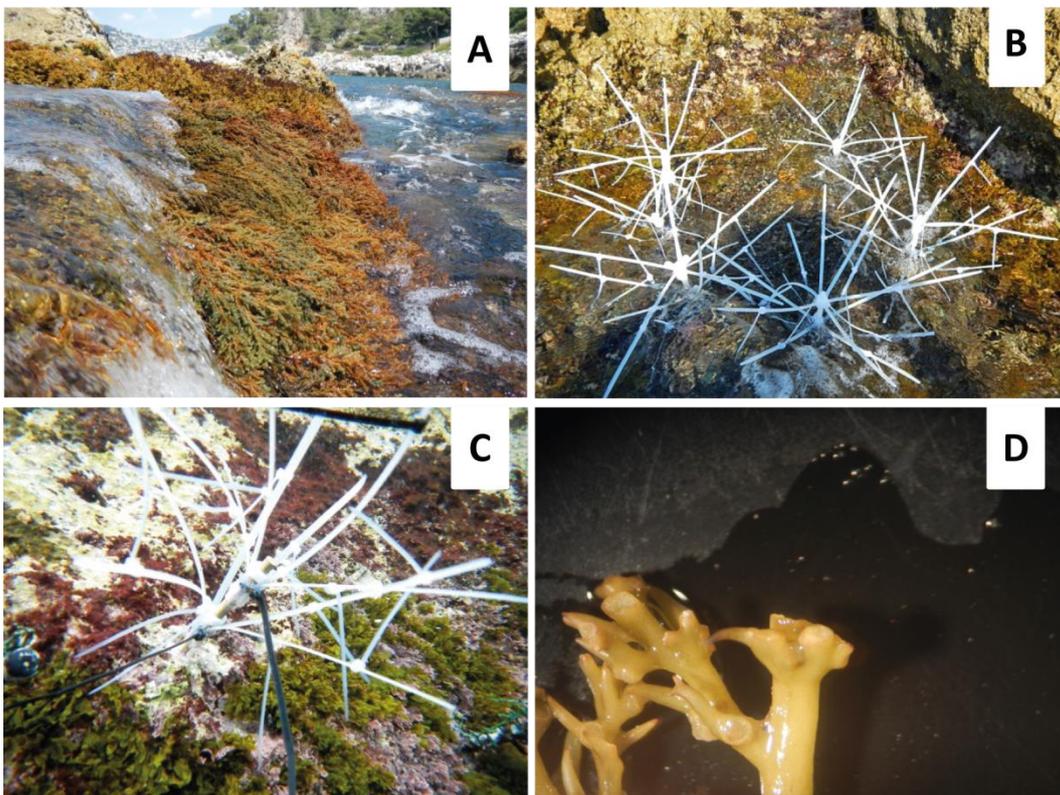


Figure 2. A: *Cystoseira stricta* belts in the infralittoral fringe; B: one of the protected blocks; C: the deterrent device used to avoid fish grazing on *C. stricta*; D: fish bites on primary and secondary *C. stricta* branches.

5.2.2 - Experimental design and data collection

In order to evaluate fish grazing on *Cystoseira stricta*, we started our experiment in March, at the beginning of the growing period. Our hypotheses were that: i) our devices were effectively able to reduce *Sarpa salpa* herbivorous pressure on *C. stricta*, ii) the decrease of *S. salpa* grazing would have resulted in a general increase in growth and reproductive potential of *C. stricta*, iii) differences between the High and the Low zone would have been reduced in the protected blocks, giving a significant interaction between the involved factors.

To test these hypotheses, an innovative herbivore deterrent system was conceived (see below). Contrarily to cages, generally used in herbivores exclusion experiments, the new devices do not need any maintenance or cleaning and do not affect light penetration. However, the installation of these deterrent devices is quite invasive. Therefore a split-plot design was planned, allowing to keep reasonably low the number of replicates, optimise the field work and reduce the impact on natural assemblages (see Anderson et al. (2008) and Jones and Nachtsheim (2009) for more information on this design).

At each site (Pointe du Rubé and Pointe de la Cuisse) we randomly identified twelve blocks (40 × 40 cm side) characterised by dense *C. stricta* belts and spaced several meters apart so as to be considered independent. Within each block we identified two vertical zones of the infralittoral fringe: one 'High', where belts are most of time exposed to air and therefore expected to be less grazed, and one 'Low', where *C. stricta* belts are more accessible to fish and subjected to higher grazing rates. At each site, the blocks were randomly associated to one of the three treatments: protected, control and artefact control. Protected blocks were enclosed with the deterrent devices, disposed as close as possible in order to limit the access to fish (Fig. 2b).

The deterrent devices consisted in a plastic threaded rod (20 cm long), on which three groups of five cable ties (18 cm long) were glued with silicon at different heights and kept stretched by a plastic bolt screwed on the rod (Fig. 2b-c). In order to discourage fish from passing through the ties, smaller ties (10 cm) were attached perpendicular to the main ones. Rods were then screwed to drop-in anchors fixed inside holes (2 cm depth) that were previously drilled into the rock along each side of the protected blocks. In the artefact control blocks, we fixed small devices, made with the same materials, so as to control for possible effect of the manipulation on *C. stricta* assemblages, but, at the same time, allow fish access.

We evaluated the fish herbivory pressure as an estimate of the number of bites on *C. stricta* branches, defining a bite as a clear cut of the primary or secondary branches (Fig. 2d). To

assess the effect of fish grazing on *C. stricta* fitness, we estimated the growth (maximum algal length: axis plus primary branches), the biomass (wet weight in mg) and the reproductive output (number of receptacles). Since *C. stricta* is a sympodial species, making the identification of the single individuals difficult, every variable was measured in a 12,5 cm² reference surface (4 cm diameter circle). The number of bites and the length of branches, were measured in March (before installing the devices), in May and June, by non-destructive counts in the field. The biomass and the reproductive output were assessed in laboratory only in June, after the collection of samples. Both the High and Low zones on the shore in the two sites were sampled in every sampling time, following an orthogonal model. During the experiment, density and size of *S. salpa* was evaluated by ten replicated transects (25 × 5 m) in our study area.

5.2.3 - Statistical analyses

Differences among treatments and zones were represented in histogram bars, for every sampling time and site, and calculated on the mean values of the algal length, number of fish bites, wet weight and number of receptacles.

Permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) based on Euclidian distance matrix was used to investigate statistical differences among the treatments and zones for each biological variable that we measured. We considered ‘Treatment’ as a fixed factor with three levels, ‘Zone’ as a fixed factor with two levels and ‘Block’ as a random factor, nested in ‘Treatment’. Within each block we had two replicates (the reference surfaces): one of which is in the High zone and one in the Low zone. As the spatial and temporal variability were not relevant for the hypotheses, we decided to perform separated analyses for every site and sampling time, also in order to avoid temporal dependence of data. We run the analyses using ‘Type I Sum of Square’, so that the terms were fitted sequentially. P-values were obtained by 9999 permutation of residuals under a reduced model. When the interaction ‘Treatment×Zone’ or the factor ‘Treatment’ were statistically significant ($p < 0.05$), pairwise tests were performed applying Monte Carlo test, because of the low number of possible permutations. Analyses were done using Primer 6 & PERMANOVA+ software package.

In order to show differences among treatments and between zones we also calculated the effect sizes on the grazing rate, growth potential, biomass and reproductive potential of *Cystoseira stricta*, in both sites. The grazing rate was calculated as the difference of number

of fish bites between June and March, the growth potential as the difference of algal branches length between June and March, and the reproductive potential as the number of receptacles multiplied by 100 and divided by the highest number of receptacles recorded in each site. Then, effect sizes on these variables and the biomass were calculated with log-response ratios (Hedges et al. 1999) for each zone in each treatment as:

$$R_t = \ln \left(\frac{E_{treatment}}{E_{control}} \right) = \ln \left(\frac{\bar{x}_{t,z}}{\bar{x}_{c,z}} \right)$$

where R_t is the log-response ratio for the treatment t , and $\bar{x}_{t,z}$ and $\bar{x}_{c,z}$ are the mean values of the grazing rate, growth potential, biomass or reproductive potential calculated respectively for the treatments ‘Protection’ or ‘Artefact control’ and the treatment ‘Control’, in each zone ($z = \text{‘High’ or ‘Low’}$).

Finally, we calculated the Pearson’s correlation between the algal length, wet weight and number of receptacles, choosing the best fit for the trend line. To calculate the correlation coefficient r , we used measures both for the protected and unprotected blocks.

5.3 - Results

5.3.1 - *Sarpa salpa* density and size

In our experimental area, *Sarpa salpa* was very abundant ($0.2 \text{ ind./m}^2 \pm 0.06$, mean \pm SE), with also high biomass values ($9.88 \text{ g/m}^2 \pm 2.14$), being most of the individuals > 14 cm length (Fig. 3).

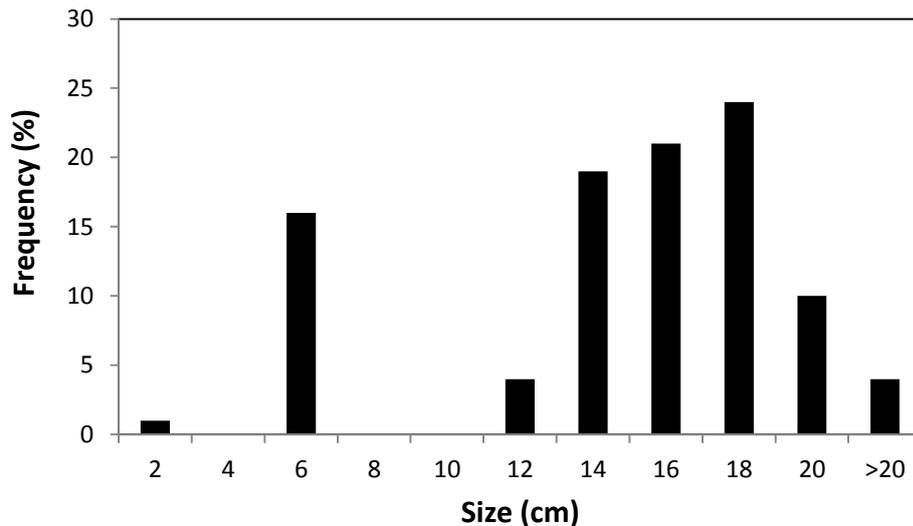


Figure 3. Size classes distribution of *Sarpa salpa* population recorded in the experimental area.

5.3.2 - Fish herbivory pressure

In March, *Cystoseira stricta* individuals were at the beginning of the growth phase bearing short branches (see next paragraph). However fish herbivory pressure (number of fish bites) was already significantly visible on *C. stricta* branches and significantly higher, in both sites, in the Low zones (5.1 ± 1.3 , mean \pm SE) than in the High zones (0.9 ± 0.3) (Fig. 4). In March, only the factor ‘Zone’ resulted statistically significant (PERMANOVA, $p < 0.01$, supplementary materials, Table S1). After four months, bites were significantly reduced in the protected blocks respect to the unprotected blocks ($p < 0.05$). A significant interaction ‘Treatment \times Zone’ ($p < 0.05$) was detected by the analysis of variance at Pointe du Rubé, showing that the number of bites in the Low zone of the protected blocks decreased to the point that no significant differences were highlighted between the two zones in June (pairwise tests, Table S1). At Pointe de la Cuisse, where the deterrent devices were less effective, the protected zones were significantly less affected by herbivory than the unprotected zones ($p < 0.05$), but the interaction ‘Treatment \times Zone’ was not statistically significant. On average, in the unprotected blocks, $10 (\pm 1.1)$ fish bites were recorded on each *C. stricta* branch, while in the protected blocks the number of fish bites in both zones varied from $1.7 (\pm 0.7)$ cm at Pointe du Rubé to $5.7 (\pm 1.2)$ cm at Pointe de la Cuisse (Fig. 4).

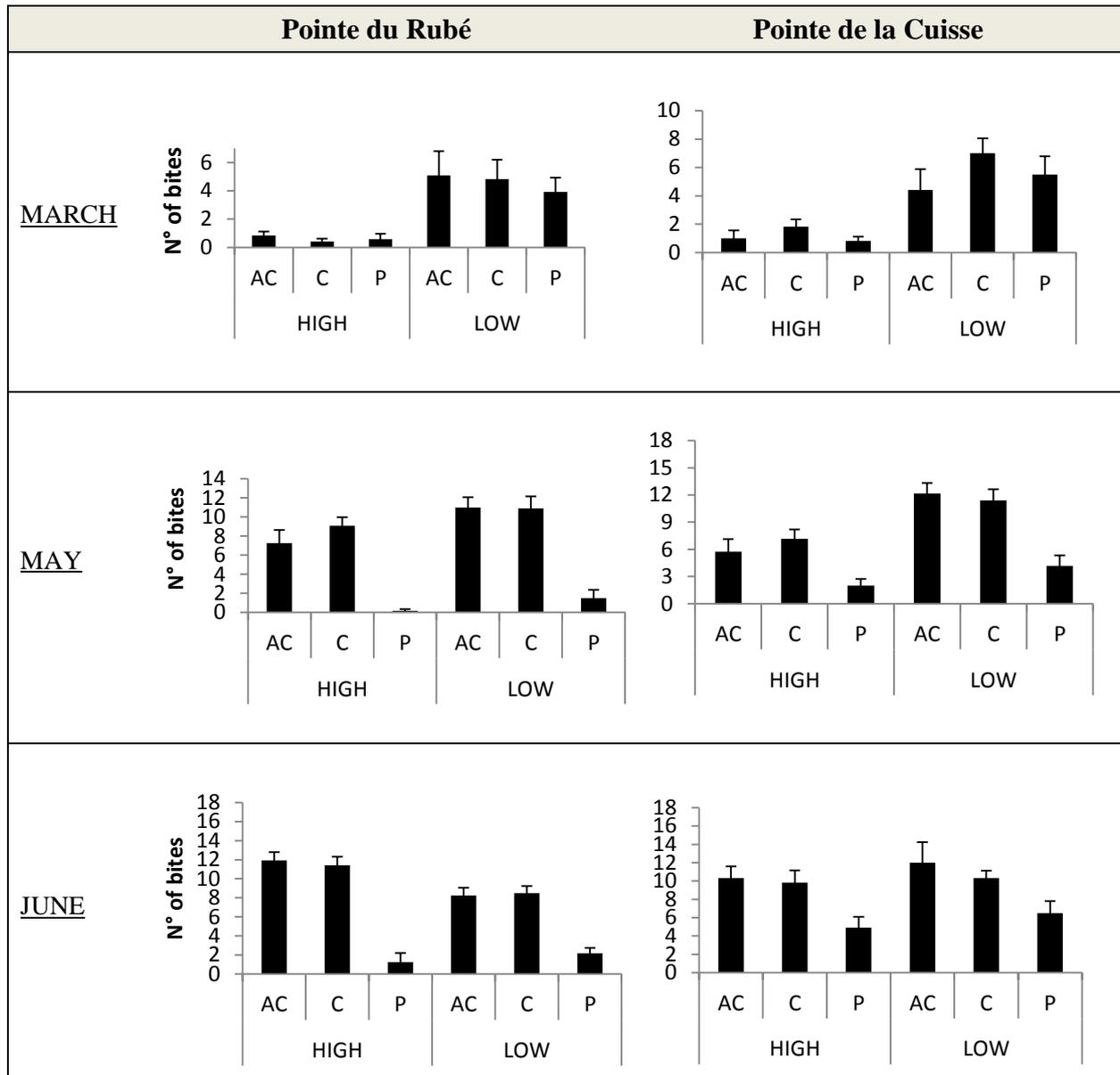


Figure 4. Fish grazing pressure. Mean number of fish bites for each zone (High and Low) and treatment in the different months and in both sites. P: protected; AC: artefact control; C: control blocks.

The grazing rate, measured as the difference of number of bites between June and March was significant in the High zone of the protected blocks at Pointe du Rubé, but not in the Low zone and at Pointe de la Cuisse (Fig. 5). This is probably due to the fact that the deterrent devices did not exclude fish totally and that at Pointe de la Cuisse storms partially damaged the setup of the experiment.

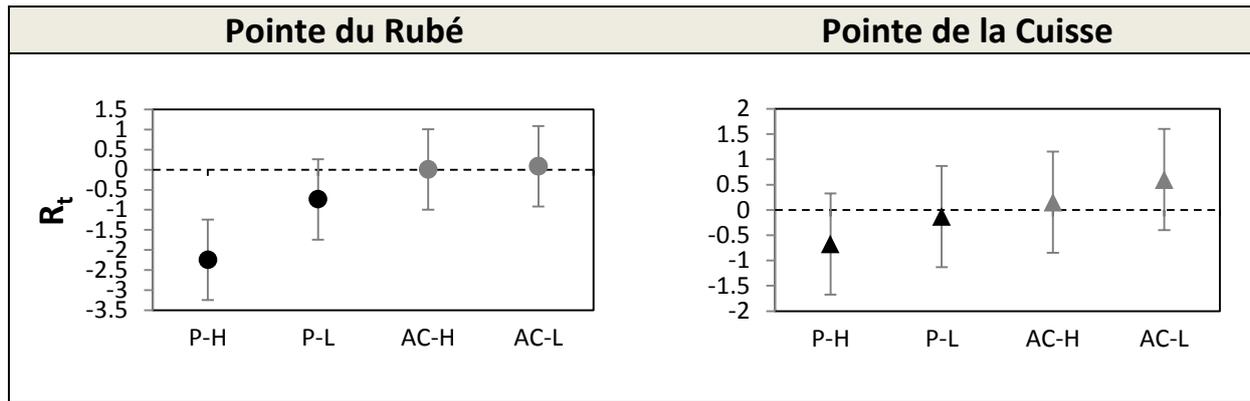


Figure 5. Effect sizes on the grazing rate obtained as difference of the number of fish bites between June and March for the two sites. R_t : the log-response ratio for each treatment. Effect sizes are significant if confidence intervals do not overlap zero line.

5.3.3 - Growth

In March, before to start the experiment, *Cystoseira stricta* individuals were at the beginning of the growth phase, bearing short primary branches. However, the individuals' branches were longer in the High zone (6.4 ± 0.6 cm, mean \pm SE) than in the Low zone (2.8 ± 0.2 cm) (Fig. 6), in both sites, as confirmed by PERMANOVA ($p < 0.01$, supplementary materials, Table S1). The same trend was observed in May, and even more in June, when *C. stricta* branches were completely developed. In addition, in these two sampling times and in both sites, also the factor 'Treatment' was statistically significant ($p < 0.05$). The pairwise tests highlighted that the 'Protection' treatment was almost always significantly higher than the 'Control' and 'Artefact control' treatments, in their turn not significantly different from each other (Table S1). In fact, the effect of protection from fish grazing was important: branches in the protected blocks reached in June, on average, 14 (± 1.1) cm in the High zone and 9.8 (± 0.7) cm in the Low zone, while in the 'Control' and 'Artefact control' treatments *C. stricta* branches were on average 6.6 (± 0.8) cm long in the High zone and 2.7 (± 0.3) cm long in the Low zone, in both sites (Fig. 6). No significant interaction 'Treatment \times Zone' was highlighted by the analysis of variance.

The growth potential, calculated as the difference in algal length between the beginning and the end of the experiment, showed a significant effect of protection for the two zones and sites (Fig. 7).

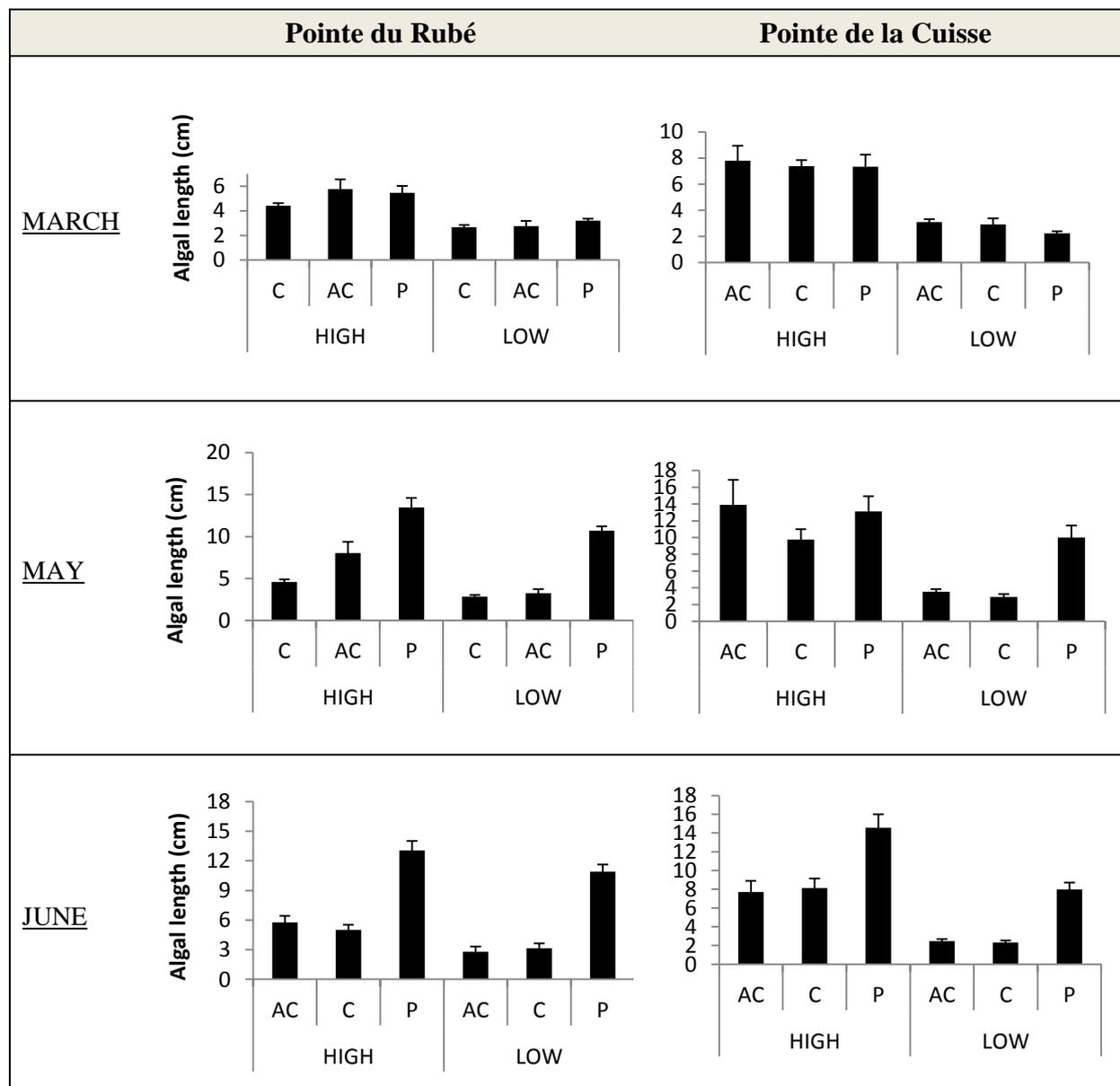


Figure 6. Growth. Mean algal length (axes plus branches) for each zone (High and Low) and treatment in the different months and in both sites. P: protected; AC: artefact control; C: control blocks. Measures were obtained in the field.

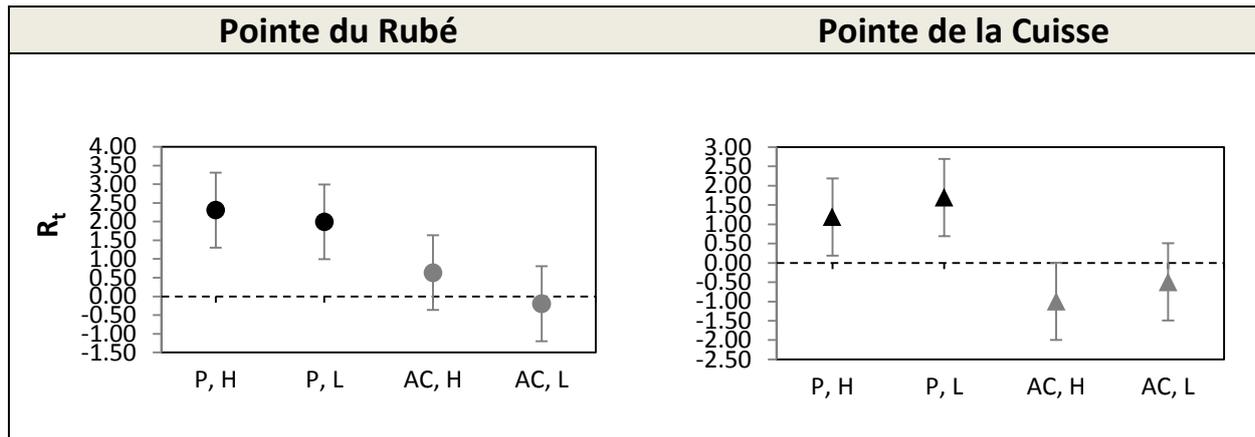


Figure 7. Effect sizes on the growth potential obtained as difference of the algal length between June and March for the two sites.

5.3.4 - Biomass

The analysis of variance on the biomass values showed a significant interaction ‘Treatment×Zone’ at Pointe du Rubé ($p < 0.01$), but the pairwise test showed that the High and Low zone were statistically different also in the protected treatment, contrarily to our hypothesis. At Pointe de la Cuisse, only the factors ‘Treatment’ and ‘Zone’ resulted significant (supplementary materials, Table S1). Globally, protected *Cystoseira stricta* individuals increased their biomass more than individuals in the unprotected blocks: the wet weight in the High zone of the protected blocks varied between $4126 (\pm 885.1)$ and $3551 \pm (583.2)$ mg (per each axis plus their branches) and in the Low zone between $2733 (\pm 424.5)$ and $1646 (\pm 192.3)$ mg, according to the different sites. In the unprotected treatments the wet weight in the High zone varied between $1934 (\pm 79.6)$ mg and $722 (\pm 55.4)$ mg, and in the Low zone, from $1780 (\pm 510.7)$ mg to $491 (\pm 66.6)$ mg, according to the site (Fig. 8). Overall, the loss in biomass due to the fish grazing was very high, being up to 86%.

The effect sizes calculated on the biomass values were significant in the protected High and Low zones only at Pointe du Rubé, because at Pointe de la Cuisse, as explained above, some devices were damaged during a storm potentially affecting our results (Fig. 9).

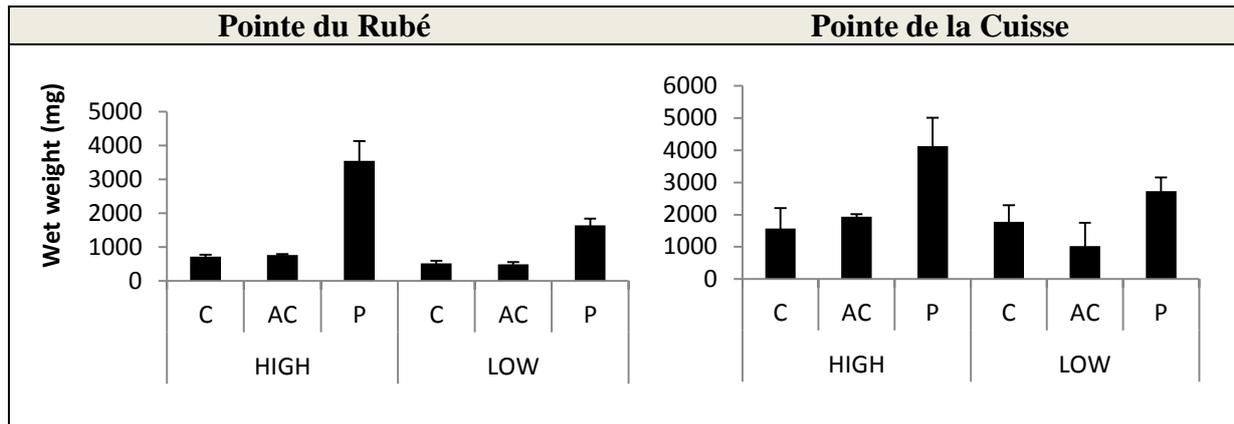


Figure 8. Biomass. Mean value of wet weight (mg) for each zone and treatment in June. Measures were obtained in laboratory, after collection of the samples in the field.

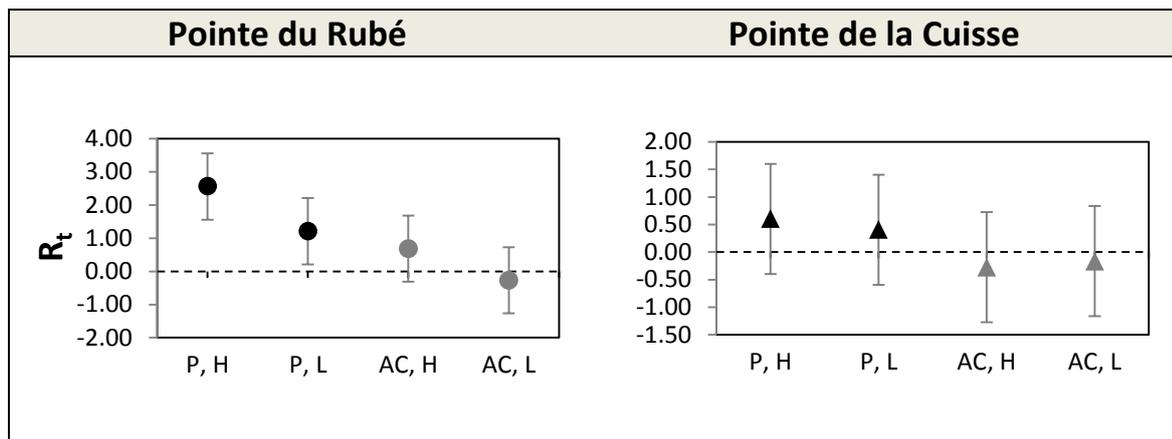


Figure 9. Effect sizes calculated on the biomass (wet weight in mg) for the two sites in June.

5.3.5 - Reproductive output

The analysis of variance on the number of receptacles showed significant interactions ‘Tr×Zo’ for both sites ($p < 0.05$). The pairwise tests confirmed that the protected treatment was statistically different from the other treatments in the two sites, but the High and Low zone were not always statistically different in the unprotected blocks, and at Pointe de la Cuisse the High and Low zones were different also in the protected blocks (supplementary materials, Table S1). Overall protected *Cystoseira stricta* individuals had a higher reproductive output respect to the unprotected ones. Indeed, on average, 115 (± 36.2) receptacles per branch were counted in the High zone, with a maximum of 690 in June at Pointe du Rubé, and 55 (± 15.7) receptacles in the Low zone of the protected blocks. On the contrary, in the unprotected blocks we recorded on average 2.4 (± 0.5) receptacles per branch in the Low zone, and from 6 (± 1.6) receptacles at Pointe Rubé to 19.3 (± 7.1) receptacles at Pointe de la Cuisse (Fig. 10), in the High zone. *C. stricta* branches in the unprotected blocks were most of time devoid of reproductive structures. The estimated loss of reproductive output was up to 97%.

The reproductive potential, expressed as the ratio of the number of receptacles in each treatment and zone on the highest number of receptacles recorded in each site, showed a clear effect of protection in both zones of the two sites (Fig. 11).

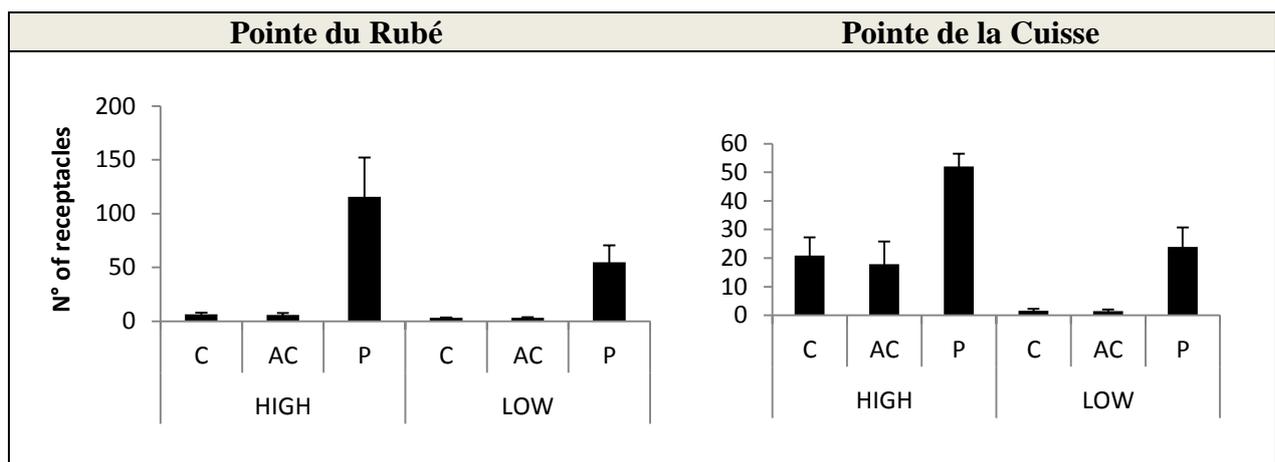


Figure 10. Reproductive output. Mean value of the number of receptacles for each zone and treatment in June. Measures were obtained in laboratory.

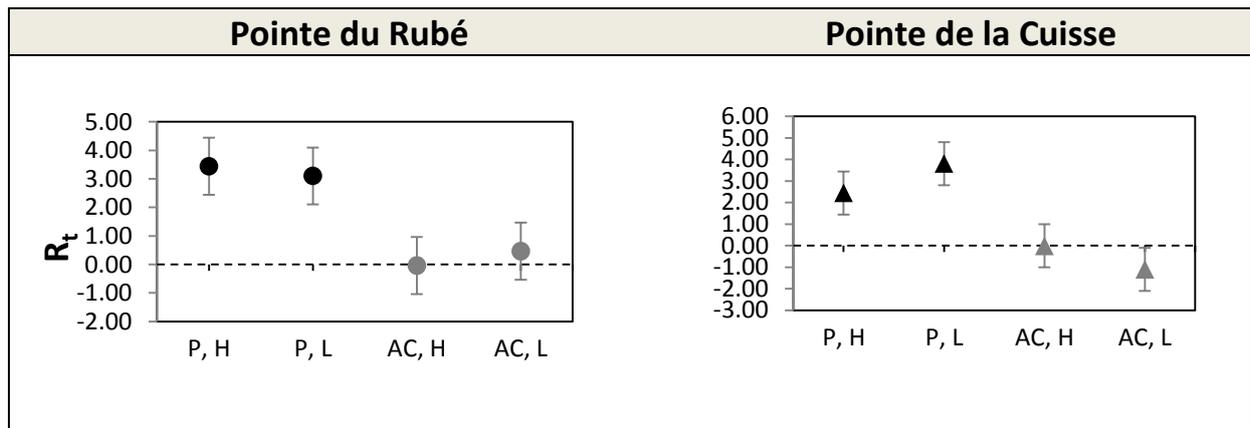


Figure 11. Effect sizes on the reproductive potential obtained by multiplying the number of receptacles in each zone and treatment by 100 and dividing it by the highest number of receptacles recorded in that site.

5.3.6 - Pearson's correlations

Pearson's correlation between algal length and wet weight revealed that this relationship is nonlinear and better described by an exponential function ($r^2 = 0.71$), while the relationship between algal length and the number of receptacles followed a polynomial function ($r^2 = 0.49$). Finally, wet weight and the number of receptacles were very positively correlated and with a linear relationship ($r^2 = 0.82$) (supplementary materials, Figure S1).

5.4 - Discussions

Marine communities are strongly regulated by top-down forces (Shurin et al. 2002). High herbivory rates generally lead to regime shifts with a collapse in production, biodiversity and ecosystem functions (Pace et al. 1999). Typically, the depletion of large brown seaweed forests and the subsequent creation of extended barren grounds is mediated by sea urchins, considered as the most effective herbivores in temperate areas (Sala and Zabala 1996, Shears and Babcock 2003, Ling et al. 2015). Recently, the depletion of algal forests has also been associated to several tropical fishes that are expanding their range in temperate areas due to climate change (Yamano et al. 2011, Vergés et al. 2014a).

The results presented in this study proved that also native herbivorous fish can have an important role in regulating macroalgal communities in temperate areas and in particular forests of large brown seaweeds. In fact, *Sarpa salpa*, the only native herbivorous fish of this Basin, was able to graze up to *Cystoseira stricta* belts in the infralittoral fringe and its grazing pressure was extremely pronounced on our experimental blocks. We recorded a very high number of fish bites on *C. stricta* branches in the unprotected blocks and a consequent reduction of algal fitness. During the maximum growing period of *C. stricta*, from March to June, grazing by salemas caused up to 78% algal growth loss. Most considerably, fish herbivory affected the biomass (up to 86% loss) and reproductive output (up to 97% loss) of *C. stricta* in the unprotected blocks. Therefore, these results suggest that the role of *S. salpa* in regulating algal forests in the Mediterranean Sea has been overlooked so far.

The infralittoral fringe is considered as a spatial refuge for some *Cystoseira* species, being less accessible to fish for its very shallow position (Vergés et al. 2009). In the present study we hypothesized that the differences in *C. stricta* fitness between the High and Low zone of the infralittoral fringe were mostly due to the herbivory pressure of *S. salpa*. Such differences would have decreased (or disappeared) in the protected zones, resulting in a significant interaction between the factors Treatment and Zone. This hypothesis was supported by our results only in one of the two studied sites (Pointe du Rubé) and for two out of four variables: the grazing pressure (number of bites) and the reproductive output (number of receptacles). In the other cases, the fitness of *C. stricta* appeared to be lower in the Low than in the High zone, although significantly higher in the protected respect to the unprotected blocks. It may be argued that some other factors, such as light and the hydrodynamism may affect *Cystoseira* fitness along the vertical gradient. However, the lack of significant interaction is more likely due to the deterrent devices that did not allow to exclude fish completely also in the protected blocks.

In any case, *C. stricta* biomass loss was very high in both the High zone (up to 80%) and the Low zone (up to 90%). In another study that investigated fish herbivory pressure on *C. stricta* in the Balearic Islands (Vergés et al. 2009), authors recorded only 10% of biomass loss due to *S. salpa*. Such difference can be explained by the density of herbivorous fish that in our study site was five times greater ($0.2 \text{ ind./m}^2 \pm 0.06$, mean \pm SE) than the one measured by Vergés and collaborators in the Balearic Islands (0.04 ind./m^2). The fish density measured in our study was comparable or higher than the ones reported in other areas of the Mediterranean (Hereu 2006, Prado et al. 2008, Giakoumi et al. 2012, Sala et al. 2012, Guidetti et al. 2014).

Recent evidences of phase-shifts from large brown macroalgae-dominated bottoms to unproductive barrens, have been associated to the spread of tropical herbivorous fishes extending their range in temperate waters (Vergés et al. 2014a). It is generally assumed that a functional mix of herbivores (i.e. browsers, scrapers, grazers) is required to drive these phase-shifts: browsers remove fronds, while scrapers and grazers remove perennial axes, holdfasts, recruits and ephemeral algae. This phenomenon has been also observed in the Eastern Mediterranean, where different species of tropical fishes (*Siganus* spp., *Sparisoma cretense*) feed on both the adult macrophytes and the earlier life-history stages (Sala et al. 2011, Vergés et al. 2014b). *S. salpa* is a browser species and therefore is not supposed to be able to cause phase shifts to barrens alone. Indeed in our experiment, *C. stricta* belts were not depleted, but strongly reduced. However, on the long term, the reduction of reproductive output may lead to the forests loss due to the insufficient production of new recruits. It is worth noting that *S. salpa* is a voracious grazer, being able to consume two times more macroalgal biomass than rabbitfish of the genus *Siganus* (Vergés et al. 2014b). We cannot exclude that its browsing effect, associated to the grazing behaviour of sea urchins, may have contributed to the formation of existing barrens in different coastal areas.

Evidences on the paramount role of native herbivorous fishes in temperate areas were also obtained in the South West Pacific. In this region, labrids are able to remove the entire primary lamina of adult kelp causing extensive biomass loss and possibly having significant effects on nutrient cycling (Andrew and Jones 1990, Jones and Andrew 1990, Taylor and Schiel 2010). Taylor and Schiel (2010), in addition, found that recruits and juveniles individuals could escape fish herbivory only in exposed sites and under dense canopies where grazing was reduced (Taylor and Schiel 2005, 2010). Also in our study we observed that environmental gradients can induce spatial variation in consumer pressure (i.e. High zone vs Low zone of the infralittoral fringe) and determine the loss of algal forests.

Natural and human driven fluctuations of marine organisms densities are common, but can often go unnoticed, especially for species non-targeted by fisheries. Local increases in *S. salpa* abundance may have occurred in the past, intensifying the herbivory pressure on marine forests. In recent decades, scientists, managers and fishermen reported an increase of salemas (author's personal observations), but long-time series are scarce and it is difficult to assess the magnitude and extent of this potential phenomenon. For instance, high salemas densities have been recorded in the Portofino tuna net (Italy) from the 50ies to the 70ies (data to be taken with caution, due to differences in fishing effort/gears, Cattaneo-Vietti (2009) and in Portugal waters in the 80ies (Ribeiro et al. 2008). An increase in salemas is plausible and may have

been fostered by different causes, such as long-term and large scale fluctuations, changes in fisheries and trophic cascades, as discussed below.

Long-term fluctuations and climate change. Global warming is driving the rise of seawater temperature (Doney et al. 2012) often associated with the spread of invasive species or the proliferation of native ones (Hellmann et al. 2008, Giakoumi 2014, Parravicini et al. 2015). Higher temperatures accelerate metabolic rates (Clarke 2003) and may favour herbivorous fishes. A striking example is Tosa Bay in Southern Japan, where the rise of water temperatures, enhanced the grazing rate of some tropical fishes, already present in that area since a century, and triggered a shift from kelps to corals (Mezaki and Kubota 2012).

Change in fishing pressure. Although salemas are traditionally fished in several areas, they have never had a high commercial value on fish markets and data on their abundance are not commonly registered. Discussions with fishermen allowed to highlight that, locally, the decreased demand for Mediterranean fishes, due to the increase of species from more productive environments such as the North West Atlantic, caused a reduction in catches of less valuable species, such as salemas. At present, in the study site, *S. salpa* is only accidentally caught with non-selective gears. The same considerations may be applicable to several regions of the world, but a coordinated collection of information would be necessary to estimate a possible decrease in herbivorous fish catches in recent decades.

On the contrary, there are evidences that *S. salpa* abundances increased significantly within MPAs (Guidetti et al. 2008, Prado et al. 2008, Raventos et al. 2009, Planes et al. 2011, Britten et al. 2014), probably favoured by fishing regulations. As an example, an increase in salemas density, following protection, has been claimed as one of the potential causes of large brown seaweeds forests depletion in the late nineties in Portofino MPA (Parravicini et al. 2013).

Decrease in *Sarpa salpa* predators. Another important driver that could have increased salema abundance is the depletion of their predators (i.e. sharks, groupers, leerfish – www.fishbase.org) due to overfishing (Sala 2004, Ferretti et al. 2008, Guidetti and Micheli 2011), but few data are available on this topic and it is not possible to confirm this cascading effect.

In conclusion, the present results suggest that the role of *S. salpa* have been underestimated so far and could have contributed, together with human impacts and natural events, to the regression of Mediterranean marine forests detected in recent decades (Airoldi et al. 2014). *Cystoseira* species bear apical reproductive structures and this feature can make forests more sensitive to fish herbivory. In fact we observed that *S. salpa* feeds preferentially on

receptacles, rich in fatty acids (Vizetto-Duarte et al. 2014). As a consequence of this selective grazing by browser fish, fast-growing, less palatable species (i.e. coralline algae or turf) can spread, leading to a shift towards less complex benthic assemblages (Sala and Boudouresque 1997, Nordemar et al. 2007). Nowadays, algal turfs are abundant worldwide and several stressors and potential interactions among them have been claimed to explain such shifts from marine forests (Strain et al. 2014). Browsers fishes herbivory may be a supplementary stressor potentially interacting with other better known impacts at different temporal and spatial scales. More information is needed on distribution, abundance and fluctuations of herbivorous fishes on temperate areas, as well as on their effect on marine forests on the long term. Such paramount information would allow to plan monitoring and management actions of herbivorous fishes. In particular, this should be done in MPAs, representing potential sanctuaries for the protection of marine forests and the associated biodiversity (Gianni et al. 2013, Smale et al. 2013).

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5.6 - Supplementary materials

5.6.1 – Tables S1.1-1.12

Table S1.1. PERMANOVA on the fish grazing pressure (number of bites) for both sites.

df	Source	MARCH						MAY						JUNE					
		Rubé			Cuisse			Rubé			Cuisse			Rubé			Cuisse		
		MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
2	Tr	1.0046	0.2117	0.822	6.2546	1.9495	0.1734	204.73	54.126	0.005	97.56	14.105	0.0063	184.29	59.651	0.0049	66.81	6.7319	0.0137
1	Zo	96.00	28.51	0.0003	117.04	27.894	0.0009	31.894	7.2592	0.02	109.8	33.403	0.0002	21.407	9.8105	0.0118	9.375	1.5283	0.2522
9	Bl(Tr)	4.7438	1.4088	0.2926	3.2083	0.7646	0.6494	3.7824	0.8609	0.5964	6.9167	2.1042	0.1463	3.0895	1.4158	0.3069	9.9244	1.6179	0.2372
2	TrxZo	0.6805	0.2021	0.8198	1.625	0.3872	0.6735	3.2546	0.7407	0.54	9.0324	2.7479	0.1202	12.088	5.5396	0.0293	0.8472	0.1381	0.8726

Table S1.2. Pairwise tests on the factor ‘Treatment’ in May and the interaction in June for Pointe du Rubé (PERMANOVA on the fish grazing pressure).

MAY					JUNE				
Factor	Groups	t	P(perm)	P(MC)	Factor	Groups	t	P(perm)	P(MC)
"Tr"	P, C	8.1093	0.0311	0.0002	"TrxZo"	Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'H' of factor 'Zone'			
	P, AC	14.631	0.0291	0.0001		P, C	7.7996	0.0312	0.0001
	C, AC	0.7864	0.659	0.4585		P, AC	8.2739	0.032	0.0001
				C, AC		0.3991	0.7745	0.6999	
				Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'L' of factor 'Zone'					
				P, C		6.862	0.0289	0.0007	
				P, AC		6.2067	0.0269	0.0009	
				C, AC		0.2316	0.8527	0.8246	
				Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'P' of factor 'Treatment'					
				H, L		0.9891	0.4349	0.3958	
				Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'C' of factor 'Treatment'					
				H, L		7.8923	0.0292	0.004	
				Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'AC' of factor 'Treatment'					
				H, L		2.4295	0.1033	0.0963	

Table S1.3. Pairwise tests on the factor ‘Treatment’ at Pointe de la Cuisse (PERMANOVA on the fish grazing pressure).

MAY					JUNE				
Factor	Groups	t	P(perm)	P(MC)	Factor	Groups	t	P(perm)	P(MC)
"Tr"	P, C	5.4251	0.0284	0.0014	"Tr"	P, C	2.9081	0.0574	0.0276
	P, AC	4.3071	0.0266	0.0054		P, AC	3.2658	0.027	0.0197
	C, AC	0.2346	0.8316	0.8215		C, AC	0.70125	0.4872	0.5058

Table S1.4. PERMANOVA on the algal growth (maximum length) for both sites.

MARCH								MAY					JUNE						
Rubé				Cuisse				Rubé			Cuisse		Rubé			Cuisse			
df	Source	MS	Pseudo-F	P(perm)															
2	Tr	1.5113	1.6841	0.2391	0.879	0.585	0.5786	154.17	57.381	0.0007	54.655	4.7038	0.0492	162.21	65.861	0.0036	100.2	21.296	0.0056
1	Zo	32.597	42.189	0.0004	135.3	67.581	0.0001	57.6	25.46	0.0013	276.04	28.254	0.0004	32.1	29.377	0.0007	207.59	95.194	0.0001
9	Bl(Tr)	0.8973	1.1614	0.4112	1.5025	0.7505	0.664	2.6867	1.1875	0.3998	11.619	1.1893	0.4079	2.4629	2.2539	0.1214	4.705	2.1575	0.1261
2	TrxZo	0.7913	1.0242	0.3838	0.2153	0.1075	0.9027	4.7713	2.109	0.1716	26.538	2.7163	0.1158	0.6384	0.5842	0.5775	0.9143	0.4192	0.6587

Table S1.5. Pairwise tests on the factor ‘Treatment’ in May and June at Pointe du Rubé (PERMANOVA on the growth).

MAY					JUNE				
Factor	Groups	t	P(perm)	P(MC)	Factor	Groups	t	P(perm)	P(MC)
"Tr"	P, C	12.39	0.0278	0.0001	"Tr"	P, C	9.0952	0.0291	0.0002
	P, AC	6.622	0.0318	0.0006		P, AC	9.3974	0.0285	0.0002
	C, AC	2.4921	0.0592	0.0508		C, AC	0.3118	0.6636	0.7604

Table S1.6. Pairwise tests on the factor ‘Treatment’ at Pointe de la Cuisse (PERMANOVA on the growth).

MAY					JUNE				
Factor	Groups	t	P(perm)	P(MC)	Factor	Groups	t	P(perm)	P(MC)
"Tr"	P, C	3.5386	0.029	0.0115	"Tr"	P, C	5.3337	0.0263	0.0018
	P, AC	1.4318	0.169	0.2038		P, AC	5.278	0.0306	0.0021
	C, AC	1.4744	0.2908	0.1917		C, AC	0.15237	0.9439	0.8867

Table S1.7. PERMANOVA on the biomass (wet weight) for both sites in June.

Rubé					Cuisse		
df	Source	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
2	Tr	2.87E+07	113.93	0.0017	2.11E+07	13.718	0.0028
1	Zo	1.32E+07	40.062	0.0003	3.37E+07	9.8587	0.0111
9	Bl(Tr)	2.52E+05	0.7659	0.6767	1.54E+06	0.4494	0.8741
2	TrxZo	1.01E+07	30.656	0.0003	4.85E+06	1.4182	0.2925

Table S1.8. Pairwise tests on the interaction 'Tr x Zo' resulted statistically significant at Pointe du Rubé (PERMANOVA on the biomass).

Factor	Groups	t	P(perm)	P(MC)
"TrxZo"	Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'H' of factor 'Zone'			
	P, C	16.4650	0.0292	0.0001
	P, AC	13.7080	0.0278	0.0001
	C, AC	2.7868	0.0264	0.0319
	Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'L' of factor 'Zone'			
	P, C	2.3774	0.0529	0.0583
	P, AC	2.6741	0.0288	0.0366
	C, AC	0.7065	0.6054	0.5020
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'P' of factor 'Treatment'			
	H, L	5.9818	0.0334	0.0093
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'C' of factor 'Treatment'			
	H, L	0.6179	0.5777	0.5720
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'AC' of factor 'Treatment'			
	H, L	4.4278	0.0391	0.0203

Table S1.9. Pairwise tests for the factor 'Treatment' at Pointe de la Cuisse (PERMANOVA on the biomass).

Factor	Groups	t	P(perm)	P(MC)
"Tr"	P, C	3.5287	0.0283	0.0133
	P, AC	5.3693	0.0274	0.0014
	C, AC	1.1731	0.3417	0.2841

Table S1.10. PERMANOVA on the reproductive output (number of receptacles) for both sites in June.

df	Source	Rubé			Cuisse		
		MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
2	Tr	44779	8.8997	0.0048	66686	40.316	0.0062
1	Zo	20817	8.1802	0.018	26611	23.18	0.0014
9	Bl(Tr)	5031	1.9771	0.1224	1654	1.4408	0.2627
2	TrxZo	18115	7.1182	0.0131	12901	11.237	0.0031

Table S1.11. Pairwise tests on the interaction at Pointe du Rubé (PERMANOVA on the reproductive output)

Factor	Groups	t	P(perm)	P(MC)
"TrxZo"	Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'H' of factor 'Zone'			
	P, C	2.8818	0.0288	0.0265
	P, AC	2.8839	0.0302	0.0293
	C, AC	9.2159E-2	0.9715	0.9290
	Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'L' of factor 'Zone'			
	P, C	2.9163	0.0291	0.0303
	P, AC	2.8271	0.0286	0.0291
	C, AC	1.0013	0.3422	0.3582
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'P' of factor 'Treatment'			
	H, L	2.7348	0.0999	0.0722
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'C' of factor 'Treatment'			
	H, L	3.4668	0.0557	0.0396
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'CA' of factor 'Treatment'			
	H, L	1.3039	0.2680	0.2760

Table S1.12. Pairwise tests on the interaction resulted statistically significant at Pointe de la Cuisse (PERMANOVA on the reproductive output).

Factor	Groups	t	P(perm)	P(MC)
"TrxZo"	Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'H' of factor 'Zone'			
	P, C	6.5329	0.0299	0.0001
	P, AC	6.751	0.0291	0.0006
	C, AC	1.74E-02	1	0.988
	Term 'TrxZo' for pairs of levels of factor 'Treatment', within level 'L' of factor 'Zone'			
	P, C	3.0097	0.0292	0.0257
	P, AC	3.0555	0.0295	0.0218
	C, AC	1.8856	0.1411	0.1063
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'P' of factor 'Treatment'			
	H, L	4.0005	0.0468	0.0285
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'C' of factor 'Treatment'			
	H, L	1.9227	0.1717	0.1559
	Term 'TrxZo' for pairs of levels of factor 'Zone', within level 'AC' of factor 'Treatment'			
	H, L	3.7278	0.0516	0.032

5.6.2 - Supplementary materials: Pearson's correlations

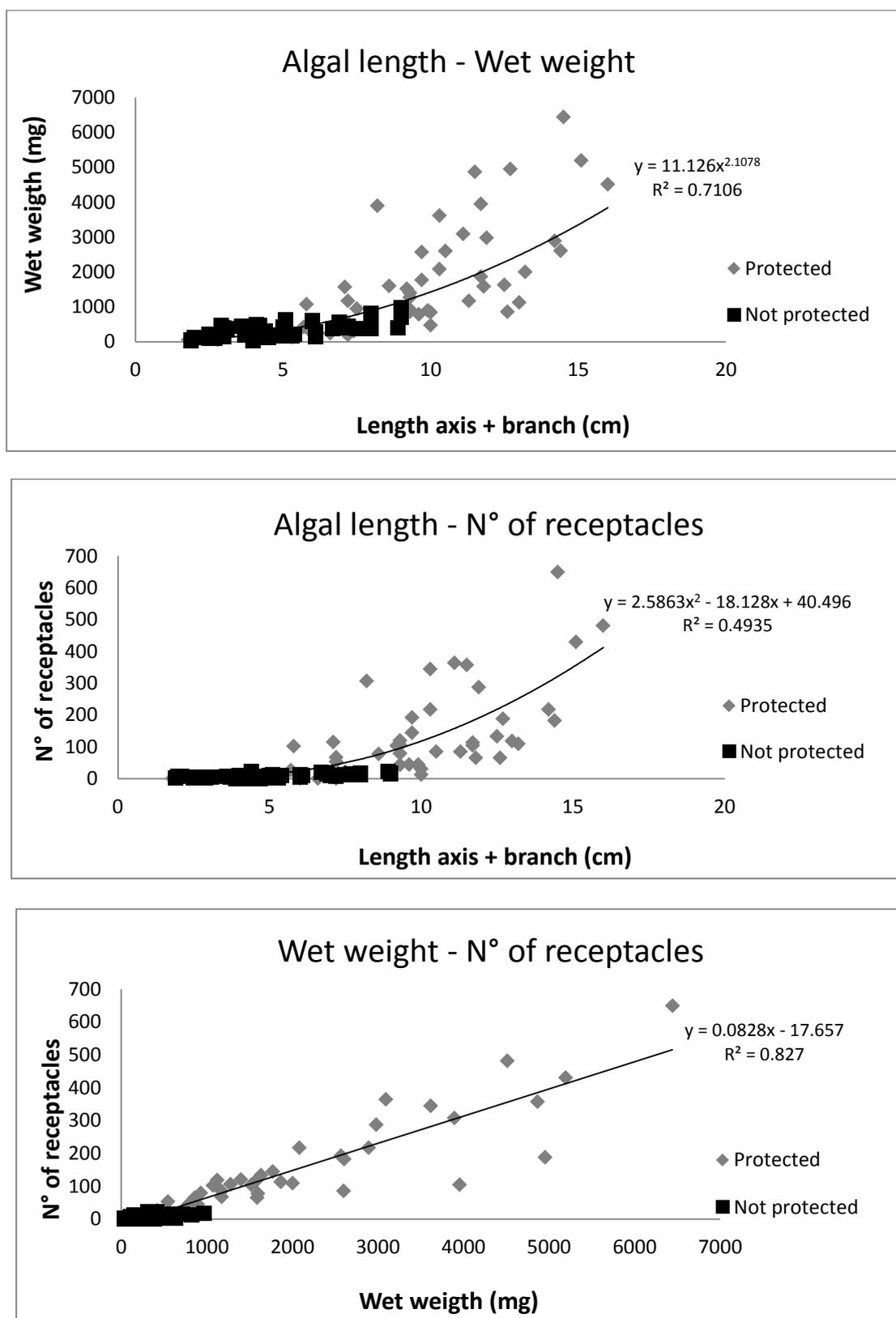


Figure S1. Pearson's correlations for the different variables of *Cystoseira stricta* that we measured in the field and in laboratory. We chose to represent the best fit of the trend line (with the higher value of the r coefficient).

Chapter 6 – General discussion

The role of herbivorous fishes is well-known in tropical areas, where several species with different feeding behaviours (browsers, grazers and scrapers) interact, affecting macroalgal communities composition and structure¹. On the contrary, in temperate areas, herbivorous fishes diversity is low and the role of fishes in regulating macroalgal communities is generally considered less important if compared to the one of herbivorous invertebrates². In the last decades, this assumption has evolved, as some range-expanding tropical herbivorous fish species are depleting marine forests in several temperate areas, causing a shift from complex benthic communities to overgrazed barren grounds¹. Few recent studies demonstrated that also native herbivorous fishes may have an important role in shaping temperate marine communities^{3,4}.

The research carried out during my PhD allowed to prove that native fish, namely *Sarpa salpa* (salema) in the Mediterranean Sea, have a paramount role in regulating marine forests of large brown seaweeds. In particular, we showed that salemas are able to affect the growth, biomass, and the reproductive potential of very shallow *Cystoseira* forests. Salemas preferentially fed on receptacles (apical reproductive structures), decreasing the reproductive output up to 97% (chapter 5), when compared to non-grazed areas. In the experiments carried out on the Saint Jean Cap Ferrat harbour dike, salemas negatively affected also the success of the restoration, consuming almost completely *Cystoseira* individuals transplanted on the artificial structure (chapter 4). This study allowed to show that salemas can be the major herbivore in very shallow Mediterranean rocky reefs, generally considered as a refuge from fish herbivory. The studies reported in this manuscript were all performed on *Cystoseira amentacea* var. *stricta*, a species forming superficial belts in the infralittoral fringe of the North Western Mediterranean Sea. In fact, since such belts are most of time out of the water, salemas grazing is considered

¹ Vergés A, Steinberg PD, Hay ME, Poore AG, Campbell AH et al 2014. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. In Proceedings of the Royal Society B Vol. 281, No. 1789, p. 20140846. The Royal Society.

² Gaines SD, Lubchenco J 1982. A unified approach to marine plant-herbivore interactions. II. Biogeography. Annual Review of Ecology and Systematics, 13, 111-138.

³ Vergés A, Alcoverro T, Ballesteros E 2009. The role of fish herbivory in structuring the vertical distribution of canopy algae (*Cystoseira* spp.) in the Mediterranean Sea. Marine Ecology Progress Series 375:1-11.

⁴ Taylor DI, Schiel DR 2010. Algal populations controlled by fish herbivory across a wave exposure gradient on southern temperate shores. Ecology 91:201-211.

discontinuous and mostly linked to sea/climate conditions (e.g. low barometric pressure, waves). The results obtained can be therefore considered as particularly conservative and it can be hypothesized that the effect of grazing pressure on some subtidal *Cystoseira* species may be even higher. Indeed, a similar experiment on *Cystoseira brachycarpa*, an upper subtidal species, performed at the end of my PhD thesis, gave results in agreement with this hypothesis (research not included in this manuscript as still in progress).

Our results suggest that the role of native herbivorous fish in driving marine forests loss has been overlooked so far, at least in the Mediterranean Sea, since they may reduce algal forests size, the recovery potential and make them more sensitive to other impacts. In addition, the consequences of forests fragmentation are likely to be more severe due a reduction in the gene flow that isolates populations⁵. This phenomenon can be then accentuated by the limited dispersal ability of most *Cystoseira* species⁶.

Fish-invertebrates interactions are well-known in the Mediterranean Sea⁷ and we can argue that herbivory of salema (browsers) and sea-urchins (grazers) may have additive effects, accelerating the process of *Cystoseira* forests depletion. Indeed, salemas feed mostly on fronds and receptacles, while sea urchins on the perennial axes. In an experiment carried out on an extensive date-mussel fishery barren ground in Montenegro, it was highlighted that *Cystoseira* recovery is possible only when both fish and sea urchins are excluded and recruitment is artificially enhanced by installing fertile receptacles (data not shown, Mangialajo et al. in preparation). This experiment represents one of the first ecological restoration attempts in areas impacted by date mussel destructive fishery, and it happened thanks to the devices conceived in my PhD (annex V).

The use of engineering methods and the conception of devices, as the one proposed here, is paramount for successful restoration actions. The research performed in my PhD allowed to prove that the ecological restoration of marine forests on existing artificial structures is feasible (chapter 4) and it is increasingly requested to enhance their ecological value. However, herbivore pressure on artificial habitats is generally higher than in natural systems⁸, thus the density of the major herbivores have to be taken into account and

⁵ Valero M, Destombe C, Mauger S, Ribout C, Engel CR et al 2011. Using genetic tools for sustainable management of kelps: a literature review and the example of *Laminaria digitata*. *CBM-Cahiers de Biologie Marine*, 52(4), 467.

⁶ Mangialajo L, Chiantore M, Susini ML, Meinesz A, Cattaneo-Vietti R et al 2012. Zonation patterns and interspecific relationships of fucoids in microtidal environments. *Journal of Experimental Marine Biology and Ecology* 412:72-80.

⁷ Ruitton S, Francour P, Boudouresque CF 2000. Relationships between algae, benthic herbivorous invertebrates and fishes in rocky sublittoral communities of a temperate sea (Mediterranean). *Estuarine, Coastal and Shelf Science* 50.2: 217-230.

⁸ Bulleri F, Menconi M, Cinelli F, Benedetti-Cecchi L 2000. Grazing by two species of limpets on artificial reefs in the northwest Mediterranean. *Journal of Experimental Marine Biology and Ecology*. 2000; 255:1-19.

regulation/exclusion actions have to be considered where necessary. Deterrent devices should be removed only when the restored forests are self-sustaining and able to resist to herbivory pressure, but further studies are needed on this topic. Scientists and engineers should work together in order to design efficient and, if possible, biodegradable devices that may be pre-installed on artificial structures before their deployment.

In order to set up guidelines for the restoration of marine forests (and avoid compensation measures as alibi of destruction), we proposed a flow-chart with reasoned procedures to undertake when a restoration action is planned (chapter 3 and annex VI). In the same chapter, the potential role of marine protected areas (MPAs) in the conservation and restoration of marine forests was also discussed.

Knowledge on marine forests forming species has improved in recent decades (chapter 2). However, most of the research is not in relation to MPAs, likely because a big proportion of the studies is not focused on conservation topics and because marine forests are not often included in MPA planning and management plans. Studies on marine forests are not evenly distributed in global MPAs. Most of the information available on kelp or furoid forests was obtained in MPAs of the developed countries, where marine forests sustain industrial activities or where their importance is recognised. A peculiar case is the Mediterranean Sea, where the identification of marine forests forming species (i.e. *Cystoseira* and *Sargassum*) is quite complex and they do not have economic interest. Our analysis showed that in this Basin the awareness of their importance is increasing, and that the scientific interest is in most cases comparable to the one of *Posidonia oceanica* meadows. These findings are encouraging for the conservation of Mediterranean marine forests, as MPAs may represent sanctuaries for the protection of existing forests, a source of propagules for nearby zones⁹ and priority sites for ecological restoration activities.

The implementation of MPAs should be carried out, taking into account marine forests distribution. Marine forests should be also included in the management plans and monitoring programs (see guidelines in annex VI), in order to promptly detect early stages of regression. Densities of herbivores (invertebrates and fishes) should be also monitored, in order to better understand the natural fluctuations of their populations and control their abundances if needed.

⁹ Couceiro L, Robuchon M, Destombe C, Valero M. 2013. Management and conservation of the kelp species *Laminaria digitata*: using genetic tools to explore the potential exporting role of the MPA "Parc naturel marin d'Iroise". Aquatic Living Resources, 26(02), 197-205.

Marine forests are sensitive to both local and global impacts, especially if they have cumulative effects¹⁰. Hence, conservation measures and recovery strategies should be urgently set up. Degraded/lost forests should be restored according to the guidelines discussed in this PhD work, keeping in mind that the conservation of the existing forests in MPAs has always to be considered as a priority¹¹.

¹⁰ Airoldi L, Beck MW 2007. Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology: An Annual Review* 45, 347-407.

¹¹ Gianni F, Bartolini F, Airoldi L, Ballesteros E, Francour P et al 2013. Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. *Advances in oceanography and limnology* 4,83-101.

Chapitre 6 – Discussion général (en français)

Le rôle des poissons herbivores est bien connu dans les régions tropicales, où plusieurs espèces avec des comportements alimentaires différents (broueteurs et pâtureurs) interagissent, affectant la composition et la structure des communautés de macro-algues¹. Au contraire, dans les régions tempérées, la diversité des poissons herbivores est faible et le rôle des poissons dans la régulation des communautés de macro-algues est généralement considéré comme moins important si on le compare à celui des invertébrés herbivores (oursins, etc.)². Au cours des dernières décennies, cette hypothèse a évolué, car certaines espèces de poissons herbivores tropicaux ont réduit l'abondance des forêts sous-marines dans plusieurs zones tempérées, provoquant un changement des communautés benthiques, qui sont passées d'un état complexe à des déserts liés au surpâturage¹. Quelques études récentes ont démontré aussi que les poissons herbivores indigènes peuvent avoir un rôle important dans les communautés de macro-algues marines des régions tempérées^{3,4}.

Les recherches menées au cours de ma thèse ont permis de montrer et confirmer que les poissons herbivores indigènes de la mer Méditerranée, notamment les Saupes (*Sarpa salpa*), ont un rôle fondamental dans la régulation des forêts marines de grandes algues brunes. En particulier, j'ai pu montrer au chapitre 5 que les Saupes sont en mesure d'influer sur la croissance, la biomasse et le potentiel de reproduction des forêts de *Cystoseira* intertidales. *Sarpa salpa* se nourrit préférentiellement des réceptacles (structures de reproduction apicales) de *Cystoseira*, ce qui diminue jusqu'à 97 % la capacité de reproduction de celles-ci par rapport aux zones protégées contre les Saupes. Dans les expériences menées sur la digue du port de Saint Jean-Cap Ferrat, les Saupes affectent aussi négativement le succès des efforts de restauration, en consommant presque complètement les *Cystoseires* transplantées sur des structures artificielles (chapitre 4). Cette étude a permis de montrer que probablement les

¹ Vergés A, Steinberg PD, Hay ME, Poore AG, Campbell AH et al 2014. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. In Proceedings of the Royal Society B Vol. 281, No. 1789, p. 20140846. The Royal Society.

² Gaines SD, Lubchenco J 1982. A unified approach to marine plant-herbivore interactions. II. Biogeography. Annual Review of Ecology and Systematics, 13, 111-138.

³ Vergés A, Alcoverro T, Ballesteros E 2009. The role of fish herbivory in structuring the vertical distribution of canopy algae (*Cystoseira* spp.) in the Mediterranean Sea. Marine Ecology Progress Series 375:1-11.

⁴ Taylor DI, Schiel DR 2010. Algal populations controlled by fish herbivory across a wave exposure gradient on southern temperate shores. Ecology 91:201-211.

Saupes sont le principal herbivore dans les récifs rocheux méditerranéens peu profonds, généralement considérés comme un refuge contre les poissons herbivores. Les études rapportées dans ce manuscrit ont toutes été effectuées sur *Cystoseira amentacea* var. *stricta*, une espèce formant des ceintures superficielles dans la frange infralittorale de la mer Méditerranée nord-ouest. Etant donné que ces ceintures d'algues sont la plupart du temps hors de l'eau (émersions dues aux marées), le broutage par les Saupes est considéré comme discontinu et la plupart du temps lié à des conditions de mer/climat. Les résultats obtenus peuvent donc être considérés comme hypothèse basse et on peut supposer que l'effet de la pression des poissons sur certaines espèces de *Cystoseires* de la zone subtidale pourrait être beaucoup plus élevé. En effet, une expérience similaire sur *Cystoseira brachycarpa*, une espèce de la zone subtidale supérieure, réalisée à la fin de ma thèse de doctorat (travaux en cours), a donné des résultats en accord avec cette hypothèse haute.

Nos résultats suggèrent que le rôle des poissons herbivores indigènes dans la destruction des forêts marines a été négligé jusqu'à présent, au moins dans la mer Méditerranée, car ils peuvent réduire la taille des forêts d'algues, le potentiel de récupération et les rendre plus sensibles à d'autres impacts, notamment anthropiques (pollution, etc.). En outre, les conséquences de la fragmentation des forêts peuvent être plus sévères en raison d'une réduction du flux de gènes qui tend à isoler les populations⁵. Ce phénomène peut être aussi accentué par la faible capacité de dispersion de la plupart des espèces de *Cystoseires*⁶.

Les interactions poissons-invertébrés sont bien connues dans la mer Méditerranée⁷ et nous pouvons affirmer que l'herbivorie des Saupes (brouteurs) et des oursins (pâtureurs) peuvent avoir des effets additifs, en accélérant le processus de disparition des forêts. En effet, les Saupes se nourrissent principalement des frondes et des réceptacles de *Cystoseira*, tandis que les oursins se nourrissent sur la partie basse, pérenne, de l'algue. Dans une expérience réalisée au Monténégro, sur un vaste désert dû à la pêche des Dattes de mer (Mollusques), pêche très destructrice, il a été montré que la reprise des *Cystoseira* n'est possible que lorsque les poissons et les oursins sont exclus de la zone et que le recrutement des *Cystoseires* est artificiellement amélioré en introduisant des réceptacles fertiles dans cette même zone (Mangialajo *et al.*, en préparation). Cette expérience, réalisée grâce aux dispositifs conçus

⁵ Valero M, Destombe C, Mauger S, Ribout C, Engel CR et al 2011. Using genetic tools for sustainable management of kelps: a literature review and the example of *Laminaria digitata*. *CBM-Cahiers de Biologie Marine*, 52(4), 467.

⁶ Mangialajo L, Chiantore M, Susini ML, Meinesz A, Cattaneo-Vietti R et al 2012. Zonation patterns and interspecific relationships of fucoids in microtidal environments. *Journal of Experimental Marine Biology and Ecology* 412:72-80.

⁷ Ruitton S, Francour P, Boudouresque CF 2000. Relationships between algae, benthic herbivorous invertebrates and fishes in rocky sublittoral communities of a temperate sea (Mediterranean). *Estuarine, Coastal and Shelf Science* 50.2: 217-230.

dans ma thèse, représente l'une des premières tentatives de restauration écologique dans des zones touchées par cette type de pêche destructrice (annexe V).

L'utilisation de méthodes d'ingénierie et la conception de dispositifs, comme celui proposé ici, est essentielle pour le succès des actions de restauration. La recherche effectuée dans ma thèse a permis de prouver que la restauration écologique des forêts marines sur des structures artificielles existantes est possible (chapitre 4) et qu'il est de plus en plus demandé d'améliorer leur valeur écologique. Cependant, la pression des herbivores sur les habitats artificiels étant généralement plus élevée que dans les systèmes naturels⁸, la densité des grands herbivores doit être pris en compte et des actions de régulation ou d'exclusion doivent être considérées, si nécessaire. Les dispositifs d'exclusion doivent être retirés lorsque les forêts restaurées deviennent autonomes et capables de résister à la pression de broutage, cependant des études supplémentaires sur ce sujet sont nécessaires. Les scientifiques et les ingénieurs doivent travailler ensemble afin de concevoir des dispositifs efficaces et, si possible, biodégradables qui peuvent être préinstallés sur des structures artificielles avant leur déploiement.

Afin de mettre en place des lignes directrices pour la restauration des forêts marines (et éviter des mesures de compensation comme alibi de destruction), nous avons proposé un organigramme avec des procédures à entreprendre lorsqu'une action de restauration est prévue (chapitre 3 et annexe VI). Dans le même chapitre, le rôle potentiel des aires marines protégées (AMPs) dans la conservation et la restauration des forêts marines a également été discuté.

Les connaissances sur les espèces formant ces forêts marines se sont améliorées au cours des dernières décennies (chapitre 2). Cependant, la plupart des recherches n'est pas en rapport avec AMPs, probablement parce qu'une grande partie des études ne se concentre pas sur des sujets de conservation et parce que les forêts marines ne sont pas souvent considérées lors de la création des AMPs ainsi que dans les plans de gestion. Les études sur les forêts marines ne sont pas réparties uniformément dans les AMPs mondiales. La plupart des informations disponibles sur les forêts de kelp ou de Fucales a été obtenue dans les AMPs des pays développés, où les forêts marines soutiennent des activités industrielles ou lorsque leur importance est reconnue. Le bassin Méditerranéen est un cas particulier puisque l'identification des espèces formant ces forêts marines (*Cystoseira* spp. et *Sargassum* spp.) est assez complexe et elles n'ont aucun intérêt économique. Notre analyse a montré que, dans ce bassin, la prise de conscience de leur importance écologique est en augmentation et que

⁸ Bulleri F, Menconi M, Cinelli F, Benedetti-Cecchi L 2000. Grazing by two species of limpets on artificial reefs in the northwest Mediterranean. *Journal of Experimental Marine Biology and Ecology*. 2000; 255:1-19.

l'intérêt scientifique est, dans la plupart des cas, comparable à celui des prairies de *Posidonia oceanica*. Ces résultats sont encourageants pour la conservation des forêts marines méditerranéennes, comme les AMPs peuvent représenter des sanctuaires pour la protection des forêts existantes, une source de propagules pour les zones voisines⁹ et des sites prioritaires pour les activités de restauration écologique.

La mise en œuvre des AMPs devrait être effectuée en tenant compte de la distribution des forêts marines. Les forêts marines devraient également être incluses dans les plans de gestion et dans les programmes de surveillance (voir les lignes directrices en annexe VI) afin de détecter rapidement les premiers stades de régression. Les densités des herbivores (invertébrés et poissons) doivent être également surveillées afin de mieux comprendre les fluctuations naturelles de leurs populations et de contrôler leurs abondances.

Les forêts marines sont sensibles à la fois aux impacts locaux et mondiaux, surtout si ils ont des effets cumulatifs¹⁰. Par conséquent, les mesures de conservation et les stratégies de rétablissement devraient être mises en place de toute urgence. Les forêts dégradées ou perdues devraient être restaurées selon les directives discutées dans ce travail de thèse, en gardant à l'esprit que la conservation des forêts existantes doit toujours être considérée comme une priorité¹¹.

⁹ Couceiro L, Robuchon M, Destombe C, Valero M. 2013. Management and conservation of the kelp species *Laminaria digitata*: using genetic tools to explore the potential exporting role of the MPA "Parc naturel marin d'Iroise". *Aquatic Living Resources*, 26(02), 197-205.

¹⁰ Airoidi L, Beck MW 2007. Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology: An Annual Review* 45, 347-407.

¹¹ Gianni F, Bartolini F, Airoidi L, Ballesteros E, Francour P et al 2013. Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. *Advances in oceanography and limnology* 4,83-101.



Annex I – Are Mediterranean MPAs protecting marine forests?

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This paper was presented at the 5th Mediterranean Symposium on Marine Vegetation held in Portoroz, Slovenia (27-28/10/2014) and published in the proceedings of the conference. It reviews the knowledge on marine forests in Mediterranean MPAs respect to the other relevant habitats or species of this Basin.

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ARE MEDITERRANEAN MPAs PROTECTING MARINE FORESTS?

Abstract

*In the Mediterranean Sea, Marine Protected Areas (MPAs) are nearly 700, covering approximately 5% of the sea surface, but merely 0.1% of the Mediterranean's total surface is included in no-take zones. Mediterranean MPAs are often established according to political or socio-economic criteria more than nature conservation aspects, and only less than half of them have a management plan or have evaluated the status and the distribution of marine habitats. Results from our literature-based research highlight that scientific studies are abundant only in few Mediterranean MPAs, generally the biggest and the long-established ones. Usually, it is often the case that on land the vegetation has a primary role in establishing protected areas. By contrast, in marine systems, the vegetation has a secondary role or in some cases is absent in the establishment of protected areas. Furthermore, in the most studied Mediterranean MPAs, there have been limited scientific research on the status of large brown algae (i.e. *Cystoseira* and *Sargassum*) forests. As a result of this lack of information, marine forests are generally not included in Mediterranean MPA management plans, making it difficult to assess their evolution and understand the potential role of MPAs in the conservation of marine forests. With this contribution, we would like to remark the importance of conducting research on marine forests of *Fucales* in MPAs that may represent priority sites for the conservation of healthy forests and for the recovery of degraded ones.*

Key-words: *Cystoseira*, marine protected areas, marine forests, algae, Mediterranean Sea

Introduction

Since many centuries Mediterranean populations have exploited coastal ecosystems for their development. In the last decades this phenomenon intensified with increased pollution, fishing, tourism and an uncontrolled coastal urbanisation (Caddy, 1993; Airoidi, 2003; Ludwig *et al.*, 2009), so that important changes have occurred on coastal ecosystems (Airoidi & Beck, 2007) but, unluckily, they are difficult to quantify due to the lack of historical data. As a result, a set of conservation measures have been conceived at the regional, national or international level to protect this biodiversity (for a review see Micheli *et al.*, 2013). Nowadays, nearly 700 Marine Protected Areas (MPAs) have been established in the Mediterranean Sea, covering nearly 5% of its surface (including the Pelagos Sanctuary, representing alone 4% of the surface), while merely 0.1% of the Mediterranean total surface is included in no-take zones. Such percentages are even smaller if we consider only the 161 MPAs of national status that cover 0.73% of the Mediterranean Sea (Gabrié *et al.*, 2012). The 33 SPAMIs (SPA/BD Protocol) usually overlap with national MPAs and therefore in the following text, we include them in the general term MPAs. However, MPAs are often established more according to political or socio-economic choices than conservation aspects (Leenhardt *et al.*, 2013), and only less than half of them have a management plan or have evaluated the status and the distribution of marine habitats, among which algal forests. Indeed, contrarily to what generally happens on land, where vegetation has a priority role in the establishment of protected

areas (e.g. many forest-reserves were established in the world to reduce deforestation), marine forests are not considered in the creation of MPAs and they are generally not targeted in monitoring programs or in the evaluation of MPA efficacy. An exception is the CARLIT index, applied in the North-Western Mediterranean and in the Adriatic Seas in the framework of the Water Directive 2000/60/EU (Ballesteros *et al.*, 2007; Mangialajo *et al.*, 2008), that use the distribution of very shallow *Cystoseira* forests to assess the ecological status of coastal shallow waters. It is now widely accepted that marine forests of large brown seaweeds (represented by the genus *Cystoseira* and *Sargassum* in the Mediterranean Sea) are some of the most important marine habitats, forming extended canopies comparable to land forests and providing refuge and subsistence for many organisms, including fish (Jones *et al.*, 1994; Ballesteros *et al.*, 1998). However, many large brown forests are considered threatened worldwide and several studies described the loss of *Cystoseira* forests in the Mediterranean Sea (for a review see Airoidi *et al.*, same issue). Only few events of natural recovery have been reported at present, and especially in MPAs (Bonaviri *et al.*, 2009; Hereu & Quintana, 2012; author's personal data). MPAs could have a strong potential for conservation of large brown algae forests because they may guarantee protection from several kinds of direct (i.e. coastal development and destructive fishing) and indirect (cascade effects of overfishing) human impacts that are the major causes of loss of these forests (Gianni *et al.*, 2013). The aim of this work was to quantify the contribution of scientific research to the study of large brown algae forests in the Mediterranean MPAs in order to estimate the degree of knowledge of this habitat compared to other major habitats and/or species.

Materials and methods

We identified Mediterranean MPAs using the MedPAN list (see Gabrié *et al.*, 2012). In our work we considered only 113 national MPAs out of 161, because we excluded coastal protected areas mainly characterised by wetlands or land, with no evidences for a real protection of the marine environment. We also considered 32 SPAMIs out of 33: we excluded the Pelagos Sanctuary because it is mostly characterized by pelagic waters. Natura 2000 sites were not considered in this work because most of them are still in the establishment process or lack a management plan, including a specific regulation for tourism or marine resources exploitation. We searched ISI Web of Science in order to quantify the number of international scientific papers produced on each MPAs. In this way we can obtain replicable results, although, unfortunately, grey literature or national papers are not considered. Papers were found searching the crossed topics:

- "name of the MPA" and
- "* protected area*" or "park" or "marine reserve*" or "nat* reserve*" or "monument" or "Mediterranean".

We selected all studies performed in the MPAs focused on the marine environment, whether or not the authors considered the protection of the site as mandatory for their study. In addition, we evaluated the number of papers on algae, on Fucales, on seagrasses and on fish combining to the previous search the following topics, respectively:

- "*alga*" or "seeweed"
- "Fucales" or "furoids" or "*Cystoseira*" or "*Sargassum*"
- "seagrass" or "*Posidonia*" or "*Cymodocea*" or "*Zostera*"
- "fish*"

In this study, we considered papers with algae, seagrasses or fish as main subject, but also papers focused on other topics and reporting some analyses or observations on algae,

seagrasses or fish. All our searches are updated to July 2014. For the entire dataset and for each MPA, we identified the papers considering the protection as mandatory for the study (hereafter MPA papers) in contrast to the general studies not considering the protection (hereafter General papers) and we calculated the relative proportions of studies on algae, Fucales, seagrasses and fish.

Results

In total, 1500 studies were carried out in the 113 MPAs (512 of which in the 32 SPAMIs), but merely 38% of them were specifically performed in those sites because they were protected (MPA papers). Studies on marine vegetation (algae and seagrasses) represented 15% of all studies and 26% of MPA papers. Papers on algae represented 9% of all studies and 14% of MPA papers (40% of which centred on Fucales). Studies on seagrasses represented about 6% of all studies (mostly on *Posidonia oceanica*) and 12% of MPA papers. Studies on fish assemblages represented 22% of all studies and 36% of MPA papers. Such percentages were coherent with the ones calculated only on SPAMIs. Only 20 out of the 113 Mediterranean MPAs with national status had at least 10 MPA papers published in international journals (henceforth it will be mentioned as Highly studied MPAs), 12 of which are also SPAMIs. In Figure 1 the amount of MPA and General papers are reported for the Highly studied Mediterranean MPAs. The totality of studies performed at Miramare and Cerbère-Banyuls MPAs were focused around the protection effect (MPA papers). A great percentage of MPA papers (> 70%) was also found for Tabarca, Tavolara-Punta Coda Cavallo, Torre Guaceto, Asinara, Port-Cros and Scandola MPAs. On the contrary, in Zakynthos, Cap de Creus, Palma Bay and Columbretes islands only 30% of studies was performed there because of the protected status of the site. The relative proportion of papers on algae (excluding Fucales), on Fucales, seagrasses and fish respect to the number of MPA papers carried out at the highly studied MPAs is reported in Figure 2.

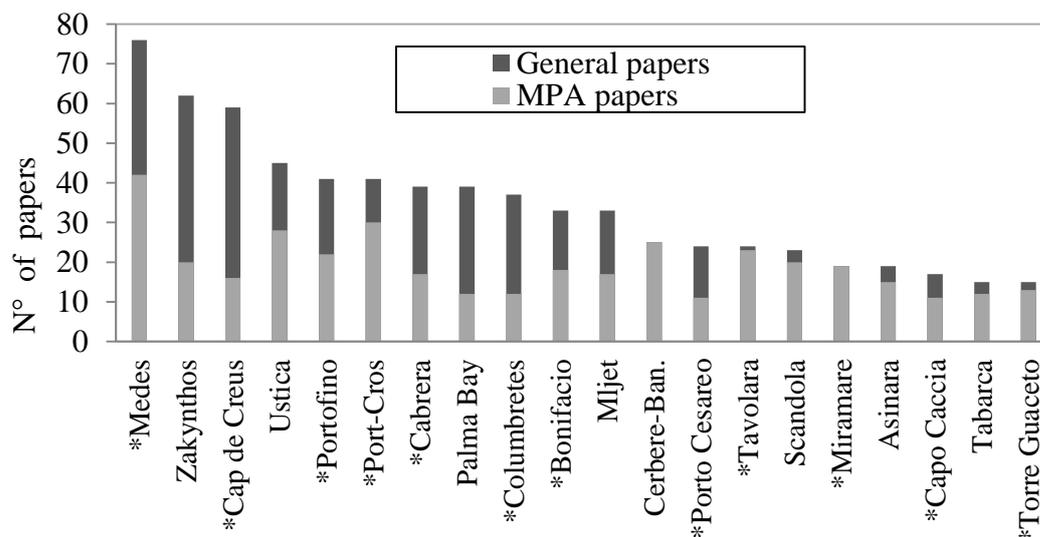


Fig 1: Amount of papers considering the protection as mandatory for the study (MPA papers) and of papers not considering the protection as mandatory (General papers) in highly studied (at least 10 MPA papers) MPAs. MPAs with an asterisk are also SPAMIs.

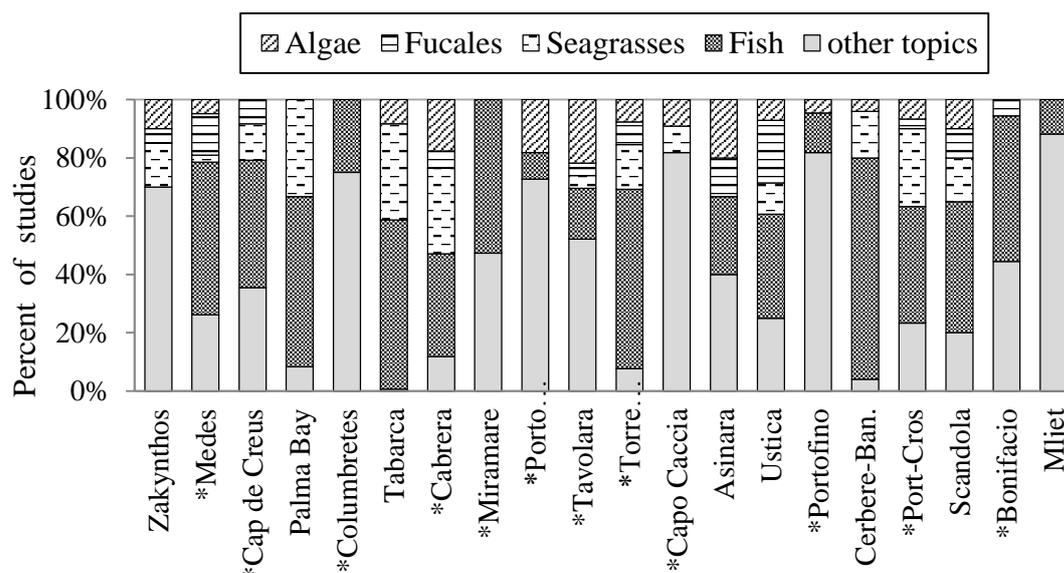


Fig. 2: Percentages of studies on algae (excluding Fucales), Fucales, seagrasses, fish and “other topics” in MPA papers (considering the protection as mandatory for the study) for highly studied MPAs (at least 10 MPA papers). MPAs with an asterisk are also SPAMIs.

The highest percentage of studies on algae, including also Fucales, was done in the protected areas of Ustica, Asinara and Tavolara (30-40%). In almost all other MPAs, papers considering algae represented less than 20%. The percentage of studies on Fucales was also lower in all MPAs (< 15%), except for Ustica (21%). Papers on seagrasses were mostly carried out in Palma Bay, Tabarca island, Cabrera archipelago and Port-Cros, where they represented nearly 30% of MPA papers. In our search we did not find any study on marine vegetation of the protected areas of Columbretes, Miramare and Mljet. In contrast, the percentages of papers on fish were almost always greater than the ones on marine vegetation (> 30%). Torre Guaceto and Cerbère-Banyuls were the MPAs with the highest percentage of studies on fish (> 60%).

Discussion

According to the Convention of Biological Diversity (reviewed in 2010), by 2020 10% of the Mediterranean Sea’s surface should be protected. In order for this to be a reality, more MPAs have to be established. However, as we showed in this work, in many national MPAs a big amount of research did not consider the protection as mandatory (General papers). This is partially due to the fact that data previous MPA establishment are lacking, so it is often difficult to assess the effect of protection on some habitats (i.e. marine vegetation). Only 20 out of 113 MPAs can be considered as highly studied MPAs. Such MPAs include the biggest and/or the long-established ones and most of them belong to France, Italy and Spain, the Mediterranean countries where most of marine research published on international journals is carried out. For instance, many North-African and Middle-East MPAs were established more than 30 years ago, but few international studies are available. Information on marine forests potentially exists in these MPAs, but it is hardly accessible, consisting in grey literature or being written in other languages than English. The highest percentage of studies carried out in Mediterranean MPAs is focused on fish assemblages, the compartment usually more facilitated by the protection (generally fishery limitations). Seagrasses, and in particular *Posidonia oceanica* meadows, were object of several studies in different MPAs, since their

ecological role is widely recognised (Personnic *et al.*, 2014). The amount of studies on *P. oceanica* was comparable to the amount of studies on all algae. Indeed, we found a low percentage of studies focused on *Cystoseira* and *Sargassum* and they were astonishingly almost absent in many well-managed MPAs where it is still possible to find dense marine forests (e.g. Tavolara-Punta Coda Cavallo, Columbretes). Ustica MPA had the higher number of papers on *Cystoseira* forests, because many studies investigated the cascade effects of sea urchins proliferations on benthos and highlighted the loss and then a gradual recovery of macroalgal assemblages (among others: Gianguzza *et al.*, 2006; Bonaviri *et al.*, 2009).

Although *Cystoseira* species are listed in the annexes of some European Conventions (Barcelona Convention, 1976 and Bern Convention, 1979), they are still unprotected and little studied. In order to ameliorate the conservation of marine forests in the Mediterranean Sea, the implementation process of MPAs has to be improved and the management has to be planned on the base of a complete and detailed habitat mapping of marine vegetation. The establishment of Natura 2000 marine sites is often accompanied by the cartography of habitats (e.g. in France), following the Habitat Directive annexes (Directive 92/43 EEC). *Posidonia oceanica*, priority species for this Directive, is therefore well documented in Natura 2000 sites and we have now good information on its health and evolution. Unluckily, large brown seaweeds (i.e. *Cystoseira* species), although mentioned in the previous Conventions, are not included in the Habitat Directive, so that the cartography done in the Natura 2000 sites usually reports merely “photophilous algae on rocky bottom”, without any specification if macroalgal communities are deserts of encrusting corallinales, filamentous algae, shrubs of photophilous algae or forests of large brown algae. In conclusion, results of our work highlighted a lack of information on marine forests in Mediterranean MPAs, especially compared to other communities (i.e. fish assemblages or *P. oceanica* meadows). Although grey literature and old papers were excluded by our search, we are confident that the results would have been comparable to the ones obtained with the search on ISI Web of Science.

MPAs can theoretically have an important role for marine forests conservation. Unluckily, the current state of knowledge does not allow assessing if present regulations in MPAs are protecting healthy marine forests and/or enhancing natural recovery of the degraded ones (Sala *et al.*, 2012). Protection of Mediterranean coastal ecosystems should be evaluated with a constant monitoring of the distribution and status of marine forests and we suggest that future research priority should be the assessment of the conservation status of Fucales in particular in the MPAs, in order to better understand the real role of present protection rules in the conservation of healthy forests and, potentially, in the restoration of damaged ones.

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PROCEEDINGS OF THE 5th MEDITERRANEAN SYMPOSIUM ON MARINE VEGETATION

Portorož, Slovenia, 27-28 October 2014

ACTES DU 5^{ème} SYMPOSIUM MÉDITERRANÉEN SUR LA VÉGÉTATION MARINE

Portorož, Slovenie, 27-28 octobre 2014

Avec le support du projet MedKeyhabitats Finance par la fondation MAVA
With the support of MedKeyhabitats project Financed by the MAVA Foundation



October 2014





Annex II – Marine forests at risk: solutions to halt the loss and promote the recovery of Mediterranean canopy-forming seaweeds

Airoldi L, Ballesteros E, Buonuomo R, Van Belzen J, Bouma TJ, Cebrian E, De Clerk O, Engelen AH, Ferrario F, Fraschetti S, **Gianni F**, Guidetti P, Ivesa L, Mancuso FP, Micheli F, Perkol-Finkel S, Serrao EA, Strain EM and Mangialajo L (2014). Marine forests at risk: solutions to halt the loss and promote the recovery of Mediterranean canopy-forming seaweeds. UNEP/MAP – RAC/SPA Proceedings of the 5th Mediterranean Symposium on Marine vegetation Portoroz, Slovenia (Portorož, Slovenia, 27-28 October 2014) LANGAR H, BOUAFIF C, OUERGI A, edits, RAC/SPA publ, Tunis, pp: 28-33; 2014.

I am co-author of this paper that was presented at the 5th Mediterranean Symposium on Marine Vegetation held in Portoroz, Slovenia (27-28/10/2014) and published in the proceedings of the conference. It synthesizes the documented regression of algal forests in the Mediterranean Sea and the perspectives of ecological restoration actions.

Laura AIROLDI, BALLESTEROS E., BUONUOMO R., VAN BELZEN J., BOUMA T.J., CEBRIAN E., DE CLERK O., ENGELEN A.H., FERRARIO F., FRASCHETTI S., GIANNI F., GUIDETTI P., IVESA L., MANCUSO F.P., MICHELI F., PERKOL-FINKEL S., SERRAO E.A., STRAIN E.M., MANGIALAJO L.
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MARINE FORESTS AT RISK: SOLUTIONS TO HALT THE LOSS AND PROMOTE THE RECOVERY OF MEDITERRANEAN CANOPY-FORMING SEAWEEDS

Abstract

Along Mediterranean coasts, canopy-forming seaweeds used to form diverse, productive and valuable “forest” habitats, but in the past decades conspicuous declines, sometimes to local extinction, have been reported in many regions. Canopies are retracting particularly close to urban areas, and are replaced by turf-forming and ephemeral algae or barrens. The persisting forests are under continued threat, and current protection measures are insufficient. We provide evidence that declines of canopy algae are dramatically extensive, and are driven by multiple local (nutrient enrichment and high sediment loads, fishing, heavy metal pollution) and global stressors (increasing temperature, high wave exposure). We also show that the combined management of local stressors (such as nutrients and sediments) would increase significantly the resilience of canopy algae to future climatic stressors, preventing their further deterioration. Finally, we discuss restoration prospects in areas where these systems have been lost. We conclude identifying the main needs to understand, guide and motivate effective conservation actions in these valuable ecosystems.

Key words: Canopy algae, habitat loss, Mediterranean Sea, multiple threats, conservation

Introduction

Along Mediterranean coasts, canopy-forming seaweeds (most frequently brown algae belonging to the order Fucales) form diverse, productive and valuable “forest” habitats. These habitats are becoming rare at local, regional and basin scales at an alarming rate (Airoldi & Beck, 2007). This is concerning because algal canopies play a key role in coastal primary production and nutrient cycling, and facilitate rich flora and fauna communities. In the past decades, algal canopies have suffered widespread and apparently irreversible loss, much of which may have gone unnoticed. Algal canopies are retracting particularly close to urban areas, and are replaced by turf-forming and ephemeral algae or sea urchin barrens, with major negative consequences for associated benthic and fish communities (Benedetti-Cecchi *et al.*, 2001). The persisting forests are under continued threat, and the benefits of current protection measures have been low.

We synthesize past research efforts aiming at quantifying the losses, and identifying what factors drive the loss or enhance the resilience of these systems. We also discuss the restoration prospects in areas where canopies have been lost and the main needs.

Materials and methods

We reviewed published primary literature and summarized it in a table. The review is organized into three sections: 1) a compilation of data on historical loss of canopies along

Mediterranean coastlines and main drivers of loss; 2) a compilation of data on known factors enhancing resilience and restoration success; 3) a discussion of gaps in the data, ecological knowledge, and protection measures for these coastal habitats and recommendations for how to address these gaps.

Results

Historical loss and main drivers

Conspicuous declines of algal canopies, sometimes to local extinction, have been reported in many regions along the coasts of Spain, France, Italy, Croatia, Albania, Greece and Turkey (Fig. 1 and Tab. 1). Along the Albères coast only 5 out of 14 species of Fucales (*Cystoseira* spp. and *Sargassum* spp.) documented as abundant in 1912 were present in 2003 (Thibaut *et al.*, 2005). Lost algal forests tend to be replaced by assemblages of lower structural complexity, such as turf-forming, filamentous or other ephemeral seaweeds, mussels or “barrens” (Mangialajo *et al.*, 2008; Connell *et al.*, 2014; Strain *et al.*, 2014). Canopy algae, turfs and barrens have been suggested to represent alternative states in shallow temperate rocky coasts under different disturbance and stress regimes (Airoidi *et al.*, 2009). There is a growing consensus and empirical evidence that these habitat shifts are driven by multiple anthropogenic stressors, including overfishing of higher trophic groups leading to outbreaks of grazers, eutrophication, excess sediment loads, coastal development, heavy metal pollution, point source pollutants such as oil spills, detergents and anti-fouling paints and invasive species (Table 1). These local anthropogenic stressors can interact negatively with environmental stressors or global climatic stressors (such as increasing temperature and CO₂) resulting in accelerated declines of canopy-algae (Perkol-Finkel & Airoidi, 2010; Asnaghi *et al.*, 2013; Olabarria *et al.*, 2013; Strain *et al.*, 2014).

Factors enhancing resilience or restoration efforts

While the proximate drivers of canopy loss are now relatively well understood, the factors that control the recovery have been more difficult to identify, and over a certain deterioration threshold, these systems may not be able to recover at all (Perkol-Finkel & Airoidi, 2010). The alternative habitat replacing lost canopies seems

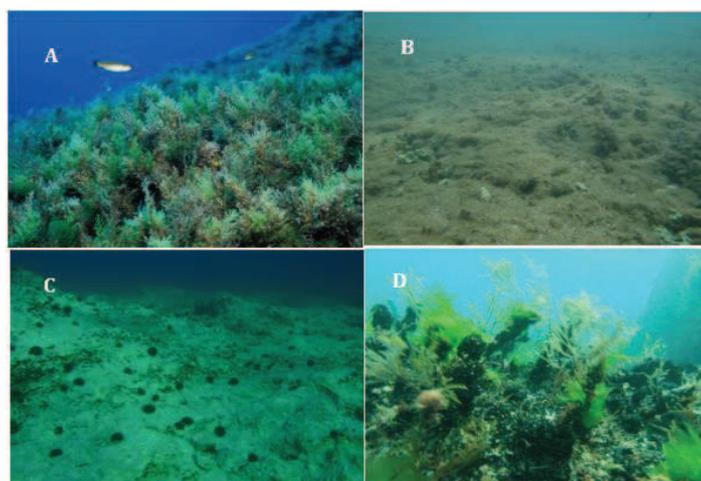


Fig. 1: Canopy algae characterise Mediterranean rocky coasts (e.g. A-*Cystoseira balearica* forest, Scandola, Corse), but many forests have been replaced by algal turfs (e.g. B- Haifa, Israel), urchin barrens (e.g. C- Porto Cesareo, Italy), or mussel beds (e.g. D - Monte Conero, Italy). Photographs by: A) E Ballesteros, B) L Airoidi, C) P Guidetti, D) L Airoidi

to play a crucial role in controlling the return to a canopy dominated state once the stressors have been removed (Airoldi *et al.*, 2009). Thus, while there are examples of recovery of canopies from outbreak of urchins when effective protection measures have been established (*e.g.* Guidetti, 2006), when algal forests become replaced by turfs, sediments, or mussels it is not yet clear what prevents the recovery of the system, other than severe recruitment failure (Perkol-Finkel & Airoldi, 2010). Recent work has shown that management of water quality and particularly sediment loads is critical for ensuring the persistence of *Cystoseira* forests (Sales *et al.* 2011, Strain *et al.* unpub.). Reduction of nutrients would provide the greatest opportunity to prevent the shift from canopy to turf algae because of the prevalence of synergistic interactions between nutrients with other local and global stressors (Strain *et al.*, 2014). If depletion of forests has already occurred over wide areas, natural recovery could be slow or even implausible (Perkol-Finkel & Airoldi, 2010). The artificial restoration of fucoids in the Mediterranean Sea has been understudied compared to kelps and fucoids in other parts of the world, but results so far suggest it could be an effective strategy (Sales *et al.*, 2011; Perkol-Finkel *et al.*, 2012; Gianni *et al.*, 2013).

Discussion

Mediterranean canopy forests are affected by many threats. The greatest impacts are associated with degraded water quality, coastal development, outbreaks of herbivores and invasive species, while effects of diseases and climate changes are uncertain. Current losses are alarming and protection is insufficient. Some key needs and opportunities for conservation and management are suggested below:

- 1) There is no comprehensive summary of the distribution of canopy forests, particularly deep sea ones, and their management is impeded by lack of knowledge on their status. Detailed habitat mapping should be given priority. The ecosystem services that these coastal habitats provide (such as nurseries for fisheries and recreation) also need to be better assessed to illustrate the costs of their loss and provide impetus and economic incentives for their protection and restoration.
- 2) An adequate evolutionary framework is needed to inform decisions on local and regional species diversity and to differentiate local extinction from species extinction. The loss of genetic biodiversity as populations undergo bottlenecks is also undescribed. New molecular tools need to be applied (RAD-seq) or developed (SNPs or microsatellites) to assess genetic diversity and link it to population resilience and ecosystem functioning, assess connectivity of populations, and study parentage and recruitment at local scales.
- 3) Like in other ecosystems (*e.g.* lakes, coral reefs, or forests) a gradual degradation of resilience paves the loss of these algal forests to alternative habitats, so that the mere restoration of environmental conditions preceding the loss may be insufficient to restore the system (Perkol-Finkel & Airoldi, 2010). Strategies for conservation of canopy forests should focus on “early-warning signals” of approaching shifts and on effective and rapid management of local stressors to maintain resilience in face of global stressors. This knowledge is presently limited for Mediterranean canopy forests, but results so far suggest that management of water quality and sediment loads would provide some of the greatest opportunities, particularly in enclosed bays or estuaries.

Tab. 1: Selected studies reporting the loss (as either percentage area lost and/or number of species lost) of canopy-forming algae in the Mediterranean Sea, suggested drivers of loss, and signs of recovery if observed. Na= no quantitative data reported

Lost species	Geographic location	Amount of loss	Driver of loss	Alternative habitat	Signs of recovery	References
<i>Cystoseira</i> spp. and <i>Sargassum acinarium</i> (as <i>S. linifolium</i>)	Monte Conero (Italy, Central Adriatic Sea)	90% (6 out of 8 sp lost)	High sediment loads/poor water quality, increased substratum instability, increased storminess	Turfs or mussels	no	(Romagnoli & Solazzi, 2003; Irving <i>et al.</i> , 2009; Perkol-Finkel & Airoldi, 2010)
<i>Cystoseira</i> spp. and <i>Sargassum</i> spp	Albères Coast (France, NW Mediterranean)	80% (9 out of 14 sp lost)	Poor water quality, overgrazing by urchins, coastal development, human trampling	Turfs, mussels or urchin barrens	no	(Thibaut <i>et al.</i> , 2005)
<i>Cystoseira</i> spp. and <i>Fucus virsoides</i>	Istrian coast (Croatia, North Adriatic Sea)	11 out of 15 sp lost	Poor water quality, overgrazing by urchins	Ephemeral algae	yes	(Munda, 1979, 2000; Zavodnik <i>et al.</i> , 2002)
<i>Cystoseira</i> spp	Genova (Italy, Ligurian Sea)	>50%	Coastal urbanisation	Algal turfs	no	(Mangialajo <i>et al.</i> , 2008)
<i>Cystoseira</i> spp	Albania	90%	Coastal development and urbanisation			(Fraschetti <i>et al.</i> , 2011)
<i>Cystoseira</i> spp. and <i>Sargassum</i> spp	Tremiti islands (Italy, South Adriatic Sea)	na	Poor water quality, overgrazing by urchins	Ephemeral algae or urchin barrens	yes	(Cormaci & Furnari, 1999; Fraschetti <i>et al.</i> , 2012)
<i>Cystoseira</i> spp	Salento Peninsula, (South Adriatic and Ionian Sea)	90%	Overgrazing by urchins	Urchin barrens	yes	(Guidetti <i>et al.</i> , 2003; Guidetti, 2006)
<i>Cystoseira</i> spp and <i>Sargassum</i> spp	Linosa Island (Italy, Sicily Channel)	60% (10 out of 15 sp lost)	Increased water temperature and changes in water circulation	Turfs and ephemeral algae	no	(Serio <i>et al.</i> , 2006)
<i>Cystoseira</i> spp	Several locations (Italy, NW Mediterranean)	90%	Coastal urbanisation, poor water quality, high sediment loads	Turfs	no	(Benedetti-Cecchi <i>et al.</i> , 2001)
<i>Cystoseira</i> spp	Several locations, (Greece and Turkey, Southern Aegean Sea)	>90%	Invasive fish	Urchin-less barrens	no	(Sala <i>et al.</i> , 2011; Giakoumi, 2014)
<i>Cystoseira crinita</i>	Cap Corse (France, NW Mediterranean)	7%	Coastal development	Turfs	no	(Sales & Ballesteros, 2010)
<i>Cystoseira crinita</i> , <i>C. barbata</i>	Maó harbour, Menorca, (Spain, Balearic Sea)	100%	Poor water quality, heavy metal pollution, and aquaculture	Ephemeral algae	Yes after transplant	(Sales <i>et al.</i> , 2011)
<i>Cystoseira zosteroides</i>	Medes Islands, (Spain, NW Mediterranean)	90%	Exceptional storm	Turfs	yes	(Navarro <i>et al.</i> , 2011)

4) Fucoids show high reproductive potential but low dispersal, which limits their natural recovery of wide lost/degraded areas. Given the extent of damage, restoration will be required in many places to meet any reasonable goals for conservation and management. Artificial restoration of *Cystoseira* forests in the Mediterranean Sea is much behind compared to other systems (i.e. seagrass beds), and much more work is needed to develop effective tools and approaches (Gianni *et al.*, 2013).

There are still opportunities for conservation of Mediterranean canopy forests. This protection should be achieved quickly because conservation is cheaper than restoration. Reducing cumulative local human impacts would represent the most effective strategy for the conservation and recovery of these systems, but, whenever this alone cannot reverse the loss, well-designed restoration projects can assist. Overall, there should be greater public, political and even scientific awareness of the extent, importance, and consequences of the loss of canopy forests, and greater commitment to motivate serious conservation and restoration actions in these highly threatened ecosystems.

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PROCEEDINGS OF THE 5th MEDITERRANEAN SYMPOSIUM ON MARINE VEGETATION

Portorož, Slovenia, 27-28 October 2014

ACTES DU 5^{ème} SYMPOSIUM MÉDITERRANÉEN SUR LA VÉGÉTATION MARINE

Portorož, Slovenie, 27-28 octobre 2014

Avec le support du projet MedKeyhabitats Finance par la fondation MAVA
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October 2014



Annex III – Census of existing *Cystoseira* species in three Mediterranean MPAs involved in the ITN-MMMPA project

In this annex a deliverable produced for the MMMPA project is reported.

A census of the existing species of Fucales was carried out in three partners MPAs: Portofino, Tavolara-Punta Coda Cavallo and Ustica island (all in Italy). The distribution and diversity of canopy forming species was recorded, compared with historical data, when available, and reported on GIS maps. Results will be delivered to MPA managers and will be useful to develop management plans for algal forests, as well as to check their evolution over time.



Monitoring of Mediterranean Marine Protected Areas (MMMPA) Initial Training Network

PROJECT DELIVERABLE

Deliverable D1.1.3 Census of existing *Cystoseira* species in
the Mediterranean MPAs involved in ITN-MMMPA

Lead beneficiary UNS

Dissemination level PU

Submission date March 2015

Grant Agreement n 290056

Project acronym MMMPA

Project title Training Network for Monitoring Mediterranean Marine
Protected Areas

Funding scheme FP7-PEOPLE-2011-ITN

1 - Introduction

In the Mediterranean Sea, large brown macroalgae of the genus *Cystoseira* and *Sargassum* (order Fucales) are represented by 36 and 9 species respectively (Gómez-Garreta *et al.* 2000; Draisma *et al.* 2010; Cormaci *et al.* 2012), living from the surface up to several meters depth (Ballesteros 1992). These species are considered ecosystem engineers (Giaccone 1973; Ballesteros 1992), because they create dense forests offering substrate, food and shelter to other algae and different species of animals (Molinier 1960; Ballesteros *et al.* 1998; Chemello and Milazzo 2002; Cheminee *et al.* 2013). However, loss of large brown seaweeds has been observed in many coastal areas where data on their past distribution are available (see Airoidi *et al.* 2014). Coastal urbanization, marine pollution and outbreak of herbivores are some of the most important factors driving marine forests degradation and loss (Verlaque 1984; Guidetti *et al.* 2003; Guidetti 2006; Airoidi and Beck 2007; Arevalo *et al.* 2007; Mangialajo *et al.* 2008). For this reason, almost all *Cystoseira* and *Sargassum* species are listed, as priority species, in two European Conventions (Barcelona Convention, 1976 and Bern Convention, 1979), and they have been monitored according to the guidelines of the Water Framework Directive 2000/60/EU (Ballesteros *et al.* 2007; Mangialajo *et al.* 2007; Asnaghi *et al.* 2009; Bermejo *et al.* 2013; Nikolic *et al.* 2013).

Marine Protected Areas (MPAs) can ensure a higher protection of marine forests than unprotected sites, since different human impacts (i.e. urbanization and many forms of illegal fishing) are absent or reduced (Mosquera *et al.* 2000; Halpern and Warner 2003). However, marine forests are not generally taken into account in the creation of MPAs and they are often not targeted in monitoring programs or in the evaluation of MPA efficacy. As a consequence, information on their distribution in MPAs is limited. Therefore, it is highly important to assess the status of marine forests in Mediterranean MPAs in order to follow their evolution and guarantee a better conservation (Gianni *et al.* 2013).

The aim of this research was to do a census of marine forests in three MPAs involved in the MMMPA ITN Project: Portofino MPA, Tavolara-Punta Coda Cavallo MPA and Ustica island MPA (Italy). In addition, the current distribution of Fucales in these MPAs was compared to the historical one, when available in scientific literature, in order to assess their evolution.

2 - Materials and methods

Information on historical distribution of Fucales in Portofino, Tavolara-Punta Coda Cavallo and Ustica island MPAs was collected by searching on the databases ISI Web of Science and Google Scholar and by asking to the MPA managers.

Successively a visual census of *Cystoseira* and *Sargassum* species was carried out in the infralittoral fringe (-/+ 0.5 m) of Portofino (June 2013), Tavolara-Punta Coda Cavallo (July-August 2013) and Ustica island MPAs (August 2014). In the latter two MPAs, also the upper-infralittoral zone was surveyed (until -3 m depth).

Specifically, in Portofino MPA, that extends around the homonym promontory for 13 km, the infralittoral fringe of the entire coastline was surveyed, but it was not possible to sample the upper-infralittoral zone due to the morphology of the coast mainly characterized by vertical cliffs. In Tavolara-Punta Coda Cavallo, the sampling areas were chosen randomly because of the high extension of the MPA. The presence of Fucales was also evaluated at “Secca del Papa” (-15/-30 m), a shoal highly popular as diving spot, where a recolonization of *Sargassum* spp. was observed recently. In Ustica island, almost the entire coastline of the island was surveyed. The sampling areas are highlighted in figures 1-4.

The infralittoral fringe was surveyed applying a simplified CARLIT method (see the box below for details). The presence and distribution of Fucales in the rockpools and in the upper-infralittoral zone was evaluated by snorkelling. Finally the survey at the “Secca del Papa” shoal, in Tavolara-Punta Coda Cavallo MPA, was done by a team of scuba divers. At this site, density of *Sargassum* spp. and *Cystoseira* spp. and the height of the main axis were estimated at three depth ranges: 15-20m, 20-25m and 25-30 m by 50 x 50 cm quadrats placed randomly ($n > 25$).

In all samplings, when it was not possible to identify the species in situ, some individuals were collected for an accurate identification. Maps and data analysis were realized using QGIS software.

The CARLIT method

The CARLIT index, applied in the North-Western Mediterranean and in the Adriatic Seas in the framework of the Water Directive (2000/60/EU), uses, among others, the distribution of very shallow *Cystoseira* forests to assess the ecological status of coastal shallow waters. In this method, the coastline is divided in sectors and the most abundant benthic communities are noted (*Cystoseira* spp., coralline algae, mussels, etc.). The presence of *Cystoseira* species (in particular *Cystoseira amentacea* var. *stricta* - hereafter *C. amentacea* - and *C. mediterranea*) is visually estimated and associated to a value of abundance (5 categories). In addition, some physical characteristics of the coast are recorded, such as the morphology (high cost/low cost/metric blocks), exposure to waves (exposed/calm), type of substrate (natural/artificial), slope of the substrate (vertical/sub-vertical/horizontal) and the presence of human impacts (see Ballesteros *et al.* 2007).

In the simplified CARLIT method that we used in these surveys, the coastline was divided in sectors of variable length depending on the bio-morphological changes of the coast and the abundance of *C. amentacea* was estimated following only three categories instead than five (1: isolated individuals; 2: dense and numerous populations; 3: algal forests forming almost continuous or continuous belts). The surveys were carried out by kayak or a small boat, proceeding very close to the coast.

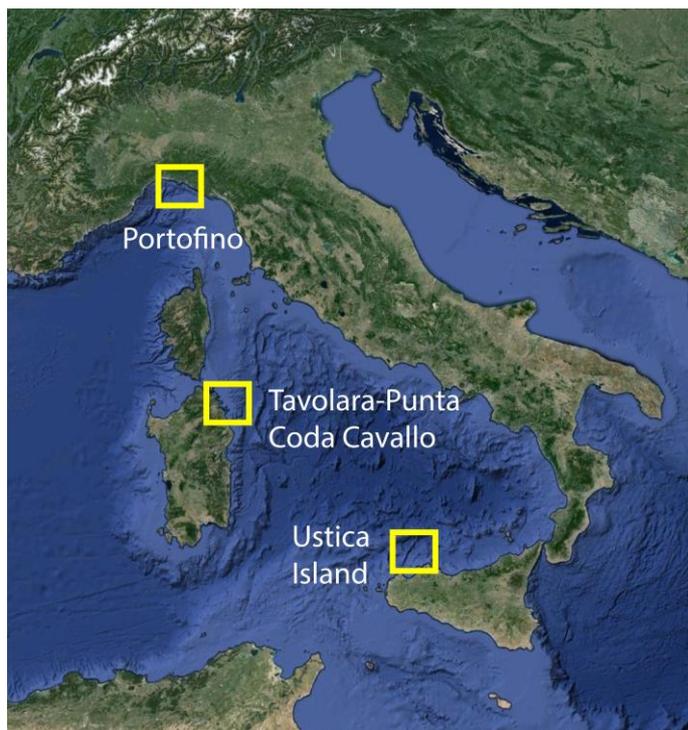


Figure 1. The three MPAs where surveys were conducted.

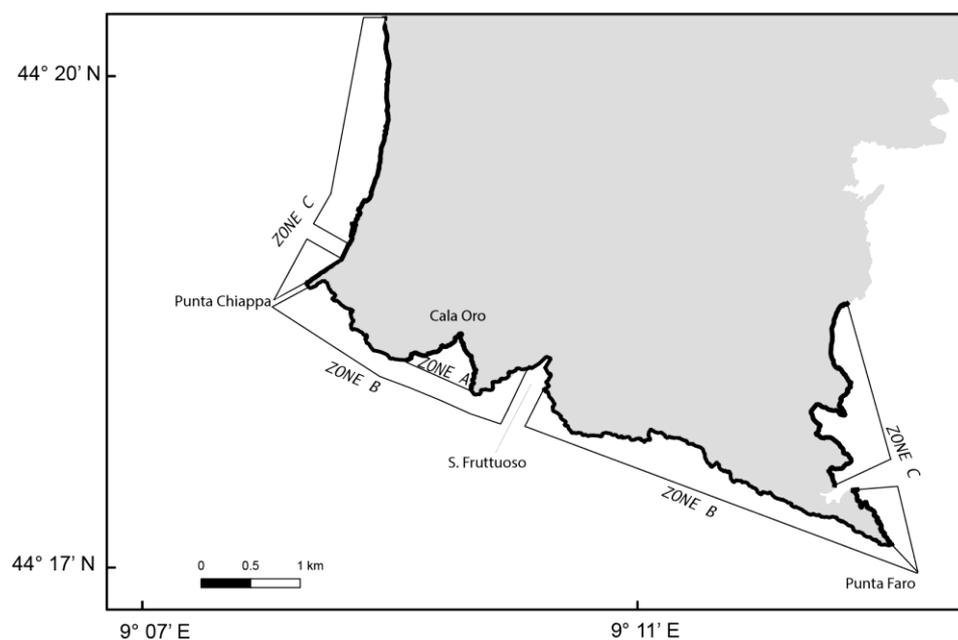


Figure 2. Portofino MPA, Liguria Region, Italy. Black lines show the sampling areas. MPA zonation is also showed in the map.

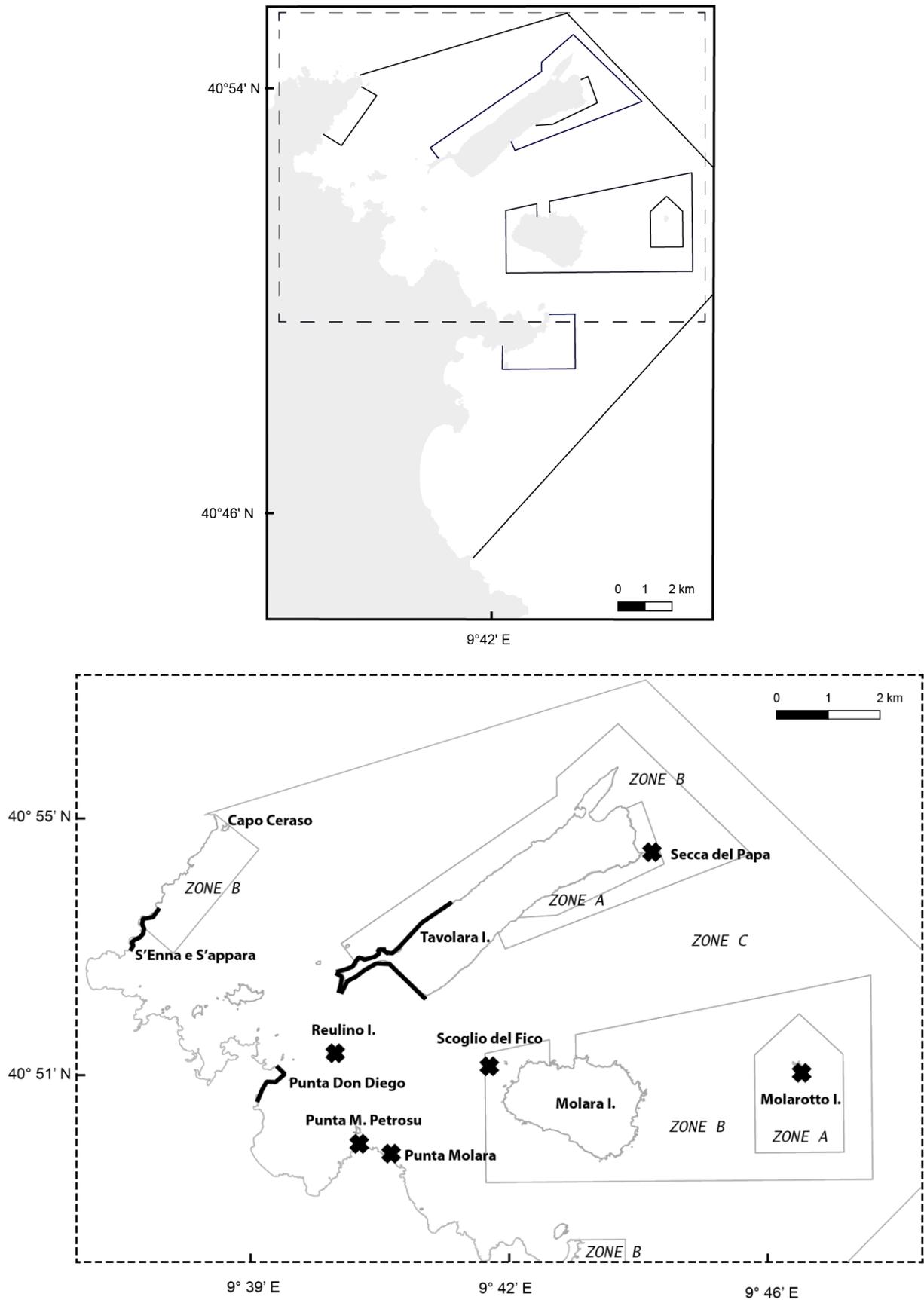


Figure 3. Tavolaria-Punta Coda Cavallo MPA, Sardinia, Italy. Black lines and crosses highlight the sampling areas. Molarotto island was entirely surveyed. MPA zonation is also showed in the maps.

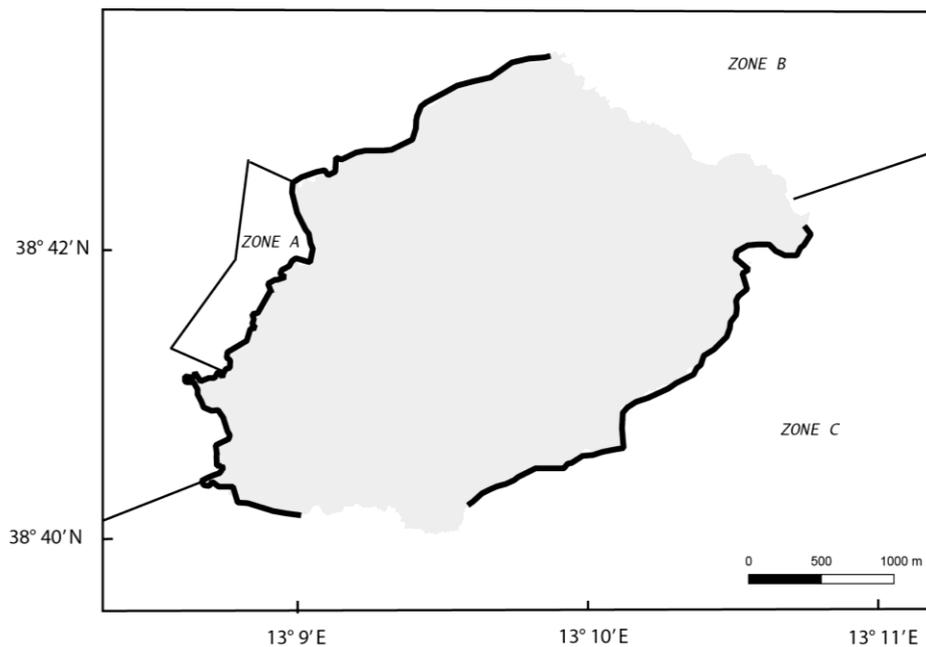


Figure 4. Ustica island MPA, Sicily, Italy. Black lines highlight the sampling areas. MPA zonation is also showed in the map.

3 - Results and discussion

3.1 - Fucales of Portofino MPA

3.1.1 - State of art

Fucales of Portofino MPA, and in particular deep species, were poorly studied in the past. The oldest observations were done by Tortonese (1958, 1961, 1962) that reported the presence of *Cystoseira compressa*, *Cystoseira amentacea*, *Cystoseira zosteroides* and *Sargassum vulgare*. These species were also observed later by Morri *et al.* (1986), Parravicini *et al.* (2013) in 1993, Mangialajo (Personal Herbarium: 1999, 2000), Schiapparelli *et al.* (2003) and Mangialajo *et al.* (2004), but they reported only a spotty presence. After 2000, studies on Fucales increased, but they focused on *Cystoseira* species of the infralittoral fringe (*C. amentacea* and *C. compressa*), mainly to evaluate the status of the water bodies following the Water Framework Directive's recommendations (Mangialajo *et al.* 2003, 2007, 2008; Asnaghi *et al.* 2009). In all these studies, the authors observed a high abundance of *C. amentacea* in the southern coast of the Portofino promontory. On the contrary, the eastern and western coasts were mainly characterized by *C. compressa*, *Dictyota*, *Corallina elongata*

and *Ulva lactuca* because of the presence of buildings, rivers, drains and a reduced wave exposure.

Rockpools of the southern coast were also surveyed by Mangialajo (2007) and high abundances of *S. vulgare*, *C. compressa* and *C. amentacea* were recorded.

A decline of *S. vulgare* forests was observed by Parravicini *et al.* (2013) that reported this species as rare in 1993 and virtually absent in 2008, probably due to the increase of herbivores.

See appendix I for further details on coverage and distribution of Fucales in Portofino MPA.

3.1.2 - Current distribution of Fucales in the MPA

The survey of the algal assemblages in the infralittoral fringe of Portofino MPA was done on 13 km of coast: the entire length of the protected area. Along the east coast of the promontory (zone C – partial reserve), the infralittoral assemblages are mainly composed by *Corallina elongata* and *Mytilus galloprovincialis*, occasionally some patches of *Cystoseira compressa* and very rare individuals of *Cystoseira amentacea* and *Sargassum vulgare* were observed (Fig. 5-6). Unluckily, it was not possible to sample in Paraggi and in Portofino bays due to the boating restrictions. After Punta del Faro, where wave exposition is higher, *C. amentacea* becomes gradually abundant and constitutes almost continuous belts. The abundance of this species is reduced only on vertical substrates, in proximity of freshwater intakes and in the inner part of S. Fruttuoso bay where it is substituted by *C. elongata*. After Punta Chiappa, *C. amentacea* is rare, mainly because wave exposition is reduced and *C. elongata*, *C. compressa* and *M. galloprovincialis* are the most abundant species (Fig. 5-6).

Cystoseira amentacea belts extend on 53% of the coastline. In the zone A (integral reserve) and in the zone B (general reserve), along the southern coast of the MPA, they are present on 94% of the sampled sectors, and in 5% and 2% of the sectors in the two zones C, along the eastern and western coasts, respectively (Fig. 5).

The comparison between information obtained in this census and previous data on *C. amentacea* (Mangialajo *et al.* 2003, 2007, 2008; Asnaghi *et al.* 2009), permitted to show that this species is still well developed in the southern side of the promontory, in particular at Punta Chiappa and in the no-take zone, as described in the previous studies. *C. amentacea* abundance decreases from the western to the eastern side of the S. Fruttuoso bay, as observed by Mangialajo *et al.* (2003).

In addition, a rockpool (44°18'16" N 9°12'0"E) on the southern coast of the promontory was surveyed. Mangialajo (2007) measured, in the same rockpool in 2006, low coverage of *C. compressa* (< 5%) and high density of sea urchins. We also measured a high density of *Paracentrotus lividus* (18 ind/m²) and only few, isolated individuals of *C. compressa* and *S. vulgare* with *Ulva*, *Hypnea musciformis* and *Dictyota* spp. MPA staff may consider to monitor this rockpool and, eventually, remove sea urchins in order to favour natural recovery of *Cystoseira* and *Sargassum* species (see Gianni *et al.* 2013).

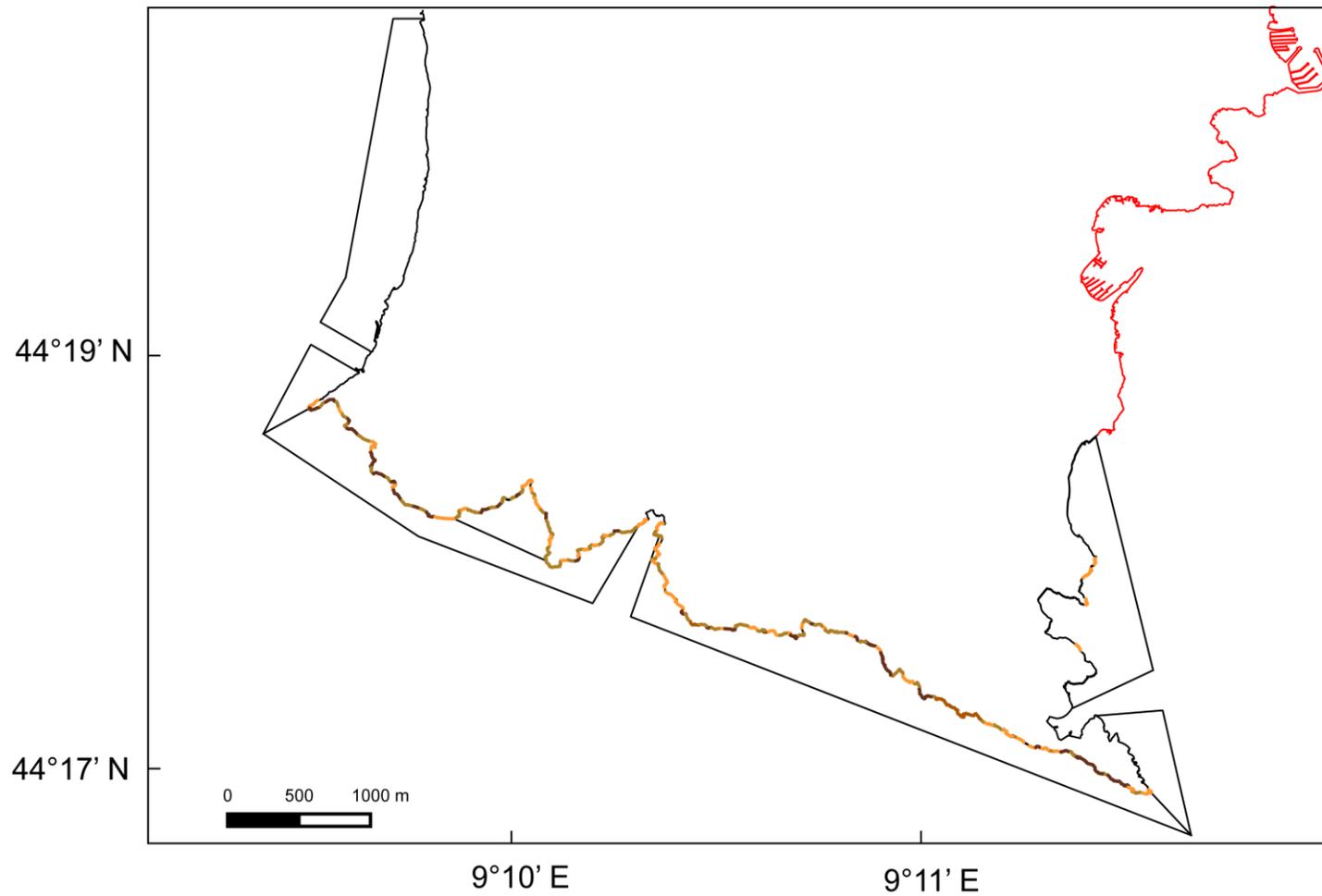


Figure 5. Portofino MPA with the relative zonation and *Cystoseira amentacea* distribution in the infralittoral fringe. The abundance of *C. amentacea* was reported in three categories (orange lines: isolated individuals; light brown lines: dense and numerous groups; dark brown lines: algal belts almost continuous or continuous). The coastline in red was not surveyed.

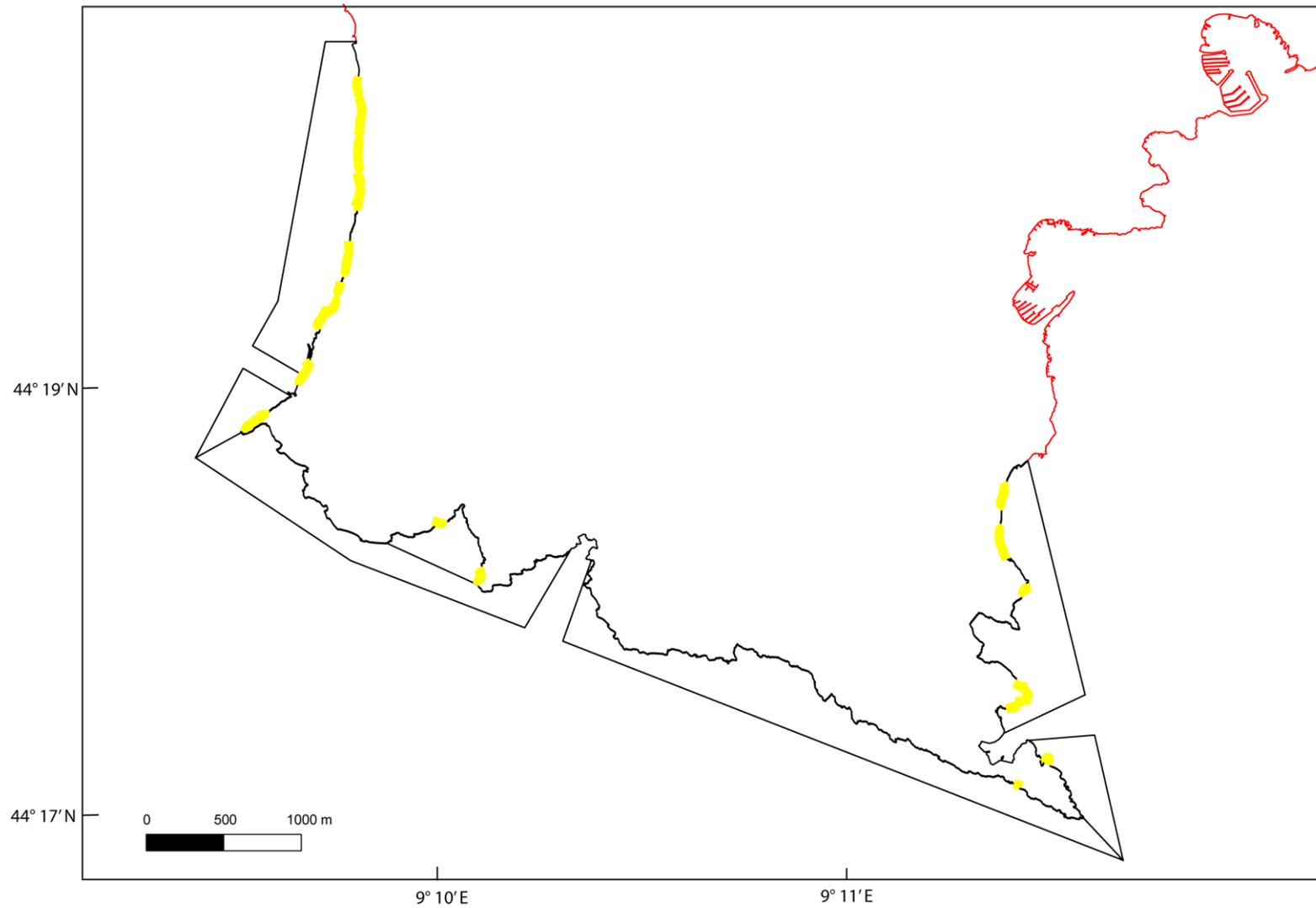


Figure 6. Distribution of *Cystoseira compressa* in the infralittoral fringe of Portofino MPA (in yellow). The coastline in red was not surveyed.

3.2 - Fucales of Tavolara-Punta Coda Cavallo MPA

3.2.1 - State of art

Few papers and reports describing past distribution of Fucales at Tavolara-Punta Coda Cavallo MPA are available in the scientific literature, and, generally, authors did not specify the sites of sampling (see appendix II).

Seven *Cystoseira* species are recorded in the literature: *Cystoseira amentacea* var. *stricta*, *Cystoseira compressa*, *Cystoseira brachycarpa*, *Cystoseira crinita*, *Cystoseira foeniculacea*, *Cystoseira foeniculacea* f. *latiramosa*, *Cystoseira zosteroides* (Cossu *et al.* 1992; Ceccherelli *et al.* 2005; UNEP(DEPI)/MED 2007; Ceccherelli and Farris 2008; Navone *et al.* 2010; Sales *et al.* 2012). Information on *Cystoseira* abundance is available only for *C. amentacea*. Continuous or almost continuous belts of this species were observed in the infralittoral fringe of Molarotto island (no-take zone) and Molara island (zone B – general reserve) (Ceccherelli *et al.* 2005; Ceccherelli and Farris 2008). On the contrary, isolated patches of individuals were observed along a small stretch of coast in the no-take zone of Tavolara island and in areas with low protection (zone C – partial reserve) of Molara island and on mainland.

Concerning the genus *Sargassum*, some authors observed *S. vulgare* in the MPA, in particular at the “Secca del Papa” diving spot (Navone *et al.* 1992; Trainito and Navone 2011). In other cases the species was not identified and just reported as *Sargassum* spp. (Modugno *et al.* 2006; UNEP(DEPI)/MED 2007). *Sargassum acinarium* was also noted at Tavolara-Punta Coda Cavallo by Solazzi in 1968 (reported in Cossu *et al.* 1992), but the exact position was not specified in the paper.

3.2.1 - Current distribution of Fucales in the MPA

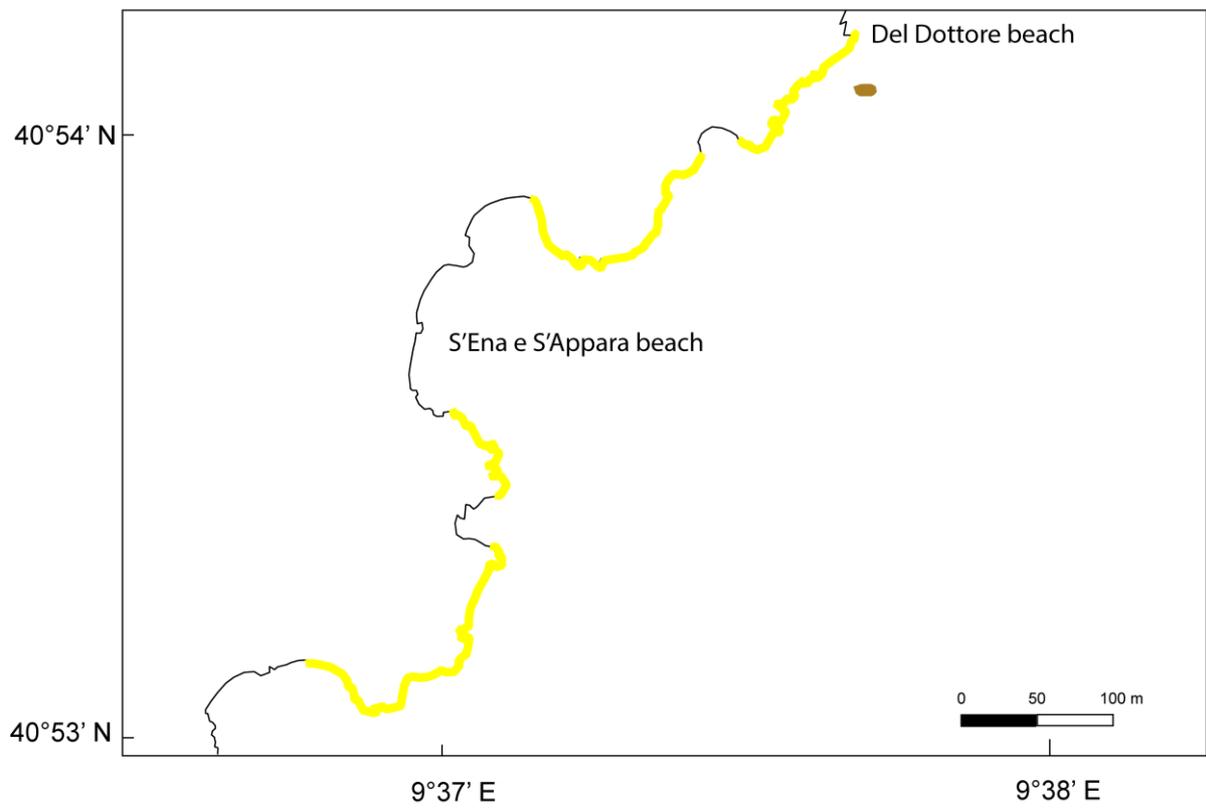
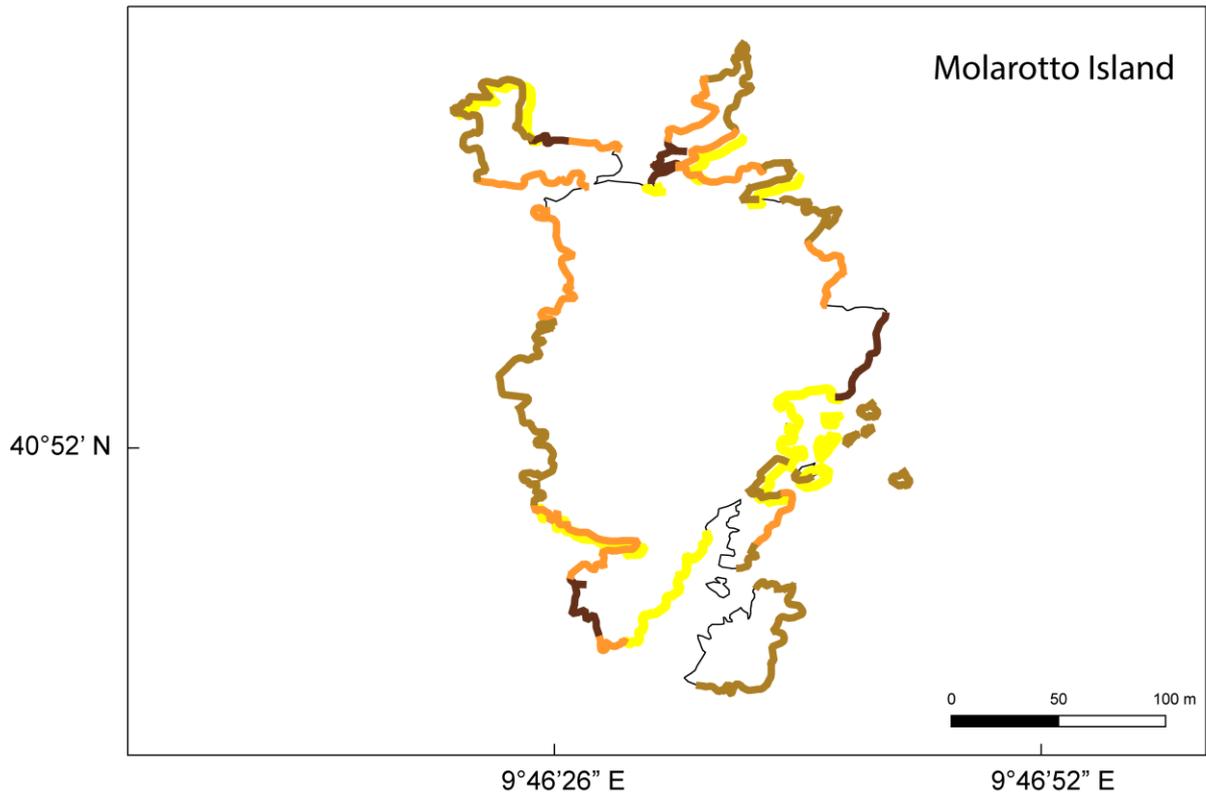
Census of Fucales at Tavolara-Punta Coda Cavallo MPA, even if not complete, allowed to describe 7 *Cystoseira* and 2 *Sargassum* species (see the check-list at the end of the paragraph). Ten km of coast were surveyed: about 80% of them are dominated by Fucales (mainly *Cystoseira* species), while 20% is characterized by other photophilic algae (e.g. *Laurencia* complex and Dictyotales). All species already described in the previous studies were observed in this census except for *Cystoseira zosteroides*, but the circalittoral zone, where this species is found, was not surveyed. Two new species were recorded: *Cystoseira barbata* and *Cystoseira compressa* var. *pustulata*.

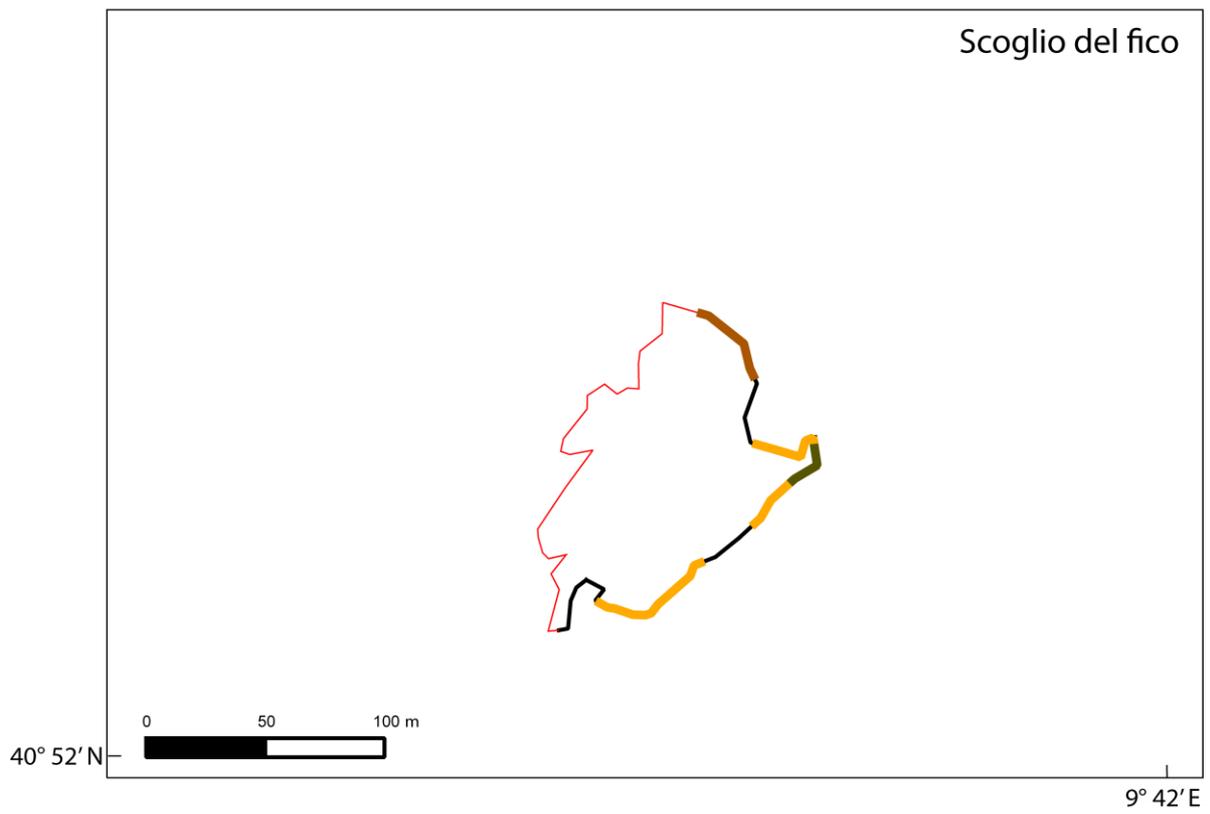
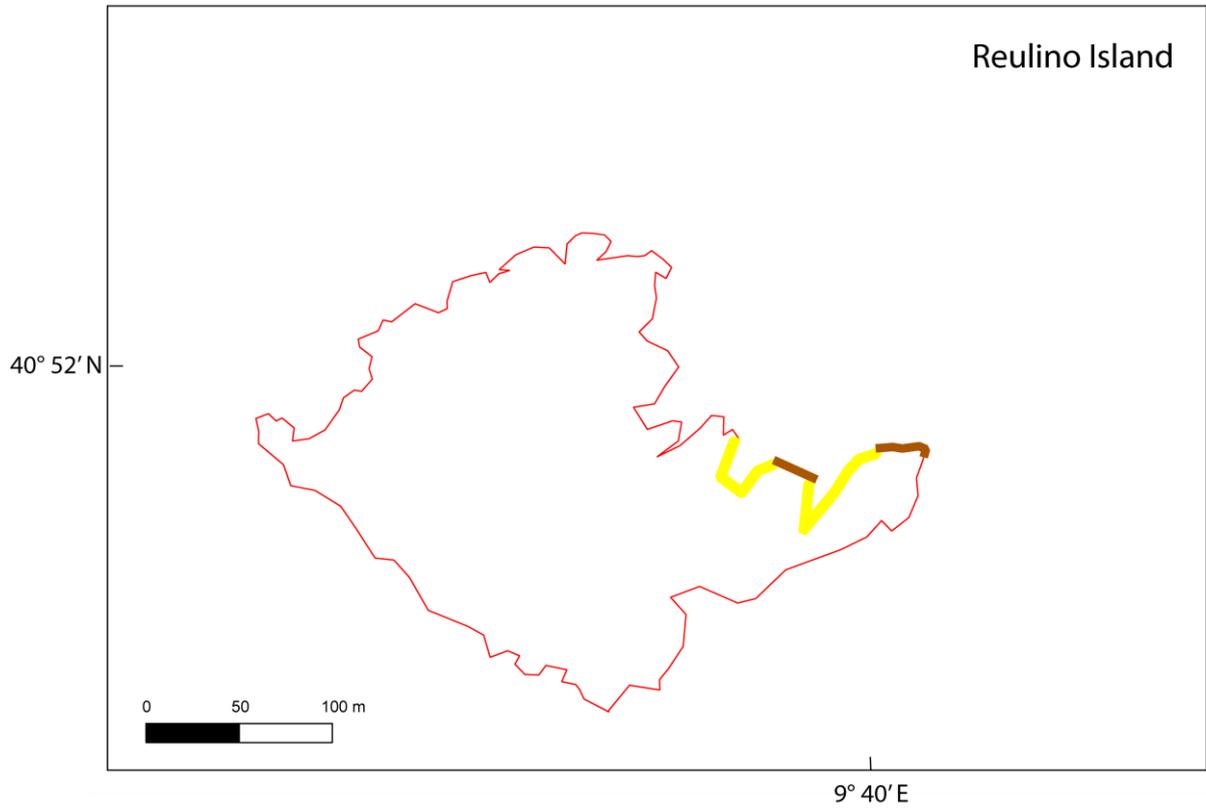
The infralittoral fringe of this MPA is mainly characterized by *Cystoseira amentacea*, associated to *Cystoseira compressa* var. *compressa*. At Molarotto island (no-take zone), *C. amentacea* is present in dense populations on the whole perimeter of the island (about 1.5 km). Only in small coves it is substituted by *Laurencia* sp., *Lithophyllum* spp. or Dictyotales (Fig. 7). A similar pattern was observed in the other surveyed areas, the most exposed to wave action, such as “Punta Spalmatore”, “Punta la Mandria”, Reulino island, “Scoglio del Fico”, “Punta Molarà” and along the north coast of Tavolara island before and after “Cala Tramontana”. On the contrary, isolated groups of individuals were observed at “Punta di Monte Petrosu”, in Costa Dorata bay and between the “S’Ena ‘e s’Appara” and “Del dottore” beaches, where *C. compressa* var. *compressa* is more abundant (Fig. 7).

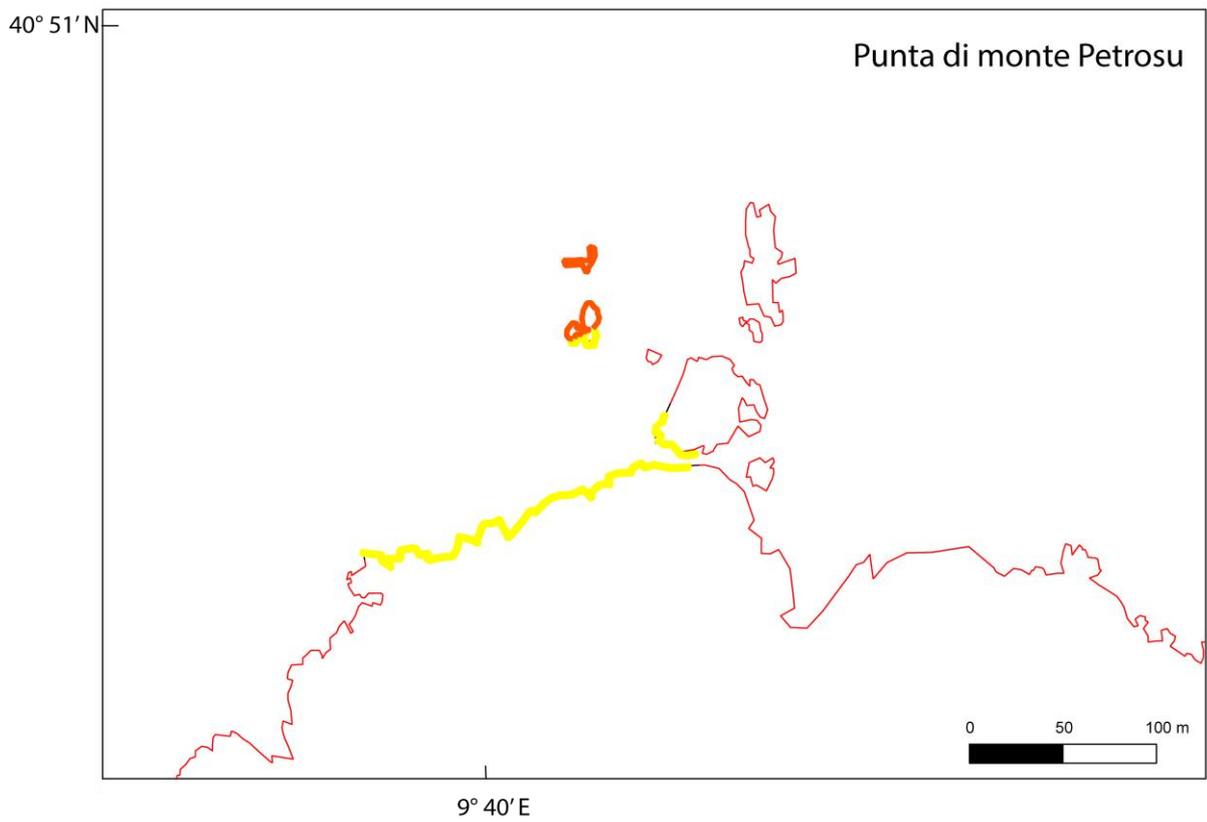
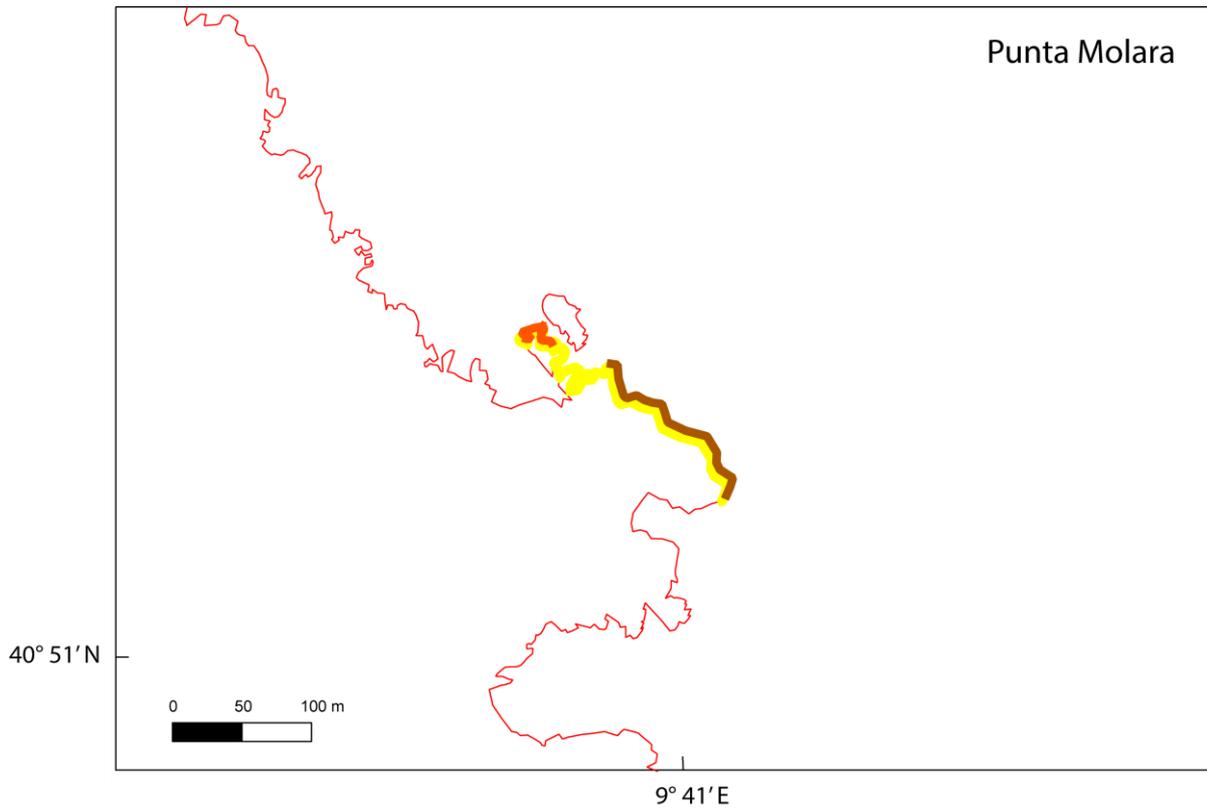
The current distribution and status of *C. amentacea* is comparable to the one evaluated in the past (Ceccherelli *et al.* 2005; Ceccherelli and Farris 2008), even if in this census a high abundance of this species was also observed in other areas. In a previous work, Guidetti *et al.* (2004) noted a difference of benthic species composition in this MPA, probably linked to the nature of the substrate, in particular more photophilic algae on granite rock and more sciaphilic algae on calcareous rocks. However, this is not the case for *C. amentacea*, being abundant both on the granitic and the calcareous rocks of Tavolara MPA.

In the upper-infralittoral zone, Fucales are also well represented (Fig. 8). The most common species are *Cystoseira brachycarpa* var. *balearica*, *C. compressa* var. *compressa* and var. *pustulata* and *Cystoseira crinita*. Occasionally, small patches or isolated individuals of other *Cystoseira* species were observed. For instance, *C. barbata* is present at “Spalmatore di Terra” (Cala Tramontana, Passetto beach), Costa Dorata bay and near “S’Ena ‘e s’Appara” beach. In the rockpools of the north coast of Tavolara island and in the harbour of the island, some individuals of *Cystoseira foeniculacea* f. *foeniculacea* were noted. Also *Cystoseira spinosa*/*Cystoseira elegans* (the phenological stage of the samples did not permit a precise taxonomic identification) was found in the harbour of Tavolara island and in Costa Dorata bay. Finally, *Sargassum vulgare* was observed at “Punta Molarà”, “Punta di Monte Petrosu”, near “S’Ena ‘e s’Appara” beach and near a site called “I forni di scirocco” on Tavolara island (Fig. 8).

Unluckily, it is not possible to do a comparison with the past distribution of these species because authors did not report the exact sites of their observations.







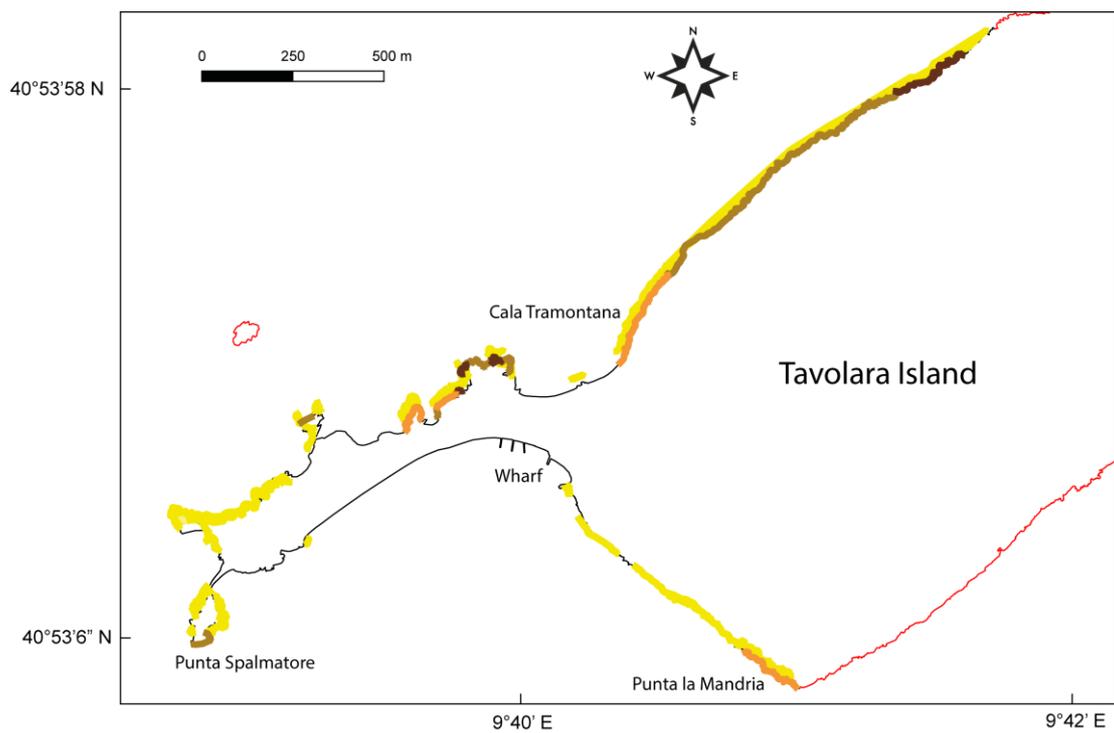
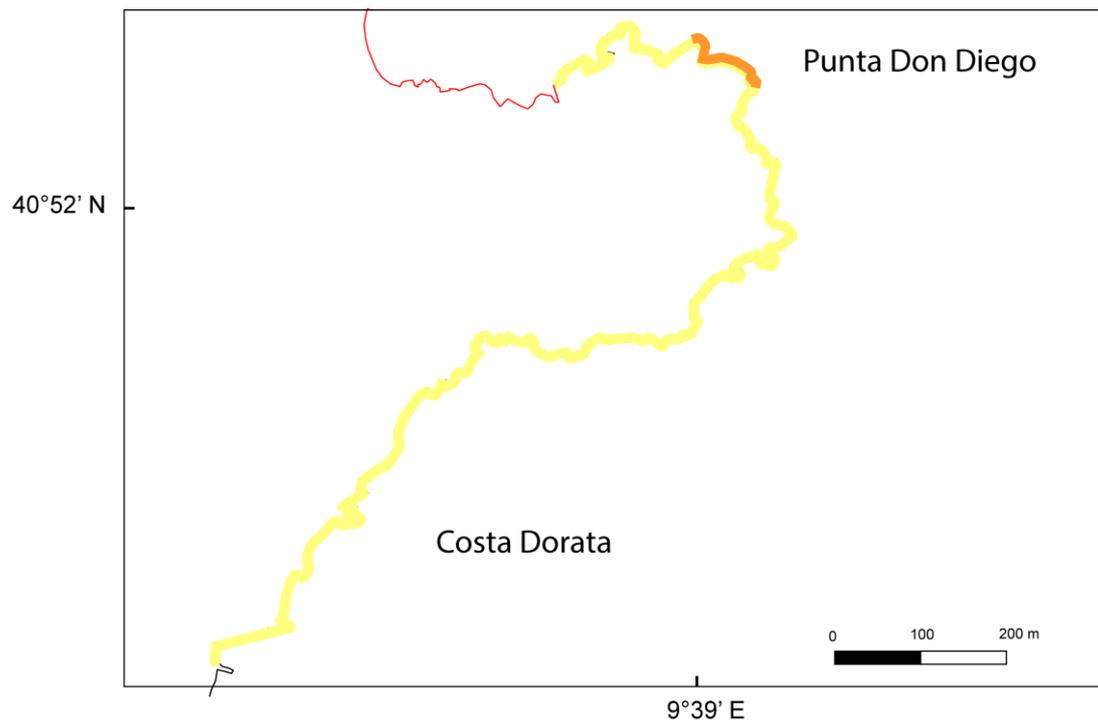
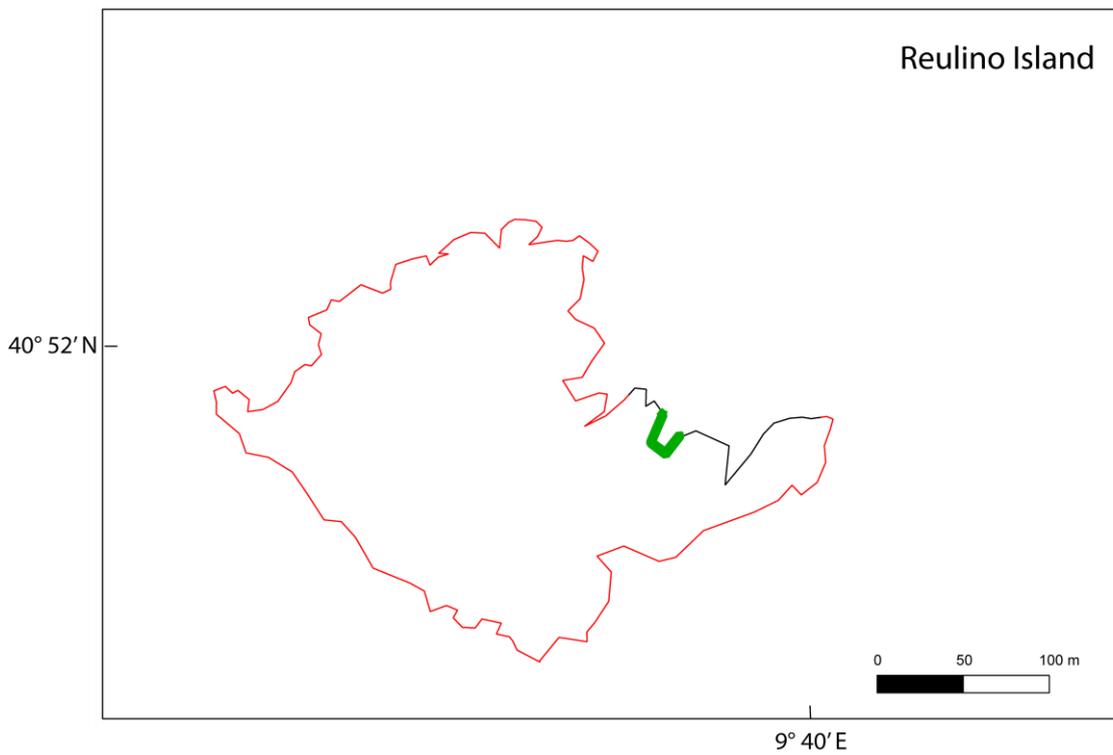
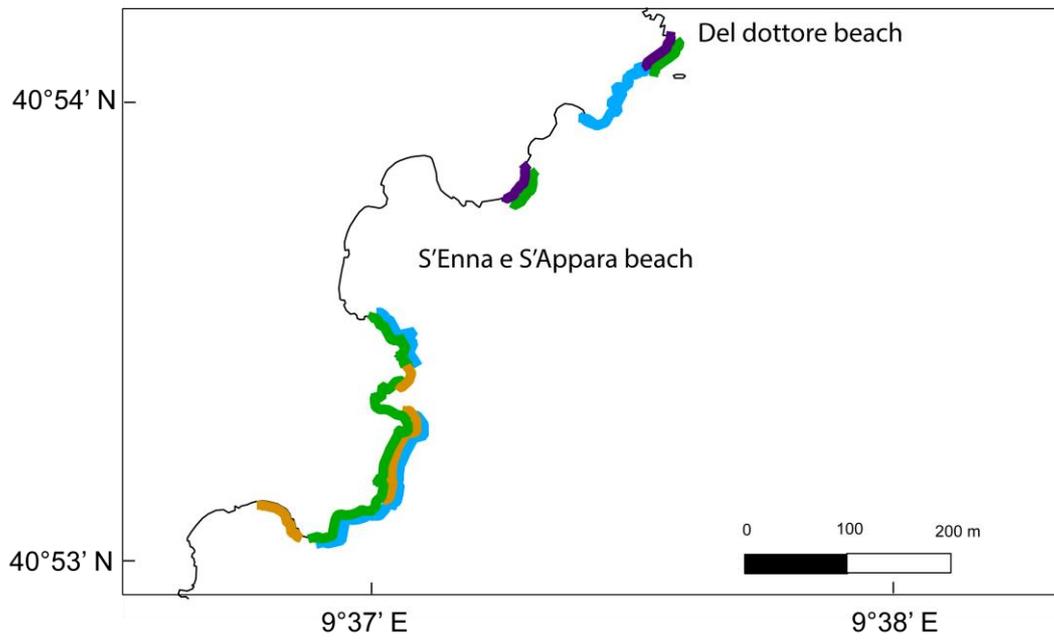
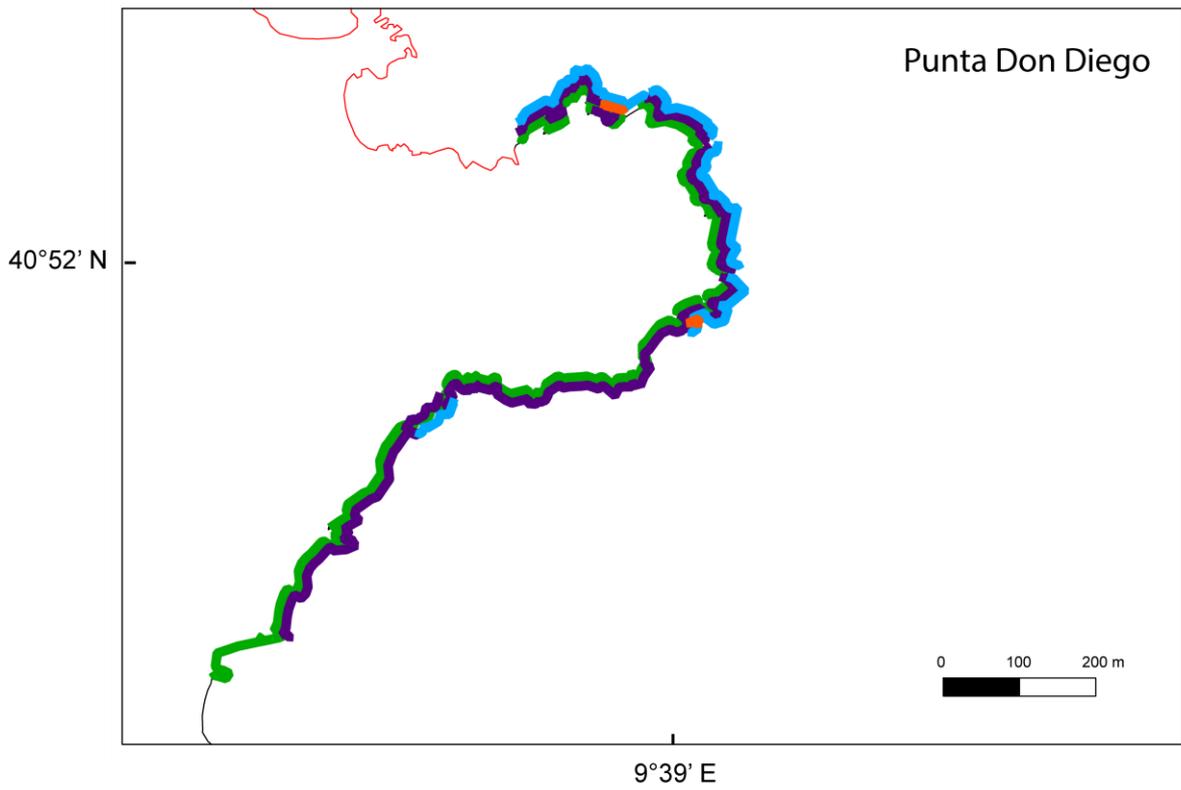
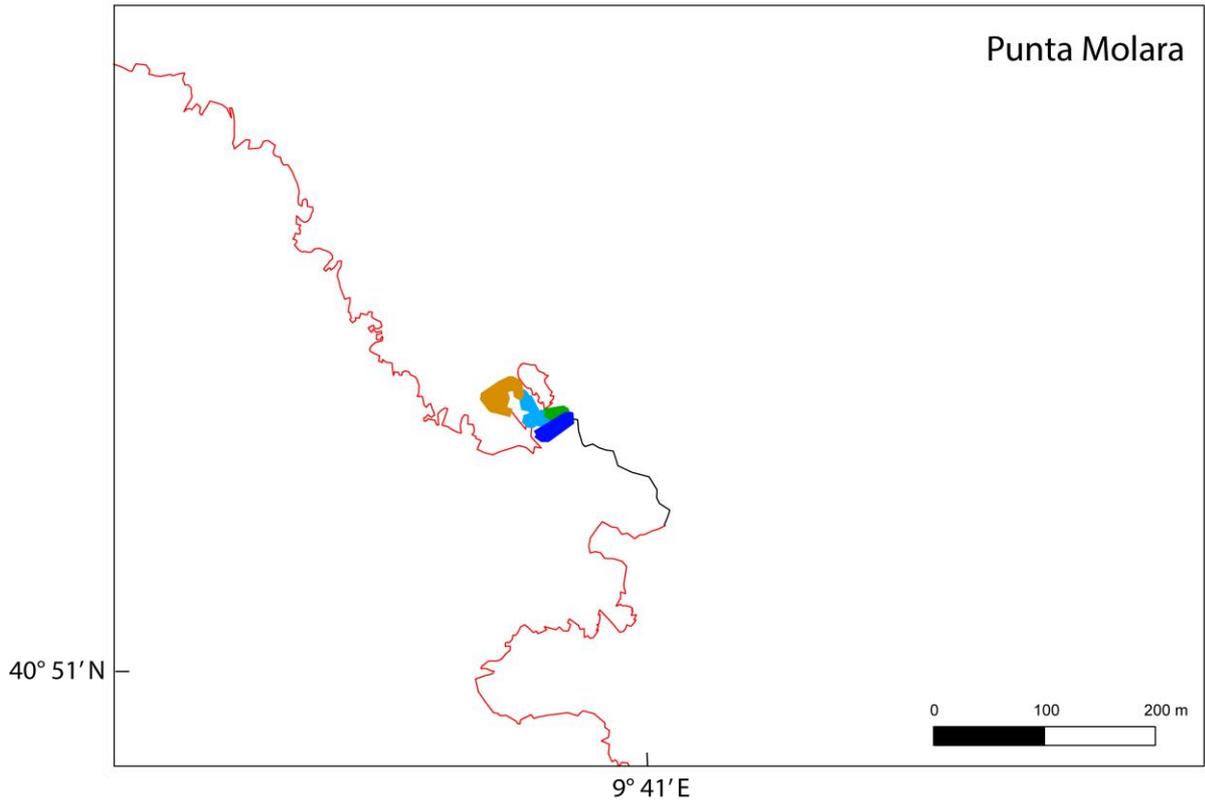


Figure 7. The surveyed coastal areas of Tavolaria Punta-Coda Cavallo MPA showing *Cystoseira amentacea* and *Cystoseira compressa* distribution in the infralittoral fringe. The abundance of *C. amentacea* was reported in three categories (orange lines: isolated individuals; light brown lines: dense and numerous groups; dark brown lines: algal belts almost continuous or continuous). Yellow lines: presence of *C. compressa*. The stretches of coast in red were not surveyed.





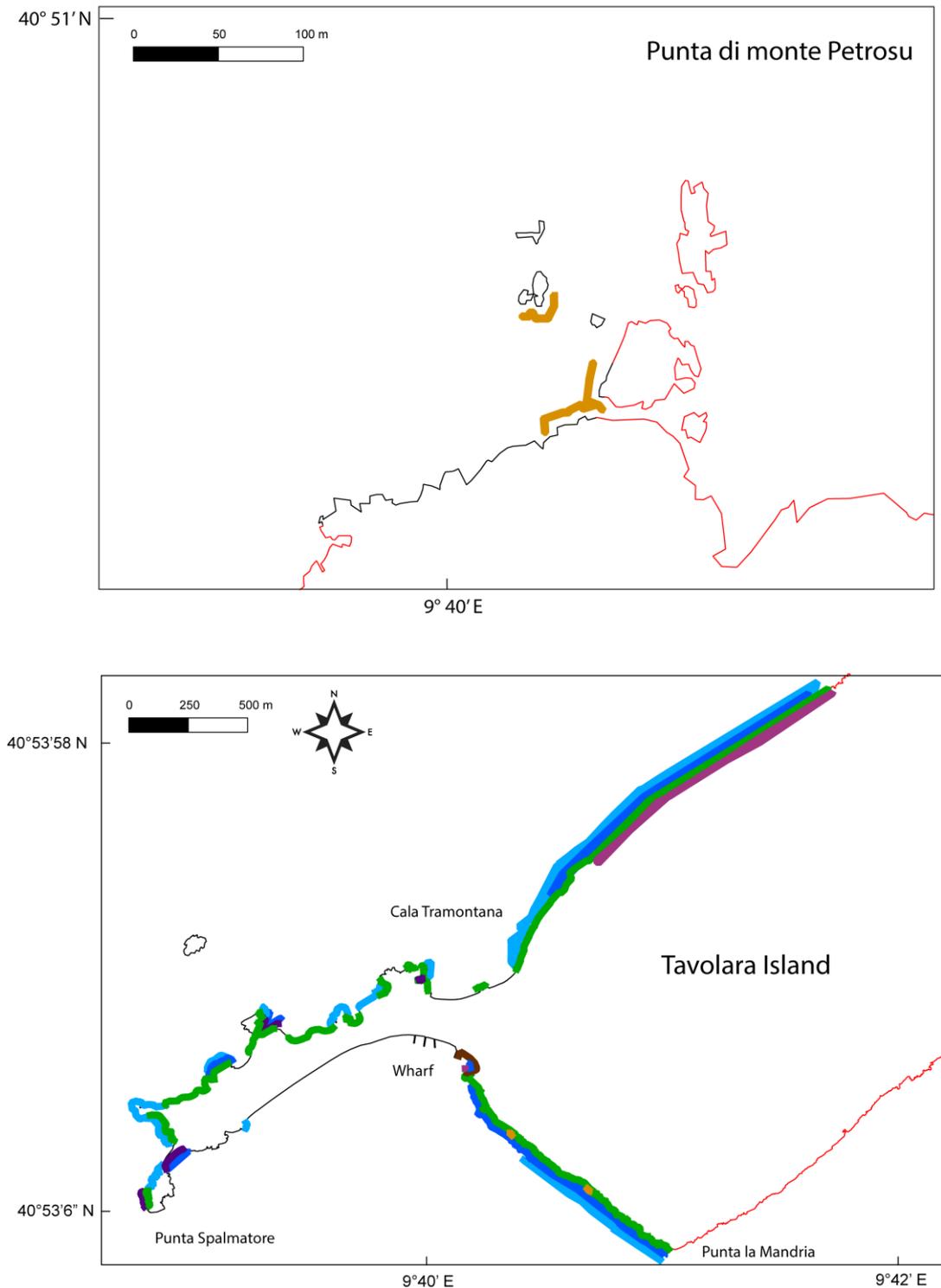


Figure 8. The surveyed upper-infralittoral zones of Tavolara Punta-Coda Cavallo MPA. The distribution of *Cystoseira* species is reported in different colours (green: *Cystoseira crinita*; blue: *Cystoseira compressa* var. *pustulata*; light blue: *Cystoseira brachycarpa*; brown: *Cystoseira spinosa*; violet: *Cystoseira barbata*; light violet: *Cystoseira foeniculacea* var. *foeniculacea*; beige: *Sargassum vulgare*). The stretches of coast in red were not surveyed.

The survey at the “Secca del Papa” shoal allowed to describe a dense population of *Sargassum* spp. from top (-16 m) until -30 m. This forest is probably composed by an association of *Sargassum acinarium* and *Sargassum vulgare*, already observed on this shoal in the past (Navone *et al.* 1992; Trainito and Navone 2011). The identification of *Sargassum* species is complicated and linked to reproductive structures: more samples are needed to define the species composition at the “Secca del Papa” shoal. Therefore, hereafter, we refer to the individuals measured in this survey as *Sargassum* spp.

Density of *Sargassum* spp. is higher at 15-20 m depth range ($8.96 \text{ ind./}0.25 \text{ m}^2 \pm 0.94$, mean \pm SE), while it decreases between -20 and -25 m ($2.7 \text{ ind./}0.25 \text{ m}^2 \pm 0.70$) and between -25 and -30 m ($0.78 \text{ ind./}0.25 \text{ m}^2 \pm 0.27$) (Fig. 9).

The mean height of the main axis is about 2 cm (1.89 ± 0.07), while the maximum recorded height was 8 cm and the minimum one was 0.5 cm. *Sargassum* size distribution shows that this population is mainly composed by young individuals with a very short main axis (Fig. 10). This may be explained by the fact that before 2004 diving boats could be anchored on top of the shoal affecting the growth of *Sargassum* spp., as showed in old pictures (Fig. 11). Unluckily, no data on *Sargassum* abundance before 2004 are available in order to do a comparison with the current status.

Only few individuals of *Cystoseira foeniculacea* f. *latiramosa* were found during the sampling ($0.3 \text{ ind./}0.25 \text{ m}^2 \pm 0.13$) and only between 16-20 m depth. The mean height of the main axis is $1.56 \text{ cm} \pm 0.25$, with a maximum value of 3 cm and minimum value of 1 cm. No size distribution analysis is possible for this species because of the low number of individuals found.

In addition, one individual of *Cystoseira compressa*, a species that generally lives in the infralittoral fringe, was noted at -16 m, on top of this shoal.

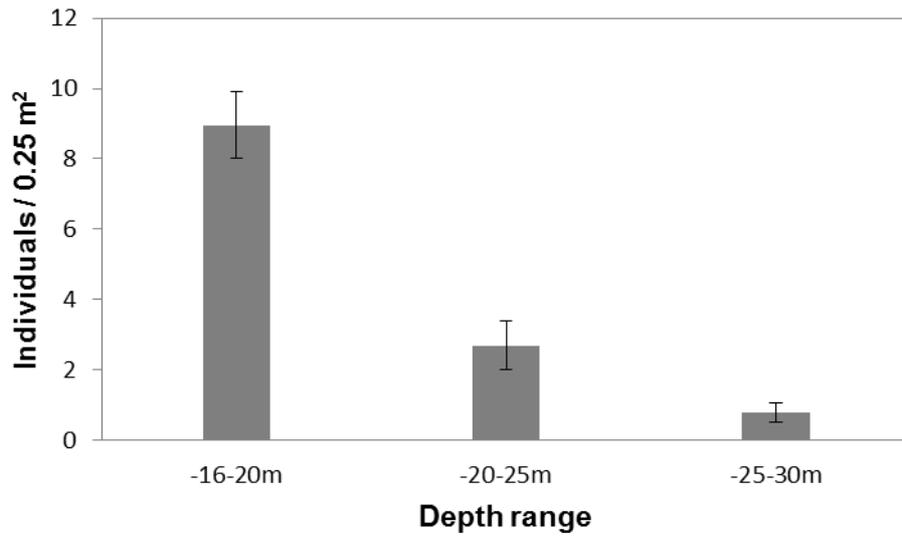


Figure 9. Mean density of *Sargassum* spp. on 0.25 m² at different depth ranges at the “Secca del Papa” shoal (n= 233 for 15-20 m depth range; n= 79 for 20-25 m depth range; n= 32 for 25-30 m depth range).

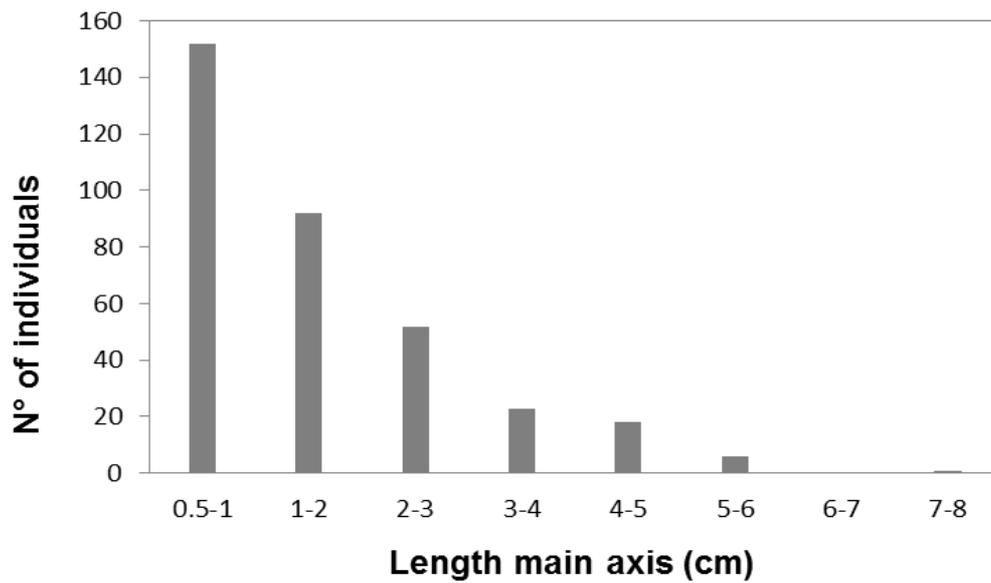


Figure 10. Size distribution of *Sargassum* population at the “Secca del Papa” shoal.

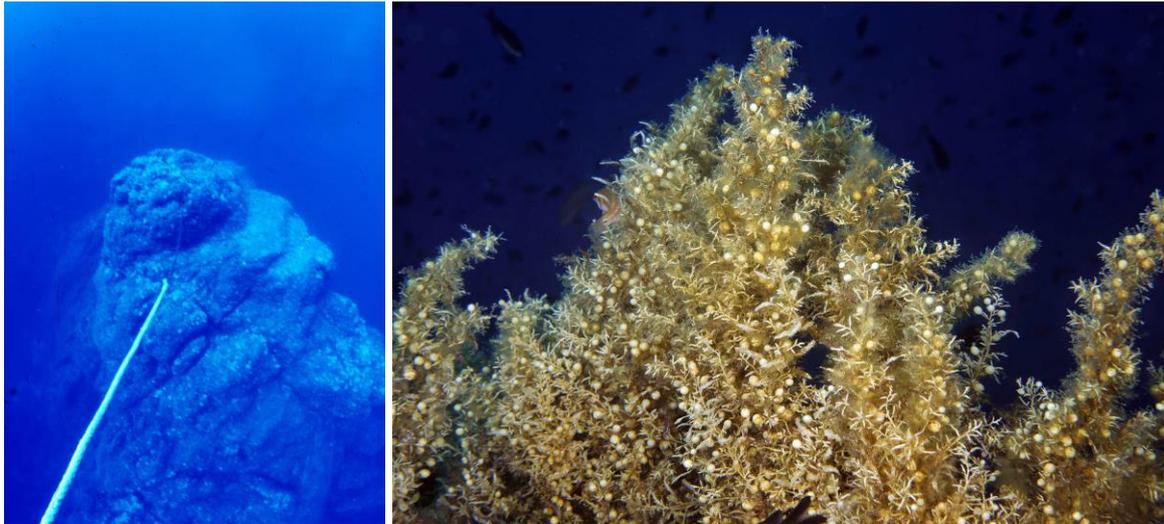


Figure 11. On the left, top of the “Secca del Papa” shoal before 2003 when anchoring was still possible. On the right, after the installation of a mooring buoy, a forest of *Sargassum* spp. started to grow and it is now abundant on the entire shoal. Photos E. Trainito.

Checklist of the observed species*

Cystoseira amentacea var. *stricta* Montagne in Durieu
Cystoseira barbata f. *barbata* (Stackhouse) C. Agardh
Cystoseira brachycarpa var. *balearica* (Sauvageau) Giaccone
Cystoseira compressa var. *compressa* (Esper) Gerloff & Nizamunddin
Cystoseira compressa var. *pustulata* Ercegovic
Cystoseira crinita Duby
Cystoseira foeniculacea f. *foeniculacea* (Linnaeus) Greville
Cystoseira foeniculacea f. *latiramosa* (Ercegovic) Gómez-Garreta
Cystoseira spinosa /*elegans* Sauvageau (identification not possible)
Sargassum acinarium (Linnaeus) Setchell
Sargassum vulgare C. Agardh

* Nomenclature according to Rodríguez-Prieto *et al.* (2013)

3.3 - Fucales of the Ustica island MPA

3.3.1 - State of art

Fucales at the Ustica island have been studied since 1960 mainly by Giaccone (see Giaccone 1969a for a review; Giaccone 1971; Giaccone *et al.* 1985), but also other authors carried out some experiments on *Cystoseira* forests and reported a spotty distribution of Fucales around the island recently (Milazzo *et al.* 2000, 2002, 2004; Drago *et al.* 2004; Report of the Project GEBECSUD 2004; Gianguzza *et al.* 2005; Catra *et al.* 2006, 2007, 2009; Gianguzza *et al.* 2010, 2013; Giaccone *et al.* 2010). In addition, historical data on the presence of the brown macroalgae *Phyllariopsis brevipes* and *Laminaria rodriguezii* are available in literature (Giaccone 1967, 1969b).

In particular, Giaccone described algal assemblages on the “Banco Apollo” shoal, localized 3 km west of the Ustica island (Giaccone 1967, 1968, 1969a, 1969b; Giaccone *et al.* 1985). A dense population of *L. rodriguezii* was observed on 1 km² of surface, from top of the shoal (-45 m) to -85 m. Together with this species, authors noted some populations of *Cystoseira spinosa*, *Cystoseira zosteroides*, *Cystoseira foeniculacea* f. *latiramosa*, *Sargassum hornschurchii* and *P. brevipes* with different density of individuals. Occasional patches of *L. rodriguezii* were also observed all along the north-west coast of the island between -50 m and -90 m depth (Giaccone 1969b).

Information on Fucales distribution in other sites of the island was reported in Giaccone (1969a, 1971) and Giaccone *et al.* (1985), but the precise location and coverage was indicated only for some species (see appendix II). Fourteen *Cystoseira* species and 2 *Sargassum* species were found. The infralittoral fringe was characterized by *Cystoseira amentacea* (40-60% of coverage), the infralittoral zone was dominated by an association of different *Cystoseira* species with coverage between 1 and 20%, like *C. spinosa*, *C. sauvageauana*, *C. brachycarpa* and *C. compressa*, while the circalittoral zone was mainly composed by *C. spinosa*, *C. zosteroides* and *C. foeniculacea* f. *latiramosa* (coverage: 2-25%).

Successive studies on Fucales at the Ustica island were carried out in the period 1996-2010 and 16 *Cystoseira* and 3 *Sargassum* species were observed. The status of *C. amentacea* was evaluated only in 2003 by Catra *et al.* (2006) and compared with the survey done by Giaccone *et al.* (1985). The authors did not find any sign of regression (see also Giaccone *et al.* 2010). All the other studies reported a spotty presence of Fucales in different sites and the authors generally observed forests of *C. brachycarpa* and *C. compressa* at 15 m depth and forests of

C. sauvageauana, *C. spinosa* and *Sargassum* sp. between -5 m and -20 m (Milazzo *et al.* 2000, 2002, 2004; Report of the Project GEBECSUD 2004; Gianguzza *et al.* 2005; Catra *et al.* 2006, 2007, 2009; Gianguzza *et al.* 2010, 2013). In the same works, the occasional presence of many other species was also reported (see annexes III and IV). However, extended barren grounds were observed in the subtidal zone of Ustica island since the institution of the MPA (in 1986). This status seems to be started with the *Paracentrotus lividus* fishing ban that induced an increase of sea urchins and, consequently, an overgrazing of the macroalgal assemblages (Gianguzza *et al.* 2006; Riggio and Milazzo 2004). In the last years, predation of the starfish *Marthasterias glacialis* seemed to reduce sea urchin abundance (Bonaviri *et al.* 2009; Di Trapani 2011; Gianguzza *et al.* 2009a, b) and potentially promote the recovery of marine forests that gradually developed in patches of tens of meters within encrusting coralline algae barrens (Agnetta *et al.* 2010; Gianguzza *et al.* 2010; Agnetta *et al.* 2013).

Finally, an analysis of the environmental pollution in the Ustica harbour allowed to observe *Cystoseira spinosa* v. *tenuior*, a new species never described for the island until then (Drago *et al.* 2004). Recently, the presence of *L. rodriguezii* and *P. brevipes* was also confirmed by Catra *et al.* (2006).

More details on the presence and distribution of Fucales at the Ustica island are reported in the appendices II and III and in the cited papers.

3.3.2 - Current distribution of Fucales and evolution

The survey at the Ustica island MPA, performed in summer 2014, allowed to describe 7 *Cystoseira* and 1 *Sargassum* species (see the check-list at the end of the paragraph). Eleven kilometers of coast were surveyed and about 90% of them is dominated by Fucales, mainly *Cystoseira* species, while the rest of the coast is characterized by other photophilic algae like *Laurencia* spp., *Corallina elongata*, *Padina pavonica* and Dictyotales (see figures 12-15). In comparison with the previous studies, a lower number of species was observed (see appendix III), but in this census the deep infralittoral and the circalittoral zones were not surveyed. However, all species of the infralittoral fringe and the upper-infralittoral zone already described in the past were also found in this survey. In addition, *Cystoseira foeniculacea* f. *tenuiramosa*, firstly described only at 20 m depth, was observed in the rockpools of the no-take zone and the zone B. *Cystoseira compressa*, *Cystoseira brachycarpa* and *Sargassum*

vulgare observed by Drago *et al.* (2004) in the Ustica harbour are still present, *Cystoseira spinosa* var. *tenuior* was not recorded in this census.

In detail, the infralittoral fringe is characterized by dense and continuous belts of *Cystoseira amentacea*, covering about 8 km of coast. Exceptionally, some individuals of *C. amentacea* and *C. compressa* were observed growing on artificial piers. *C. compressa* var. *compressa* or other algae like *Corallina elongata*, that generally substitute *C. amentacea* when the water quality is poor (Mangialajo *et al.* 2008), are abundant only in areas with a low wave exposition (i.e. small bays, beaches, harbours), not favorable for *C. amentacea* development. Therefore, the presence and abundance of *C. amentacea* in Ustica island seems mainly linked to the morphology of the coast, without any relation with the MPA zonation. In addition, no sign of regression of *C. amentacea* population have been observed, as reported by Catra *et al.* (2006). However, the authors did not specify the sampling sites and it is not possible to do a precise comparison between the current status and the past one.

The upper-infralittoral zone is mainly characterized by patchy forests of *C. brachycarpa* var. *balearica*, generally associated with *C. compressa* (var. *pustulata* and var. *compressa*), as described in the studies realized in the last decade (see the papers of Gianguzza and Milazzo). In the no-take zone and zone B of the MPA, *C. brachycarpa* forests are also associated with forests of *C. spinosa* and *C. sauvageauana*. On the contrary, in the zone C, only few isolated individuals of *C. spinosa* and *C. sauvageauana* were observed, probably because of the geomorphology of the coast characterized by cliffs.

In the most wave-exposed rockpools, *C. brachycarpa*, *C. compressa* var. *pustulata*, *C. spinosa* and *C. sauvageauana* are abundant, together with *C. foeniculacea* f. *tenuiramosa*. Scattered individuals of *S. vulgare* were observed only in some rockpools or bays where light intensity is lower.

Marine forests below 3 m depth were not object of this research, but the comparison between historical data collected by Giaccone and data collected recently by other authors (Milazzo *et al.* 2000, 2002, 2004; Report of the Project GEBECSUD 2004; Gianguzza *et al.* 2005; Catra *et al.* 2007, 2009; Gianguzza *et al.* 2010, 2013; Giaccone *et al.* 2010), showed that many species like *C. brachycarpa*, *C. compressa*, *C. spinosa*, *C. sauvageauana*, *C. foeniculacea* f. *latiramosa*, *C. foeniculacea* f. *tenuiramosa* and *Sargassum* spp. are still present with high coverage. No recent information on *C. zosteroides* distribution is available from literature, but Giaccone *et al.* (2010) reported that well-structured populations of this species are still present. No detailed studies were carried out at the “Banco Apollo” shoal recently.

Finally, it is worthwhile to note that two patches of *Caulerpa racemosa*, covering few square meters, were observed only in a rockpool near Cala Sidoti (38°42' 23.51" N 13° 9' 34.23" E) and in front of the cliff at north-east of Cala Madonna, at 2 meters depth. *Caulerpa racemosa* is a particularly invasive species, able to cover the substrate in short-time and reduce benthic biodiversity (Piazzi *et al.* 2001); therefore, it is highly recommended to remove it during this first stage of invasion.

As described above, the species diversity and distribution of Fucales at the Ustica island seem stable in recent decades. However, Fucales distribution in the Ustica island may have locally changed in terms of abundance and species distribution and in particular due to the outbreak of herbivores that altered the underwater landscape of the protected zone. A natural recovery of macroalgal forests seems to occur presently (see above), and this MPA represents one of the few cases of reported natural recovery of *Cystoseira* forests; more studies replicated over time would be necessary to better understand this process.

At the moment, no relevant sources of direct impacts seem to affect Fucales populations at the Ustica island. The desalination plant placed in the zone C does not seem to have any effect on *Cystoseira* forests. It was not possible to assess if the wastewater treatment plant, localized to the other side of the island, is affecting *Cystoseira* abundance. The potential source of pollutants represented by the harbour does not seem to impact *Cystoseira* and *Sargassum* forests that are abundant in proximity of it (nevertheless it was not possible to find a species observed few years ago inside the harbour). Other possible sources of impact for *Cystoseira* forests, that may be considered by the managers of the MPA, are the pollution by pleasure boating (in particular in some sheltered bays), the proliferation of invasive species (i.e. *Caulerpa racemosa*) and trampling by tourists, especially in some beaches of the no-take zone where bathing is allowed and where small populations of *C. foeniculacea* f. *tenuiramosa* are located.

Checklist of the observed species*

Cystoseira amentacea var. *stricta* Montagne in Durieu
Cystoseira brachycarpa var. *balearica* (Sauvageau) Giaccone
Cystoseira compressa var. *compressa* (Esper) Gerloff & Nizamuddin
Cystoseira compressa var. *pustulata* Ercegovic
Cystoseira foeniculacea f. *tenuiramosa* (Erceg.) Gómez Garreta
Cystoseira sauvageauana Hamel
Cystoseira spinosa Sauvageau
Sargassum vulgare C. Agardh

* Nomenclature according to Rodríguez-Prieto *et al.* (2013)

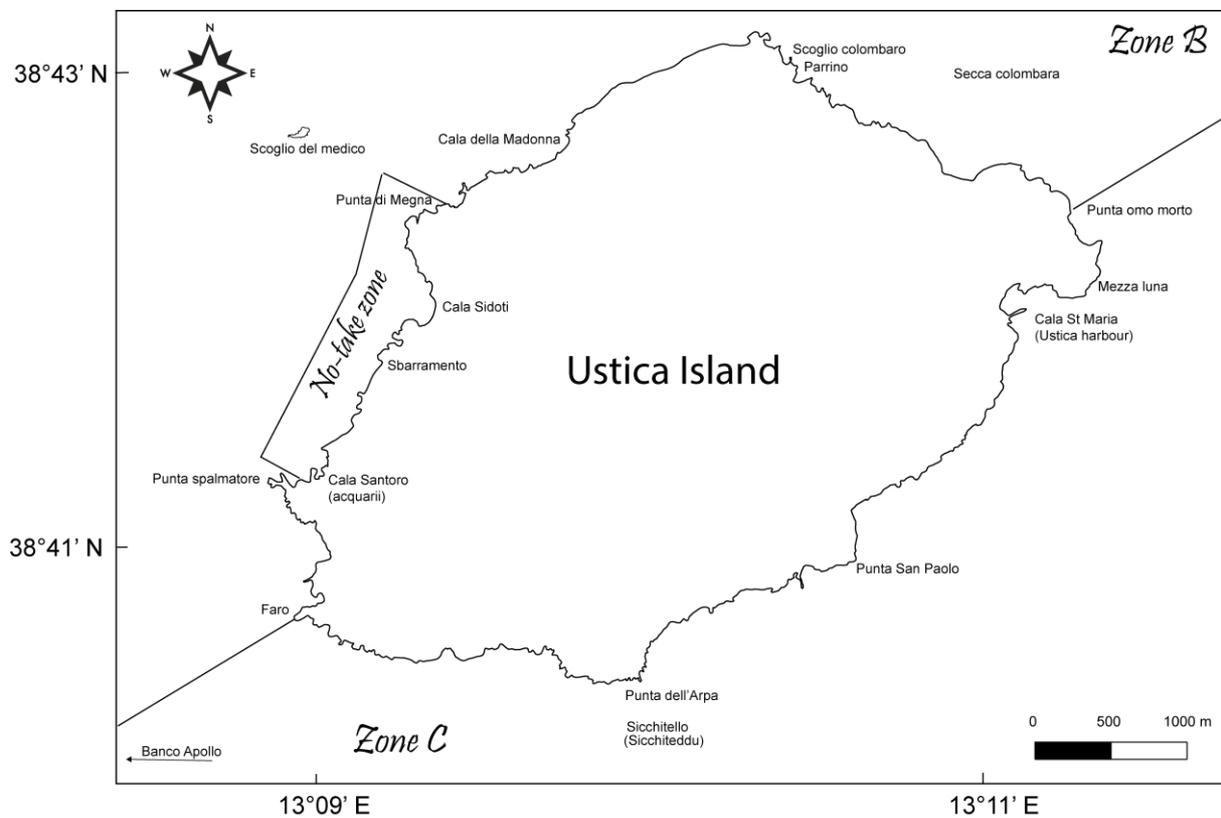


Figure 12. A map of the Ustica island with the name of the localities and the MPA zonation.

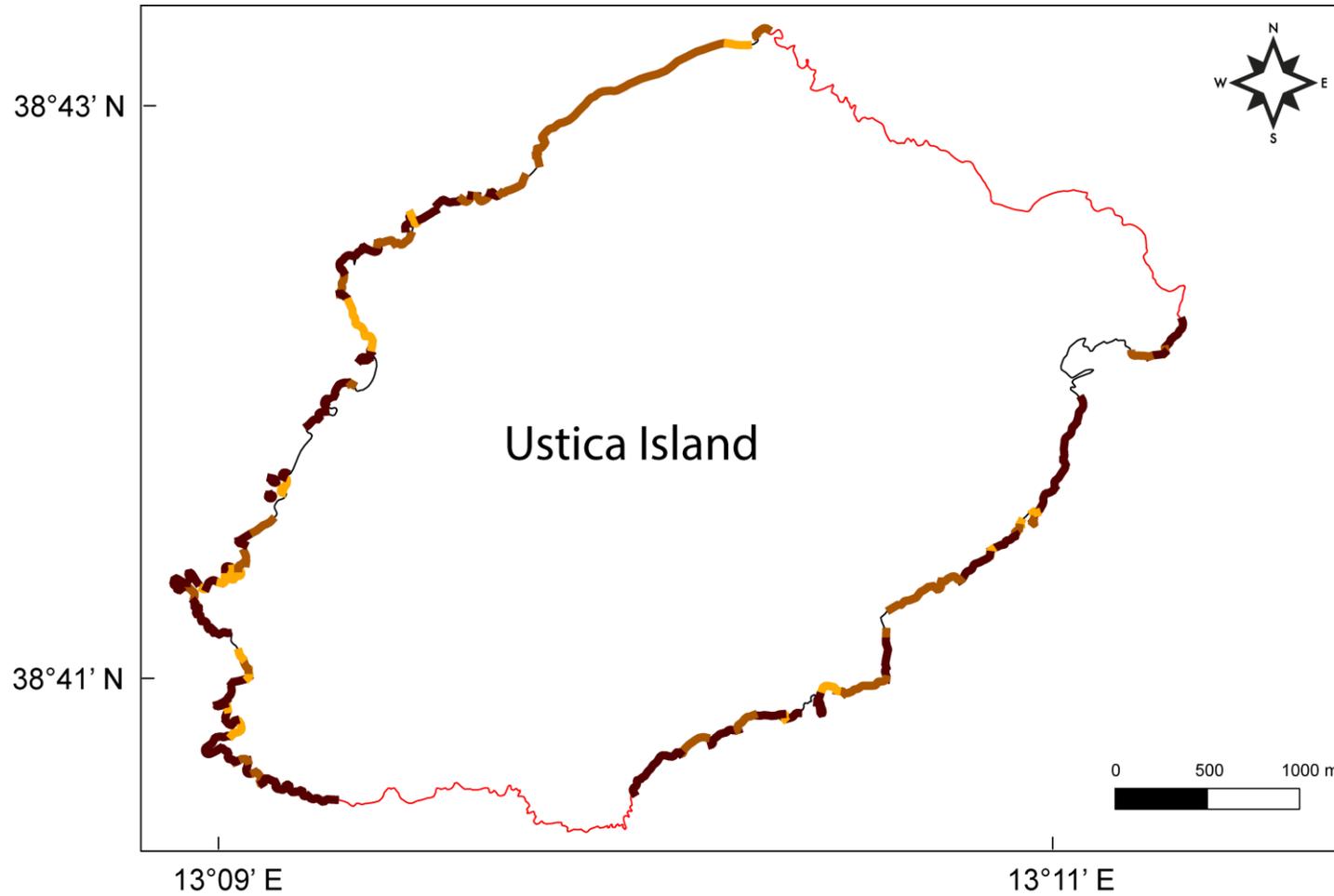


Figure 13. Distribution of *Cystoseira amentacea* var. *stricta* in the infralittoral fringe of Ustica island MPA. During the survey, the coastline was divided in sectors according to the geomorphological features. The abundance of *C. amentacea* was reported in three categories, here represented in different colours (orange lines: isolated individuals; light brown lines: dense and numerous groups; dark brown lines: algal belts almost continuous or continuous). The stretches of coast in red were not surveyed.

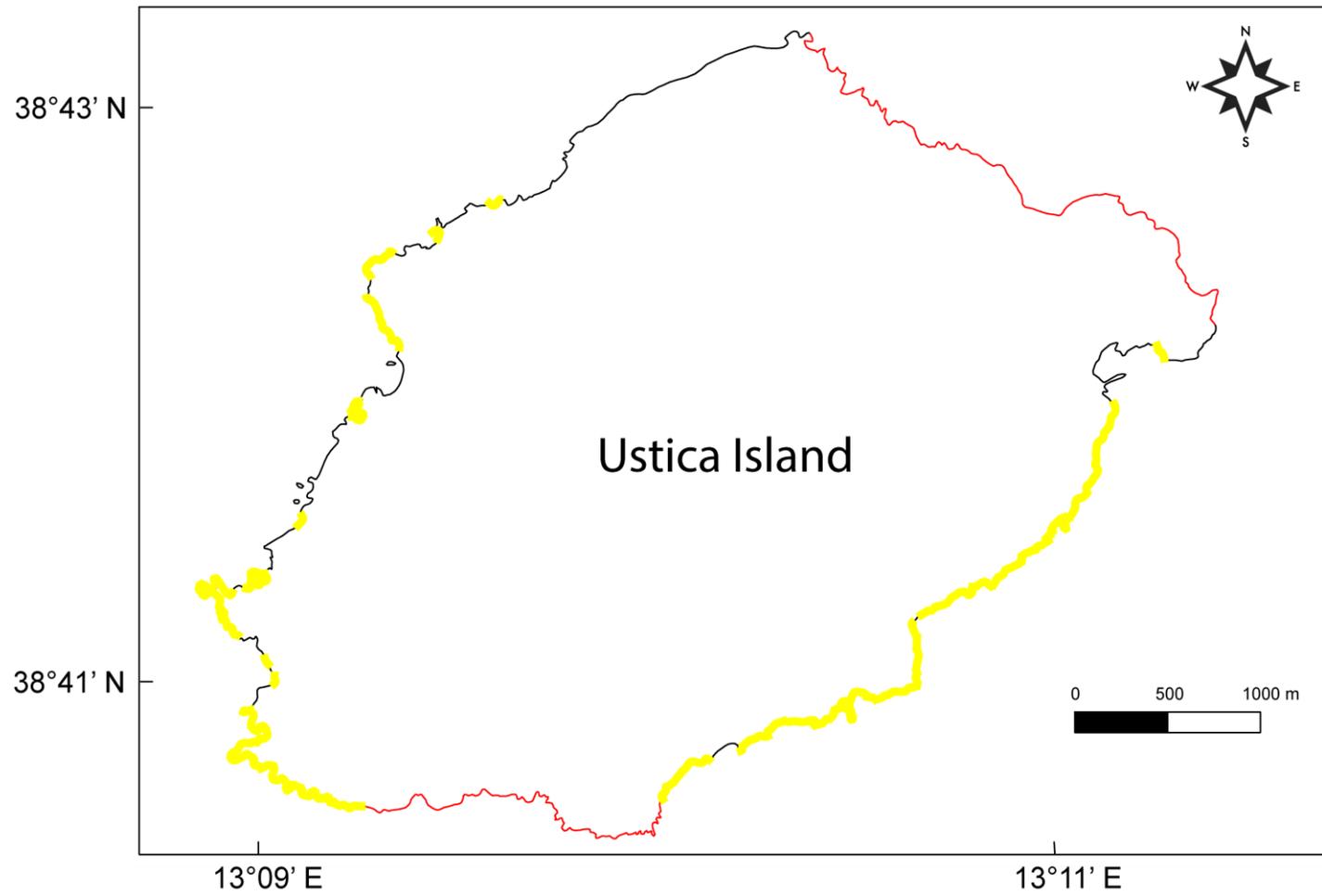


Figure 14. Distribution of *Cystoseira compressa* (in yellow) in the infralittoral fringe of Ustica island MPA. The stretches of coast in red were not surveyed.

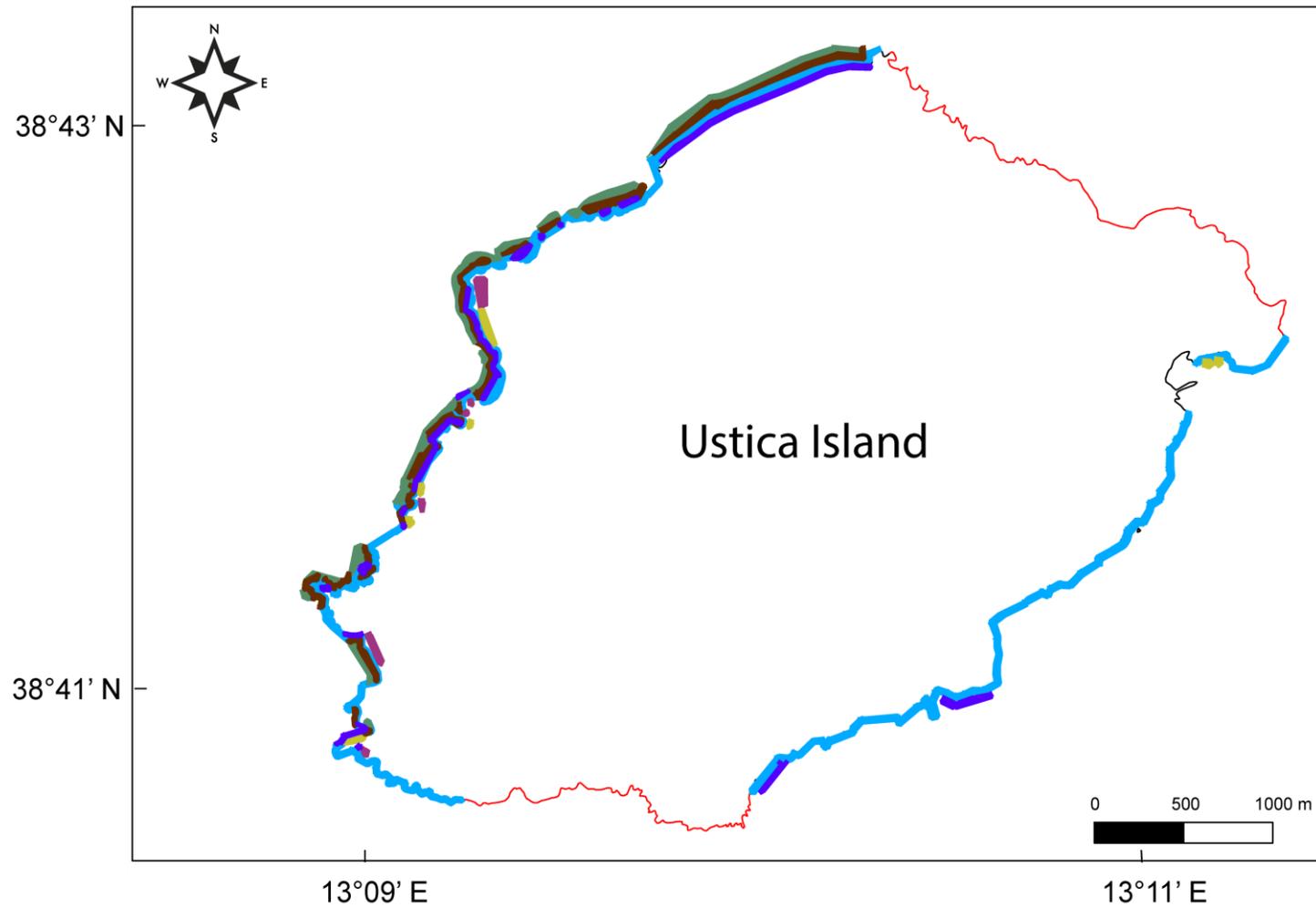


Figure 15. Distribution of *Cystoseira* species in the upper infralittoral zone and rockpools of Ustica island MPA. Green: *Cystoseira sauvageauana*; blue: *Cystoseira compressa* var. *pustulata*; light blue: *Cystoseira brachycarpa*; brown: *Cystoseira spinosa*; light violet: *Cystoseira foeniculacea* var. *foeniculacea*; beige: *Sargassum vulgare*. The stretches of coast in red were not surveyed.

4 - Conclusion

These surveys carried out in Portofino, Tavolara-Punta Coda Cavallo and Ustica island allowed to obtain detailed information on the current status and distribution of marine forests in these MPAs. Unluckily, this work highlighted a lack of historical data on marine forests that does not allow a reliable analysis of their evolution in recent decades. This is due to the fact that marine forests are poorly considered in the studies and therefore they have been less studied in MPAs than other species or habitats, like fish assemblages, coralligenous and seagrasses. This means that we do not know neither if the current regulations of MPAs are protecting healthy marine forests and/or eventually enhancing natural recovery of the degraded ones (Gianni and Mangialajo 2014). The evolution of marine forests in Ustica island MPA is an example of how protection enforcement (i.e. ban of sea urchins harvesting) can have deleterious effects on marine vegetation when the ecological dynamics are not considered as a whole. The long-term studies performed in Ustica Island MPA allowed to describe this phenomenon as much as, for the first time, a natural recovery of marine forests. In many other places, where regular monitoring of marine vegetation is not regularly carried out, we may have had similar phenomena, without even notice it.

Since almost all *Cystoseira* and *Sargassum* species are protected by European Conventions, conservation of marine forests should represent an important goal of protected areas. In order to achieve such goal, the implementation process of MPAs has to be improved and the management has to be planned on the basis of a complete and detailed habitat mapping of marine vegetation. Therefore, the surveys conducted in this study represent a starting point for future monitoring of Fucales in these three MPAs. In particular, it is recommended to check human impacts constantly, but also natural events, like exceptional storms or the increase of herbivores population, in order to detect first signs of regression and proceed with protection measures and/or restoration actions (Gianni *et al.* 2013).

5 - References

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7 - Appendices

7.1 - Appendix I

List of studies carried out in Portofino MPA and reporting Fucales distribution.

<i>Species</i>	<i>Year</i>	<i>Distribution/Status</i>	<i>Source</i>	<i>Comments</i>
<i>Cystoseira amentacea var. stricta</i>	before 1986	High density at Punta Chiappa; at Punta del Faro together with <i>Corallina elongata</i>	Morri <i>et al.</i> (1986)	
<i>Cystoseira amentacea var. stricta</i>	2000	Punta Chiappa and S Fruttuoso bay. It is present in the 44.6% of sectors (21.4% of the sampled coast); continuous belts for 36.3% of sectors (16.6% of the sampled coast); few individuals in 23.5% of sectors (4.8% of the sampled coast)	Mangialajo (2000)	
<i>Cystoseira amentacea var. stricta</i>	before 2004		Mangialajo <i>et al.</i> (2004)	
<i>Cystoseira amentacea var. stricta</i>	before 1962		Mangialajo <i>et al.</i> (2004)	Cited by Tortonese (1962)
<i>Cystoseira amentacea var. stricta</i>	before 1961		Mangialajo <i>et al.</i> (2004)	Cited by Tortonese (1961)
<i>Cystoseira amentacea var. stricta</i>	before 1958		Mangialajo <i>et al.</i> (2004)	Cited by Tortonese (1958)
<i>Cystoseira amentacea var. stricta</i>	2006	Rockpool 1, abundance: 5.1-25%	Mangialajo (2007)	General rockpools size: >1 m ² and > 50 cm deep)
<i>Cystoseira amentacea var. stricta</i>	May 2003	Punta Chiappa	Luisa Mangialajo's Herbarium	
<i>Cystoseira amentacea var. stricta</i>	February 2000	Punta Chiappa	Luisa Mangialajo's Herbarium	

<i>Cystoseira amentacea var. stricta</i>	August 2004	zone B	Luisa Mangialajo's Herbarium	
<i>Cystoseira amentacea var. stricta</i>	Before 2003	Abundant at Punta Chiappa (90%), west side of S Fruttuoso (60%), decreasing towards the east side of S Fruttuoso (40%). Present in 97.9% of the sectors. In general 72.8% covered coastline	Mangialajo <i>et al.</i> (2003)	CARLIT (20m long sectors)
<i>Cystoseira amentacea var. stricta</i>	2006-2007	More than 90% of the sectors is present (different cover classes)	Asnaghi <i>et al.</i> (2009)	CARLIT (20m long sectors)
<i>Cystoseira amentacea var. stricta</i>	2004-2005	Abundant (no-take zone)	Mangialajo <i>et al.</i> (2007)	CARLIT (20m long sectors).
<i>Cystoseira amentacea var. stricta</i>	May 2004	Cover 50%; reported also dry weight, holdfast cover, axis length, branches length (see the paper)	Mangialajo <i>et al.</i> (2008)	10 independent 20 m transects.
<i>Cystoseira compressa</i>	Before 2003		Mangialajo <i>et al.</i> (2004)	
<i>Cystoseira compressa</i>	May 2004	Cover less than 10%; reported also dry weight, holdfast cover, axis length, branches length (see the paper)	Mangialajo <i>et al.</i> (2008)	10 independent 20 m transects.
<i>Cystoseira compressa</i>	2000	Punta Chiappa and S Fruttuoso bay. In the 37.8% of sectors (10% of the sampled coast)	Mangialajo (2000)	
<i>Cystoseira compressa</i>	May 2000	S Fruttuoso bay	Luisa Mangialajo's Herbarium	
<i>Cystoseira compressa</i>	february 2000	Punta Chiappa	Luisa Mangialajo's Herbarium	
<i>Cystoseira compressa</i>	Before 2003	More abundant at the east side of St Fruttuoso (25%). At Punta Chiappa and west side of St Fruttuoso (15%). In general 35% covered coastline. Present in 35% of the sectors. No very abundant at low	Mangialajo <i>et al.</i> (2003)	CARLIT (20m long sectors).

hydrodynamic places				
<i>Cystoseira compressa</i>	2009-2010	Punta Chiappa; 0.4 % coverage	Asnaghi <i>et al.</i> (2010)	
<i>Cystoseira compressa</i>	2004-2005	Scattered and rare (no-take zone)	Mangialajo <i>et al.</i> (2007)	CARLIT (20m long sectors).
<i>Cystoseira compressa</i>	2006	Rockpool1, abundance: 25.1 - 50%. Rockpool2, abundance: <5%. Rockpool3, abundance: 25.1 - 50%. Rockpool4, abundance: 75.1-100%. Rockpool5, abundance: 25.1 - 50%. Rockpool6, abundance: 5.1 - 25%.	Mangialajo (2007)	General rockpools size: >1 m ² and > 50 cm deep)
<i>Cystoseira zosteroides</i>	before 1958		Mangialajo <i>et al.</i> (2004)	Cited by Tortonese (1958)
<i>Cystoseira zosteroides</i>	Before 2004		Mangialajo <i>et al.</i> (2004)	
<i>Cystoseira zosteroides</i>	June 2003	Cala Oro, at 12 m on horizontal rocky surface	Schiaparelli <i>et al.</i> (2007)	lat. 44 18.857 N, long. 9 9.787 E
<i>Cystoseira zosteroides</i>	November 1999	Punta della torretta (-20 m)	Luisa Mangialajo's Herbarium	
<i>Sargassum vulgare</i>	2006	Rockpool1, abundance: 5.1-25%. Rockpool6, abundance: 25.1-50%	Mangialajo (2007)	General rockpools size: >1 m ² and > 50 cm deep)
<i>Sargassum vulgare</i>	1993 and 2008	Rare in 1993; virtually absent in 2008 (5 m depth). The authors also reported that <i>Sargassum</i> was abundant from 1950's to 1980's (see references in the paper number 18 and 33-38) and showed photos from "Punta del Faro" of 1981 and 2009.	Parravicini <i>et al.</i> (2013).	

<i>Sargassum vulgare</i>	before 1958		Mangialajo <i>et al.</i> (2004)	Cited by Tortonese (1958)
<i>Sargassum vulgare</i>	2000	Punta Chiappa, San Fruttuoso; surface	Luisa Mangialajo's Herbarium	
<i>Sargassum vulgare</i>	before 2004		Mangialajo <i>et al.</i> (2004)	
<i>Sargassum vulgare</i>	before 1986	Up to 10-15 m. Also at Punta Carega 7 m	Morri <i>et al.</i> (1986)	

7.2 - Appendix II

A list of papers that reported past distribution and status of *Cystoseira* and *Sargassum* species in Tavolara-Punta Coda Cavallo MPA.

<i>Species</i>	<i>Year</i>	<i>Distribution/Status</i>	<i>Source</i>	<i>Comments</i>
<i>Cystoseira amentacea</i> v. <i>stricta</i>	2002	Abundant at Molarotto and Molarata (zone B). Not present in the zone B at Capo Ceraso and in the zone C at Punta La Greca and Molarata	Ceccherelli <i>et al.</i> (2005)	
<i>Cystoseira amentacea</i> v. <i>stricta</i>		Abundant at Molarotto and Molarata (zone B), but not continuous belts. Bigger holdfast for individuals present at Molarotto. Coverage 100%. Occasional at Tavolara (no-take zone), Punta di Monte Petrosu, Capo Ceraso and Molarata (zone C). Not present at Porto San Paolo, Capo Coda Cavallo, at Salina Bamba and Porto Istana.	Ceccherelli and Farris (2008)	
<i>Cystoseira amentacea</i> v. <i>stricta</i>		In the no-take zone and zone B	Navone <i>et al.</i> (2010)	
<i>Cystoseira amentacea</i>		Conservation status: excellent	UNEP(DEPI)/MED , Meeting of MAP Focal Point, Madrid, 2007.	
<i>Cystoseira brachycarpa</i>		Conservation status: excellent	UNEP(DEPI)/MED , Meeting of MAP Focal Point, Madrid, 2007.	
<i>Cystoseira brachycarpa</i>	2012	Capo Coda Cavallo (zone C) -0.5 m	Luisa Mangialajo's Herbarium	
<i>Cystoseira compressa</i>	1968		Cossu <i>et al.</i> (1992)	

<i>Cystoseira compressa</i>		Conservation status: excellent	UNEP(DEPI)/MED , Meeting of MAP Focal Point, Madrid, 2007.	
<i>Cystoseira crinita</i>	1884		Cossu <i>et al.</i> (1992)	
<i>Cystoseira crinita</i>	2012	Capo Coda Cavallo (zone C) -0.5 m	Luisa Mangialajo's Herbarium	
<i>Cystoseira crinita</i>	2007/2008		Sales <i>et al.</i> (2012)	
<i>Cystoseira crinita</i>		Conservation status: excellent	UNEP(DEPI)/MED , Meeting of MAP Focal Point, Madrid, 2007.	
<i>Cystoseira foeniculacea</i>	1968		Cossu <i>et al.</i> (1992)	
<i>Cystoseira foeniculacea</i> f. <i>latiramosa</i>	2012	Secca del Papa 1 diving spot	Mangialajo's Herbarium	
<i>Cystoseira zosteroides</i>		No-take zone	Navone <i>et al.</i> (2010)	
<i>Cystoseira zosteroides</i>		Conservation status: excellent	UNEP(DEPI)/MED , Meeting of MAP Focal Point, Madrid, 2007.	
<i>Cystoseira</i> sp.		Diving spots: Tedja Liscia, Occhio di Dio and Secca Arresto, well rapresented. Not present at Secca del Papa	Modugno <i>et al.</i> (2006)	
<i>Sargassum acinarium</i>	1968		Cossu <i>et al.</i> (1992)	Reported by Solazzi (1968)

<i>Sargassum vulgare</i>	Conservation status: excellent	UNEP(DEPI)/MED , Meeting of MAP Focal Point, Madrid, 2007.	
<i>Sargassum vulgare</i>	Secca del Papa 1 and 2 diving spots	Trainito and Navone (2011)	Information obtained from the document "Piano di Gestione Volume A Quadro di riferimento ambientale 2006, pag 180".
<i>Sargassum vulgare</i>	Secca del Papa diving spot	Navone <i>et al.</i> (1992)	
<i>Sargassum sp.</i>	Diving spots: Secca Arresto, rare; Secca del Papa, discreet presence. Not present at Occhio di Dio and Tedja Liscia.	Modugno <i>et al.</i> (2006)	
<i>Sargassum spp.</i>	In the sublittoral zone. Conservation status: excellent	UNEP(DEPI)/MED , Meeting of MAP Focal Point, Madrid, 2007.	

7.3 - Appendix III

All studies found in literature that described the presence of Fucales and Laminariales in the Ustica island MPA are listed below. It is also reported algal abundance and location, if described in the papers.

<i>Species</i>	<i>Year</i>	<i>Distribution/Status</i>	<i>Source</i>	<i>Comments</i>
<i>Cystoseira stricta</i>	1962-1969	Ustica island	Giaccone (1969a)	
<i>Cystoseira stricta</i>	Jun-69	Scoglio del Medico (coverage 60%); Mezza Luna (coverage 40%)	Giaccone (1971)	
<i>Cystoseira stricta</i>	1983	Infralittoral fringe (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira amentacea v. stricta</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira caespitosa (C. brachycarpa balearica)</i>	1962-1969	Ustica island	Giaccone (1969a)	
<i>Cystoseira balearica</i>	1983	Infralittoral zone (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira balearica v. claudiae</i>	1983	Infralittoral zone (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira brachycarpa</i>	1983	Infralittoral zone	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira brachycarpa</i>	1996	Punta di Megna (no-take zone): at -3, -5, -10 m with % of coverage: 26-50%. Parrino (zone B): at -5, -10 m with % of coverage: 26-50%. Punta dell'Arpa (zone C): at -1 m with % of coverage: <25%	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira brachycarpa v. balearica</i>	1996	Punta di Megna (no-take zone): at -1m with % of coverage: > 50%. Punta dell'Arpa (zone C): at -1 m with % of coverage: <25%, -3 and -15 m with % of coverage: 26-50%, -5 and -10 m with % of coverage: >50%	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira brachycarpa v. balearica</i>	2000	Sbarramento; reported dry weight, % of coverage before and after trampling experiments. Upper infralittoral zone	Milazzo <i>et al.</i> (2004)	see the report for the % of coverage

<i>Cystoseira brachycarpa v. balearica</i>	2000	Sbarramento; reported dry weight and % of coverage before and after trampling experiments. Upper infralittoral zone	Milazzo <i>et al.</i> (2002)	see the report for the % of coverage
<i>Cystoseira brachycarpa</i>	2001	Cala St Maria port, in front of the Banchina Barresi, some meters deep	Drago <i>et al.</i> (2004)	see the map in the paper for the sites
<i>Cystoseira brachycarpa v. brachycarpa</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira brachycarpa v. claudiae</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira brachycarpa v. balearica</i>	2004	Cala Sidoti, upper infralittoral zone	Gianguzza <i>et al.</i> (2005)	038'42.50N; 013'09.00E
<i>Cystoseira brachycarpa</i>	2004	-10 m, high percentage of coverage	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira brachycarpa v. balearica</i>	2004	-10 m, high percentage of coverage	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira brachycarpa v. brachycarpa</i>	2007	Site T1S1, south of Ustica, at -7 m deep, rare on few rocks. Site T2S1 few individuals at -8 m	Catra <i>et al.</i> (2009)	T1S1 38° 41' 30"N - 13° 10' 28"E; T2S1 38° 41' 37"N - 13° 10' 38"E
<i>Cystoseira brachycarpa v. balearica</i>	2007	Sbarramento and Acquario sites (no-take zone). Patches of 1.5 m in diameter. -1m deep	Gianguzza <i>et al.</i> (2010)	
<i>Cystoseira brachycarpa v. balearica</i>	2007-2008	Two sites in the no take area and two sites in the take area (zone C), Southern Ustica. Up to 70% of coverage. -4-6 m deep. Patch of 1.5 m	Gianguzza <i>et al.</i> (2013)	see the map in the paper for the sites
<i>Cystoseira compressa</i>	1962-1969	Ustica island	Giaccone (1969a)	
<i>Cystoseira compressa</i>	1983	Infralittoral zone (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira compressa</i>	1996	Punta di Megna (no-take zone): at -3 m with % of coverage: 26-50%. Parrino (zone B): at -1 m with % of coverage: >50%. Punta dell'Arpa (zone C): at -1, -5, -10 m with % of coverage: 26-50%, -3 m with % of coverage: >50%, -15 m with % of coverage: <25%	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira compressa</i>	2001	Cala St Maria port, infralittoral fringe, banchina barresi	Drago <i>et al.</i> (2004)	see the map in the paper for the sites
<i>Cystoseira compressa</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	

<i>Cystoseira compressa</i>	2004	-10m	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira compressa</i>	2007	Sbarramento and Acquario sites (no-take zone). Patches of 1.5 m in diameter. -1 m deep	Gianguzza <i>et al.</i> (2010)	
<i>Cystoseira compressa</i>	2007-2008	Two sites in the no take area and two sites in the take area (zone C), southern Ustica. Up to 70% of coverage. -4-6 m deep. Patch of 1.5m	Gianguzza <i>et al.</i> (2013)	see the map in the paper for the sites
<i>Cystoseira crinita</i>	1962-1969	Ustica island, infralittoral zone	Giaccone (1969a)	
<i>Cystoseira elegans</i>	1962-1969	Ustica island, infralittoral zone	Giaccone (1969a)	
<i>Cystoseira elegans</i>	1996	Punta di Megna (no-take zone): at -15 m with % of coverage: <25%.	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira elegans</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira foeniculacea f. tenuiramosa</i>	1962-1969	Ustica island, infralittoral zone	Giaccone (1969a)	
<i>Cystoseira foeniculacea f. tenuiramosa</i>	1996	Punta di Megna (no-take zone): at -15 m with % of coverage: <25%.	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira foeniculacea f. tenuiramosa</i>	2004	-20 m	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira foeniculacea f. latiramosa</i>	1964	On top of Banco Apollo shoal (-40-45 m), % of coverage in the paper	Giaccone (1967)	At the Banco Apollo shoal: temperature: 14° C all year, strong currents. Map of the Banco Apollo shoal with the distribution of the species in the paper
<i>Cystoseira foeniculacea f. latiramosa</i>	1969	Banco Apollo, Coverage 1% between -15-65 m.	Giaccone (1969b)	
<i>Cystoseira foeniculacea f. latiramosa</i>	1962-1969	Ustica island, infralittoral zone	Giaccone (1969a)	
<i>Cystoseira foeniculacea f. latiramosa</i>	1969	Secca Colombara (coverage 5%, -25-35 m); Sicchiteddu (coverage 5%, -20-30 m); Scoglio Colombaro (coverage 2%, -30-40 m); Punta Homo Morto (coverage 5%, -20-30 m)	Giaccone (1971)	
<i>Cystoseira foeniculacea f. latiramosa</i>	1983	Circolittoral zone (1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira foeniculacea f.</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	

latiramosa

<i>Cystoseira foeniculacea f. latiramosa</i>	2004	-10 m; -20 m	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira funkii</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira humilis</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira jabukae</i>	2004	rare: -20 m	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira montagnei (species inquirenda)</i>	1962-1969	Ustica island	Giaccone (1969a)	
<i>Cystoseira pelagosae</i>	1983	Infralittoral zone (1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira sauvageauana</i>	1983	10-20 m deep, at lower depths it is substituted by <i>C. brachycarpa</i> , particularly at Secca della Colombara	Giaccone <i>et al.</i> (1985)	
<i>C. sauvageauana v. polyoedematis (C. sauvageauana)</i>	1983	Infralittoral zone (1-20%)	Giaccone <i>et al.</i> (1985)	
<i>C. sauvageauana v. polyoedematis (now C. sauvageauana)</i>	1996	Punta di Megna (no-take zone): at -15 m with % of coverage: >50%. Parrino (zone B): at -3, -5, -10, -15 m with % of coverage: >50%.	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira sauvageauana</i>	1996	Punta di Megna (no-take zone): at -10 m with % of coverage: <26% and -15 m with % of coverage: 26-50%.	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira sauvageauana</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira sauvageauana</i>	2004	-10m; -20 m. High % of coverage	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira spinosa</i>	1964	On top of the Banco Apollo shoal (-40-45 m), together with <i>Laminaria rodriguezii</i> on light exposed rocks, % of coverage in the paper	Giaccone (1967)	At the Banco Apollo shoal: temperature: 14° C all year, strong currents. Map of the Banco Apollo shoal with the distribution of the species in the paper
<i>Cystoseira spinosa</i>	1968	Coverage 15%, circalittoral zone	Giaccone (1968)	
<i>Cystoseira spinosa</i>	1969	Banco Apollo shoal, coverage 1% between 15-65 m. Biomass at -62 m: 0.2 kg/1 m ²	Giaccone (1969b)	
<i>Cystoseira spinosa</i>	1962-	Infralittoral zone, Ustica	Giaccone (1969a)	

	1969	island		
<i>Cystoseira spinosa</i> (incl. <i>montagnei</i>)	1968-1969	Punta San Paolo (coverage 5%, -45-55 m); Scoglio della Chiesa (coverage 10%, -30-40 m); Secca Colombara (coverage 20%, -20-30 m); coverage 25%, -30-40 m); Scoglio Colombaro (coverage 10%, -30-40 m)	Giaccone (1971)	
<i>Cystoseira spinosa</i>	1983	Circalittoral zone (21-40%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira spinosa</i>	1996	Punta di Megna (no-take zone): at 10 m with % of coverage: 26-50%.	Milazzo <i>et al.</i> (2000)	
<i>Cystoseira spinosa v. spinosa</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira spinosa v. spinosa</i>	2004	-20m	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira spinosa v. squarrosa</i> (now <i>C. squarrosa</i>)	1983	Infralittoral zone (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Cystoseira spinosa v. squarrosa</i> (now <i>C. squarrosa</i>)	2004	-20 m	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Cystoseira spinosa v. tenuior</i>	2001	Cala St Maria port	Drago <i>et al.</i> (2004)	see the map in the paper for the sites
<i>Cystoseira spinosa v. compressa</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Cystoseira spinosa v. compressa</i>	before 2007	At Secchitello shoal, on coralligenous, high % of coverage, sublittoral zone	Catra <i>et al.</i> (2007)	
<i>Cystoseira zosteroides</i>	1964	On top of Banco Apollo shoal (40-45 m), % of coverage in the paper	Giaccone (1967)	At the Banco Apollo shoal: temperature: 14° C all year, strong currents. Map of the Banco Apollo shoal with the distribution of the species in the paper
<i>Cystoseira zosteroides</i>	1968	Coverage 10%, circalittoral zone	Giaccone (1968)	
<i>Cystoseira zosteroides</i>	1969	Banco Apollo shoal, coverage 1% between 15-65m	Giaccone (1969b)	
<i>Cystoseira zosteroides</i>	1962-1969	Ustica Island, Infralittoral zone	Giaccone (1969a)	
<i>Cystoseira zosteroides</i>	1968-1969	Punta San Paolo (coverage 20%, 45-55m); Scoglio del Medico (2%, 45-55m); Mezza Luna (3%, 45-55m)	Giaccone (1971)	
<i>Cystoseira zosteroides</i>	1983	Circalittoral zone (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	

<i>Cystoseira zosteroides</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Sargassum hornschurchii</i>	1964	On top of the Banco Apollo shoal (40-45 m), coverage % in the paper	Giaccone (1967)	At the Banco Apollo shoal: temperature: 14° C all year, strong currents. Map of the Banco Apollo shoal with the distribution of the species in the paper
<i>Sargassum hornschurchii</i>	1968	Circalittoral zone on coralligenous, few individuals	Giaccone (1968)	
<i>Sargassum hornschurchii</i>	1969	Banco Apollo shoal, coverage 1% between 15-65m	Giaccone (1969b)	
<i>Sargassum hornschurchii</i>	1962-1969	Ustica island	Giaccone (1969a)	
<i>Sargassum hornschurchii</i>	1969	Scoglio della Chiesa (coverage 10%, 30-40m); Sicchiteddu (coverage 10%, 20-30m)	Giaccone (1971)	
<i>Sargassum hornschurchii</i>	1983	Infralittoral and circalittoral zone (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Sargassum hornschurchii</i>	before 2007	Secchitello shoal, on coralligenous, high % of coverage	Catra <i>et al.</i> (2007)	
<i>Sargassum vulgare</i>	1962-1969	Ustica island	Giaccone (1969a)	
<i>Sargassum vulgare</i>	1983	Infralittoral (coverage 1-20%)	Giaccone <i>et al.</i> (1985)	
<i>Sargassum vulgare</i>	2001	Cala St Maria port, infralittoral fringe of banchina barresi	Drago <i>et al.</i> (2004)	see the map in the paper for the sites
<i>Sargassum sp.</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Sargassum sp.</i>	2004	(-10, 20 m)	Report of the Project GEBECSUD 2004	see the report for the % of coverage
<i>Sargassum acinarium</i>	2003	Ustica island	Catra <i>et al.</i> (2006)	
<i>Laminaria rodriguezii</i>	1964	Banco Apollo shoal. Between 45 and 85 m deep in the western slope and between 55 and 65 m deep in the eastern slope (150 m ²). A dense population between 55 and 75 m deep, it extends for 1 km ² of surface. Also few isolated individuals at Scoglio del Medico (30 m deep) and at Secca della Colombara (45 m deep). Detailed description in the paper	Giaccone (1967)	At the Banco Apollo shoal: temperature: 14° C all year, strong currents. Map of the Banco Apollo shoal with the distribution of the species in the paper

<i>Laminaria rodriguezii</i>	1968	Coverage 90%, circalittoral zone	Giaccone (1968)
<i>Laminaria rodriguezii</i>	1969	Banco Apollo shoal and north-west coast of Ustica island, discontinuous belts between 50-90 m deep. At the Banco Apollo shoal: biomass at -62 m: 2.94 kg/1 m ² . Coverage 80% between 15-65m	Giaccone (1969b)
<i>Laminaria rodriguezii</i>	1962-1969	Ustica island	Giaccone (1969a)
<i>Laminaria rodriguezii</i>	1983	Circalittoral zone (coverage 21-40%)	Giaccone <i>et al.</i> (1985)
<i>Laminaria rodriguezii</i>	2003	Banco Apollo shoal	Catra <i>et al.</i> (2006)
<i>Phyllariopsis brevipes</i>	1969	Banco Apollo shoal. Coverage 1% between 15-65 m	Giaccone (1969b)
<i>Phyllariopsis brevipes</i>	1962-1969	Ustica island	Giaccone (1969a)
<i>Phyllariopsis brevipes</i>	2003	Ustica island	Catra <i>et al.</i> (2006)

7.4 - Appendix IV

Species of Fucales observed in the different periods of sampling at Ustica island MPA.

	1960-1970 (Giaccone)	1983 (Giaccone <i>et al.</i> 1985)	1996-2000 (Milazzo <i>et al.</i> 2000, 2002, 2004)	2000-2010	2014 (Gianni)
<i>Cystoseira amentacea</i> <i>v. stricta</i>	+	+		+ (Catra <i>et al.</i> 2006)	+
<i>Cystoseira balearica</i>		+			
<i>Cystoseira balearica v.</i> <i>claudiae</i>		+			
<i>Cystoseira</i> <i>brachycarpa</i>		+	+	+ (Drago <i>et al.</i> 2004; Report of the Project GEBECSUD 2004)	+
<i>Cystoseira</i> <i>brachycarpa v.</i> <i>balearica</i>	+		+	+ (Report of the Project GEBECSUD 2004; Gianguzza <i>et al.</i> 2005, 2010, 2013)	
<i>Cystoseira</i> <i>brachycarpa v.</i> <i>brachycarpa</i>				+ (Catra <i>et al.</i> 2006, 2009)	
<i>Cystoseira</i> <i>brachycarpa v claudiae</i>				+ (Catra <i>et al.</i> 2006)	
<i>Cystoseira compressa</i>	+	+	+	+ (Drago <i>et al.</i> 2004; Report of the Project GEBECSUD 2004; Catra <i>et al.</i> 2006; Gianguzza <i>et al.</i> 2010, 2013)	+
<i>Cystoseira crinita</i>	+				
<i>Cystoseira elegans</i>	+		+	+ (Catra <i>et al.</i> 2006)	
<i>Cystoseira</i> <i>foeniculacea f.</i> <i>tenuiramosa</i>	+		+	+ (Report of the Project GEBECSUD 2004)	+
<i>Cystoseira</i> <i>foeniculacea f.</i> <i>latiramosa</i>	+	+		+ (Report of the Project GEBECSUD 2004; Catra <i>et al.</i> 2006)	
<i>Cystoseira funkii</i>				+ (Catra <i>et al.</i> 2006)	

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<i>Cystoseira humilis</i>				+ (Catra <i>et al.</i> 2006)	
<i>Cystoseira jabukae</i>				+ (Report of the Project GEBECSUD 2004)	
<i>Cystoseira montagnei</i> (species inquirenda)	+				
<i>Cystoseira pelagosae</i>		+			
<i>Cystoseira sauvageauana</i>		+	+	+ (Report of the Project GEBECSUD 2004; Catra <i>et al.</i> 2006)	+
<i>Cystoseira spinosa</i>	+	+	+		+
<i>Cystoseira spinosa</i> v. <i>compressa</i>				+ (Catra <i>et al.</i> 2006, 2007)	
<i>Cystoseira spinosa</i> v. <i>spinosa</i>				+ (Report of the Project GEBECSUD 2004; Catra <i>et al.</i> 2006)	
<i>Cystoseira spinosa</i> v. <i>tenuior</i>				+ (Drago <i>et al.</i> 2004)	
<i>Cystoseira squarrosa</i>		+		+ (Report of the Project GEBECSUD 2004)	
<i>Cystoseira zosteroides</i>	+	+		+ (Catra <i>et al.</i> 2006)	
<i>Sargassum hornschuchii</i>	+	+		+ (Catra <i>et al.</i> 2007)	
<i>Sargassum acinarium</i>				+ (Catra <i>et al.</i> 2006)	
<i>Sargassum vulgare</i>	+	+		+ (Drago <i>et al.</i> 2004)	+
<i>Sargassum</i> sp.				+ (Report of the Project GEBECSUD 2004; Catra <i>et al.</i> 2006)	
<i>Laminaria rodriguezii</i>	+	+		+ (Catra <i>et al.</i> 2006)	
<i>Phyllariopsis brevipes</i>	+			+ (Catra <i>et al.</i> 2006)	



Annex IV – Conservation of macroalgal specimens

All *Cystoseira* and *Sargassum* specimens collected during the surveys were fixed with a method reported by Cormaci et al (2003)*. This method is advisable for macroalgae that have tough axes, therefore it is particularly suitable for Fucales and Laminariales, even if they were previously fixed in formalin. Algae treated with this procedure maintain the same consistency and flexibility that they had when alive, as well as all the internal structures are not altered.

The first step consists in plunging algae in a solution made of 6 parts of seawater, 4 parts of glycerine and 1 ml of phenol for every litre of solution.

After at least 10 days, specimens have to be moved in a solution made of 3 parts of seawater, 7 parts of glycerine and 1 ml of phenol for every litre of solution. Algae should remain in this solution for 10-15 days.

Successively, algae should be dripped, rubbed delicately with a cloth to remove any excess of the solution and left for one week under a fume hood. They can be preserved in plastic bags with labels reporting species, site, depth and date of collection and the name of the collector. It is advisable to prepare a big amount of the solutions, that can be stored and reused several times.



Figure A: one step of the method. **Figure B:** two *Cystoseira* samples after the treatment.

*Cormaci M, Furnari G, Giaccone G 2004. "Macrofitobenthos". In: Gambi MC, Dappiano M (Eds.), "Mediterranean marine benthos: a manual of methods for its sampling and study", *Biologia Marina Mediterranea*, n. 10, Vol. 11 (Suppl. 1), pp. 217-266.

List of species of Fucales collected during the surveys in different Mediterranean MPAs.

Species	Locality	Date
<i>Cystoseira compressa</i>	Secca del Papa1, -15m, Tavolara island, Italy	August 2013
<i>Cystoseira foeniculacea</i> var. <i>latiramosa</i>	Secca del Papa1, -20m, Tavolara island, Italy	August 2013
<i>Cystoseira barbata</i>	Cala Tramontana, -1m, Tavolara island, Italy	August 2013
<i>Cystoseira spinosa/elegans</i>	Punta Don Diego, -1m, Sardinia, Italy	August 2013
<i>Cystoseira brachycarpa</i>	Cala Tramontana, -1m, Tavolara island, Italy	August 2013
<i>Cystoseira crinita</i>	Cala Tramontana, -1m, Tavolara island, Italy	August 2013
<i>Cystoseira foeniculacea</i> var. <i>foeniculacea</i>	rockpool, north coast of Tavolara island, Italy	August 2013
<i>Cystoseira compressa</i> var. <i>pustulata</i>	Punta la mandria, -1m, Tavolara island, Italy	August 2013
<i>Cystoseira spinosa/elegans</i>	Tavolara island harbour, -1m, Italy	August 2013
<i>Cystoseira foeniculacea</i> var. <i>foeniculacea</i>	Ustica island, zone B, -1m, close to the lighthouse, Italy	August 2014
<i>Cystoseira compressa</i>	Ustica island, zone B, -1m, close to the lighthouse, Italy	August 2014
<i>Cystoseira spinosa</i>	Ustica island, zone B, -1m, close to the lighthouse, Italy	August 2014
<i>Cystoseira compressa</i> var. <i>pustulata</i>	Ustica island, zone B, -1m, close to the lighthouse, Italy	August 2014
<i>Cystoseira funkii</i>	Secca della colombara, Ustica island, Italy	August 2014
<i>Cystoseira crinita</i>	Secca della colombara, Ustica island, Italy	August 2014
<i>Sargassum vulgare</i>	Punta la mandria, -1m, Tavolara island, Italy	August 2013
<i>Sargassum vulgare</i>	Porto Istana, -1m, Sardinia, Italy	August 2013
<i>Sargassum</i> spp.	Secca del Papa1, -15m, Tavolara island, Italy	August 2013



Annex V – A new conceived herbivores-exclusion system

To test the hypothesis of fish herbivory pressure on intertidal *Cystoseira* belts (chapter 5), an exclusion system had to be conceived (Fig. 1). The most used method to exclude fishes in ecological experiments are cages. However, cages require a periodical cleaning to avoid that fouling reduces light intensity under the nets. We designed a new anti-herbivore device that act as deterrent for *Sarpa salpa* and, at the same time, does not affect *Cystoseira* growth (Fig. 2-3).



Figure 1. A picture showing *Cystoseira* belts protected by the devices. Photo Bartolini F.

Several prototypes were developed with different materials, in order to find a good compromise among effective salemas exclusion, resistance to waves and sunlight penetration. The construction of these devices was time-consuming and not particularly low-cost (see Chapter 5 for details), but they were conceived in order to be easily manipulated, fixed and removed in the field.

Since restoration actions in the future should consider herbivorous fish exclusion, we suggest that devices like the one designed for this experiment may be developed. They should be improved, especially by using low-cost and possibly biodegradable materials. In case new man-made structures (breakwaters, piers, etc.) have to be gardened, it is suggested to build artificial structures provided with deterrent devices since the beginning. Scientists and engineers should share their knowledge in order to develop effective deterrent devices and limit fish herbivorous pressure in both natural and artificial substrates. This would guarantee a better conservation of existing forests, permit the restoration of the lost ones and the forestation of man-made structure.



Figure 2. *Cystoseira stricta* individuals completely developed in the protected plots after few months. Outside protected plots, *Cystoseira* individuals were highly grazed, as showed in the picture on the right. Photo Bartolini F and Mangialajo L.

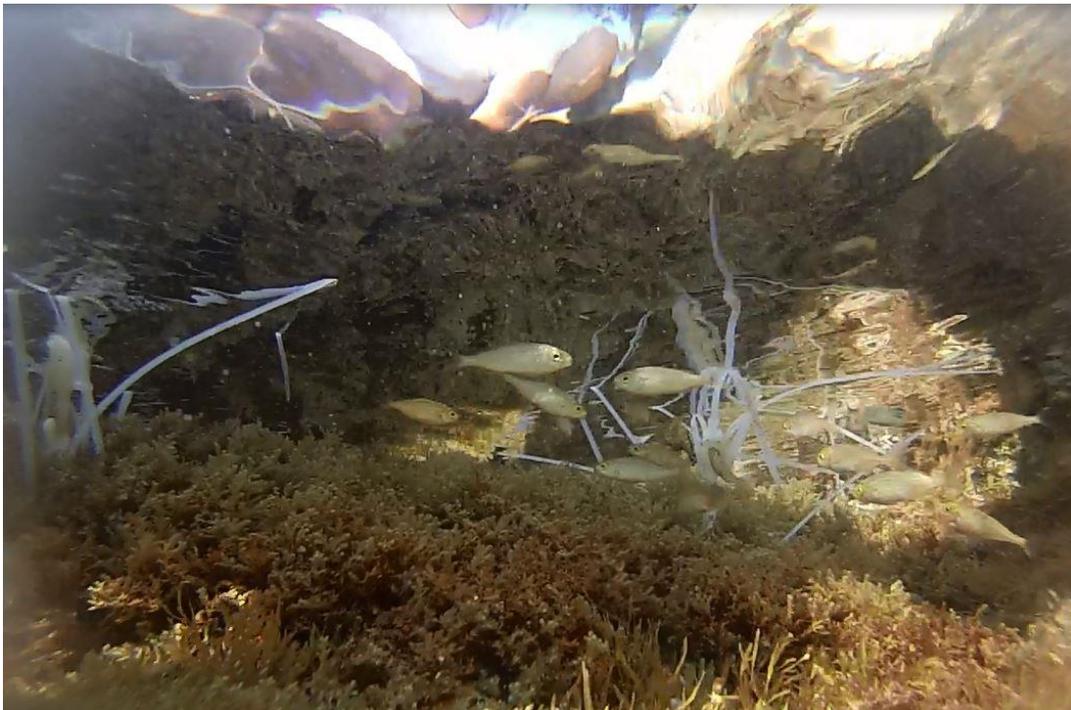


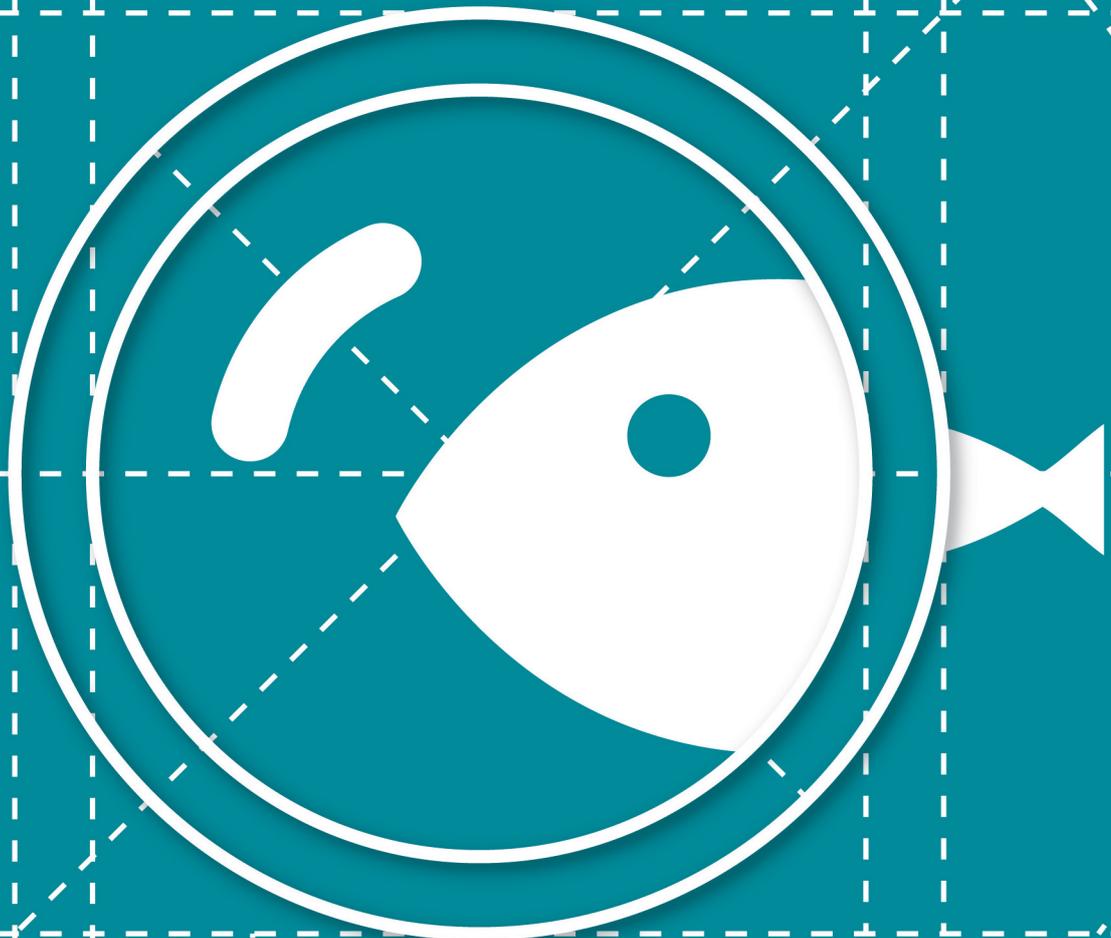
Figure 3. Salemas were observed feeding only outside the experimental plots, but never inside them: the deterrent devices effectively reduced fish access and their grazing. Screenshot from a short video by Laurent M.



Annex VI – Communication and outreach

In the framework of the MMMPA project, I realized some communication and outreach materials; the most important are reported in this annex:

- 1) Practical guidelines for monitoring Mediterranean marine forests, addressed to the MPA managers and researchers. It is the first chapter of a booklet with guidelines on the different topics faced by MMMPA fellows. The full booklet is available here: <http://www.mmmpa.eu/publications.asp>
- 2) A short movie on the importance of MPAs. This movie was shared with several researchers, on the main social networks, and it will be presented to different film festivals.
- 3) A vulgarization poster made for the Science Festival at the University of Nice (October 2014), explaining the importance of *Cystoseira* forests to the general public.



MONITORING MEDITERRANEAN MARINE PROTECTED AREAS

*A set of guidelines to support
the development of management plans*



MONITORING MEDITERRANEAN MARINE PROTECTED AREAS

*A set of guidelines to support the development
of management plans*

With the participation of:



Edited by MMMPA Supervisory Board

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LARGE BROWN ALGÆ FORESTS

MONITORING MEDITERRANEAN
MARINE PROTECTED AREAS

GUIDELINES FOR THE CONSERVATION, MONITORING AND RESTORATION OF CYSTOSEIRA FORESTS IN THE MEDITERRANEAN SEA

Fabrizio Gianni^{1,2} and Luisa Mangialajo^{1,2}

In this document we propose guidelines with simple and replicable methods that may be applied by MPA managers in order to evaluate the distribution and status of marine forests, and, eventually, restore them.

Why focus on marine forests?

In the Mediterranean Sea, marine forests of large-brown seaweeds are mostly formed by the genus *Cystoseira* and *Sargassum* (order Fucales), distributed from the surface up to several meters depth (Ballesteros, 1992). Most of the species belonging to these genus are ecosystem engineers, because they create unique habitats offering substrate, food and shelter to other algae and a large amount of invertebrates and fish (Ballesteros et al., 1998). Based on their ecology and zonation, we can differentiate species forming belts in the very shallow infralittoral fringe, forests in rock-pools (photophilous species thriving in rock-pools), shallow subtidal forests (photophilous species thriving in the upper infralittoral zone) and deep forests (sciaphilous forests thriving in the circalittoral zone) (Fig. 1).

However, loss of Mediterranean forests has been observed in many coastal areas. Coastal urbanization, marine pollution and outbreak of herbivores (i.e. sea urchins and herbivorous fish) are some of the most important factors affecting marine forests (for a review see Mineur et al., 2015). For this reason, almost all Mediterranean *Cystoseira* and *Sargassum*

species are listed in two European Conventions (Barcelona Convention, 1976 and Bern Convention, 1979), but very few tangible focused actions have been carried out so far for their conservation, monitoring and management, especially as concern the assessment of marine forests distribution or the establishment of marine protected areas (MPAs). An exception is the cartography of *Cystoseira* belts in the infralittoral fringe performed to assess the ecological status of coastal waters using the CARLIT index, under the Water Framework Directive (WFD) 2000/60/EU (Ballesteros et al., 2007, Mangialajo et al., 2007). This index is applied in the North-Western Mediterranean and in the Adriatic Sea, but it is often performed only on limited stretches of the rocky coastlines. In addition, most of the marine forests distribution in the subtidal zone is still largely unknown, also because the cartography needed for the institution of the Natura 2000 sites only reports ‘photophilous algae on rocky bottom’, without any distinction among deserts of encrusting corallinales, turf-forming algae, shrubs of erect algae or forests of large-brown seaweeds.

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Therefore, it is extremely important to increase our knowledge on marine forests, updating maps on their distribution, following their evolution over time and, if necessary, considering restoration (Gianni *et al.*, 2013). These actions are particularly important in MPAs, in order to give the managers the tools necessary to conserve existing marine forests or the restoration of damaged ones, allow the survival of many other associated organisms, including some species of fish, and detect impacts that may affect rocky-bottom communities.

Here we propose some guidelines with simple and replicable methods that may be applied by scientists/MPA managers in order to evaluate the distribution and status of marine forests.



Monitor *Cystoseira* forests is important because:

- They produce oxygen.
- They are reproductive and nursery habitats.
- They export organic matter to other systems.
- You can early detect impacts affecting rocky bottoms communities.

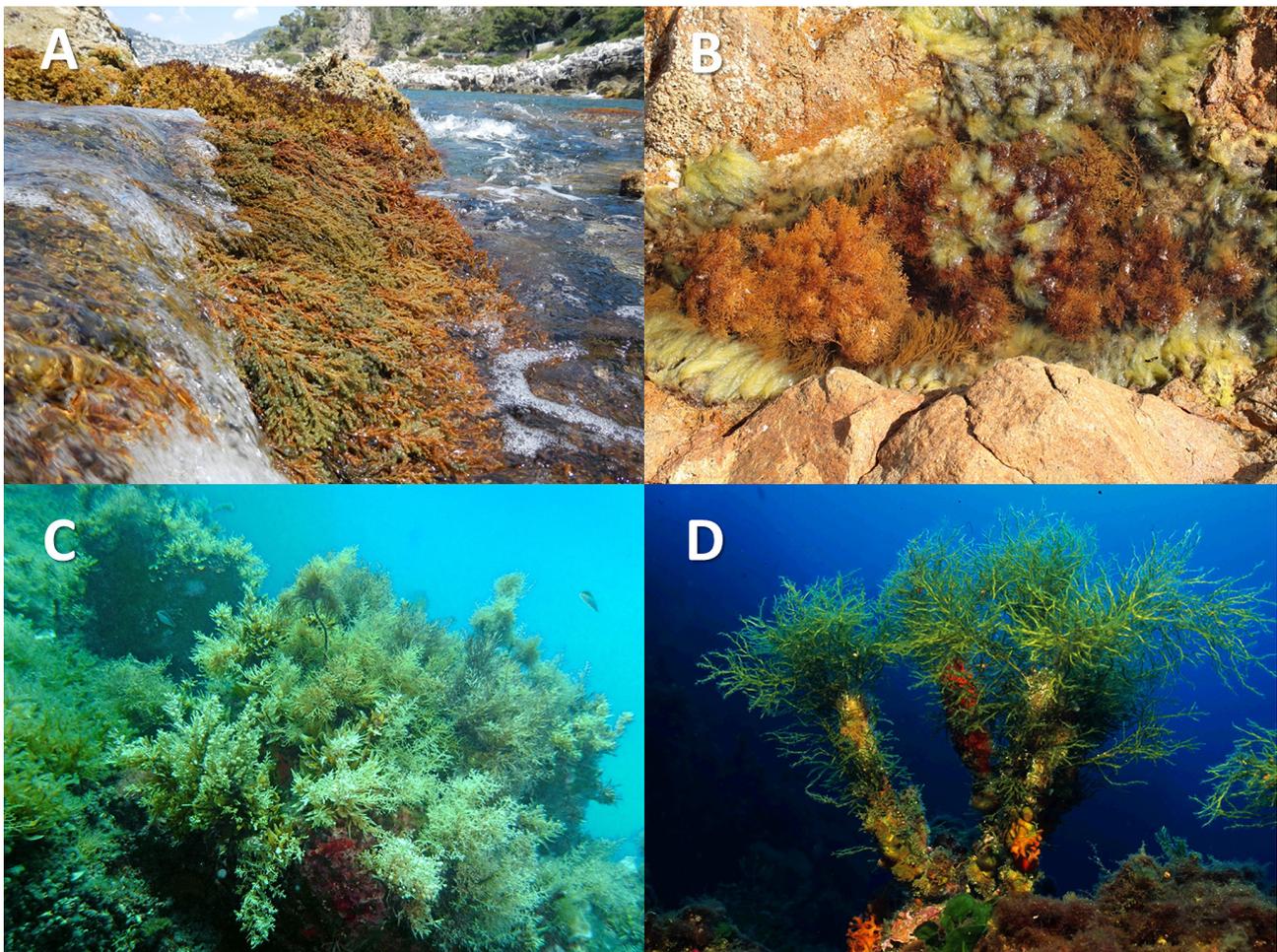


Figure 1. *Cystoseira* forests in the infralittoral fringe (A), in rock-pools (B), in the infralittoral zone (C) and in the circalittoral zone (D). (Photos A: Gianni, F.; B: Parisi, L.; C: Mangialajo, L.; D: Ballesteros, E.).



*Cystoseira species should become
a conservation priority in the
future context of Mediterranean
Sea management*

How to conserve, monitor and, eventually, restore marine forests

In order to conserve, monitor and, if required, restore marine forests, we suggest following a few steps allowing to plan the adequate actions. These steps can be performed by trained MPA staff, because they are easy to apply and involve low-cost techniques. At the beginning of the monitoring/restoration process, experts can help to train MPA staff and with the identification of some species.

Are marine forests present in the area?

In order to enhance conservation of marine forests the first gap to fill is the lack of knowledge on their distribution in MPAs. In some cases, information on Fucales distribution in the target areas is already available from past surveys, so that a literature search should be performed first. Expert judgment can be useful in this phase. Then, a detailed cartography has to be carried out in order to have information on *Cystoseira* presence and distribution in MPAs. The presence and distribution of very shallow species (species forming belts and the forests in rock-pools) can be evaluated by a small pneumatic boat/kayak and/or snorkelling. Scuba diving is generally used for both shallow and deep subtidal species, but progress has been made with the use of remote control engines (cameras, ROVs).

It is recommended to map the entire surface of the protected area, in order to have complete information on the rocky-bottom communities present in the MPA and address future management actions in the best way. During surveys, it is essential to georeference data on species distribution, using a GPS tracker or detailed orthophoto maps for very shallow species. This procedure will permit to create georeferenced and detailed maps in GIS environment that could be used by MPA managers as baseline maps in order to evaluate marine forests evolution over time and manage potentially impacts affecting these important habitats.

What is the conservation status of marine forests?

Once marine forests distribution is available, their conservation status should be assessed with non-destructive techniques.

The status of *Cystoseira* belts in the infralittoral fringe can be evaluated with linear transects as for the CARLIT index calculation (Ballesteros et al., 2007, Nickolić et al., 2013). The coastline is mapped and *Cystoseira* abundance is visually estimated and associated to a value corresponding to three categories (1: isolated individuals; 2: dense and numerous populations; 3: algal forests forming almost continuous or continuous belts). The survey is carried out by two operators, proceeding very close to the coast in kayak or by a small pneumatic boat. Transects can be coupled to replicated quadrats (20 × 20 cm) randomly placed into *Cystoseira* belts in order to estimate the percentage of coverage. This last method can be also applied to monitor the status of Fucales in rock-pools and potentially may be coupled to an assessment based on Braun-Blanquet abundance classes.

Cystoseira populations of the infralittoral and circalittoral zones are assessed with transects performed by a team of scuba divers (Perkol-Finkel and Airolidi, 2010). We suggest to do a rough estimation of the forests covering the rocky bottom by using 25 m transects. Along each transect, changes in rocky bottom communities/habitats (e.g. *Cystoseira* canopies, mosaic of different species, seagrass meadows, turfs, barren grounds, etc.) are recorded at a small scale of variability (20–50 cm). If *Cystoseira* canopies are present, randomly quadrats (50 × 50 cm side) can be performed inside the forests to assess the status: the density of individuals and the height of the axes is estimated for species with a single axis (monopodial species), while the percentage of coverage and/or the number of axes and/or the maximal height is estimated for species with multiple axes at the base (sympodial species). Estimation of biomass can be obtained by applying conversion factors to some features of the individuals (e.g. axis length for the monopodial species, coverage, etc.). If sea-urchins barren grounds are present, it is important to note them and eventually assess the density of individuals based on size classes.

Is an action necessary to protect marine forests?

In case *Cystoseira* stands are in regression or are lost, the first step is to investigate the causes of such decline and, whenever possible, manage the sources of the impact that threatens or generated the loss of the forests (e.g. water discharge, herbivores overgrazing).

If a restoration action is deemed necessary to enhance *Cystoseira* recovery, we suggest to choose a non-destructive forestation method. However, being the restoration of marine forests still at an experimental stage, it may be necessary at the beginning of these actions a collaboration with experts. Several approaches are available, depending on the species and the environmental conditions (see Gianni *et al.*, 2013 for details). The most easy-to apply methods are the installation of fertile receptacles in the target areas or the interception of embryos, but more sophisticated methods, such as the culture of embryos/juveniles in laboratory can be planned.

Based on the scientific literature (see Gianni *et al.*, 2013 for a review) and following the results of the studies we performed (see below), it appears important to set up herbivores exclusions to avoid high grazing rates at least in the first phases of the restoration.

Regular monitoring of marine forest

All forests thriving in the MPA (healthy, suffering or recently restored) should be regularly monitored, in order to detect any human impact at the first stages of development (e.g. proliferations of herbivores) or assess the success of the restoration action.

In the case of healthy forests, the same techniques proposed for the assessment of their conservation status should be applied. In the case of monitoring following a restoration action, different variables can be measured, like density and mortality of recruits or adults and/or fertility of the individuals. If possible such variables should be compared to healthy forests in order to understand when the restored forest matches the features of the natural ones and can be considered self-sustaining.

We suggest to monitor *Cystoseira* forests once a year during spring (the season of maximal growth of the primary branches). All these methods are cheap and can be coupled to other monitoring activities performed by the MPA staff in order to reduce the costs.

A flow-chart, proposed in a recent review on marine forests (Gianni *et al.*, 2013), resumes hypothetical conservation, monitoring and non-destructive restoration actions to undertake, and highlighting the paramount role that MPAs should play for the protection of marine forests (Fig. 2).

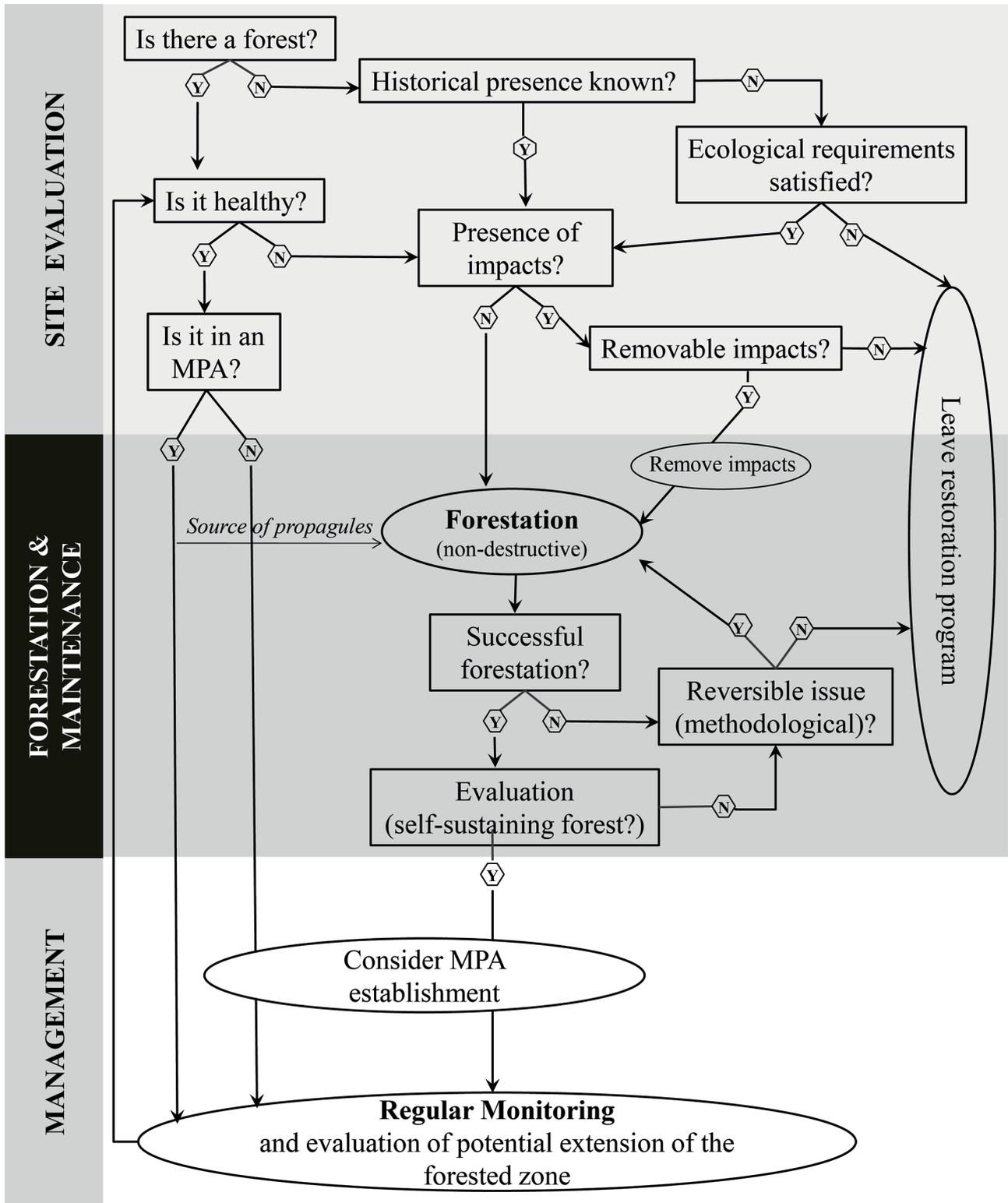


Figure 2. Conservation, monitoring and forestation of *Cystoseira* species in the Mediterranean Sea should follow some practical steps to be successful (modified by Gianni et al., (2013)).

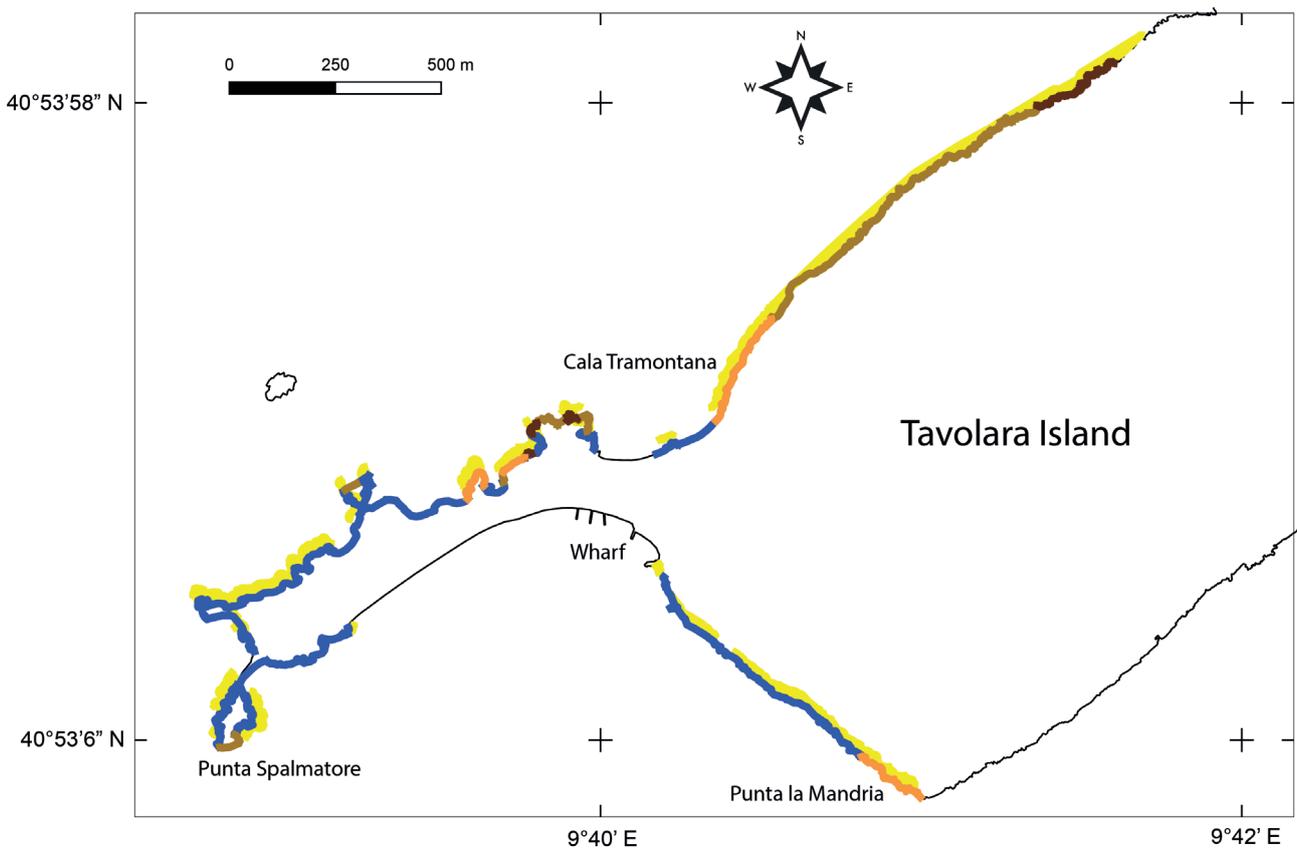


Figure 3. A stretch of coast of Tavolara Punta Coda Cavallo MPA in Italy, showing the distribution of *Cystoseira compressa* (in yellow) and *Cystoseira amentacea* (orange lines: isolated individuals; light brown lines: dense and numerous groups; dark brown lines: algal belts almost continuous or continuous; blue lines: absence).

Practical applications and examples

In the framework of the Programme ITN-MMMPA (International Training Network on Monitoring Mediterranean Marine Protected Areas), we assessed shallow marine forests distribution in three Mediterranean MPAs: Portofino, Tavolara–Punta Coda Cavallo and Ustica island (Italy) in spring/summer 2013 – 2014. Information on historical distribution of Fucles in these MPAs was, firstly, collected by searching in the scientific literature and asking the MPA managers. The research revealed a general lack of knowledge on marine forests distribution and highlighted the necessity to do a cartography in such MPAs. Intertidal macroalgal communities were surveyed applying a simplified CARLIT method (as described above), while Fucles in rock-pools and in the upper-infralittoral zone were assessed by snorkelling.

For instance, in Tavolara–Punta Coda Cavallo MPA, *Cystoseira* and *Sargassum* are well represented: we observed up to eleven taxa including new species,

never described in the MPA. Overall, up to 90% of the surveyed coastline is covered by Fucles. *Cystoseira* forests of the infralittoral fringe are characterized by almost continuous belts and density of the canopies is mostly linked to the physical features of the coast (e.g. wave exposure, morphology). Upper-infralittoral forests are also abundant and continuous along the coasts of the MPA, formed by a mosaic of different species.

Finally, data were georeferenced in GIS maps (Fig. 3) that will be provided to the managers in order to inform them on the presence of Fucles in their MPAs and support decisions. The surveys conducted in this study represent a starting point for future monitoring of Fucles and for checking their evolution in these three MPAs.

Concerning ecological restoration research, several experiments were carried out in the French Riviera with the aim to improve *Cystoseira* restoration

techniques. Our studies showed that herbivorous fish, very likely *Sarpa salpa*, were the main herbivores able to reduce the restoration success of *Cystoseira amentacea* in the infralittoral fringe of artificial structures. Such results were confirmed by experiments in tanks. Subsequently, the effect of fish grazing was also quantified on natural *Cystoseira* populations, highlighting an important loss of growth and reproductive potential.

Our studies demonstrated that herbivorous fish are highly responsible to reduce the success of *Cystoseira* restoration and severely graze on natural populations. Likely, their role in regulating very shallow macroalgal assemblages has been overlooked so far. Even if we cannot state it with the current knowledge, *Sarpa salpa* population in the Mediterranean Sea has probably increased in the last decades due to the overfishing of its predators, and in particular in MPAs (Prado et al., 2008). We suggest that future conservation and restoration actions of marine forests, also in MPAs, take into account herbivorous fish exclusion or regulation by means of devices to protect forests, including *Sarpa salpa* in target fishing species and favouring the recovery of top-predators.

Conclusions

In the future context of Mediterranean Sea management, ecologically relevant and sensitive species, as *Cystoseira*, should become a conservation priority. The awareness on the importance of marine forests of large-brown seaweeds should be raised and cartographies should be performed, especially in MPAs where information is scarce, but also in non-protected sensitive areas where *Cystoseira* forests are still healthy and deserve attention. Then, on the base of a complete and detailed habitat mapping of marine vegetation, a regular monitoring of such forests should be included in MPA management plans in order to evaluate first signs of regression due to local human impacts and/or ecological dynamics. Restoration plans can be considered to enhance *Cystoseira* recovery when necessary and if all the conditions for a successful restoration are guaranteed. However, the conservation of the existing pristine forests has always to be considered as priority, since it is the most effective tool for conservation and it represents the only way for preserving older marine forests that are still present in some remote zones of the Mediterranean Sea.

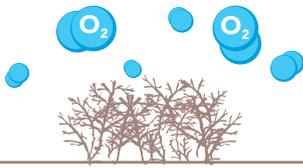
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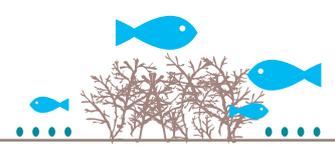
MONITORING CYSTOSEIRA FORESTS

Monitoring and restoration of *Cystoseira* forests in Marine Protected Areas of the Mediterranean Sea

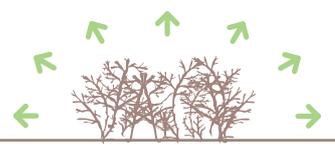
WHY MONITOR IT?



oxygen producers



reproductive and nursery habitats



they export organic matter

HOW TO MONITOR IT?

1 ARE THERE ALGAE FORESTS?



LITERATURE SEARCH

for prior available information



DETAILED CARTOGRAPHY

expanding on found literature



PUT DATA INTO GIS

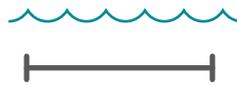
for automated processing

2 WHAT IS ITS CONSERVATION STATUS?



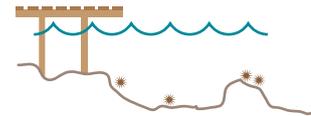
FORESTS FORMING BELTS AT THE WATER SURFACE LEVEL

CARLIT method + quadrats for density/coverage



SUBTIDAL FORESTS

Line transects + quadrats for density/coverage



ENVIRONMENTAL VARIABLES

human impacts, sea urchins density, substrate morphology, etc.

3 IS THERE ACTION REQUIRED?



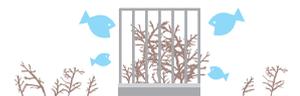
INVESTIGATE CAUSES OF REGRESSION

e.g. water discharge, herbivores overgrazing



NON-DESTRUCTIVE FORESTATION

from installing fertile receptacles to laboratory cultures



HERBIVORE EXCLUSION

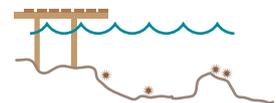
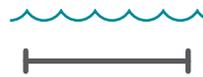
to avoid high grazing rates

4 FURTHER MONITORING



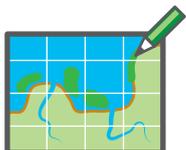
YEARLY MONITORING

Preferably in spring

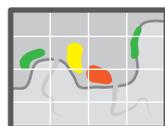


SAME METHODS AS INITIAL ASSESSMENT

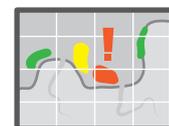
WHAT TO EXPECT



Detailed cartography of marine forests in the MPA



Health status of marine forests



Need for impacts management and/or forests restoration



The book of Marine Protected Areas

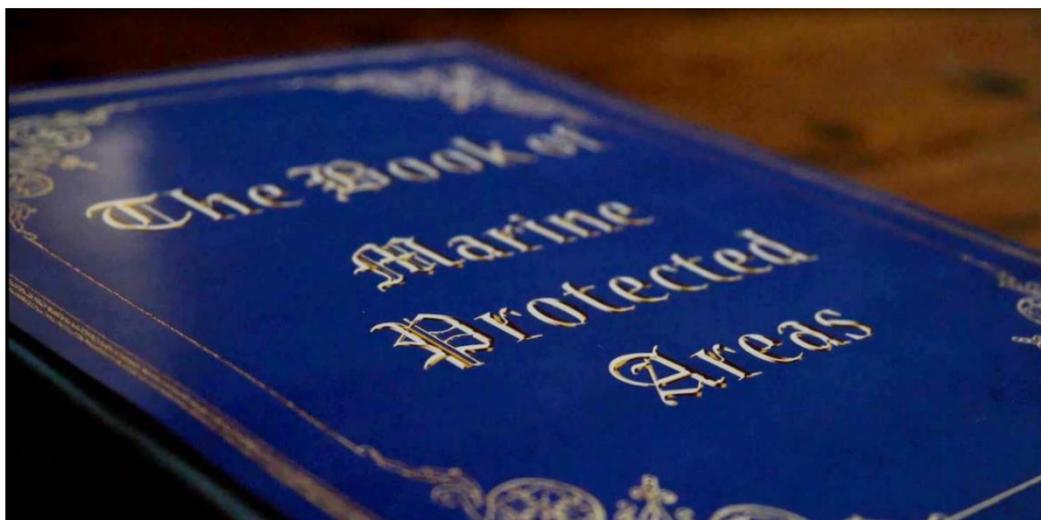
Authors: Fabrizio Gianni and Giulia Prato

Filming and editing: Kévin Peyrusse

Drawing: Céline Barrier

Voice: Sébastien Pruneta

<https://vimeo.com/149648126>



This short movie wants to tell a true story of depletion of the Mediterranean Sea following the increase of human population and fish demand. From healthy environments at the beginning of the century, fish stocks overexploitation led to ecosystem shifts in many coastal areas, where important macroalgal habitats were overgrazed by sea urchins, and replaced by marine deserts. A succession of drawings and videos show how well-managed marine protected areas, where the collaboration among managers, stakeholders and scientists is achieved, represent one of the best solutions to prevent and, sometimes reverse, this negative trend and restore our sea.

Subtitles available in english, french, italian, spanish, greek, arabic, turkish, and croatian.

Special thanks to Emna Lamine, Patricia Marti Puig, Amos Joshua, Sylvaine Giakoumi, Vasiliki Markantonatou, Ana Markic, Yaprak Arda for helping us with subtitles.

Thank you to WWF Mediterranean and to all researchers of the ECOMERS laboratory, in particular to Luisa Mangialajo, Paolo Guidetti, Patrice Francour, Claudia Scianna and Antonio Di Franco for the useful suggestions.



Les Forêts marines de *Cystoseires*



Que sont les *Cystoseires* ?

Les *cystoseires* sont des grandes algues brunes formant des forêts marines en Méditerranée entre la surface et plusieurs dizaines de mètres de profondeur. Nombreux organismes vivent et se reproduisent dans ces forêts.

Malheureusement, les forêts de *Cystoseira* sont en train de disparaître à cause des nombreuses activités humaines. Les chercheurs de l'Université Nice Sophia Antipolis étudient le rôle des Aires Marines Protégées (AMP) dans leur conservation ainsi que la restauration écologique de zones dégradées.



Les chercheurs estiment l'état de santé des forêts marines dans les AMP

Une *Cystoseira* transplantée sur une digue et protégée contre les poissons herbivores

