



*Doctoral Thesis*

# Mediterranean macroalgal forests under threat:

## The effects of ongoing climate change and design of restoration methods

*Jana Verdura Brugarola*  
2021

Universitat  
de Girona

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TESI DOCTORAL

**MEDITERRANEAN MACROALGAL FORESTS UNDER  
THREAT: THE EFFECTS OF ONGOING CLIMATE  
CHANGE AND DESIGN OF RESTORATION METHODS**

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**Mediterranean macroalgal forests under threat: The effects of  
ongoing climate change and design of restoration methods**

**El boscos de macroalgues amenaçats de la Mediterrània: els efectes del  
canvi climàtic i disseny de mètodes de restauració**

Memòria presentada per optar al títol de Doctora per la Universitat de Girona

Programa de Doctorat en Medi ambient

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2021

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Verdura J (2021). Mediterranean macroalgal forests under threat: The effects of ongoing climate change and design of restoration methods. PhD thesis. Universitat de Girona.

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Cover design: Laura López: @laulopezarts

A l'avi Joan i a l'avi Tòbal.

A les meves àvies Loles i a la Tia Maria.

Als meus pares i a en Rixi.

A l'Adri.



## Agraïments

I avui, contenta, però a la vegada amb certa tristor, en començar a escriure aquestes paraules me n'adono que aquesta etapa està arribant al final. Tot comença el dia 7 de març de 2012, quan l'Emma em contesta el mail per fer les pràctiques de la carrera al CEAB. Amb una gran desconeixença, aquest va ser el meu primer contacte amb el món de la ciència. Al cap d'un temps anant i venint, un dia, sense prèviament haver-me plantejat fer una tesi i encara no sé molt bé com, m'hi vaig decidir.

Sembla impossible que hagi passat tant de temps. Aquesta etapa ha estat una gran experiència, una experiència inoblidable! Quantes coses apreses, viscudes i compartides! Campanyes, viatges, seminaris, congressos, hores sota l'aigua i encara més davant l'ordinador. El millor, però, és la gran quantitat de persones que, cada una a la seva manera i algunes potser sense ser-ne conscients, m'heu acompanyat i fet costat durant aquests anys. És per això que aquesta tesi porta un trosset de cadascú de vosaltres, sou els que heu fet possible que arribés fins aquí! Moltes gràcies a tots i a totes.

Emma, sense la teva confiança, res de tot això hauria estat possible. Gràcies per tant! Tinc tantes coses a agrair-te! Com sempre en fem broma, no sé si va ser per l'hoquei o per les circumstàncies del moment, però que em contestessis aquell mail, per mi, va ser una gran sort. Gràcies per creuar-te al meu camí i obrir-me la porta al món de la recerca! Un món que m'has ensenyat a viure amb entusiasme, amb equip i des d'una manera molt humana. Gràcies també per ser-hi sempre, no només com a directora, sinó també com a companya i com amiga! Sempre disposada a escoltar i donar-me el millor consell, tant a nivell científic com a nivell personal. Com m'agraden les nostres xerrades sobre qualsevol tema, fins i tot sota l'aigua. La llibertat que m'has donat en tot moment m'ha permès participar en tot allò que se m'ha proposat, la qual cosa m'ha fet aprendre i gaudir al màxim durant aquesta etapa. Quantes hores que hem passat assegudes l'una al costat de l'altra corregint manuscrits (també a vegades comprant sandàlies a Privalia) i explicant-me el perquè de cada modificació. Quina paciència la teva, però que útil que ha estat i quant m'ha ajudat a progressar. M'has ensenyat a anar una mica més enllà dels resultats, mirant-los des de perspectives més àmplies. Quantes coses compartides: immersions, campanyes, congressos, sopars, compres... on el bon rotllo hi ha estat sempre present. Que bé ens ho hem passat! La teva energia inesgotable i les teves ganes fan que treballar amb tu sigui productiu i divertit. Gràcies per tants i tants riures, anècdotes i pels teus "despistes" que només de pensar-hi m'agafen atacs de riure sola davant l'ordinador.

També et vull donar les gràcies per considerar-nos i pensar sempre en nosaltres, en el nostre present i en el nostre futur. Ha estat un plaer créixer i treballar al teu costat i en el teu equip. Un equip que he tingut la sort de veure com de mica en mica es feia gran en tots els sentits, i que ha estat, per mi, un equip de persones i professionals immillorable! Com sempre et dic, vull que siguis la meva “jefa” indefinidament!

I com no, milions de gràcies a tu també, Kike! Contactar amb l'Emma també em va permetre conèixer-te i treballar amb tu. Recordo els inicis al teu despatx, ensenyant-me a identificar espècies del coral·ligen, i després d'aquella primera immersió a l'imperial, vam començar a compartir campanyes, sobretot d'invasores, fins que vas acabar essent el codirector de la meva tesi sobre Cystoseires. Crec que ets la persona amb qui més hores he passat sota l'aigua! Ha estat tot un privilegi! D'aquí cap allà, Menorca, Formentera, Cabrera, Albània i Açores, fins i tot a l'aigua dolça dels llacs del Pirineu! Compartint el dia a dia a la Mola, a Can Marroig o al “Gimnasio”, tot i que si puc triar em quedo amb el Plan B! Quins llocs tan bonics m'has ensenyat, les millors immersions, coves o cales, sempre des del teu vessant i la teva expertesa naturalista. Quant he après! Llàstima que la meva memòria no pugui retenir tot el que m'has explicat ni tots els noms d'espècies que m'has ensenyat, però, tot aquest coneixement transmès forma part de mi. I a part de treballar, com hem rigut i que bé que hem menjat! Crec que em quedo amb les rotges xapades per la meitat, tot i que també podria triar els ceps, els ous de reig, les múrgoles o els rogers de Tossa, sempre amb un bon vinet! Tot i que també m'agrada molt el moment quelis amb sobrassada! Quina tela amb les revisions maratonianes dels comentaris dels referees a Tossa quasi esmorçant, dinant i sopant! Gràcies per la teva paciència, pels teus consells i per les infinites correccions sempre amb el teu segell d'exigència, exactitud i rigor científic. Pel teu “voilà” poques hores després d'enviar-te quelcom i per les discussions i les llargues correccions, però alhora divertides, via Skype d'aquest darrer any. Gràcies també per ser-hi sempre.

Emma i Kike, m'heu permès créixer en tots els aspectes. Treballant amb vosaltres he après moltíssim, he viscut un munt de bones experiències i he conegut gent molt enriquidora. He estat molt afortunada! Gràcies per la vostra ajuda incondicional i per voler sempre el millor per mi! Heu estat i sou molt més que els meus directors de tesi. Entre tot el que hem compartit s'ha forjat una relació professional i personal que jo tant i tant valoro i que em fa molt feliç. Per mi heu estat un tàndem perfecte! De veritat, gràcies i gràcies a tots dos!

Una altra part molt important d'aquesta tesi és el Marine Forest Team! Quin superequip!



Va ser només començar la beca, i puf! Va arribar en Jorge! Qué suerte que viniste para siempre! Des del primer dia et vas convertir en el meu company de tesi! Si algú m'ha patit, diria que tu ets el que més! Va ser arribar i començar a fer-ho tot junts, a "tope" amb els experiments, cap a Cala Estreta o Port de la Selva a contar Cystos (aguantant-nos l'un a l'altre per no xocar amb les pedres), campanyes, mostrejos fugaços a les gàbies de Formentera, congressos, discussions d'estadística, viatges, sopars i tot el que fes falta! M'has ajudat en tot moment, gràcies per estar-ne sempre disposat! Sempre amb la teva meticulositat i perfecció! Tot i que jo vaig començar una mica abans, hem fet la tesi en paral·lel, compartint fins i tot l'última etapa de clausura (llàstima que hagi estat a distància), has estat un gran suport en tot moment i un immillorable company i amic de viatge! I a mitja tesi, va arribar en Raül, per enèsima vegada, després de la carrera i el màster, tornaries a formar part del meu dia a dia! I que bé no? No en dubtava gens! Tan diferents, però tan amics. I et vas afegir a tot: a contar Cystos, a les campanyes, a les discussions d'estadística, i després a Colòmbia, al road-trip amb "parada a Venècia" i mil coses més. Tot i que em vas robar una mica en Jorge, en alguns moments podria dir que semblàvem un matrimoni a tres bandes, això sí, sempre els dos contra mi...! Però que bé m'ho he passat amb vosaltres, quant hem après i quant m'heu ajudat! M'ha encantat compartir el doctorat amb vosaltres!

Sònia i Alba! També heu estat clau en aquesta tesi! Sempre tan properes i preparades per ajudar i fer les coses fàcils. Des de contar reclutes a contar adults aquí i allà, sempre amb riures assegurats. Alba, gràcies per la teva sinceritat i el teu ordre, amb les coses sempre sota control! Aportant el teu punt de seny entre tots nosaltres. Per la teva ajuda a resoldre temes burocràtics de la universitat i per saber com funciona tot. M'encanta quan la teva expressió facial parla per tu! Sònia, gràcies per fer qualsevol cosa fàcil i agradable, sempre posant el teu toc artístic que tant m'agrada! Com trobo a faltar les nostres xerrades durant els trajectes a la uni, i com m'agrada compartir experiments amb tu. També has patit força els meus dilemes vitals, gràcies per escoltar-me sempre i pels teus consells.

I una mica més tard, arribava la Cris! Crec que cap de les dues havia imaginat que compartiríem el teu primer i el meu últim any de tesi. Que bé! Que bé que ens hem entès en tan poc temps. Anem alhora, allà on no arriba una, arriba l'altra! La comunicació i la transparència és el nostre fort! Durant aquest últim any, hem estat l'una l'ombra de l'altra. Gràcies per la teva franquesa i per la teva naturalitat. Has estat una peça clau en aquesta última etapa de la tesi. Animant-me, ajudant-me i compartint amb mi el dia a dia al CEAB quan la barreja "pandèmia-final de tesi" limitaven els meus dies a poc més de casa-CEAB-casa-CEAB. Sempre preparada per escoltar i comentar qualsevol cosa, conversar sobre ciència, sobre la vida i sobre el nostre futur. Totes

dues sabem que ens queda molt camí per recórrer juntes! Quina sort haver coincidit, t'has convertit en una bona amiga!

I després s'incorporava en Jordi! La teva simpatia la vaig percebre des del primer dia que et vaig conèixer, que ja fa uns anys... Entusiasta i curiós per definició! Vam començar compartint dinars al CEAB, on sempre aportes la teva alegria i energia per tot el que fas! Després vam compartir campanyes i cursos de busseig, fins que vam anar a petar al mateix moment a l'altra punta del món! Sydney, quina experiència! Com em vau cuidar! La Valeria, en Bru (el meu "precioso") i tu, us vau convertir en la meva Aussie Family, els "Top 4", on la tieta Jana era sempre benvinguda! Vale, com m'agrada la teva sinceritat, clara i directe, sense pèls a la llengua. Que bona! Com vam riure! Sempre recordarem el: "Aparca" en modo Gus i el "Bon Natal" 2017 a Apollo Bay a la nostra *suit* familiar!

Ha estat un gust fer la tesi envoltada de tots vosaltres! Us podria escriure tantes coses a tots... Heu estat puntals en aquest camí i us heu convertit en molt més que companys de feina! Com ja sabeu, m'encanteu!

També vull donar les gràcies a tota la gent del CEAB, perquè tot i fer la tesi a la UdG, són moltíssimes les hores que hi he passat. Gràcies a tots els que formeu part del dia a dia del CEAB, amb qui he compartit àpats, discussions, converses i rialles a la cuina, a la terrassa o als passadissos. Vosaltres també formeu part d'aquesta tesi! Maria Elena (Mari Helen) aviat em vaig convertir en la teva acompanyant de seguiment de Cystos. Port, Cala Estreta o Montjoi, ens perdiem dia sí dia també, però teníem temps de xerrar de tot i més! Que bé que ens ho passàvem! Gràcies per la teva amabilitat, pel teu riure i per la teva passió per les Cystos! Va ser un molt bon començament! Un dels moments top, és "l'expedició" a Albània! Maria, Teresa, Toni, Pep, Luisa, Kike i Emma gràcies per fer-me sentir una més des del primer dia! Com vaig aprendre, riure i disfrutar. Vaig al·lucinar! Teresa, va ser un plaer aprendre a contar pussi amb tu! Maria, gràcies per la teva simpatia i amabilitat des del moment 0. Gràcies també als Forest Rangers Formentera amb el súper equipo Media. Va ser una supercampanya, llàstima que no hagi pogut incloure els resultats a la tesi! Maria i Ramón, gràcies per fer del Rael casa nostra, i per la meva primera travessia Formentera-Palamós.

María (López), vas ser una de les primeres que vaig conèixer al CEAB, les dues a la comuna analitzant fotos! Qui ens havia de dir que hi compartiríem tants anys! Des del principi les nostres xerrades ja prometien, no sé qui xerra més, si tu o jo, però m'encanten! Gràcies per integrar-me des del primer moment. Per les tantes converses que podrien ser infinites i per tenir les millors respostes a totes les preguntes. Per la teva súper organització i la teva energia

en organitzar i compartir! Et vas convertir en la primera comercial de Can Xicoy, inaugurant la sucursal del CEAB. I d'una comuna, vaig passar a l'altra, on hi vaig trobar la Marta! Una altre cop de sort! En pocs dies i després d'uns quants "post it" de companya de despatx a companya de despatx, et vas convertir en "la meva Compi". A partir de llavors, començava una gran relació d'amistat que ens portaria a compartir mil coses, fins i tot a l'altra punta del món! És tan guai tenir-te, ets la festa en persona! Pon una Compi en tu vida! Gràcies per ser-hi sempre i ajudar-me a solucionar qualsevol dubte i problema! Us vas convertir en el meu duo preferit! Quan no estava al meu despatx, volia dir que era al vostre! Sempre heu estat disposades a escoltar els meus dilemes vitals (sovint, repetides vegades els mateixos), aconsellar-me i ajudar-me a decidir! Ara, tot i que no ens veiem tant, tenim l'excusa de Makara, que tot i que crec que no faria falta, és un "seguro" de connexió entre nosaltres! Cèlia, també hi ets des de l'inici. Estic contenta de com la nostra relació s'ha intensificat aquests últims anys, però des de sempre m'ha agradat compartir amb tu. El teu riure m'encanta i sóc molt fan de la teva sinceritat i de la teva manera pràctica de veure les coses! Mil gràcies pels teus súper inputs de final de tesi! Marta, Maria, Cris i Cèlia: hem format un grup d'amigues polifacètic complementat quasi a la perfecció (jajaja) que convida a compartir un munt de coses més. No en tinc cap dubte.

Roger, gràcies pels teus atacs de riure per qualsevol xorrada, m'has fet riure molt! A la que ens deixin, vull un dels teus bailoteos de la "Boy Band"! Potser sóc una mica repetitiva, però el moment samarreta d'imperi sempre serà un dels meus top. Vicente, ens vam anar fent amics entre quinieles, euromillons i xerrades. Llàstima que no ens va tocar mai res... però d'aquells moments en queda una bona amistat! Gràcies per ajudar-me a planificar el dipòsit de la tesi i pel teu, "tranquila, déjame ver", quan entro al teu despatx esverada perquè no trobo la informació que busco! Turón! La teva arribada em va fer molta il·lusió, va ser un input d'energia i rialles! Gràcies per les teves ganes i la teva capacitat organitzativa, esquiades, sortides de busseig, pàdels (tot i que sempre era baixa) o el que faci falta, i per sempre trobar algun bon moment per explicar-nos la vida. Mateu, sort que em vas venir a rescatar a Formentera, després d'aquella tempesta! Et dec una immersió en condicions! Gràcies també a en Buñuel i a en Joan. Fer qualsevol cosa amb tots vosaltres és festa i diversió assegurada! Els dinars i les sobretaules, on sovint també s'hi afegien en Xevi, en Jan, i més tard en Mario i l'Adri, eren sinònims de desconexió i rialles. Gràcies també a en Jordi Pagès, perquè conversar amb tu és sempre un plaer, a l'Ibor i a en Rudi, uns indispensables. M'agradaria agrair també a en Xavi Turón per les campanyes a Cabrera, i a la Iosune i en Rafel Coma per la seva ajuda amb les esponges i gorgònies del coral·ligen. Gràcies a l'equip

“Caulerpa”. Simone, per transmetre aquesta passió per les Cystos. Estic segura que algun dia sabrem quina és quina! Xavi Torras, per les converses que es creen al teu voltant, sigui dinant o sigui al passadís. Gràcies també a l’equip d’Administració, a en Ramón i a en Manel. A l’Angel, essencial, gràcies per ajudar-nos a muntar i desmuntar el que faci falta! A la Kenny i a la Fina, pels seus bons dies i bones nits!

També m’agradaria agrair a la Marta Sales, per cedir-me les dades i deixar-me explicar la bonica història de la nostra barbata de Teulera i pels moments compartits a Menorca. Gràcies també a la Núria Teixidó per la teva ajuda i confiança a l’inici de tot. I a en Mikel pel seu pla d’estudis clar i concís: 1r cicle a Barcelona i 2n cicle a la Laguna. Vas ser el primer guia de la meva carrera, i la vas ben encertar.

També vull donar les gràcies a tota la gent de la UdG. A la Marta i a la resta de l’equip del GR-MAR. Sílvia, per la teva ajuda en aconseguir el que calgui per fer el que toca en cada moment. Pilar Eijo, gràcies per facilitar la burocràcia. Jordi Bou, per resoldre’m mil dubtes. Alba, per encapçalar el projecte a Islas del Rosario que va ser per tots una experiència inoblidable. Ander, per la teva il·lusió en participar-hi. Gràcies també a en David, a la Irene, a la María i a tots els altres doctorands per les estones compartides. També vull agrair a tots els estudiants de pràctiques, TFG i TFM que han passat i col·laborat amb nosaltres, gràcies pel vostre granet de sorra. Vull agrair també el suport dels membres del grup de recerca MedRecover, especialment a en Quim, en David, la Cristina, en Diego i a la Paula. També a tots els doctorands amb qui he coincidit, en especial a en Pol, a l’Eneko, a l’Alba i a l’Anabel.

I am also very grateful to Adriana Vergés for giving me the opportunity to collaborate with your research group during my international internships. Especially George and Shannen, I appreciate your help and kindness, I will always remember the fieldwork sampling Crayweed, where I learned a lot about seaweeds and about Australia. Jervis Bay Team, I was very lucky to share with you this experience, you contributed enormously in making it very special and unforgettable.

Sortint del món acadèmic, ara és el torn dels meus incondicionals. Els de casa. Gràcies als meus pares! Per donar-m’ho tot. Per sempre ajudar-me a aconseguir tot el que em proposi, per creure en mi i fer-me costat en tot. Perquè m’heu ensenyat a ser conscient de les meves virtuts però també de les meves mancances o defectes. Per ensenyar-me a viure la vida amb alegria i a estimar i cuidar les persones que m’envolten. Per construir les bases de la persona que sóc. Per ensenyar-me tantes coses. Per fer-me descobrir el meravellós món submarí i animar-me a conèixe’l més a fons! Quantes coses bones ens ha portat la nostra Calella! Si tanco els ulls, ens

veig a tots 4 al Portet amb el tub, les ulleres i els “patos” amunt i avall buscant pops i fent peixeres! Mama, per estar sempre pendent del que pugui necessitar i ajudar-me en tot. Papa, per sempre empenyem a millorar. Rixi, gràcies pel teu humor, la teva energia i la teva passió per la natura! Tan diferents, però estimant-nos per sobre de tot! Gràcies a tots tres per ser incondicionals. I fa poquet arribava la Lau, també apassionada per la vida animal i. Gràcies pel teu saber escoltar i interès amb el que faig, és un plaer estar i compartir amb tu.

Aquí també hi entra una pota fonamental, l'Adri! Gràcies per ser el meu complement perfecte. La teva paciència ha estat clau. T'ha tocat viure cada dia i cada moment d'aquesta etapa, els d'eufòria i alegria, però també els de nervis, decepció i estrès. Gràcies per la teva confiança i per fer del nosaltres el benestar i la calma que en certs moments em són tan necessaris. Com m'agraden les nostres escapades que ens omplen d'energia i aire fresc. Per aquest estil de vida lligat al teu projecte professional que, malgrat el sacrifici, tant enriqueix el nostre dia a dia. Crec que hem estat capaços de compaginar els nostres projectes individuals, tot i que podríem dir que les nostres professions no són les ideals per fer-ho. Gràcies per créixer amb mi i per estimar-me tant. Una part molt gran d'aquesta tesi, també és teva.

Gràcies també a tota la meva família! A l'avi Tóbal, sempre patint i preguntant: ara ja se't quedaran a treballar per sempre a Blanes? Perquè tot i no entendre massa bé de què va la meva feina, sempre estar-ne pendent, i escoltar amb atenció les meves explicacions. Tia Maria, la meva companya de riures! Sempre comparant les meves fotos amb els documentals de la 2, i dient-me com de bonica que és la vida sota el mar! Tia Fina, gràcies per ser-hi sempre i per animar-me a fer realitat el que em proposi. T'has escarrassat a entendre el món de la ciència i has estat pendent en tot moment de què em faltava per acabar la tesi! Gràcies també a la Família Brugarola! Que guais que sou! Avi Joan, per ensenyar-me a estimar, a com és d'important la família i a compartir-ho tot! Per estar orgullós de mi fes el que fes. Tia Magda, per cuidar-nos tant a tots. Tiu Pedro, per estar disposat a fer el que calgui. Tia Montse, per estar sempre disposada a compartir i a ajudar. Teta, perquè tot i ser l'única que tinc, ets la millor cosina del món. Per compartir tant amb mi. Isma, per haver estat el meu company de cursos de submarinisme i pels riures espontanis que origines! Jordi, pel teu entusiasme i energia. Alba, pel teu tarannà. Joan, pel teu sentit de l'humor. Mariona, pel teu riure contagiós. Gere, per recordar-nos sempre com ens estimes. Cris, pel teu saber estar. I com no, a les petites (o ja no tant) de la família, Mar, Bruna, Jana, Abril, Greta i Nina per ser encantadores i l'alegria de casa! Tenim pendent l'acampada familiar 2.0. Gràcies també a la “tia” Anna i a la Ceci, que jo sé que esteu sempre pendents.

Moltes gràcies també a tota la meva família “postissa” que fa tant de temps que compartiu i seguíu el meu camí. Elena, per estar pendent dels meus èxits, estar-ne orgullosa i animar-me a anar més enllà. Ferran, també hem crescut al teu costat, n’hem compartit de tots colors, gràcies per les teves paraules sempre tan sinceres, també per les nostres xerrades (entre elles, les de festa Major!), per tot el compartit i per com t’agrada fer-ho. Gràcies també al tiet Joan, per estimar tant als teus nebots i nebodes. Rosor, per la teva estima i per ser tan propera. A l’Ignasi, l’Emma i el tiet Fernan. Ferran i Magda, moltes gràcies per seguir-me i animar-me sempre de prop. Gràcies pel suport i l’interès de tots els del Clan dels Altés, en especial a l’Elena i a l’Ivan, per ser tan propers. També vull agrair a la Pat aquests anys compartits de riures, àpats, escapades, compres per Marràqueix i cafès, sempre tan natural i sincera, també m’encanta conversar amb tu.

Geor, Jur i Ingrid, per ser les de sempre i per tenir-vos tan a prop. Geor, saps percebre què necessito en cada moment, gràcies per ser-hi sempre des de fa tant temps. M’encanten els nostres atacs de riure! Jur, per les teves visites sorpresa d’ànims o de “despedida” i per estar pendent de mi, sobretot quan he estat a l’altra punta del món. També per ser la meva companya de festes Majors. Ingrid, per la teva màxima paciència i el teu saber escoltar. M’encanta estar amb vosaltres. Gràcies per estar sempre preparades per fer teràpia. I a “la traca” al “completo”, amb en Ferran, l’Anna, en Lluís, la Maria, l’Albert i la Sara, per tots els moments compartits i sobretot per les escapades que són un plus d’energia necessari per carregar les piles! Haurem de tornar a agafar el ritme dels 100 cims, on s’afegiran els petits Uri i Elna que estan per venir! Carles i Geor, per les millors truites de patates! Arnau, perquè tot i la distància i que no ens veiem molt, la nostra amistat i confiança em continua agradant tant com sempre. Gràcies també a les KT i a tota la gent amb qui les he compartit, són un parèntesi de 10 dies inexplicables, de riures, diversió i desconexió.

Ane eta Amaia, nire Tinersisters. ¡Porqué fuisteis esenciales en una de las mejores etapas de mi vida! ¡Conectamos a la perfección! Y ahora, aunque separadas por unos cuantos kilómetros, sé que os tengo cerca. Rita, porque también tuviste que ver en mis decisiones en cuanto a la ciencia. Gràcies també al Petit Comitè de l’autònoma per tots els moments a la gespa i al costat del bar i pels retrobaments, que tot i no poder venir sempre, tant m’agraden. Ju, per la setmaneta fantàstica al Mar Roig que em va fer reviure moments de la UAB. Lau Lo, gràcies per posar el teu toc artístic a la tesi i fer-ho tan fàcil, amb una conversa telefònica i quatre fotos n’hi va haver prou. M’encanta com ha quedat, no en dubtava gens, els dibuixos dels teus apunts (essencials per molts) ja apuntaven maneres!! Gràcies.

Per últim, gràcies també a les amistats de l'hoquei, Maria i família, Roca i Marta, gràcies per aquesta amistat que s'ha creat entre "stiks", patins, "entrenos", sopars i festes. Ha estat un plaer jugar al vostre costat tots aquests anys. Roca i Marta sou culpables d'infinits atacs de riure durant aquests últims anys al Vilassar, Roca tu potser més! També vull agrair al Club Vilassar Hoquei, per com cuida l'hoquei femení i perquè m'ha permès compaginar la tesi amb aquest esport que tant estimo, fins el dia d'avui. Gràcies també al meu equip actual, sou genials! Heu estat una via d'escapament molt important!

No m'agradaria haver-me oblidat de ningú. Si fos el cas, tot i que em sabia molt de greu, podeu estar segurs que també us podria agrair moltes coses.

Moltíssimes gràcies a tots i a totes, heu estat i sou essencials!

Arenys de Munt, abril del 2021.





La Dra. Emma Cebrian i el Dr. Enric Ballesteros, ambdós del Centre d'Estudis Avançats de Blanes (CEAB-CSIC),

CERTIFIQUEM:

Que el treball titulat “*Mediterranean macroalgal forests under threat: The effects of ongoing climate change and design of restoration methods*”, que presenta la Sra. Jana Verdura i Brugarola com a compendi de publicacions per a l'obtenció del títol de doctora, és idoni per ser presentat sota aquest format.

La doctoranda ha participat activament en el desenvolupament del treball de recerca associat a cadascun d'aquests articles, així com en la seva elaboració, fets que demostren la seva rellevant contribució en cada un d'ells. En concret, la seva participació en cada un dels articles ha estat la següent:

- Plantejament de les hipòtesis i objectius
- Planificació i execució dels experiments, tan pel que fa a la feina de camp com al laboratori, i seguiments biològics i censos
- Presa i anàlisi de dades
- Redacció dels articles i seguiment del procés de revisió dels mateixos i en la redacció i discussió dels resultats de les diferents publicacions que es detallen a continuació, fets que demostren la seva rellevant contribució a cada una d'elles.

I, perquè així consti i tingui els efectes oportuns, signem aquest document.



Directora de la tesi  
Dra. Emma Cebrian i Pujol



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Girona, abril del 2021

This PhD thesis is presented as a compendium of publications. It includes three publications, two of them already published and the other one is under revision.

**List of manuscripts of this thesis, organized by chapters, with indication of journal impact factor (IF), quartile (Q) and publication stage.**

CHAPTER 1: **Jana Verdura**<sup>1,2</sup>, Jorge Santamaría<sup>1</sup>, Enric Ballesteros<sup>2</sup>, Dan A. Smale<sup>3</sup>, Maria Elena Cefali<sup>4</sup>, Raül Golo<sup>1</sup>, Sònia de Caralt<sup>1</sup>, Alba Vergés<sup>1</sup>, Emma Cebrian<sup>1,2</sup> (2021). Local-scale climatic refugia offer sanctuary for a habitat-forming species during a marine heatwave. *Journal of Ecology* 109 (4): 1758-1773. doi: 10.1111/1365-2745.13599. IF (2019): 5.762, Q: Q1, Stage: Published.

CHAPTER 2: **Jana Verdura**<sup>1,2</sup>, Jorge Santamaría<sup>1</sup>, Raül Golo<sup>1</sup>, Enric Ballesteros<sup>2</sup>, Joaquim Garrabou<sup>5</sup>, Simonetta Frascchetti<sup>6,7</sup>, Luisa Mangialajo<sup>8</sup>, Ljiljana Ivesa<sup>9</sup>, Simone Farina<sup>7</sup>, Emma Cebrian<sup>1,2</sup>. Intraspecific variability in thermal tolerance shapes future declines in macroalgal forests. IF (2019): 8.555, Q: Q1, Stage: Under revision in *Global Change Biology*

CHAPTER 3: **Jana Verdura**<sup>1,2</sup>, Marta Sales<sup>4</sup>, Enric Ballesteros<sup>2</sup>, Maria Elena Cefali<sup>4</sup>, Emma Cebrian<sup>1,2</sup> (2018). Restoration of a canopy-forming alga based on recruitment enhancement: Methods and long-term success assessment. *Frontiers in Plant Science* 9:1832. doi: 10.3389/fpls.2018.01832. IF (2019): 4.402, Q: Q1. Stage: Published.

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## **Financial Support**

I have had the financial support of a 3-year fellowship of the University of Girona (IF-UdG 2016), and a travel fellowship also financed by the University of Girona. The research conducted on this thesis has been supported by the European Union projects MERCES (No. 689518) and AFRIMED (No. EASME/EMFF/2017/ 1.2.1.12/S4/01/SI2.789059), the Spanish Ministry ANIMA project (No. CGL2016-76341-R, MINECO/FEDER, UE) and the Intramural CSIC-PIE project (202030E180).



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## LIST OF ABBREVIATIONS

AUC: Area Under the Curve

C: Carbon

CTA: Classification Tree Analysis

Df: Degrees of freedom

GBM: Gradient Boosting Machines

GLM: Generalized Linear Model

GLMM: Generalized Linear Mixed Model

IST: *In Situ* Sea Temperature

LMM: Linear Mixed Model

MDS: Multidimensional Scaling

MHW: Marine Heatwave

MM: Mixed Model

N: Nitrogen

NOAA: National Oceanic and Atmospheric Administration

NW: North-Western

PAM: Pulse Amplitude Modulated

PAR: Photosynthetically Active Radiation

PSII: Photosystem II

PVC: Polyvinyl Chloride

RCP: Representative Concentration Pathways

ROC: Receiver Operating Characteristic

SD: Standard Deviation

SDM: Species Distribution Models

SE: Standard Error

SRE: Surface Range Envelop

*s.l.*: *sensu lato*

SST: Sea Surface Temperature

UNESCO: United Nations Educational, Scientific and Cultural Organization

UV: Ultraviolet

VIF: Variance Inflation Factor

$\chi^2$ : Chi-square



## RESUM

Els boscos de macroalgues representen un dels hàbitats més productius i diversos del planeta. A la mar Mediterrània, les espècies del gènere *Cystoseira sensu lato* (que inclou *Cystoseira* C. Agardh, *Ericaria* Stackhouse i *Gongolaria* Boehmer) dominen els fons rocosos submareals de les àrees ben preservades, on formen comunitats d'elevada complexitat. Tanmateix, des de finals del segle XX, aquestes comunitats estan patint importants regressions arreu de la Mediterrània, principalment a causa de la destrucció de l'hàbitat, dels canvis en la qualitat de l'aigua i de la sobrepastura de garotes. En conseqüència, aquestes comunitats sovint són substituïdes per comunitats més simples i menys productives, dominades per espècies oportunistes o més resistents a les condicions d'estrès, que alhora impedeixen l'assentament de reclutes de *Cystoseira s.l.* Per tant, en molts casos, el fet de revertir les condicions a nivells previs a la pertorbació, no necessàriament implica la recuperació natural dels boscos de *Cystoseira s.l.*, i llavors, la restauració activa esdevé l'única alternativa factible per recuperar aquestes poblacions.

Actualment, l'escalfament gradual i els episodis excepcionals de temperatura elevada causats pel canvi climàtic, representen una amenaça global pels boscos de macroalgues. Tanmateix, els factors i processos a escala local i regional poden modificar els gradients climàtics i, en última instància, condicionar les respostes de les poblacions de

macroalgues a l'escalfament. Tot i la manca d'evidències de l'impacte de l'escalfament en poblacions de *Cystoseira s.l.*, tenint en compte les pertorbacions que han patit històricament aquestes poblacions i els escenaris climàtics que es preveuen per a la Mediterrània, és prioritari determinar com el canvi climàtic pot afectar aquestes espècies.

En el marc d'aquesta tesi s'han abordat dos objectius principals ben diferenciats. En primer lloc, s'estudia com el canvi climàtic pot afectar les poblacions de *Cystoseira s.l.* En segon lloc, es proposen dues noves tècniques de restauració no invasives per poblacions de *Cystoseira s.l.*

El primer objectiu s'aborda mitjançant experiments manipulatius en condicions controlades, dades observacionals ecològiques i ambientals obtingudes *in situ* al llarg del temps i l'ús de models de distribució. Concretament, a partir d'un experiment de termo-tolerància es determinen els efectes directes de la temperatura a escala d'individu per a *Ericaria crinita*. D'altra banda, mitjançant l'anàlisi de les dades recollides *in situ*, s'estudia com els factors locals poden modular les respostes de les poblacions a episodis excepcionals de temperatura elevada. Finalment, de manera experimental, es caracteritza la termo-tolerància de vuit poblacions d'*Ericaria crinita* provinents de diferents règims tèrmics, que juntament amb l'ús de models de distribució ens permeten predir la vulnerabilitat d'aquesta espècie enfront a futurs escenaris climàtics. Els nostres resultats mostren que l'escalfament pot afectar greument la supervivència d'*Ericaria crinita*, especialment durant les primeres etapes de vida. També suggereixen que l'heterogeneïtat ambiental a escala local modula la resposta de les poblacions enfront a episodis excepcionals d'elevada temperatura possibilitant l'existència de refugis climàtics. Finalment, l'experiment de termo-tolerància mostra que les poblacions d'*Ericaria crinita* de les zones més fredes són les menys tolerants a l'estrès tèrmic, demostrant l'existència de variabilitat intraespecífica en la tolerància tèrmica d'aquesta espècie. Les nostres prediccions mostren que aquesta variabilitat defineix la resposta de

l'espècie a l'escalfament, i posen de manifest la importància de considerar-la a l'hora de predir la vulnerabilitat de les espècies en front el canvi climàtic.

En relació al segon objectiu, es dissenyen i s'apliquen dues tècniques de restauració basades en l'obtenció de nous reclutes (directament *in situ* i fent-los créixer *ex situ* en condicions de laboratori) a partir de branques fèrtils de poblacions donants. A més, s'estableix un seguiment a mitjà termini per tal d'avaluar l'èxit de la restauració. Sis anys després i amb una única acció de restauració, ambdues tècniques han resultat ser exitoses (en termes de cost i benefici) per recuperar poblacions de *Gongolaria barbata*.

En conclusió, els nostres resultats mostren els efectes potencials de l'escalfament en les poblacions d'espècies superficials de *Cystoseira s.l.*, i assenyalen la rellevància dels factors i processos locals, els quals poden definir la resposta d'aquestes poblacions a les tendències globals de canvi climàtic. A més, es proporcionen noves eines esperançadores per tal de restaurar localment aquests hàbitats amb èxit. En general, els nostres resultats són rellevants i aplicables a futurs plans de gestió i conservació a escala local per garantir la persistència d'aquests boscos de macroalgues en la Mediterrània.





## RESUMEN

Los bosques de macroalgas constituyen uno de los hábitats más productivos y diversos del planeta. En el Mar Mediterráneo, las especies del género *Cystoseira sensu lato* (que incluye a *Cystoseira* C. Agardh, *Ericaria* Stackhouse y *Gongolaria* Boehmer) dominan los fondos rocosos submareales de áreas bien preservadas, donde forman comunidades complejas. Sin embargo, desde finales del siglo XX estas comunidades están sufriendo importantes regresiones en todo el Mediterráneo, principalmente debido a la destrucción del hábitat, a los cambios en la calidad del agua y a la excesiva herbivoría por parte de los erizos. Consecuentemente, estas comunidades son, a menudo, sustituidas por comunidades más simples y menos productivas, dominadas por especies oportunistas y más resistentes a las condiciones de estrés, que a su vez impiden el asentamiento de los reclutas de *Cystoseira s.l.* Por lo tanto, en muchas ocasiones, el hecho de revertir las condiciones a niveles previos a la perturbación no va asociado con la recuperación natural de los bosques de *Cystoseira s.l.* y entonces, la restauración activa se convierte en la única alternativa factible para recuperar estas poblaciones.

Actualmente, el calentamiento gradual y los episodios excepcionales de temperatura elevada derivados del cambio climático, representan una amenaza global para los bosques de macroalgas. No obstante, los factores y procesos a escala local y regional pueden modificar los gradientes climáticos y, en última instancia, condicionar las

respuestas de las poblaciones de macroalgas al calentamiento. Aunque no existen evidencias del impacto del calentamiento en poblaciones de *Cystoseira s.l.*, si consideramos las perturbaciones que históricamente han sufrido estas poblaciones, así como los escenarios climáticos que se prevén para el mar Mediterráneo, determinar cómo el cambio climático puede afectar estas especies es de gran importancia.

En el marco de esta tesis se han abordado dos objetivos principales bien diferenciados. En primer lugar, se estudia como el cambio climático puede afectar a las poblaciones de *Cystoseira s.l.* En segundo lugar, se proponen dos nuevas técnicas de restauración no invasivas para poblaciones de *Cystoseira s.l.*

El primer objetivo se aborda mediante experimentos manipulativos en condiciones controladas, datos ecológicos y ambientales obtenidos *in situ* a lo largo del tiempo y el uso de modelos de distribución. Concretamente, a partir de un experimento de termo-tolerancia se determinan los efectos directos de la temperatura a nivel de individuo para *Ericaria crinita*. Por otro lado, mediante el análisis de los datos recogidos *in situ*, se estudia como los factores locales pueden modular las respuestas de las poblaciones a episodios excepcionales de temperaturas elevadas. Finalmente, de manera experimental, se caracteriza la termo-tolerancia de ocho poblaciones de *Ericaria crinita* provenientes de diferentes regímenes térmicos que juntamente con el uso de modelos de distribución nos permiten predecir la vulnerabilidad de esta especie frente a futuros escenarios climáticos. Nuestros resultados muestran que el calentamiento puede afectar gravemente a la supervivencia de *Ericaria crinita*, especialmente durante las primeras etapas de vida. Además, también sugieren que la heterogeneidad ambiental a escala local modula la respuesta de las poblaciones a episodios excepcionales de elevada temperatura posibilitando la existencia de refugios climáticos. Finalmente, el experimento de termo-tolerancia muestra que las poblaciones de las zonas más frías son las menos tolerantes al estrés térmico, lo que demuestra la existencia de variabilidad intraespecífica en la tolerancia térmica de esta especie. Nuestras predicciones muestran que esta variabilidad

define la respuesta de la especie al calentamiento, y subraya la importancia de considerarla para predecir la vulnerabilidad de las especies frente al cambio climático.

En relación al segundo objetivo, se diseñan y se aplican dos técnicas de restauración basadas en la obtención de nuevos reclutas (directamente *in situ* y haciéndolos crecer *ex situ* en condiciones de laboratorio) a partir de ramas fértiles de poblaciones donantes. Además, se establece un seguimiento a medio plazo para evaluar el éxito de la restauración. Seis años después y con una única acción de restauración, ambas técnicas han resultado ser exitosas (en términos de coste y beneficio) para recuperar poblaciones de *Gongolaria barbata*.

En resumen, nuestros resultados muestran los efectos potenciales del calentamiento en las poblaciones de especies superficiales de *Cystoseira s.l.*, y revelan la importancia de los factores y procesos locales, ya que pueden definir la respuesta de estas poblaciones a las tendencias globales de cambio climático. Además, se proporcionan nuevas herramientas prometedoras para restaurar localmente estos hábitats. En general, nuestros resultados son relevantes para informar planes de gestión y conservación a escala local para salvaguardar la persistencia de estos bosques de macroalgas mediterráneos.



## SUMMARY

Macroalgal forests represent some of the most productive and biodiverse habitats on Earth. In the Mediterranean Sea, species of the genus *Cystoseira sensu lato* (including *Cystoseira* C. Agardh, *Ericaria* Stackhouse and *Gongolaria* Boehmer) dominate the well-preserved subtidal rocky habitats where they form complex macroalgal communities. However, since the end of the twentieth century, they have suffered important and widespread regressions across the Mediterranean Sea, mainly due to habitat destruction, changes in water quality and overgrazing by sea urchins. As a result, they are often replaced by simpler and less productive communities dominated by opportunistic and stress-resistant taxa that prevent *Cystoseira s.l.* settlement. Thus, the return to pre-disturbed conditions does not necessarily imply the natural recovery of *Cystoseira s.l.* stands, being active restoration the only feasible alternative to assist the recovery of these populations.

Nowadays, climate change, especially gradual warming and acute marine heatwaves (MHWs), represents a global threat for macroalgal forests. However, local and regional factors and processes can modify climatic gradients and ultimately define responses of seaweed populations to warming. In this context, while no studies have reported evidence of warming impacts for *Cystoseira s.l.* forests, taking into account that *Cystoseira s.l.* populations have been historically impacted and the expected climate

change scenarios for the Mediterranean Sea, it is of paramount importance to know how climate change can affect these species.

Within the framework of this thesis two well-differentiated main objectives have been addressed. First, we aim at understanding how climate change may affect *Cystoseira s.l.* populations. Secondly, we design and experimentally test two new non-invasive restoration techniques for *Cystoseira s.l.* populations.

In order to address the first goal, a manipulative thermo-tolerance experiment has been performed under controlled conditions to study the direct effects of temperature on *Ericaria crinita* at the individual-level. Moreover, in-situ observational ecological and environmental data have been analysed in order to elucidate how local factors may modulate between-population responses to MHWs. Finally, by means of another manipulative experiment under controlled conditions, we have characterized the thermo-tolerance of eight populations of *Ericaria crinita* coming from different thermal regimes. This information has then been combined with modelling approaches to forecast species response under a future climate scenario. Our results show that warming may severely impair the survival of *Ericaria crinita*, especially at early-life stages. Moreover, they suggest that local-scale environmental heterogeneity mediate population responses to MHWs highlighting the potential for local scale climatic refugia. Finally, we find intraspecific variability in the thermo-tolerance responses of *Ericaria crinita*, with populations from coldest areas being less tolerant. Our predictions show that such variability shapes species response to warming, which highlights its importance when forecasting species vulnerability under future climate change scenarios.

Regarding the second objective, we have designed two restoration techniques based on obtaining new recruits (directly *in situ* and growing them *ex-situ* in laboratory conditions) from fertile branches of donor populations. We have also applied the designed techniques and we have established a mid-term monitoring program to assess

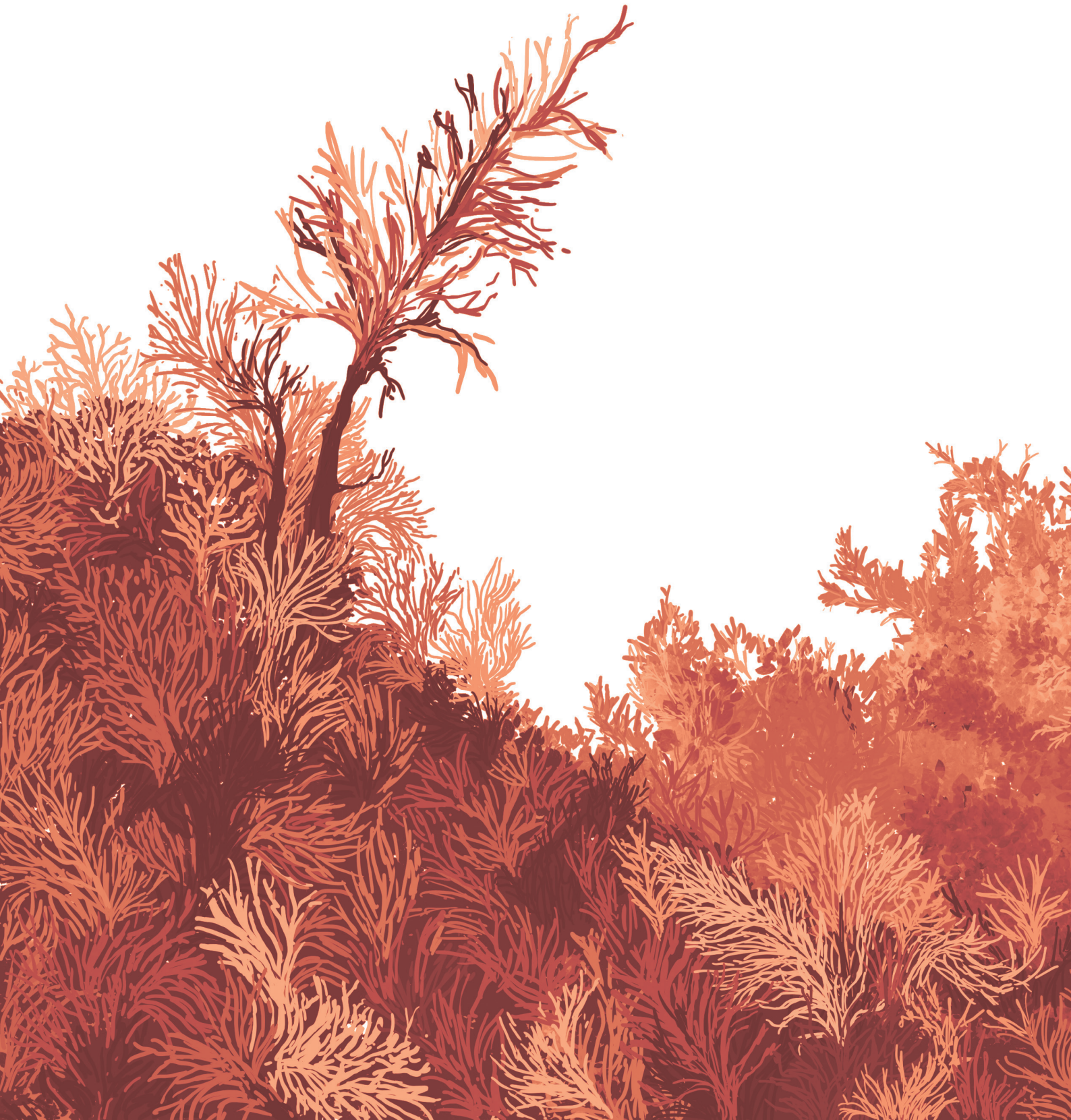
the restoration success. Both techniques have resulted to be cost-effective to recover *Gongolaria barbata* populations after 6 years and with only one restoration action.

In summary, our results show the potential effects of warming on populations of shallow *Cystoseira s.l.* species, pointing out the relevance of local environmental factors and processes ultimately defining the response of these populations to global trends of climate change. Moreover, we also offer new and promising tools to locally restore these habitats. Overall, our findings are relevant in order to inform local-scale management and conservation plans for safeguarding the persistence of these Mediterranean macroalgal forests.





# General Introduction and Objectives





## MACROALGAL FORESTS

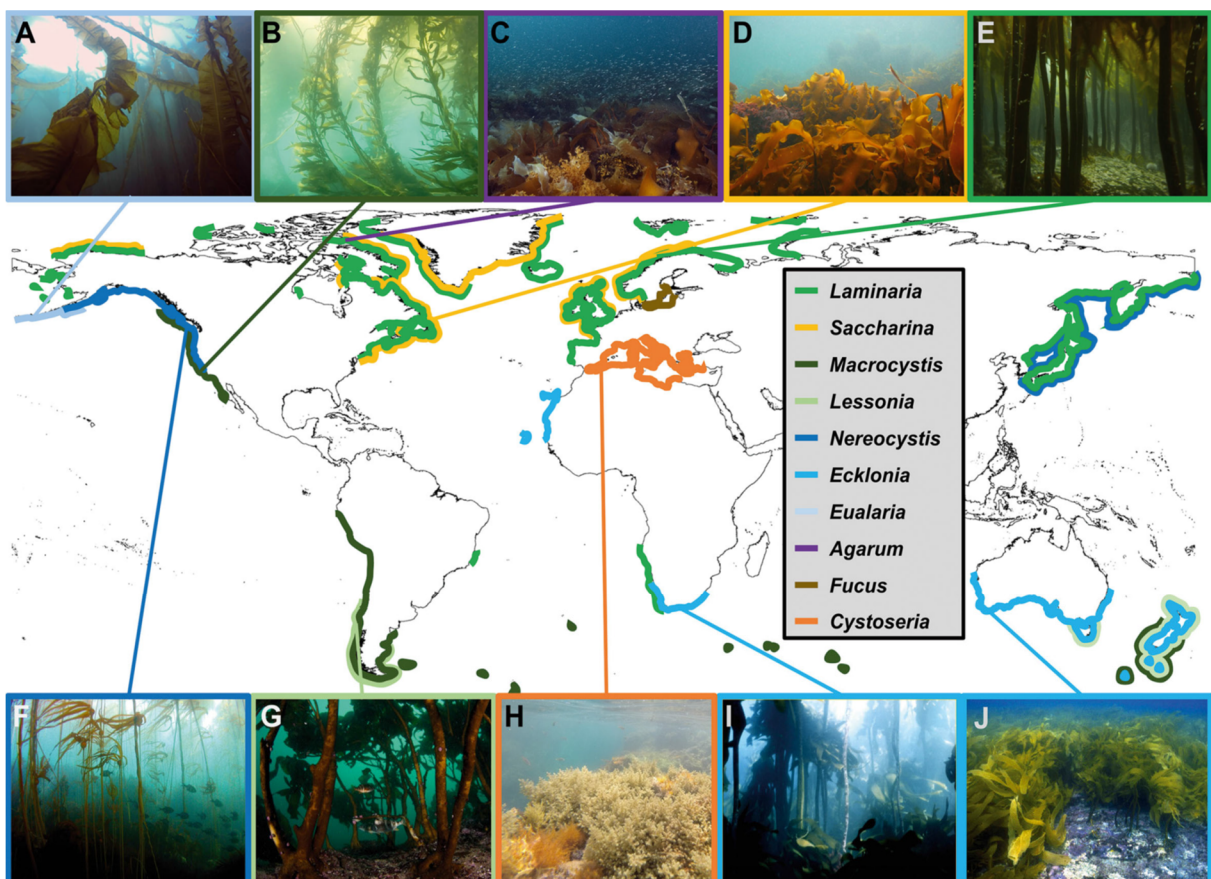
Large canopy-forming algae of the orders Laminariales, Tylopteridales, Desmarestiales and Fucales can create extensive and structurally complex underwater stands which are very similar to terrestrial forests (Figure 1)(Steneck et al., 2002, Steneck and Johnson, 2013, Coleman and Wernberg, 2017).



**Figure 1** | Marine macroalgal forests A) *Lessonia vadosa* at Isla de los Estados, Tierra del Fuego B) *Ericaria crinita* in Mallorca C) *Macrocystis pyrifera* at Isla de los Estados, Tierra del Fuego and D) *Sargassum vulgare* in Columbretes Islands. Photographs by Manu San Félix (A) and Enric Ballesteros (B, C, D).

These forests commonly dominate the intertidal and subtidal rocky bottoms of most temperate, subpolar and polar regions of the world, from the lower shore down to depths of about 120 m (Feldmann, 1934; Giaccone, 1969). They can also be an important component of coastal marine habitats in tropical and subtropical regions (fucoïds like *Sargassum* and *Turbinaria*) (Pérès and Picard, 1964; Steneck and Johnson, 2013; Boudouresque et al., 2016; Wernberg and Filbee-Dexter, 2019; Gouvêa et al.,

2020)(Figure 2). Canopy-forming macroalgae are considered habitat-forming species (Jones et al., 1994; Steneck et al., 2002) as they generate a three-dimensional structure that modifies some environmental factors, such as light and turbulence, thereby creating understory conditions favourable for a myriad of associated species (Jackson and Winant, 1983; Gerard, 1984; Reed and Foster, 1984), which find habitat, food and shelter at different life stages (Feldmann, 1937; Giaccone, 1973; Mann, 1973; Dayton, 1985; Ballesteros et al., 2009; Teagle et al., 2017). Understory assemblages are generally dominated by a great diversity of algae (mainly red, but also brown and green algae) whereas mobile and sessile animals (e.g. herbivores, predators, detritivores or suspensivores) are abundant within the forests, all of them playing an important role in the functioning and structure of these ecosystems (Boudouresque et al., 2016; Teagle et al., 2017).



**Figure 2** | Approximate distribution of key seaweed genera around the world. Image from Wernberg and Filbee-Dexter, 2019, photographs (photo credits in parentheses) show: (A) *Eualaria fistulosa* (Pike Spencer); (B) *Macrocystis pyrifera* (Jenn Burt); (C) *Saccharina latissima* and *Agarum clathratum*

(Frithjof Küpper); (D) *Saccharina latissima* (Kira Krumhansl); (E) *Laminaria hyperborea* (Thomas Wernberg); (F) *Nereocystis luetkeana* (Jared Figurski); (G) *Lessonia trabeculata* (Alejandro Pérez Matus); (H) *Cystoseria* spp. (Albert Pessarrodona); (I) *Ecklonia maxima*; (J) *Ecklonia radiata* (both Thomas Wernberg).

In effect, seaweed forests enhance and support diverse food webs and concentrate and magnify secondary production (Smale et al., 2013), representing some of the most productive and biodiversity-rich ecosystems on Earth (Mann, 1973). Marine forests underpin a broad range of valuable ecosystems goods and services, such as nutrient cycling, coastal protection, nursery grounds and habitat and protection for commercial species (Steneck et al., 2002; Smale et al., 2013; Bennett et al., 2016).

### THREATS FACING WORLD'S MACROALGAL FORESTS

Human influence on Earth has been intensified since the mid-20<sup>th</sup> century (Waters et al., 2016), with growing human population and activities (such as urbanization, industrialization and transformation of land and coasts), which are currently changing the global biodiversity at an unprecedented rate (Folke et al., 2002; Elmqvist et al., 2003; Millennium Ecosystem Assessment (MEA), 2005a; Crain et al., 2008).

Among anthropogenic stressors, climate change is expected to become the strongest driver of change in biodiversity at a global scale (Bellard et al., 2012). Currently, worldwide species are responding to climate change through geographic range shifts (Parmesan and Yohe, 2003; Sunday et al., 2012; Poloczanska et al., 2013; Pecl et al., 2017), changes in demographic rates (Selwood et al., 2015) or developing acclimatization and adaptation mechanisms (Hoffmann and Sgró, 2011; Sanford and Kelly, 2011). As a consequence, climate change is driving a global redistribution of species and reorganization of natural systems. Such changes may disrupt species composition and interactions between species (e.g. prey-predator asynchrony in space, loss of structural species), thus triggering cascading effects that have far-reaching

implications at the community or ecosystem-level (Wernberg et al., 2013; Vergés et al., 2014a, 2019) with important ecological and socio-economic consequences (Pecl et al., 2017).

In this context, like many other complex ecosystems, extensive losses of seaweed forests have been documented in many places around the globe under pressure from different anthropogenic drivers operating and interacting across local and global scale (Thibaut et al., 2005; Airoidi and Beck, 2007; Wernberg et al., 2011; Krumhansl et al., 2016; Smale et al., 2019; Smale, 2020). Historically, habitat loss, changes in water quality and herbivores outbreaks have been the main drivers of the decline of macroalgal forests (Thibaut et al., 2005; Airoidi and Beck, 2007; Araújo et al., 2016; Filbee-Dexter and Wernberg, 2018). More recently, the number of reports of seaweed decline linked to chronic gradual warming and acute extreme warming events, such as marine heat waves (MHWs), has markedly increased (Wernberg et al., 2011; Tanaka et al., 2012; Smale, 2020; Wernberg, 2021).

However, in contrast to many other species around the world, e.g. coral reefs and seagrasses (Pandolfi et al., 2003; Waycott et al., 2009), global declines of seaweed forests are small on average, with region-specific and system-specific responses to global change (Krumhansl et al., 2016). This fact, as also suggested by other authors, reflects that local and regional drivers play an important role in driving patterns of seaweed populations declines (Helmuth et al., 2006b; Krumhansl et al., 2016; Friedlander et al., 2020).

### **MACROALGAL FORESTS IN THE MEDITERRANEAN SEA**

The Mediterranean Sea, is the largest (2.5 millions of square km) and deepest (approximately 1,500 m on average and 5,267 m as the maximum) semi-enclosed sea on Earth and constitutes one of the major hot spots on marine species diversity (Margalef, 1985; Boudouresque, 2004; Coll et al., 2010). Related to its closed geography and its

elongated shape through longitude, the Mediterranean Sea is characterized by an extreme tide reduction, oligotrophic and transparent waters and relatively high salinity and average water temperature (Ros et al., 1985). Despite presenting certain similarities to subtropical seas, such as low nutrient concentration and relatively high irradiance, their seasonal variability brings the Mediterranean Sea closer to temperate seas (Zabala and Ballesteros, 1989).

These particular conditions, however, limit the development of hermatypic corals as well as kelps (order Laminariales) (Zabala and Ballesteros, 1989). In fact, only two species of kelp are reported from the Mediterranean. The Atlantic species *Laminaria ochroleuca* is only present close to the entrance of the Atlantic and the western coasts of Africa affected by the flow of Atlantic waters and in some deep areas of the Strait of Messina (Sicily) (Giaccone, 1969, 1972; Ballesteros and Pinedo, 2004). On the other hand, the endemic *Laminaria rodriguezii* forms extensive but critically endangered forests between 65 and 90 m depth (Giaccone, 1967, 1969; Bo et al., 2011; Joher et al., 2012). In contrast, furoid species of the genus *Cystoseira sensu lato* (Fucales, Heterokontophyta; including also the recently reinstated genera *Gongolaria* and *Ericaria*) are the dominant canopy-forming seaweeds in Mediterranean sublittoral rocky environments (Sauvageau, 1912; Feldmann, 1937; Ercegović, 1952; Giaccone and Bruni, 1973; Rodríguez-Prieto et al., 2013).

Species identification of *Cystoseira s.l.* based on morphological characters is difficult due to the high morphological plasticity of the species, and in some cases, morphology alone may be insufficient to differentiate species for which complementary molecular tools may be needed (Rožić et al., 2012; Orellana et al., 2019). Based on DNA analysis, Draisma et al. (2010) demonstrated the polyphyly of the Mediterranean species of the genus *Cystoseira*, which was resolved into 3 well supported clades (Draisma et al., 2010; de Sousa et al., 2019). Afterwards, Orellana et al. (2019) assigned the three clades to three different genera based also on morphological characters: *Cystoseira* C. Agardh, 1820, *Carpodesmia* Greville, 1830 and *Treptacantha* Kützing, 1843. Very recently, Molinari-

Novoa and Guiry (2020) revised the reinstatement of the genera proposed by Orellana et al., 2019, concluding that *Gongolaria* Boehmer, 1760 and *Ericaria* Stackhouse, 1809 have priority over *Treptacantha* Kützing and *Carpodesmia* Greville, respectively. Accordingly, *Cystoseira*, *Gongolaria* and *Ericaria* seem to be the correct names for the three clades (Molinari-Novoa and Guiry, 2020).

*Cystoseira*, *Gongolaria* and *Ericaria* are widely distributed in the Mediterranean and adjoining Atlantic coasts (Roberts, 1978; Amico, 1995). Although it is thought that these genera were originated in the Thetys Sea during de Mesozoic, the current Mediterranean species may have either originated in the Atlantic Ocean or diverged in the Mediterranean after the Messinian salinity crisis (around 5.3 million years ago) (Oliveras-Pla and Gómez-Garreta, 1989; Amico, 1995), when the Mediterranean basin replenished with Atlantic waters after being desiccated during 0.7 million years (Hsü et al., 1973; Krijgsman et al., 1999; Garcia-Castellanos et al., 2009). The speciation process seems to continue nowadays (Ercegović, 1952; Roberts, 1978; Amico, 1995; Draisma et al., 2010) as recently demonstrated by studies like the one related to the clade *Ericaria amentacea-tamariscifolia-mediterranea* (Bermejo et al., 2018). The Mediterranean Sea is considered the focus of speciation for *Ericaria* and *Gongolaria* (Oliveras-Pla and Gómez-Garreta, 1989; Rodríguez-Prieto et al., 2013).

*Cystoseira*, *Ericaria* and *Gongolaria* species can form dense canopies up to 1 m high that extend from exposed to sheltered conditions and from the upper infralitoral to the upper circalitoral zone (0 to more than 50 m depth) (Giaccone, 1973; Giaccone and Bruni, 1973; Ballesteros, 1988, 1990a, 1990b; Ballesteros et al., 2009; Sales et al., 2012). Despite showing smaller sizes, *Cystoseira s.l.* assemblages exhibit functional properties similar to those exhibited by kelps or larger fucoids dwelling in temperate oceans, and therefore, they give rise to habitats that can be compared to miniature underwater forests (Figure 2). *Cystoseira s.l.* forests are amongst the most productive habitats of the Mediterranean Sea (Ballesteros, 1989) and represent the highest level of the Mediterranean seaweed complexity (Ballesteros, 1988, 1989, 1990a, 1990b; Clayton, 1990). In fact, these



communities are spatially organized in 4 different strata: an encrusting basal layer mainly but not only formed by encrusting calcareous algae; a caespitose or turf layer formed by calcareous or soft sciaphilic algae; a shrubby layer of middle canopies formed by arborescent or shrub algae; and an arboreal layer of upper canopy made by *Cystoseira s.l.* (and occasionally *Sargassum* spp. or kelps) with their epiphytes (Giaccone, 1969; Ros et al., 1985; Templado et al., 2012). As a result, these communities are home to a large number of associated species, which in turn support numerous trophic networks and high biodiversity (Molinier, 1960; Boudouresque, 1972; Ballesteros, 1992; Ballesteros et al., 2009; Thiriet et al., 2016; Piazzini et al., 2018; Pinna et al., 2020).

*Cystoseira s.l.* forests are distributed along the entire infralittoral and upper circalittoral zones, but, different species dominate the canopy at various bathymetric levels, resulting in forests of different height and age (depending on the species and site) (Giaccone, 1973; Giaccone and Bruni, 1973; Sant and Ballesteros, 2021). Their vertical zonation mainly depends on light intensity but also, hydrodynamics, temperature, resistance to desiccation, nutrient availability, and grazing (Feldmann, 1937; Giaccone and Bruni, 1973; Ballesteros, 1989; Ballesteros and Zabala, 1993; Delgado et al., 1995; Vergés et al., 2009; Sant and Ballesteros, 2021). While only a few species of *Cystoseira s.l.* can thrive towards the upper circalittoral zone, as we get closer to the surface, more species become dominant in shallow protected or exposed habitats (Giaccone, 1973; Ballesteros, 1988, 1990a; Ballesteros et al., 1998; Hereu et al., 2008; Sales and Ballesteros, 2009). At a geographical scale, some species are very restricted in their spatial distribution, while others are distributed throughout the entire Mediterranean Sea (Cormaci et al., 1992; Ribera et al., 1992).

### **THREATS AND STATUS OF *CYSTOSEIRA S.L.* FORESTS**

In coastal areas of the Mediterranean human civilizations and natural environment have co-evolved over several millennia. Therefore, due to a long history of intense human

impacts and modifications, the Mediterranean coastal areas are nowadays among the most severely degraded systems worldwide (Lotze et al., 2011).

Like other seaweeds around the world, *Cystoseira s.l.* species are highly sensitive to human disturbances and their interactive effects across regional and local scales. Since the end of the 20th century, declines of *Cystoseira s.l.* forests have been widely reported in different areas of the Mediterranean Sea (Cormaci and Furnari, 1999; Thibaut et al., 2005, 2015; Bianchi et al., 2014; Mariani et al., 2019). Consequently, and considering their important role as canopy-forming species, all the Mediterranean species, but *C. compressa*, have been listed under the Annex II of the Barcelona Convention (UNEP/MAP, 2013) as species that are considered threatened or endangered and need protection measures.

Such declines have been mainly attributed to the increasing coastal urbanization which is responsible of changes in water quality (i.e. loads of sediments, nutrients or pollution) (Munda, 1974, 1982; Rodríguez-Prieto and Polo, 1996; Soltan et al., 2001; Airoidi, 2003; Arévalo et al., 2007; Mangialajo et al., 2008; Pinedo et al., 2015) and habitat destruction (Gros, 1978; Thibaut et al., 2005), but also to overgrazing by sea-urchins and invasive fish (Verlaque, 1987; Sala et al., 1998, 2011, 2012; Vergés et al., 2014b). However, although some efforts have been done to understand the direct effect of such stressors on *Cystoseira s.l.* species (Irving et al., 2009; de Caralt et al., 2020), in many cases, the direct causes of such declines are not well understood, being the interactive effects of local and global stressors the most likely drivers.

In places where these habitats have disappeared they tend to be substituted by simpler and less productive habitats that can better endure the novel stressful conditions, such as mussel beds, algal turf and barren grounds, hence impacting the provision of ecosystem functions and services (Bellan-Santini, 1965; Munda, 1993; Cormaci and Furnari, 1999; Mangialajo et al., 2008; Pinedo et al., 2013; Iveša et al., 2016).

Nonetheless, extinctions are not happening everywhere, and *Cystoseira s.l.* populations are outstanding in areas with low human pressures (Hereu et al., 2008; Sales and Ballesteros, 2009, 2010; Blanfuné et al., 2016, 2019; Thibaut et al., 2016a; Medrano et al., 2020b). However, whereas some populations have been shown to naturally recover after a population decline (e.g. Iveša et al., 2016; Ricart et al., 2018), usually this is not the case (Sales et al., 2011). Often, despite the implementation of conservation efforts, most degraded systems are not able to recover. *Cystoseira s.l.* species show a low dispersal capacity mainly because of the large size of their zygotes (100-120 µm) (Guern, 1962; Clayton, 1992; Capdevila et al., 2018b). Therefore, patchy distributions may represent a serious challenge for the natural recovery of the extinct populations, particularly if fragmented populations are spatially more distant than species' dispersal capacity. It is in these cases, when always subsequent to the identification and mitigation of the stressor, active restoration emerges as the only tool to recover these locally extinct populations.

In the Mediterranean, *Cystoseira s.l.* species restoration actions have been mainly focused on adult transplantation (Falace et al., 2006; Susini et al., 2007; Sales et al., 2011). Given the threatened or endangered status of most *Cystoseira s.l.* species, less invasive restoration techniques are required, such as recruitment enhancement without adult translocation (Gianni et al., 2013). Although obtaining recruits from fertile material has been explored for some furoid (Falace et al., 2018) no protocols have been yet provided for *Cystoseira s.l.* restoration actions.

On the other hand, the Mediterranean Sea is very sensitive to recent and future climate change (Giorgi, 2006; Belkin, 2009; Lejeusne et al., 2010). Mediterranean sea water temperatures are steadily increasing and are expected to exceed global rates by 25% (Lionello and Scarascia, 2018), while the Mediterranean is also very susceptible to MHWs (Hobday et al., 2016; Oliver et al., 2018), which are projected to intensify in frequency and severity in the coming decades (Darmaraki et al., 2019b, 2019a; Oliver et al., 2019). In this context, temperature-driven range shifts of several species (Francour

et al., 1994; Astraldi et al., 1995; Bianchi and Morri, 2003; Chevaldonné and Lejeusne, 2003; Lejeusne and Chevaldonné, 2005; Azzurro et al., 2019) and numerous mass mortality episodes of benthic invertebrates (Cerrano et al., 2000; Perez et al., 2000; Linares et al., 2005; Garrabou et al., 2009, 2019; Cebrian et al., 2011; Rivetti et al., 2014, 2017) have been reported during the last decades. Despite of that, and unlike other seaweed forests around the world, for which extensive and numerous losses have been directly or indirectly attributed to chronic or acute warming impacts (Serisawa et al., 2004; Wernberg et al., 2011, 2013; Tanaka et al., 2012; Smale and Wernberg, 2013; Voerman et al., 2013; Filbee-Dexter et al., 2016; Vergés et al., 2016; Smale, 2020), there are no studies that clearly relate warming impacts to regressions of *Cystoseira s.l.* populations in the Mediterranean Sea.

In this line, some studies suggest that high temperatures may significantly impact at least the early life-stages of *Cystoseira s.l.* or the production of chemical defences, under laboratory condition (Mannino et al., 2016; Capdevila et al., 2018a). However, the impacts of chronic and acute thermal stress and their interaction with other stressors on historically impacted and already fragmented *Cystoseira s.l.* populations and their associate communities are still uncertain.

Climate change impacts canopy-forming seaweeds at multiple life history stages (Helmuth et al., 2006b; Ladah and Zertuche-González, 2007; Harley et al., 2012; Andrews et al., 2014; Capdevila et al., 2018a; Savva et al., 2018) and represents a global threat for these species (see references above). Given the expected climate change scenarios for the Mediterranean Sea, and the current impaired status of most *Cystoseira s.l.* forests, it is of paramount importance to know which are the potential effects of thermal stress on *Cystoseira s.l.* populations, and to understand the potential role that local factors can play in the resulting *Cystoseira s.l.* declines under chronic warming and MHWs. This information will be key for stablishing pro-active and local-management plans, which have been identified as primordial to enhance ecosystem resistance and resilience to buffer future climate change impacts (O’Leary et al., 2017).

## OBJECTIVES

This thesis is focused on species of the genus *Cystoseira s.l.* inhabiting shallow and sheltered areas. We have selected shallow and sheltered environments mainly because they support a high diversity of *Cystoseira s.l.*, but also because human stressors, such as pollution or climate change, can be magnified due to their low water renewal. Thus, these environments can serve as sentinels to assess climate change impacts on *Cystoseira s.l.* populations (Vinagre et al., 2018). On the other hand, the fact that these areas have been a target for the development of human, and often polluting, infrastructures (e.g. harbours, marinas, cities), has led to the disappearance and degradation of most *Cystoseira s.l.* forests growing on these environments. Only in some cases, in which the driver of extinction has been mitigated or removed, these environments may represent suitable areas for undertaking restoration plans.

The extensive knowledge of the research team in which this thesis has been carried out, e.g. on the biology, ecology, diversity, distribution and state of conservation of *Cystoseira s.l.* forests (Ballesteros, 1989, 1990b, 1990a, 1992; Ballesteros et al., 1998, 2007, 2009, 2014; Verlaque et al., 1999; Sant, 2003; Sales and Ballesteros, 2010, 2012, 2009; Sales et al., 2011, 2012; Cefali, 2018), has served as a base to develop it. Based on this knowledge, the most suitable study areas, species and populations have been identified and selected to answer and solve the questions raised in the different objectives. The Catalan coast, the coast of Menorca (Balearic Islands), and different localities throughout the Mediterranean Sea, have been the study areas, while *Ericaria crinita* and *Gongolaria barbata* (previously *Cystoseira crinita* and *C. barbata*) have been selected as target species (Box 1).

This thesis raises two main objectives. First, we aim at understanding how climate change may affect *Cystoseira s.l.* populations. More specifically, we determine the effects of warming on *Cystoseira s.l.* individuals and we try to elucidate how local factors and intraspecific variability may modulate population responses to climate change. The

second goal, is to design and experimentally test two non-invasive restoration techniques to re-establish self-sustaining *Cystoseira s.l.* populations.

This thesis is structured in three chapters. **Chapter 1** and **2** are focused on studying the effects of climate change on *Cystoseira s.l.* populations, while **Chapter 3** is focused on restoration methods. The detailed specific objectives of the different chapters are:

**Chapter 1** tests the direct effects of warming on different life-stages of *Ericaria crinita* at the individual-level. In parallel, it tries to determine between-site variability in relation to the intensity of climate-driven stressors, specifically a marine heatwave (MHW). We study the dynamics of *Ericaria crinita* populations that were differently affected by a regional MHW in summer 2015. The field observations are combined with the thermo-tolerance experiment to elucidate mechanisms underlying the variation in population-level responses to warming.

After having tested the negative implications of warming on *E. crinita* populations, as well as the importance of local-scale variability, the main goal of **Chapter 2** is to accurately predict the vulnerability of *E. crinita* to future warming trends considering the intraspecific responses to warming. In order to achieve this final objective, we first experimentally assess the intraspecific variability to thermal stress of *E. crinita* populations living under different temperature regimes and across the entire range of the species distribution. Then, the experimental information is combined with modelling to predict the vulnerability of *E. crinita* under the projected RCP 8.5 scenario for 2050.

Finally, given the impaired conservational status of most *Cystoseira s.l.* species throughout the Mediterranean Sea and considering their important ecological role, in **Chapter 3** we propose two non-invasive restoration techniques based on recruitment enhancement. The two techniques are applied at two different sites with the final goal of restoring a population of *Gongolaria barbata* (= *Cystoseira barbata*), which was unable to recover naturally even 30 years after the removal of the pressure which brought the

population to become extinct. We also establish a midterm-monitoring program on restored and reference populations aiming at defining optimal and feasible indicators of restoration success.

The work in the different chapters combines field observations (**Chapter 1** and **3**), field experiments (**Chapter 3**), controlled experiments in the laboratory (**Chapter 1, 2** and **3**) and species distribution modelling (**Chapter 2**). The different specific objectives of each chapter are summarised in Figure 3.

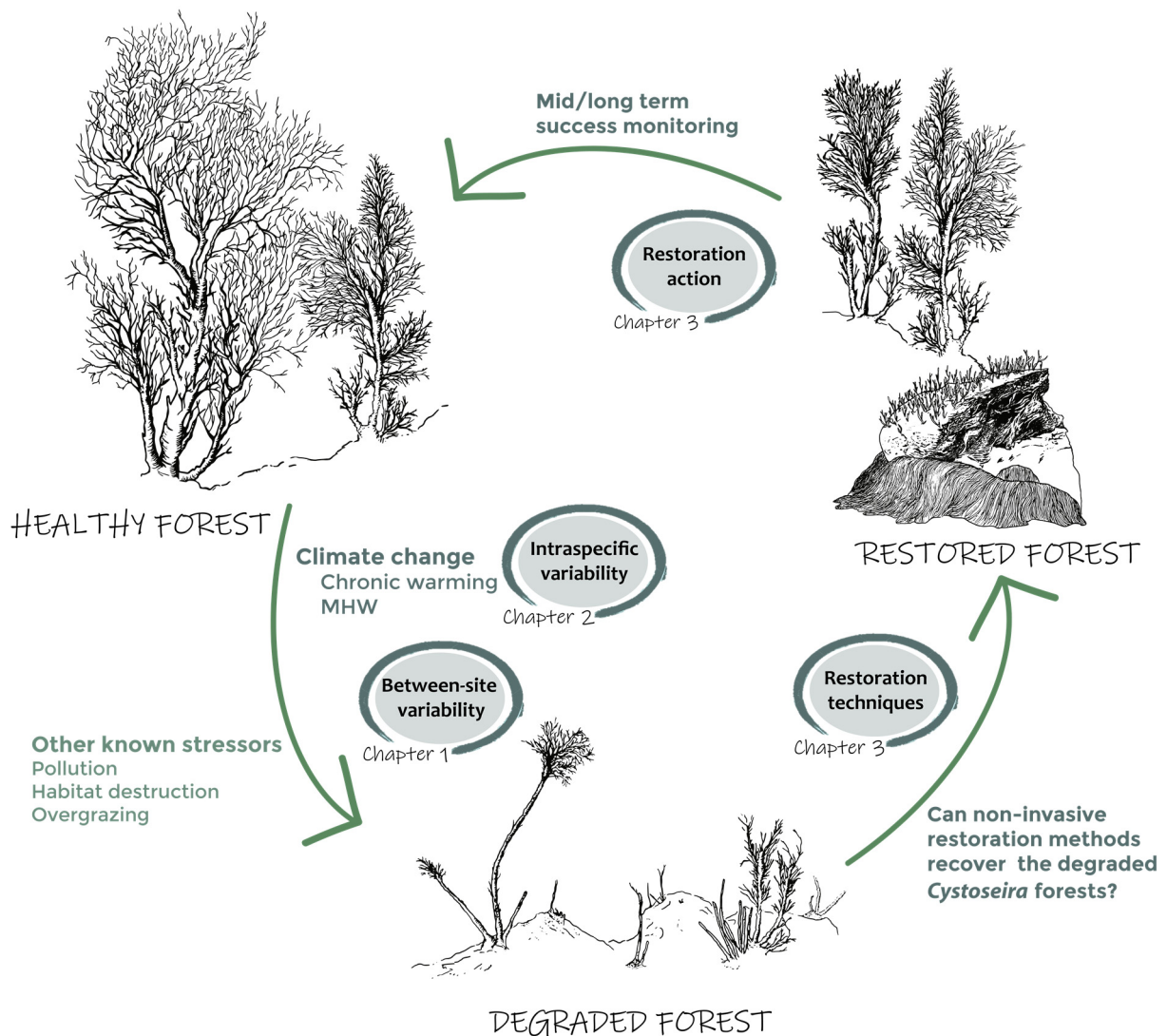


Figure 3 | Diagram of the specific objectives of the different chapters.

### BOX 1: Target Species

*Ericaria crinita* (Duby) Molinari-Novoa & Guiry (= *Cystoseira crinita* Duby) (a) is a perennial seaweed formed by a discoid base from which several erect axes grow (Cormaci et al., 2012) making a dense canopy of up to 30 cm. Apex of the axes are prominent and covered with spines. Tophules and aerocysts are absent. Primary branches are cylindrical, with small spines at the base. Secondary and tertiary branches are also cylindrical, very thin and normally without spinose appendages. Reproductive structures can be present all year around but are better developed and more abundant from April to November (Gómez-Garreta et al., 2000; Rodríguez-Prieto et al., 2013; Silva, 2019). The growth pattern is seasonal as described in Ballesteros, (1992) and Sales and Ballesteros, (2012). It is distributed throughout the entire Mediterranean Sea (Ribera et al., 1992; Sales et al., 2012), where it is mainly restricted to the upper sublittoral zone in relatively sheltered and well-illuminated environments (Molinier, 1960; Sales and Ballesteros, 2009, 2010, 2012).

*Gongolaria barbata* (Stackhouse) Kuentze [= *Cystoseira barbata* (Stackhouse) C. Agardh] (b) is a perennial seaweed with a ramified monopodial cauloid, attached to the substratum by a circular disc. The apex of the axes is smooth and very prominent, and tophules are absent (Cormaci et al., 2012). Small aerocysts are arranged in chains, often near the receptacles. Primary branches are well-developed, especially in spring. Fertile plants are present in all seasons of the year, although they are more abundant in spring and early summer (Gómez-Garreta et al., 2000; Falace and Bressan, 2006; Rodríguez-Prieto et al., 2013). It typically develops in shallow and sheltered environments across the Mediterranean Sea, (Sales and Ballesteros, 2009). However, in the Spanish coast the species is currently only known from Menorca.

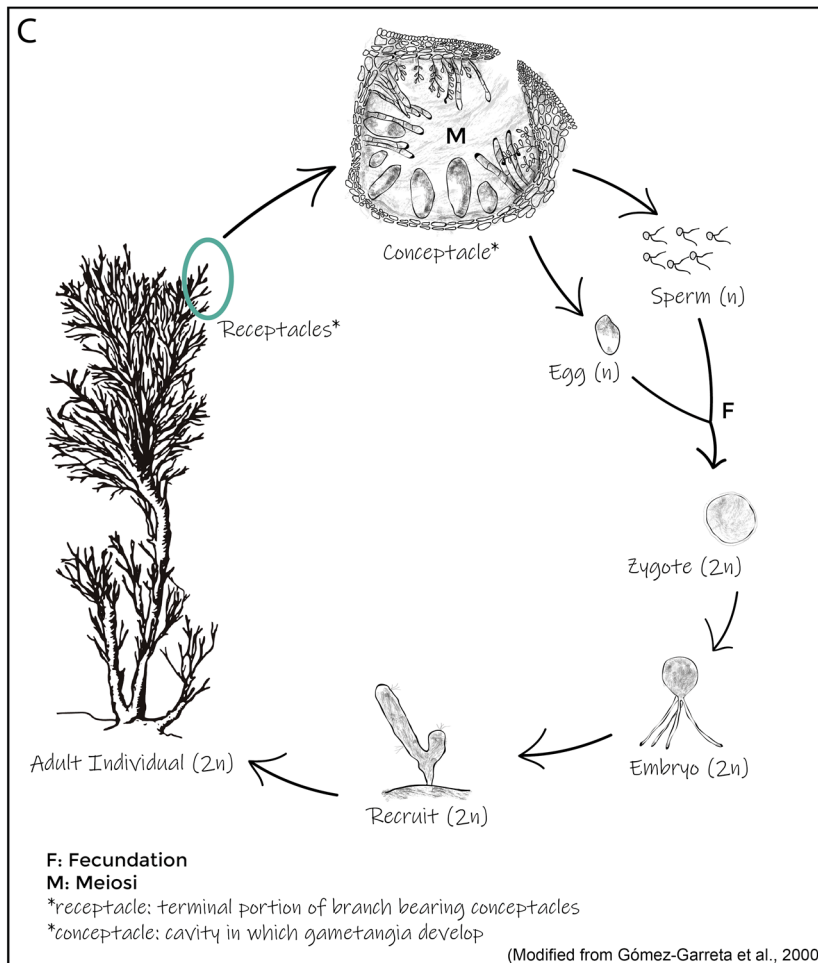
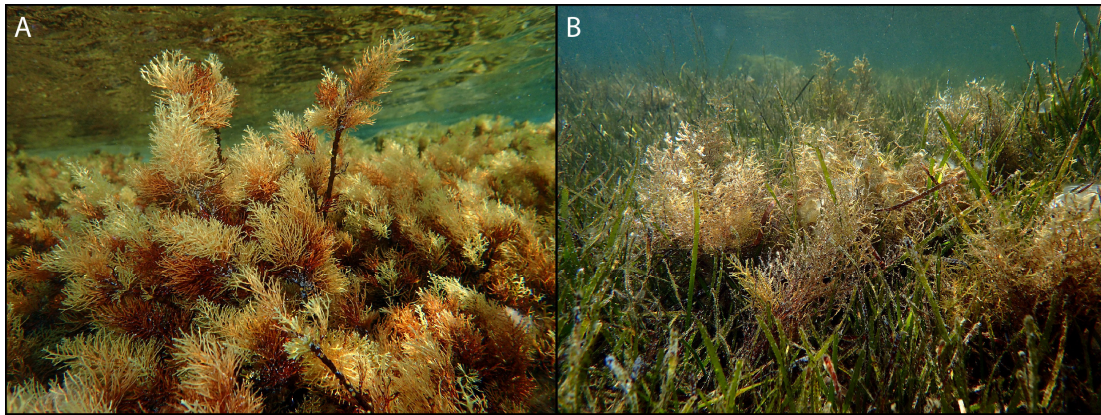
Both species are endemic from the Mediterranean sea, classified as threatened under the Barcelona Convention (UNEP/MAP, 2013). The reduction of its distribution is strongly correlated with human development (Thibaut et al., 2005, 2015; Blanfuné et al., 2016; Mariani et al., 2019).

**Life cycle and reproduction of *Cystoseira s.l.* species (c).** *Cystoseira s.l.* species present a sexual reproduction from monoecious individuals, with male and female gametes housed within the same conceptacle, which are grouped in receptacles (Guern, 1962; Rodríguez-Prieto et al., 2013). Reproduction is oogamic, with large non-motile eggs and biflagellate sperm (Gómez-Garreta et al., 2000). Fertilization is external, giving place to a large and free-



living zygote (~70–130  $\mu\text{m}$ ) which rapidly sinks to the bottom where it is fixed during the first 12–72 hours (Gros, 1978; Orfila, 2014; Falace et al., 2018).

The life cycle of *Cystoseira s.l.* species is monophasic diploid. The meiosis takes place during the formation of gametes ( $n$ ) and the zygote ( $2n$ ) develops into the only diploid vegetative phase of the life cycle (Gómez-Garreta et al., 2000; Rodríguez-Prieto et al., 2013).





**Local-scale climatic refugia offer sanctuary for a habitat-forming species during a marine heatwave.**



**Jana Verdura**, Jorge Santamaría, Enric Ballesteros, Dan A. Smale, Maria Elena Cefali, Raül Golo, Sònia de Caralt, Alba Vergés, Emma Cebrian (2021). Local-scale climatic refugia offer sanctuary for a habitat-forming species during a marine heatwave. *Journal of Ecology* 109 (4): 1758-1773. doi: 10.1111/1365-2745.13599.

<https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2745.13599>

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## Abstract

1. Gradual climate change and discrete extreme climatic events have driven shifts in the structure of populations and the distribution of species in many marine ecosystems. The most profound impacts of recent warming trends have been generally observed at species' warm edges and on large conspicuous species. However, given that different species and populations exhibit different responses to warming, and that responses are highly variable at regional scales, there is a need to broaden the evidence to include less conspicuous species and to focus on both local and regional scale processes.

2. We examined the population dynamics of canopy-forming seaweed populations situated at the core range of their distribution during a regional marine heatwave (MHW) event that occurred in the Mediterranean Sea in 2015, to determine between-site variability in relation to the intensity of the MHW. We combined field observations with a thermo-tolerance experiment to elucidate mechanisms underlying observed responses.

3. Despite our study populations are located in the species core range, the MHW was concomitant with a high mortality and structural shifts in only one of the two surveyed populations, most likely due to differences in habitat characteristics between sites (e.g. degree of shelter and seawater transfer). The experiment showed high mortalities at temperatures of 28 °C, having the most severe implications for early life stages and fertility, which is consistent with warming being the cause of population changes in the

field. Crucially, the regional-scale quantification of the MHW (as described by satellite-derived SSTs) did not capture local-scale variation in MHW conditions at the study sites, which likely explained variation in population-level responses to warming.

4. *Synthesis*. Enclosed and semi-enclosed seas, such as the Mediterranean Sea, often highly impacted by human perturbations, are also global hotspots for ocean warming and are highly susceptible to future MHWs. Our findings highlight that local-scale variability in the magnitude of extreme climatic events can lead to local extinctions of already fragmented populations of habitat-forming seaweeds, even towards the species' core range. However, our results highlight the potential for local-scale climatic refugia, which could be identified and managed to safeguard the persistence of canopy-forming seaweeds.

**Keywords:** canopy-forming seaweeds, *Carpodesmia*, climate change, climatic refugia, coastal ecosystems, *Cystoseira*, *Ericaria*, marine heatwaves.

**Intraspecific variability in  
thermal tolerance shapes future  
declines in macroalgal forests.**



**Jana Verdura**, Jorge Santamaría, Raül Golo, Enric Ballesteros, Joaquim Garrabou, Simonetta Frascetti, Luisa Mangialajo, Ljiljana Ivesa, Simone Farina, Emma Cebrian. Intraspecific variability in thermal tolerance shapes future declines in macroalgal forests. Under revision in *Global Change Biology*.

The sections and distribution of the chapter are presented as established by the journal's editorial.

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## Abstract

In response to anthropogenic climate change, the geographic distributions of many species are shifting. Accurate forecasting of species responses to warming is of particular importance in marine ecosystems, where temperature plays a central role in shaping species distribution. Environmental conditions and biological processes, such as physiological plasticity and/or local adaptation, may result in diverse intraspecific responses to thermal stress. However, most models assume that species responses are homogeneous throughout their distributional range, and the responses of whole populations to warming remain poorly understood. In this study, we aim to predict the vulnerability of a canopy-forming seaweed, *Ericaria crinita*, to future warming trends throughout the Mediterranean Sea, while taking into consideration the regional, intraspecific variability in thermal tolerance found in this species. First, we experimentally assess the thermo-tolerance response of individuals taken from eight populations in areas with different temperature regimes, covering an area of about 2000 km. Then, this information is combined with a modelling approach to accurately predict the vulnerability of this species under the RCP 8.5 scenario for 2050 across its entire geographical distribution. Our results show a clear intraspecific variability in the temperature tolerance thresholds among populations, with populations from the coldest zones of the species' distribution being those that have the lowest temperature tolerance thresholds. Predictive modelling tends to suggest that future warming will lead to similar levels of vulnerability among populations regardless of their geographical distribution.

Our results indicate, however, that disregarding the intraspecific variability in temperature tolerance may result in an under- or overestimation of species vulnerability to future warming at the regional scale, thus highlighting the importance of fine-scale data in efforts to obtain more realistic predictions of species responses.

**Keywords:** intraspecific variability, thermal tolerance, macroalgal forests, climate change, species distribution modelling, environmental variability, fucales, *Cystoseira*.

## INTRODUCTION

Current anthropogenic climate change is eroding ecosystem resilience and driving a redistribution of species on a global-scale (Parmesan and Yohe, 2003; Chen et al., 2011; Lenoir and Svenning, 2015; Poloczanska et al., 2016). As climate changes, species either shift their distribution to track optimal environmental conditions through migration or colonization, else they persist *in situ* through phenotypic acclimatization and evolutionary adaptation or in the absence of either mechanism, they become locally extinct (O' Connor et al., 2012). Geographic range shifts in response to chronic and acute climate-driven stressors have already been reported and more are predicted to occur in a large number of marine and terrestrial species (Chevaldonné and Lejeune, 2003; Parmesan, 2006; Garrabou et al., 2009; Carvalho et al., 2010; Chen et al., 2011; Urban, 2015; Wiens, 2016; Martínez et al., 2018; Smale, 2020). Such changes may disrupt species composition and structure, as well as interactions between species (e.g. prey-predator asynchrony in space, loss of structural species), thus triggering cascading effects that have far-reaching implications at the community or ecosystem-level (Wernberg et al., 2013; Vergés et al., 2014a, 2019) with important ecological and socio-economic consequences (Pecl et al., 2017).

In terrestrial ecosystems, the geographic ranges of species appear to be highly complex and driven by a large number of factors, e.g., temperature, precipitation, moisture, solar irradiance, biotic interactions, etc. (Badik et al., 2015; Fei et al., 2017; Advani et al., 2019). In marine ecosystems, although they are far less studied, it is known that temperature plays a central role in shaping species distribution patterns (Miranda, 1931; Niell, 1977; van den Hoek, 1982; Yarish et al., 1986; van den Hoek et al., 1990). Population extinctions and declines due to warming have been reported twice as frequently in the ocean as on land (Pinsky et al., 2019) and numerous marine species have shifted their distribution ranges in response to chronic and acute warming impacts (Wernberg et al., 2011; Somero, 2012; Poloczanska et al., 2013; Wiens, 2016). Such warming impacts are

of particular concern when they affect habitat-forming species, whose disappearance may cause the decline or collapse of the entire ecosystem they support (Smale and Wernberg, 2013; Wernberg et al., 2013, 2016; Hoegh-Guldberg et al., 2017; Hughes et al., 2017; Smale, 2020). Therefore, considering the current and future trends of climate change, the early detection of climate change hotspots or refuge areas for species and populations, and the early prediction of potential species range shifts, have become urgent priorities in efforts to effectively and proactively guide the management and preservation of biodiversity (Carvalho et al., 2010; Urban, 2015).

A species distribution model (SDM) correlates current environmental conditions with distributional records of species to produce predictive maps of habitat suitability and allow projections to be made of a species' response to environmental changes (Pearson and Dawson, 2003). These tools have been widely used to forecast the potential consequences of climate change on the distribution of species (Araújo et al., 2006; Urban, 2015), to predict changes in geographical patterns of biodiversity (Wohlgemuth et al., 2008; Pineda and Lobo, 2009) and the spread and risk of invasive species (Beaumont et al., 2009; Herborg et al., 2009; Padalia et al., 2014), and to design and support management and conservation strategies (Early et al., 2008; Carvalho et al., 2010; Embling et al., 2010; Guisan et al., 2013). Recently, alternative strategies (e.g. hybrid SDMs) that correlate SDMs with biological knowledge (such as physiological limits obtained, for example, from experiments simulating stress conditions) are gaining recognition, since the robustness of the predictions may be significantly increased (Kearney and Porter, 2009; Buckley et al., 2010). Nevertheless, their use for forecasting marine species responses to climate change is still not very common (but see, Franco et al., 2018; Martínez et al., 2015; Rodríguez et al., 2019). Additionally, there is growing evidence that past and present thermal variation across a species' distributional range may favour intraspecific variation in temperature thresholds by means of biological processes, such as physiological plasticity and/or local adaptation (Genner et al., 2004). In fact, both processes are commonly found in natural populations (Stillman, 2003;

Kawecki and Ebert, 2004; Howells et al., 2011, 2013; Sanford and Kelly, 2011), and their role in shaping populations' thermal responses and species distribution patterns has been demonstrated for several species (Valladares et al., 2014), in particular, for sessile organisms with limited dispersal capacity, such as canopy-forming macroalgae (King et al., 2018 and references therein). Intraspecific tolerance thresholds are beginning to be included in studies in terrestrial ecosystems to predict species range shifts (Valladares et al., 2014; Benito-Garzón et al., 2019; Peterson et al., 2019; Razgour et al., 2019). However, in marine ecosystems, species have largely been treated as homogenous units. This has led to an assumption of uniform responses throughout the distribution ranges of such species (Sunday et al., 2012; Martínez et al., 2015; Franco et al., 2018; Rodríguez et al., 2019), which may have resulted in biases in the prediction outputs (Valladares et al., 2014; Benito-Garzón et al., 2019). In this context, what is required to accurately assess species responses' to climate change are studies that can link intraspecific thermal divergences and future warming scenarios (King et al., 2018; Benito-Garzón et al., 2019).

Long-term increases in temperature and recurrent marine heatwaves (MHWs) have led to numerous population declines or extinctions, and range shifts, involving many canopy-forming macroalgae around the world (Wernberg et al., 2011; Díez et al., 2012; Tanaka et al., 2012; Voerman et al., 2013; Witman and Lamb, 2018; Casado-Amezúa et al., 2019; Thomsen et al., 2019; Smale, 2020; Wernberg, 2021). Canopy-forming seaweeds such as fucoids and kelps are the main foundation species of shallow rocky bottoms in temperate, subpolar and polar regions, where they play a fundamental role in the structure and functioning of coastal habitats (Giaccone and Bruni, 1973; Steneck et al., 2002; Schiel and Foster, 2006; Wernberg and Filbee-Dexter, 2019). In the Mediterranean Sea, seawater temperatures are steadily increasing and are expected to exceed global rates by 25% (Lionello and Scarascia, 2018), while MHWs have been projected to intensify in the coming decades (Darmaraki et al., 2019a, 2019b). In this context, climate warming has also emerged as a real threat for Mediterranean canopy-

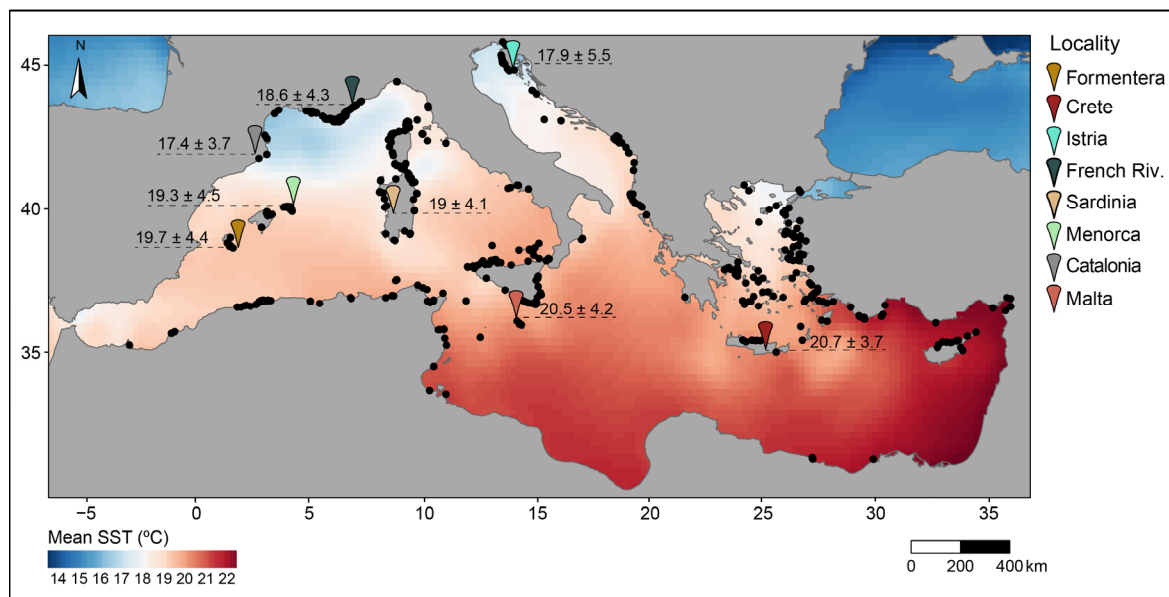
forming species (Verdura et al., 2021). However, very few studies have attempted to understand the responses of these ecosystems to the projected scenarios of climate change. As is the case with many other macroalgae, most Mediterranean canopy-forming species are widely distributed across different thermal regions, which suggests some degree of intraspecific thermal divergence – a common pattern in marine macrophytes (King *et al.*, 2018). As mentioned above, such divergence needs to be taken into consideration in order to obtain an accurate understanding of future trends.

In this context, our objective was two-fold: i) to predict the vulnerability of a canopy-forming species to future warming trends, while accounting for the intraspecific variability in thermal tolerance across regions, and ii) to highlight the implications of neglecting such variability. We combine laboratory experiments that test intraspecific variability to thermal stress across the entire range of species distribution, together with a modelling approach. Our target species is the canopy-forming alga, *Ericaria crinita*, which is distributed throughout the Mediterranean Sea and thrives under different thermal regimes. To do so, first we characterize local adjustments to thermal stress by experimentally assessing the thermo-tolerance response of individuals from eight different populations living under different temperature regimes, across the entire range of the species distribution. We hypothesize that the populations from the coldest locations are less thermo-tolerant than the populations situated in the warmest locations. Then, the physiological information gathered is used to forecast the species' vulnerability under the RCP 8.5 scenario, for 2050. Finally, to highlight the importance of using local thermal adjustments when forecasting species responses to climate change, projection outputs across the entire distribution range of the species that consider either a homogeneous thermal response or the local thermal adjustments, are compared.

## MATERIALS AND METHODS

### Target species

*Ericaria crinita* (Duby) Molinari-Novoa & Guiry (= *Cystoseira crinita* Duby) is a canopy-forming macroalga of the order of Fucales, which can measure up to 40 cm. Its life cycle is monophasic diploid (Rodríguez-Prieto et al., 2013). Forests dominated by *E. crinita* mainly develop in the upper sublittoral zone, on shallow, well-illuminated and sheltered rocky reefs (Ballesteros, 1992; Gómez-Garreta et al., 2000; Rodríguez-Prieto et al., 2013). *Ericaria crinita* has been selected because it is widespread in the Mediterranean Sea (Fig. 1) and because there is previous evidence of warming effects on this species (Verdura et al., 2021).



**Figure 1** | Annual mean sea surface temperature (SST in °C) in the Mediterranean Sea (1982-2019 period, data from NOAA). Black dots show the current records of *Ericaria crinita* throughout the Mediterranean Sea. The location of target *E. crinita* populations is indicated on the map and the average SST (annual mean ± SD; 2000-2019 period) is detailed for each location.

### **Temperature effects and the role of local thermal regimes on *Ericaria crinita* survival**

#### *Study site and algal collection*

The Mediterranean Sea is the largest semi-enclosed sea on Earth (Boudouresque, 2004) and presents a marked biogeographic and oceanographic heterogeneity (Bethoux, 1979; Bosc et al., 2004; Bas, 2009), in which thirteen biogeographical sectors have been recognized (Pérès and Picard, 1964; Bianchi and Morri, 2000). To test whether populations from different thermal regimes may respond differently to thermal stress, eight populations of *E. crinita* were selected from localities presenting different historical thermal regimes and separated by hundreds of kilometres (Figure 1; Supporting Information Table S1). Crete, Malta and Formentera were selected as warmer areas; Menorca, and Sardinia as mild thermal regimes at the core range of the distribution; and Catalonia, Istria and the French Riviera as colder areas.

Twenty individuals were collected (together with the holdfast) at each locality between June and early July 2018. After collection, the individuals were carefully cleaned to remove macro-epiphytes and/or organic matter and were kept in plastic bags in cold and dark conditions at 100% humidity and placed in isothermal boxes with cold storage accumulators for their transport to the laboratory facilities at the *Centre d'Estudis Avançats* in Blanes (CEAB-CSIC, Spain). All samples arrived in perfect health at the facilities between 24-36 hours after collection. Once in the laboratory, individuals were gently washed and placed in acclimation tanks at 22 °C with circulating natural seawater and maintained at this temperature until the beginning of the experiment.

#### *Experimental design*

Adult individuals from all the selected populations were subjected to 4 different temperature conditions (26°C, 27°C, 28°C and 29°C). The two lowest temperatures are commonly reached at present during summer periods, while the highest temperatures were chosen to represent the extreme conditions that the Mediterranean Sea might experience in the coming decades, especially in the warmer areas. Darmaraki et al.



(2019b) indicates an average increase in the Mediterranean SST of 3°C and temperature anomalies (SST<sub>99Q</sub>) of 4°C by 2100 under the RCP 8.5 scenario. Each temperature treatment consisted of 3 independent sets of eight aquariums (8 L) [4 temperatures x 3 sets (replicates) x 8 aquariums; Supporting Information Figure S1] and each set was connected to a buffer tank. Temperature conditions were achieved by heating the seawater in the buffer tank by means of water temperature controllers (Teco TK 500) from which water was pumped continuously into the aquariums. One individual from each of the 8 populations was randomly placed in an individual aquarium of each set. Initially, temperature was raised by 1°C per day, beginning at 22°C, until the target temperature conditions were reached and at that moment the experiment began. Photoperiod was variable, coinciding with the natural light conditions during the season in which the experiment took place. The experiment lasted for 3 months, and was conducted from mid-July to mid-October 2018.

We tested the effects of thermal stress on individual performance by means of biomass variation. Each specimen was periodically weighed, once every 7 or 10 days, resulting in a total of 14 sampling times. Prior to weighing, specimens were partially dried using filter paper to standardize wet weight measurements and minimize weight variation due to seawater. Biomass variation at each sampled time (BV<sub>t</sub>), considered as percentage of biomass lost, was calculated with the following formula:

$$BV_t = \left( \frac{W_t - W_b}{W_b} \right) \times 100$$

where  $W_t$  is the weight of the specimen at the sampling time “t” and  $W_b$  is the weight of the specimen at the beginning of the experiment (time 0).

### *Statistical analysis*

To test whether populations collected from the 8 different localities responded differently to thermal stress, we fitted a Linear Mixed Model (LMM) with *temperature* and *locality* as fixed factors and *time* as a crossed random factor. A second random term,

*the identity of each individual* as nested factor within *aquarium set*, was fitted in order to address: i) the lack of independence between repeated measurements of the same individuals over time, and ii) the individuals grouped within an aquarium set. To complete the analysis, and with the aim of comparing the response of the different populations in each temperature condition at the conclusion of the experiment, a LMM with *locality* as a fixed factor and *aquarium set* as a random factor was fitted for each temperature condition with only the final (i.e., end-of-experiment) values of weight loss being considered. To determine the effect of fixed factors, Type II Wald  $X^2$  tests were applied to each fitted model. Finally, for those fixed factors presenting significant effects, Tukey post-hoc tests were used for multiple comparisons.

All the analyses were conducted in the statistical environment R (R Core Team, 2019). Models were fitted using the “lmer” function from the *lme4* package. The p-values were obtained by means of Wald  $X^2$  tests using the “Anova” function from the *car* package (Fox and Weisberg, 2019) and the function “lsmeans” from the *lsmeans* package (Lenth, 2016) was used to perform post-hoc Tukey tests. All graphics were generated with the *ggplot2* package (Wickham, 2016).

### **Species distribution models**

#### *Georeferenced data for *Ericaria crinita**

Presence and absence records for *Ericaria crinita* were gathered from a number of sources: i) academic literature from 1980 onwards, found at the ISI Web of Knowledge and Google Scholar by using the key words: Mediterranean seaweeds, *Cystoseira crinita* and *Carpodesmia crinita* (for all references, see Supporting Information Annex S1); ii) the online databases Global Biodiversity Information Facility (GBIF, <https://www.gbif.org/>) and Ocean Biodiversity Information System (OBIS, <https://obis.org/>); and iii) personal communications with M. Sales, M. Orlando-Bonaca, A. Badreddine, S. Pinedo, L. Bahbah, S. Beqiraj and D. Serio, along with the authors’ own knowledge from field observations. A total of 774 presences and 4921 absences

where compiled and georeferenced onto a map using the function “*qplot*” in R, and Google Earth was then used to check and correct any erroneous locations (Supporting Information Figure S2). The compilation included the whole Mediterranean Sea and was restricted to bathymetric depths shallower than 10 m, thus accounting for the depth ranges usually reported for this species.

### *Predictor variables selection*

A total of 20 raster data grids of ocean surface environmental data were downloaded from different sources following indications of the importance of predictor variables in Fabbrizzi *et al.* (2020). Most of these variables were downloaded from the global marine repository, Bio-ORACLE (<https://www.bio-oracle.org/>) (Assis *et al.*, 2017b; Tyberghein *et al.*, 2012), whereas substrate data was downloaded from the EMODNET Seabed Habitats portal in the form of a Spatial Polygons Data frame (<https://www.emodnet-seabedhabitats.eu>) which was then transformed into raster data using the “*raster*” function from the package *raster* (Hijmans *et al.*, 2015) in R. The *Distance-to-Port* raster for the Mediterranean Sea was created following the script: R-port-distance-calculator, available in [Github.com/tsunghao-huang](https://github.com/tsunghao-huang). All variables were rescaled to 0.05 decimal degrees, restricted to the area of study and, when necessary, re-projected to the WGS84 datum. To select the variables, analysis of both the Pearson correlation and the Variance Inflation Factor (VIF) were performed. When two or more variables were auto-correlated ( $r^2 > 0.70$ ) and/or the VIF was higher than 5, the most relevant predictor was selected, taking into account the importance of the predictors for the biogeography of the target species (Lüning, 1990). Also, when possible, maximum and minimum values were preferred to the mean values due to their higher physiological influence (Martínez *et al.*, 2015) (See variables in Table 1).

### *Habitat suitability*

Since presence-absence data was available, we were able to build species distribution models using the *BIOMOD2* package for R (Thuiller *et al.*, 2009).

**Table 1** | Predictor variables initially considered for the model, with their source and abbreviated name. The selected predictor variables included in the SDM are highlighted in bold.

Name	Source	Abbreviated name
1 <b>Calcite concentration</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	Calcite
2 <b>Current velocity</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	CurrentVel
3 <b>Distance to port</b>	Calculated based on: R-port-distance-calculator	Port_Dist
4 <b>Distance to shore</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	DistSh
5 <b>Light diffusion</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	Difus
6 <b>Maximum sea surface temperature</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	TempMaxMonthAvg
7 <b>Mean sea surface temperature</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	TempRange
8 <b>Nitrate concentration</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	Nitrate
9 <b>Phosphate concentration</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	Ppmean
10 <b>Slope of the seafloor</b>	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	Slope
11 <b>Substrate type</b>	EMODNET geology portal	Substrate
12 Chlorophyll mean concentration	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	Chlorophyll
13 Dissolved Oxygen mean concentration	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	DisOx
14 Surface pH	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	pH
15 Maximum Primary production	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	MaxPP
16 Mean Primary production	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	MeanPP
17 Mean surface Salinity	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	SST
18 Mean surface silicate	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	Silicate
19 Average maximum month temperatures	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	AvgMaxMonth
20 Temperature range	<a href="https://www.bio-oracle.org/">Bio-ORACLE repository (https://www.bio-oracle.org/)</a>	RangeTemp

Additionally, to improve model performance, three groups of pseudo-absences were generated by using a 5-km circle-shaped buffer around the presences. These pseudo-absences were then mixed with the real absences to construct the models. For the model construction, 4 algorithms were used: Generalized Linear Models (GLM), Gradient Boosting Machines (GBM), Classification Tree Analysis (CTA) and Surface Range Envelop (SRE) and ten cycles were performed with each of them. 70% of the data was used for model training and 30% for model evaluation. The performance of the models was evaluated with the predicted area under the curve (AUC) of the receiver operating characteristic (ROC) plot (Fielding and Bell, 1997; Manel et al., 2001; Thuiller et al., 2005). Only models with a high predictive accuracy ( $AUC > 0.7$ ) were assembled to build the habitat suitability prediction (Swets, 1988). The results of the species distribution model were transformed from habitat suitability indexes into binary maps (presence/absence or suitable/unsuitable) using the optimal threshold that maximizes the ROC scores as a cut-off value (Cantor et al., 1999; Liu et al., 2005).

### *Projections*

For the projections, we selected, for the year 2050, the most pessimistic of the four Representative Concentration Pathways – RCP 8.5 – which assumes a substantial increase in emissions until the end of this century. The corresponding future monthly maximum averaged SST values were downloaded from the Bio-ORACLE online repository (<https://www.bio-oracle.org/>).

We then built an accurate prediction of the vulnerability of *E. crinita* to future warming trends that takes intraspecific variability into account. The Mediterranean Sea was divided into eight different geographic regions according to the temperature conditions representative of each area. One reference population, represented by those that were studied in the experiment, was attributed to each region, to establish the response to warming conditions (see Figure 5 of results section). Then, we projected the vulnerability of the species within each region using the thermal response of the

respective reference population for each region. To do so, the thermal response of each population (LMM predictions) was individually combined with the raster of forecasted temperature conditions for its corresponding region. Afterwards, in order to restrict the projected distribution of *E. crinita* to those areas presenting suitable environmental features for the presence of the species, the previously created suitability map (see section 3.3) was used as a mask. The final map of the future thermal vulnerability of *E. crinita* across the whole Mediterranean Sea was obtained by integrating the vulnerability maps from each region.

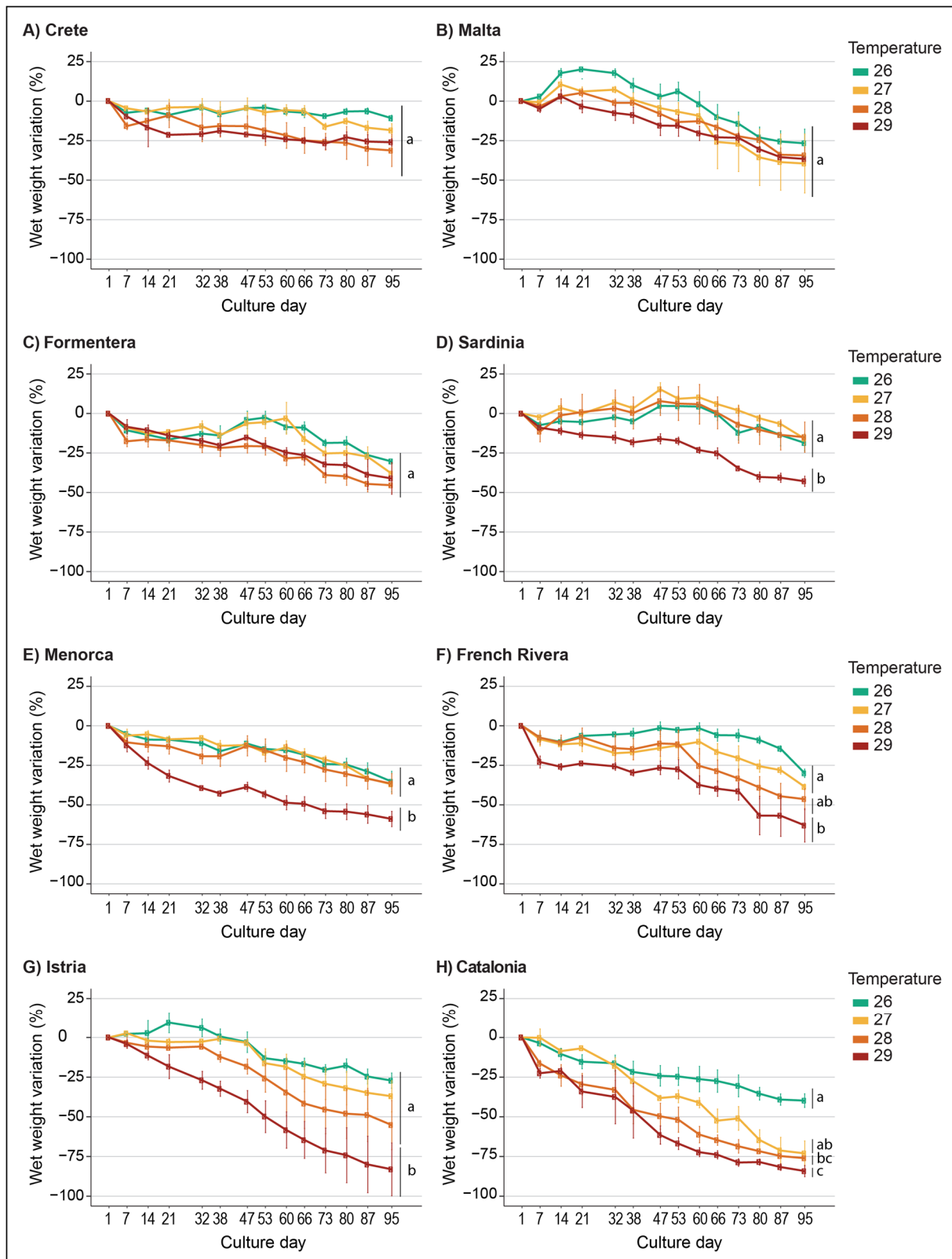
Finally, in order to highlight the importance of the assumed thermal response of a given species when predicting future scenarios, we built eight different vulnerability maps. Each map was built assuming that the thermal response of each of the studied populations was a homogenous response across the entire distribution range of the species. Vulnerability maps were obtained using the same procedure detailed above.

For the predictive vulnerability maps, different degrees of vulnerability were established according to the implications that the biomass loss during the experiment had on the specimens.

## RESULTS

### **Temperature effects and the role of local thermal regimes on *Ericaria crinita* survival**

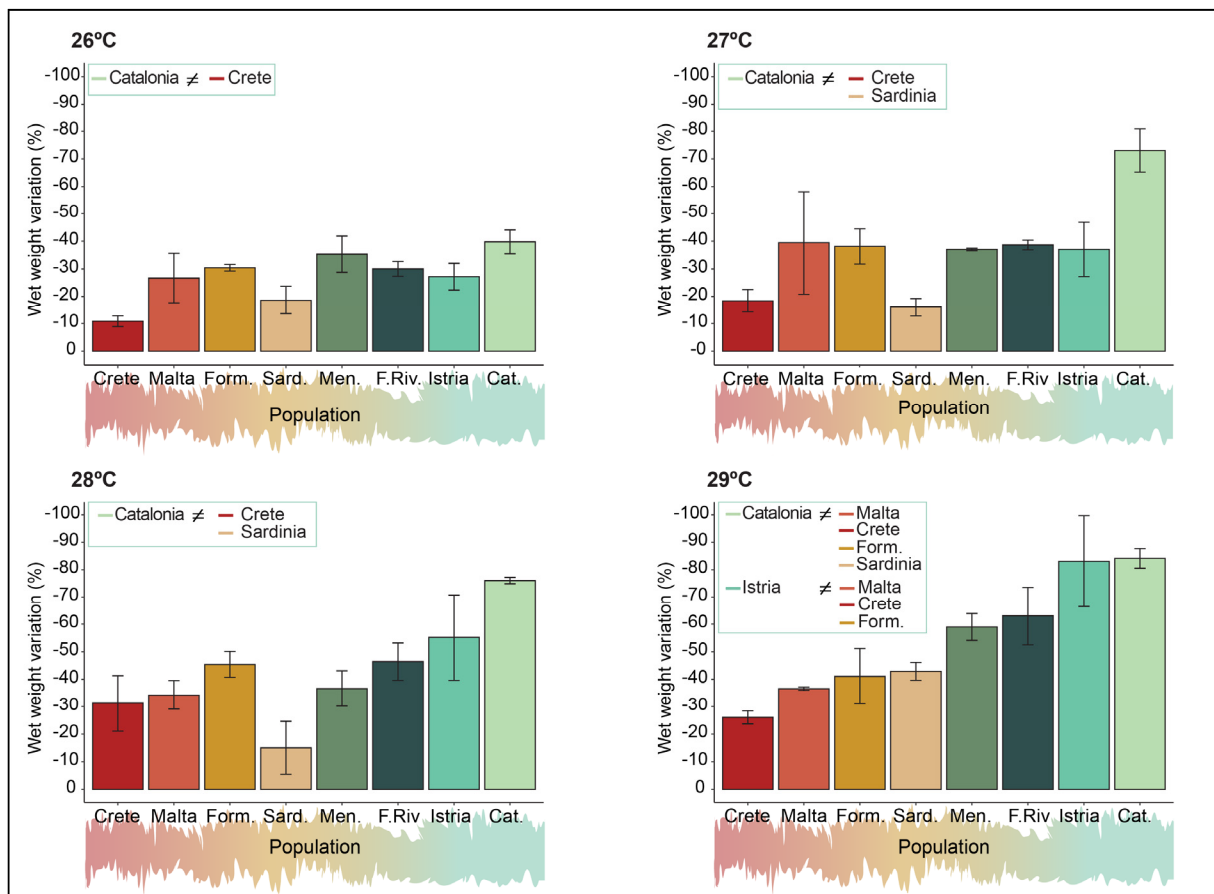
The Wald  $X^2$  test of the fitted LMM showed a significant interaction of temperature and locality (temperature \* locality; p-value < 0.05; Table 2), indicating that specimens from different populations showed a differential response, in terms of biomass loss (%), to thermal stress. As expected, specimens from colder regions showed lower thermo-tolerance thresholds than specimens from warmer regions (Figures 2 and 3; Supporting Information Figure S3). In specimens from warmer areas (Crete, Malta, Formentera and Sardinia), values of biomass loss never exceeded 50 % at any temperature condition (Figure 2a-d), while in the core and cold areas (Menorca, French Rivera, Istria and



**Figure 2** | Temperature effects on the wet weight variation (mean  $\pm$  SE) of *Ericaria crinita* individuals of the eight different populations studied, over the 95 days of exposure to the different thermal experimental conditions. Significant differences between thermal experimental conditions (p-values from Tukey's test with 95% confidence intervals) are indicated with letters for each graph.

Catalonia), thermal stress resulted in significantly higher biomass losses (> 50 % in Menorca and French Rivera, >75% in Istria and Catalonia; Figure 2e-h).

When comparing the response between populations to the different thermal thresholds, differences were more evident at higher temperatures. Specifically, at 29 °C, specimens from colder areas lost significantly more biomass than those from warmer areas. For example, specimens from Istria and Catalonia lost more than 75% of biomass, while those from Crete or Malta lost less than 35% of biomass. In contrast, at 26 °C, specimens from all populations performed similarly, with the exception of those from Catalonia vs. those from Crete (Figure 3; Supporting Information Figure S3).



**Figure 3** | Temperature effects on the wet weight variation (mean ± SE) at the end of the experiment for *Ericaria crinita* individuals of the different populations for each thermal experimental condition. Significant differences between localities at each temperature condition (p-values from Tukey’s test with 95% confidence intervals) are indicated for each graph. The colour gradient on the x-axis is a graphic support to arrange the populations in relation to their thermal regime of origin (from warm to cold thermal regimes).



**Table 2** | Summary of the results from analyses of deviance (Wald  $\chi^2$  test) for the fitted model that tests the influence of the fixed factors *Temperature* and *Locality* on wet weight variation in adults. Chi-square values ( $\chi^2$ ), degrees of freedom (Df) and *p* values are reported for each factor and their interaction.

Response	$\chi^2$	Df	<i>p</i>
Temperature	93.04	3	<0.001 ***
Locality	143.17	7	<0.001 ***
Temp * Loc	33.29	21	0.043 *

### Species distribution models

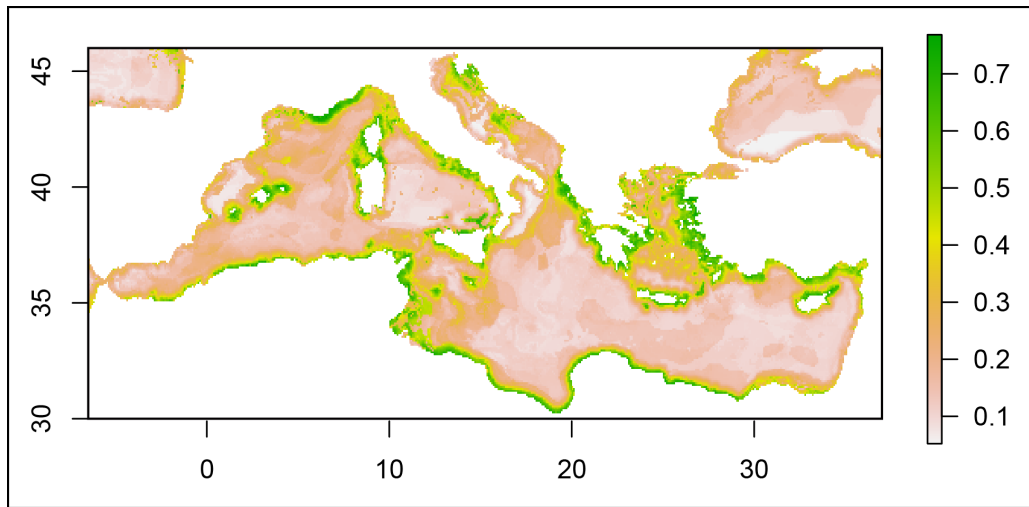
#### *Predictor variables selection and habitat suitability map*

The distribution models that were built included the most important predictors, which were: Calcite, Current velocity, Light diffusion, Distance to shore, Nitrate, Phosphate, Slope, Substrate, Maximum temperature, Mean temperature and Distance to port (Table 1).

To build the current habitat suitability prediction, only CTA, GBM and GLM models were assembled, since their AUC values indicated that these models had fair to excellent predictive accuracy. SRE models were excluded due to their poor model performance (AUC < 0.7). The average AUC value of the habitat suitability prediction (AUC = 0.93) indicated an excellent predictive accuracy.

The current habitat suitability map for *E. crinita* predicted a wide longitudinal range, encompassing coastal areas in the entire Mediterranean Sea. Several areas, such as the Balearic Islands, French Provence, Tunis, Libya and Turkey, among others, were identified as areas that host suitable environments for the presence of *E. crinita* (Figure 4). In contrast, for the entire continental coast of Spain (except for a few points along

the Catalan coast), Israel, Lebanon and the southern Adriatic coast of Italy, there was a much lower probability of finding suitable environments for the presence of *E. crinita*.



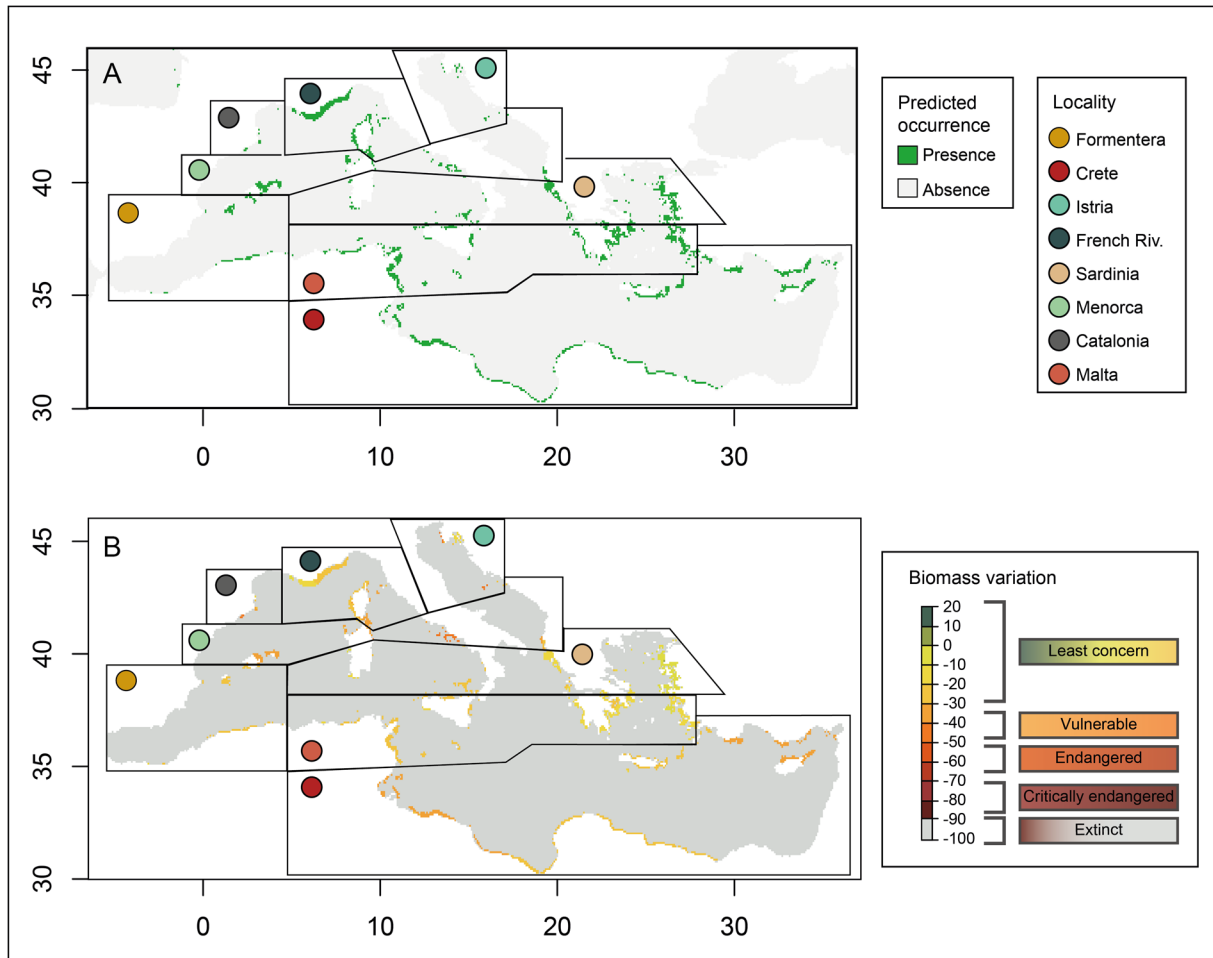
**Figure 4** | Habitat suitability map for *Ericaria crinita* in the Mediterranean Sea. The different colours along the shorelines indicate the predicted probability of occurrence.

### Projections

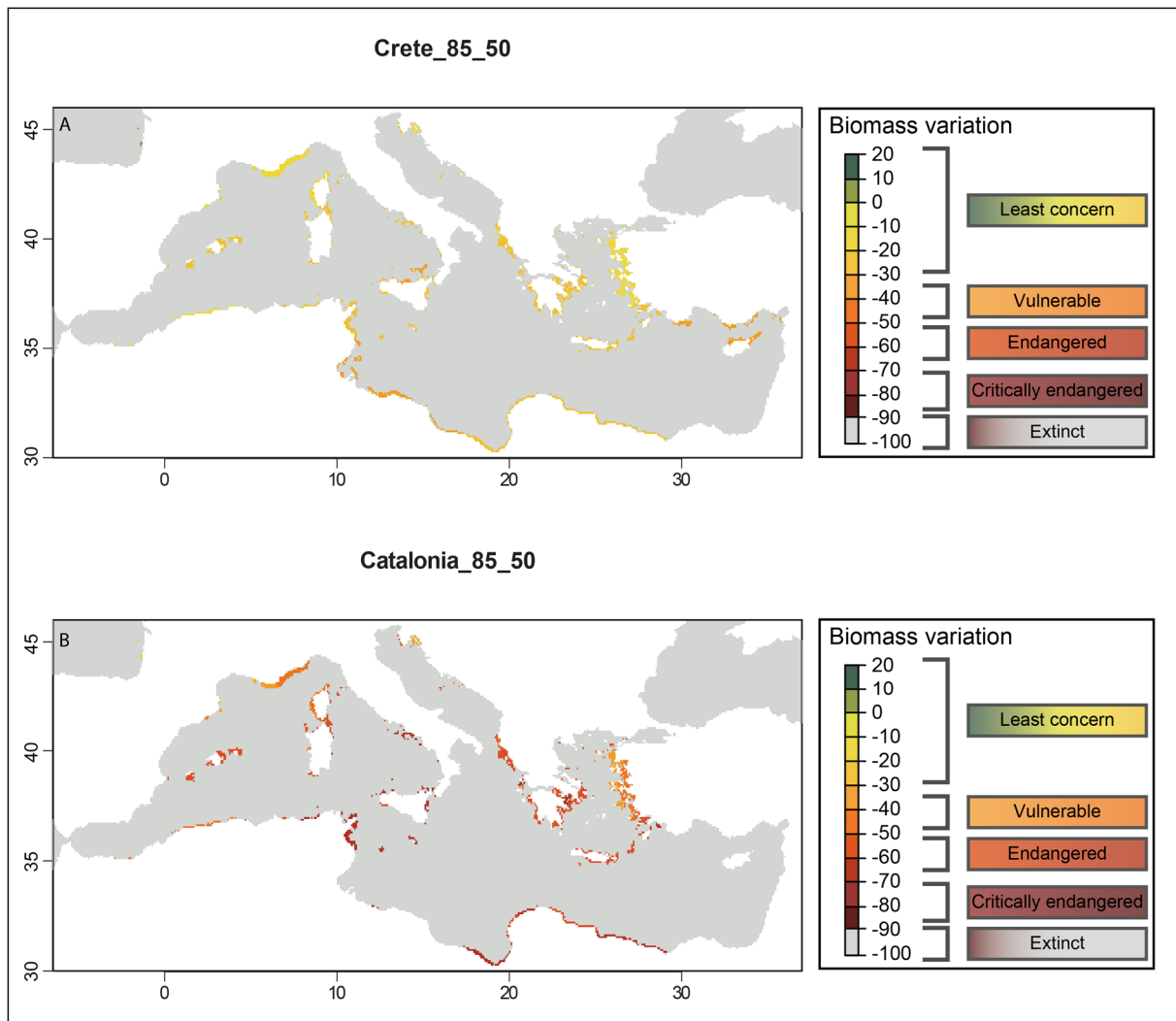
The accurate *E. crinita* vulnerability map, built by integrating the intraspecific thermo-tolerances, and projected under the RCP 8.5 scenario for 2050 throughout the Mediterranean Sea, showed higher vulnerability for the Catalan, Istrian, Dalmatian and Eastern Italian (Tyrrhenian Sea) coastal populations, where projections indicated biomass losses of 50%, which may compromise the viability of these populations. However, mild vulnerability was projected for populations in the areas of Cyprus, the Anatolian coast, the northern Balearic Islands, the Strait of Bonifacio, the Gulf of Gabès (Tunisia) and the surroundings of Tripoli (Libya), with losses of around 40%; while low vulnerability was projected for the other regions (projected losses of less than 30%), such as the French Riviera, Greece and Turkey (Figure 5).

Nevertheless, when we applied a homogeneous thermo-tolerance response of the species, the different vulnerability maps forecasted for *E. crinita* suggested highly contrasting scenarios, depending on which population response was used to build them

(Figure 6 and Supporting Information Figure S4). For example, projections that assumed the thermal sensitivity of populations from the warmer areas in the Mediterranean Sea, such as Crete (Figure 6A), delivered a less worrying scenario for *E. crinita* populations. Projected biomass losses were always less than the 30-40% in the warmer areas of the Mediterranean Sea (South-East), with even lower vulnerability projections (biomass losses of 20-30 %) in the colder regions (Figure 6A). However, when projections assumed the thermal tolerance of populations from the colder areas, such as Catalonia, projection outputs were much more alarming (Figure 6B). In this case, the warmer regions of the species' distribution were the most drastically affected, with populations in Cyprus, the Anatolian coast, eastern Tunisia and Libya projected to be extinct by 2050 under the RCP 8.5 scenario. Vulnerability was, nevertheless, also very high for all other areas, with projected biomass losses of at least 60%, which could seriously compromise the viability of *E. crinita* populations (Figure 6B).



**Figure 5 | A)** Current map of the potential distribution of *Ericaria crinita* in the Mediterranean Sea divided into eight different geographical regions in accordance with their historical thermal regimes. The different coloured circles refer to the reference population of each region. The green colour along the shorelines indicates the predicted occurrence of *E. crinita*. **B)** Accurate projection of *E. crinita* distribution under future SST, projected for 2050 under the RCP 8.5 scenario, taking into account the intraspecific thermo-tolerance response. The specific response of each population has been used as a reference to predict the vulnerability of the species within its thermal region. Different degrees of population vulnerability are established according to the implications that the biomass loss in the experiment had on the specimens.



**Figure 6** | Projection of *Ericaria crinita* distribution combining future SST projected for 2050 under the RCP 8.5 scenario and assuming the thermal-tolerance threshold obtained in the experiment for A) the Crete population (warm thermal regime) and B) the Catalonia population (cold thermal regime).

## DISCUSSION

We have found a clear intraspecific variation in the temperature tolerance thresholds among *E. crinita* populations throughout its distribution range (2000 km longitude). Confirming our hypothesis, populations inhabiting cold thermal regimes showed a lower temperature tolerance than those inhabiting warmer conditions, which showed more limited impacts when exposed to the same temperatures. The thermo-tolerance experiment revealed that exposure to temperatures of 27°C already affected populations of *E. crinita* very differently, with core and warm populations tolerating this temperature

perfectly, whereas the population from the colder thermal regime (Catalonia) was greatly impacted. These differences became more pronounced at higher temperatures. In short, our experiment demonstrated that populations present locally-specific thermal adjustments, in accordance with the marked intraspecific divergences in temperature tolerances reported for other seaweeds (Bennett et al., 2015; Saada et al., 2016; King et al., 2019; Liesner et al., 2020).

Local adaptation and/or physiological plasticity might be involved in the intraspecific variability of *E. crinita*. However, further complementary methodological studies are needed in order to clarify the underlying nature of such variability. In this sense, studies assessing the genetic connectivity and variation between populations, using transcriptomics or performing reciprocal transplant experiments (Merilä and Hendry, 2014; Donelson et al., 2019), would provide a better understanding of the role that biological processes and neutral evolutionary forces might have on macroalgal responses to increasing temperatures (Bennett et al., 2019; Donelson et al., 2019).

In any case, whatever the nature of this intraspecific response is, taking it into account in species distribution models is essential if we are to obtain accurate and realistic responses to ongoing warming. When we considered the intraspecific variability, the predicted thermal vulnerability for the species showed no strong relationship with the thermal gradient of the species' distribution. Although the predicted vulnerability of *E. crinita* was generally low (< 30% of biomass loss; Figure 6), more vulnerable populations (40-50 % of biomass loss) were detected, either in core and cold areas of the species' distribution. Hence, our results suggest that future warming may lead to a similar vulnerability of populations regardless of its geographical distribution. These results contrast with the general pattern of species moving poleward under current climate warming (Martínez et al., 2018; Smale et al., 2019), which implicitly assumes the existence of constant tolerance thresholds throughout a species' distribution range and, therefore, that core-range populations are less vulnerable to warming than those from the warmer, rear edges (Sunday et al., 2012). We have provided evidence of how

intraspecific variability may override this widely accepted pattern and drive non-intuitive patterns of distribution change under future climate warming.

At the same time, we have shown how prediction outputs vary according to the assumed thermo-tolerance response of a given species. Simulations that took into account intraspecific variation delivered either less alarming or more alarming results compared to simulations that assumed – as the species' homogeneous response – the thermo-tolerance of a cold-area population (e.g. Catalonia) or that of a warm-area population (e.g. Crete), respectively (Figure 5 and 6). Consequently, vulnerability predictions can be easily over- or underestimated, and this may lead to widely disparate and erroneous recommendations for local-scale management and site prioritization (e.g. local scale climatic refugia).

It should be noted that, in this study, we have only used temperature as an explanatory factor influencing the geographic distribution of *E. crinita*. However, although temperature can be used to predict range shifts with a high degree of confidence (van den Hoek, 1982; Lüning, 1990; van den Hoek et al., 1990; Blanchette et al., 2008), the interaction of temperature with a number of other factors (e.g. pollution, habitat destruction) cannot be disregarded (Schiel et al., 2004; Díez et al., 2012; Brown et al., 2013; Côté et al., 2016; Gissi et al., 2021). Thus, incorporating the variability of all these factors at local spatial scales and across different temporal scales would be also important (Bates et al., 2018). However, gathering this information is a complex and difficult task that requires substantial knowledge of the species, the environment and the human pressures, which is rarely available. Filling these gaps is challenging, but may significantly enhance our ability to more realistically predict species responses to current and forecasted ocean warming (Assis et al., 2017a; Bates et al., 2018).

As the Mediterranean Sea is a geographically enclosed system, the opportunities for species distribution changes in response to changing temperatures, as well as to other stressors, are reduced. It is expected, therefore, that there will be an erosion of resilience

in many populations, and even local extinctions within the next few decades (Lejeusne et al., 2010; Peters et al., 2015). Several Mediterranean macroalgal forests have long been subject to perturbations by recurrent local stressors (e.g. pollution or overgrazing). As a result, the current conservational status of most Mediterranean fucales (which includes *Ericaria crinita*) is defined as *endangered* or *threatened* (UNEP/MAP, 2013). Our results indicate that the rapid rates of warming expected in the Mediterranean Sea, coupled with the limited possibilities for expanding its distribution, will set its macroalgal forests on trajectories toward local extinction. We would argue that there is an urgent need to develop conservation and management strategies to preserve these emblematic forests. The forecasts produced here can serve as the early detection of the most and least resistant and resilient populations, and can help identify the areas where the most serious impacts of warming are most likely to occur. All such information can be highly valuable for making informed decisions relating to pro-active local and global action plans that explicitly address climate change (Rilov et al., 2020; Gissi et al., 2021). In fact, local-scale, strict conservation of resistant macroalgal forests that reduce the impact of other local stressors, along with re-enforcement by warm-resistant individuals, has been identified as the most important strategy for fostering ecosystem resilience (O’Leary et al., 2017). Our results may be also relevant in terms of the prioritization of large-scale sites within the framework of restoration, by enabling the identification of potential climate refugia.

In conclusion, while research on the distribution of intraspecific variability among populations continues around the world, this study shows that species responses to thermal stress can depend on the temperature regimes where populations develop. Information on this variability in relation to climate change vulnerability is crucial for decisions on effective conservation measures. Fine-scale information on species responses and their geographical variation is of paramount importance if we are to better predict the responses of marine species to ocean warming.

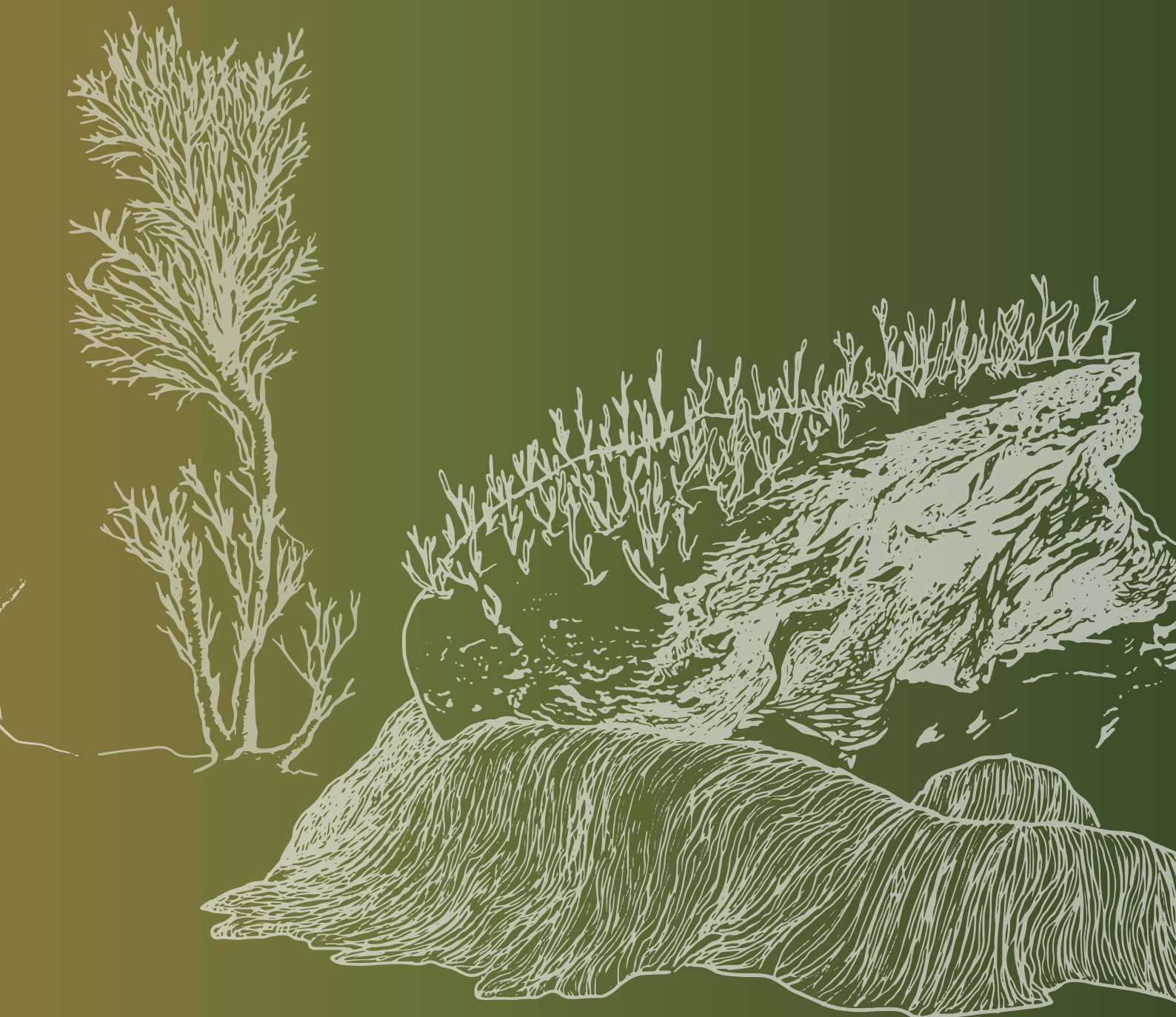


## Acknowledgements

We acknowledge the Environmental Department of the Council of Palamós for providing the permits to conduct the field work. Financial support has been provided by European Union's Horizon 2020 Research and Innovation Program, MERCES Project (No. 689518), the European Union's EMFF Program, AFRIMED (No. EASME/EMFF/2017/1.2.1.12/S4/01/SI2.789059), the Spanish Ministry Project ANIMA (No. CGL2016-76341-R, MINECO/FEDER, UE) and University of Girona (MPCUdG2016/097). This output reflects only the authors' view and the European Union cannot be held responsible for any use that may be made of the information contained therein. This work was also supported by an IF-UdG grant and a contract within the project Intramural CSIC-PIE (202030E180) to J. Verdura, a "La Caixa" Foundation fellowship (ID 100010434) with code (LCF/BQ/DE17/11600001) to J. Santamaría and a FPI fellowship (BES-2017-079907; ANIMA Project) to R. Golo. J. Verdura, J. Santamaría, J. Garrabou and E. Cebrian are members of the consolidated Research Group MedRecover ([www.medrecover.org](http://www.medrecover.org); 2017 SGR 1521, Generalitat de Catalunya). The authors declare no conflict of interests.



**Restoration of a canopy-forming  
alga based on recruitment  
enhancement: Methods and  
long-term success assessment.**



**Jana Verdura**, Marta Sales, Enric Ballesteros, Maria Elena Cefali, Emma Cebrian (2018). Restoration of a canopy-forming alga based on recruitment enhancement: Methods and long-term success assessment. *Frontiers in Plant Science* 9:1832. doi: 10.3389/fpls.2018.01832.

The publication of this article can be found in the section of the Published Work of this thesis. The sections and distribution of the chapter are presented as established by the journal's editorial, and only the shape and size of the font have been edited to unify the thesis format. The genus name of the model species has been modified in accordance with the recent reinstatement of the genus to be consistent throughout the thesis.

## Abstract

Marine forests dominated by macroalgae have experienced noticeable regression along some temperate and subpolar rocky shores. Along continuously disturbed shores, where natural recovery is extremely difficult, these forests are often permanently replaced by less structured assemblages. Thus, implementation of an active restoration plan emerges as an option to ensure their conservation. To date, active transplantation of individuals from natural and healthy populations has been proposed as a prime vehicle for restoring habitat-forming species. However, given the threatened and critical conservation status of many populations, less invasive techniques are required. Some authors have experimentally explored the applicability of several non-destructive techniques based on recruitment enhancement for macroalgae restoration; however, these techniques have not been effectively applied to restore forest-forming fucoids. Here, for the first time, we successfully restored four populations of *Gongolaria barbata* (i.e., they established self-maintaining populations of roughly 25 m<sup>2</sup>) in areas from which they had completely disappeared at least 50 years ago using recruitment-enhancement techniques. We compared the feasibility and costs of active macroalgal restoration by means of *in situ* (wild-collected zygotes and recruits) and *ex situ* (provisioning of lab-cultured recruits) techniques. Mid/long-term monitoring of the restored and reference populations allowed us to define the best indicators of success for the different restoration phases. After 6 years, the densities and size structure distributions of the restored populations were similar and comparable to those of the natural reference

populations. However, the costs of the *in situ* recruitment technique were considerably lower than those of the *ex situ* technique. The restoration method, monitoring and success indicators proposed here may have applicability for other macroalgal species, especially those that produce rapidly sinking zygotes. Recruitment enhancement should become an essential tool for preserving *Cystoseira sensu lato* forests and their associated biodiversity.

**Keywords:** conservation, cost-effective restoration, *Cystoseira*, Fucales, human impacts, marine forests, recruitment enhancement, seaweed restoration

## INTRODUCTION

Canopy-forming brown macroalgae, such as kelps (Laminariales) and fucoids (Fucales), are habitat-forming species in the intertidal and subtidal zones of most temperate and subpolar regions (Steneck et al., 2002; Schiel and Foster, 2006). These macroalgae create structurally complex communities that have several similarities with terrestrial forests (Dayton et al., 1984, 1992; Reed and Foster, 1984; Ballesteros et al., 2009; Gianni et al., 2013). In addition to playing a crucial role in coastal primary production and nutrient cycling, these marine forests increase the three-dimensional complexity and spatial heterogeneity of rocky bottoms, providing food, shelter, nurseries and habitat for many other species (e.g. fish, invertebrates and other algae); thus, they host high biodiversity (Mann, 1973; Seed and O'Connor, 1981; Dayton, 1985; Graham, 2004; Schiel and Foster, 2006).

Compared to many other structurally complex ecosystems around the world, marine forests are suffering from a small global decline on average, despite large regional variation in both the direction and magnitude of the changes, meaning that while global declines are small on average, local-scale declines can be severe (Krumhansl et al., 2016). In many areas, the cumulative impacts of different human pressures, such as habitat destruction, pollution, overgrazing, invasive species and ocean warming, have largely disturbed canopy-forming macroalgae in recent decades (Steneck et al., 2002; Thibaut et al., 2005; Airoidi and Beck, 2007; Connell et al., 2008; Ling et al., 2009; Vergés et al., 2014a, 2016; Wernberg et al., 2016). As a result, vast underwater marine forests have gone missing from many coastal areas and are being replaced by simpler and less productive communities dominated by opportunistic taxa (such as turfs or barrens) (Benedetti-Cecchi et al., 2001; Thibaut et al., 2005; Connell et al., 2008; Mangialajo et al., 2008; Ling et al., 2009; Smale and Wernberg, 2013; Vergés et al., 2014a; Valdazo et al., 2017). Although some giant kelp populations have been shown to recover quickly from local- to large-scale disturbances (Dayton et al., 1992; Edwards, 2004), this is not always

the case for other giant kelp populations, not for other kelps (e.g. Dayton 1973) or fucoids (Coleman et al., 2008; Sales et al., 2011; Smale and Wernberg, 2013). The low dispersal abilities of zygotes and/or spores have been blamed for the lack of furoid population recovery (Kendrick and Walker, 1991; Chapman, 1995; Dudgeon and Petraitis, 2001). In these cases, and when populations have become extinct, natural recovery is almost impossible, and active restoration emerges as the only tool to recover these missing forests (Stekoll and Deysher, 1996; Terawaki et al., 2003; Falace et al., 2006; Susini et al., 2007; Sales et al., 2011; Campbell et al., 2014).

The Mediterranean Sea, a marine biodiversity hotspot, has experienced large alterations in its ecosystems (Coll et al., 2010; Lotze et al., 2011). Marine forests dominated by species of the genus *Cystoseira sensu lato* (Fuciales) are widespread on well-preserved Mediterranean rocky bottoms (Giaccone, 1973; Ballesteros, 1988, 1990a, 1990b; Ballesteros et al., 1998, 2009; Zabala and Ballesteros, 1989; Sales et al., 2012). Despite not reaching the size of kelp or some other fucoids, *Cystoseira s.l.* species produce a dense canopy (rarely > 1 m) creating a “forest-like” assemblage, with species growing in the understory that are not found without their presence. This is the reason we talk about *Cystoseira s.l.* forests.

Some *Cystoseira s.l.* forests have severely declined in recent decades (Cormaci and Furnari, 1999; Thibaut et al., 2005; Serio et al., 2006; Blanfuné et al., 2016). Since zygotes of *Cystoseira s.l.* species are very large (around 100-120µm) and exhibit low dispersal abilities (Guern, 1962; Clayton, 1992), transplantation techniques have been used as a tool for environmental mitigation (Falace et al., 2006; Susini et al., 2007; Perkol-Finkel et al., 2012; Robvieux, 2013).

However, since most *Cystoseira s.l.* species are considered threatened or endangered by the Barcelona Convention (Annex II) (UNEP/MAP, 2013), individual transplants from remaining populations are undesirable, and therefore, less invasive restoration actions are required (see Gianni et al., 2013 for a review). As a result, new recruits of certain



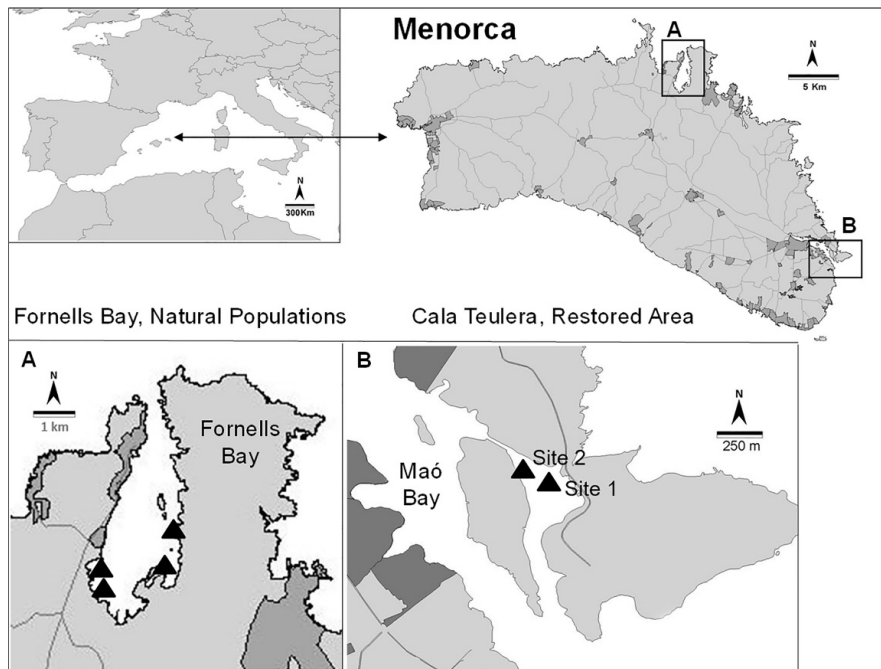
furoid species have been artificially obtained and monitored for one year (Stekoll and Deysher, 1996; Terawaki et al., 2003; Yatsuya, 2010; Yu et al., 2012; Falace et al., 2018), introducing the possibility of recruitment enhancement as a new strategy for restoring *Cystoseira s.l.* populations.

In this context, the general objective of this study is to provide and experimentally test non-destructive restoration methods that can lead to the establishment of self-sustaining *Cystoseira s.l.* populations and to describe the proper success indicators for the different restoration stages. Specifically, we describe two techniques using *in situ* and *ex situ* recruitment enhancement aimed at restoring populations of *G. barbata*, and the success of each is assessed by comparing restored and reference populations over six years. Moreover, because the success and broad-scale application of a restoration technique also depends on its cost feasibility, we also describe this key piece of information.

## MATERIALS AND METHODS

### Species and study site

This study focuses on the species *Gongolaria barbata* (Stackhouse) C. Agardh (= *Cystoseira barbata*), which typically develops in shallow and sheltered environments (Sales and Ballesteros, 2009) across the Mediterranean Sea. The reduction in its range is strongly correlated with human development (Thibaut et al., 2005, 2015; Bologna and Sava, 2006), and the species is classified as threatened under the Barcelona Convention (United Nations Environment Programme/Mediterranean Action Plan [UNEP/MAP], 2013). These features make *G. barbata* a perfect target species for restoration in places from which it has disappeared.



**Figure 1** | Location of the reference populations (A) and the restored area (B).

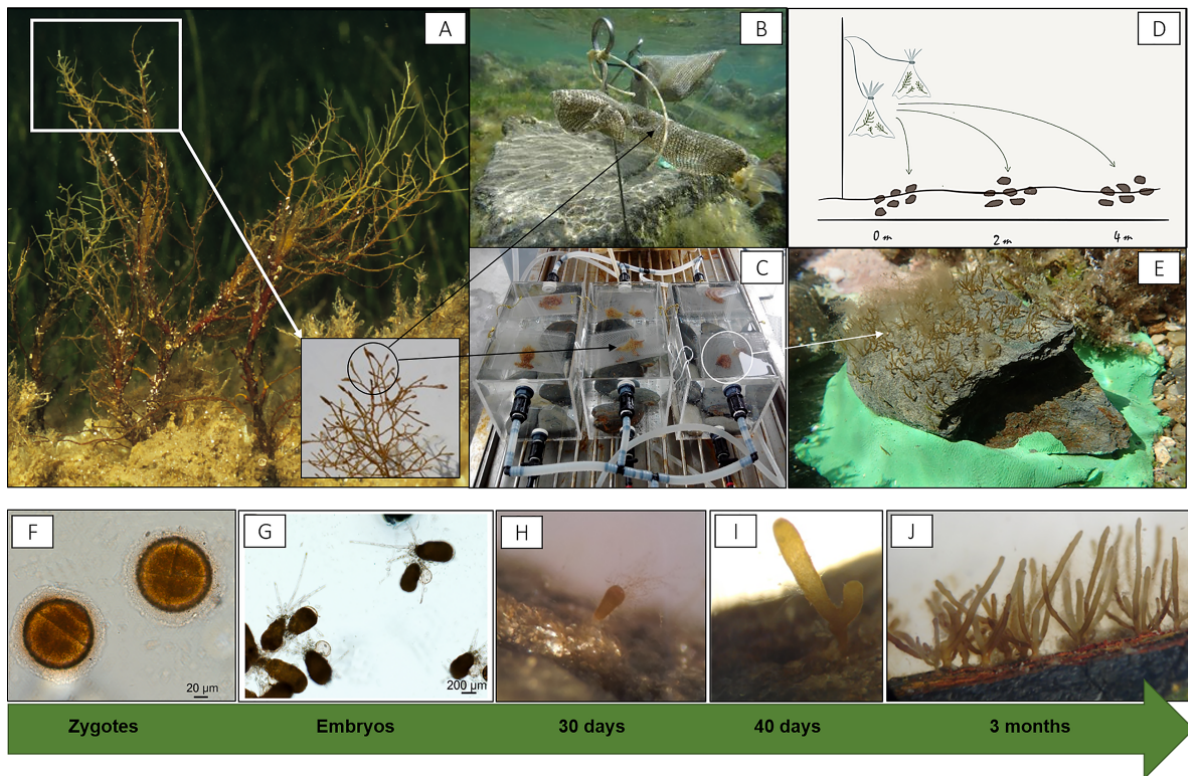
This study was conducted in Menorca (Balearic Islands, NW Mediterranean), which has been a UNESCO Biosphere Reserve since 1993. Most coastal areas in Menorca are well preserved and have limited urbanisation. The coastal water quality is high, so the extent and cover of habitats dominated by *Cystoseira s.l.* species is outstanding (Sales and Ballesteros, 2009). *Gongolaria barbata* naturally makes small patches (usually around a few square meters) in very sheltered and shallow environments. This species is extremely rare not only in Menorca but also in other Mediterranean areas (Gómez-Garreta et al., 2000) because there are very few places matching its environmental requirements, with the exception of the northern Adriatic Sea. *Gongolaria barbata* is present in Fornells Bay (Menorca), one of the few places where the environmental conditions are suitable for its development. However, this species was reported from Cala Teulera ( $39^{\circ}52'40.64''$  N,  $4^{\circ}18'22.03''$  E; Bay of Maó, Figure 1) in the XVIII century (Rodríguez-Femenías, 1888), but it disappeared from this area due to direct dumping of urban and industrial sewage into the bay during the 1970s, leading to impaired water quality. A sewage outfall was built in 1980, and waste waters were diverted into the open

sea (Hoyo, 1981). However, no recovery of the *G. barbata* populations was detected during the next 30 years (Sales et al., 2011). Nevertheless, Cala Teulera still shelters a reduced meadow of the seagrass *Cymodocea nodosa* and some stands of *Cystoseira compressa* var. *pustulata* and *Cystoseira foeniculacea* f. *tenuiramosa*. In contrast, Fornells Bay (40°2'10.12" N, 4°7'43.24" E; Figure 1) continues to be characterised by low human influence and extensive sheltered seagrass meadows (e.g., *Posidonia oceanica*, *C. nodosa*, *Zostera noltii*) (Delgado et al., 1997) and healthy *Cystoseira s.l.* spp. forests, including the only preserved *G. barbata* populations from Menorca (Sales and Ballesteros, 2009). For this reason, the stands in Fornells Bay were selected as donor populations to restore two different sites in Cala Teulera (Figure 1).

### Applied restoration techniques

Two different restoration techniques involving *in situ* and *ex situ* recruitment enhancement were experimentally tested to promote *G. barbata* recovery. Both techniques are considered non-destructive since they only rely on harvesting a small proportion (< 5%) of reproductive fertile branchlets from wild individuals. Both donor and restored sites were situated between depths of 0.2 m and 1 m. *In situ* recruitment consisted of collecting fertile apical branchlets (March 2011) from the donor populations (Fornells Bay) that were then transported to the restoration sites and placed in dispersal bags that were 8 cm wide and 10 cm long (Figure 2A, B) and made of 36% fibreglass and 64% PVC with a mesh size of 1.20 x 1.28 mm.

Bags were tied to a pick and directly fixed at a vertical distance of 0.25 cm from the bottom using a hammer (Figure 2B). Eight bags (two for each pick) containing approximately twenty fertile receptacles each were placed at each of the two selected restoration sites at distances of 2-3 m from each other. At both sites, six natural flat schist stones with similar surface areas (approximately 0.04 m<sup>2</sup>) were collected, cleaned of organisms and sediment and randomly placed in radii from 0.1 m to 4 m around the dispersal bags to promote *G. barbata* settlement. We used stones adjacent to our study



**Figure 2** | Experimental setup and zygote development into recruits. A: Fertile thalli and branchlets from natural populations, B: dispersal bags placed *in situ*, C: dispersal bags placed in culture tanks (*ex situ*), D: dispersion range capacity under *in situ* recruitment, and E: placement of *ex situ* recruits in the area to be restored. Zygote and embryo development into recruits from *ex situ* cultures (F to J). F: Zygotes (1 day), G: embryos adhered to the substrate by rhizoids (1 week), H: embryos developing into recruits (1 month, 200-400 µm), I: first branching of the recruit (1.5 months, 400-600 µm), and J: fully developed recruits (3 months, 5-15 mm).

areas, and not from the same area, to avoid disturbing the study site when cleaning the stones from organisms and sediment. The stones were cleaned to provide free substrate and avoid competition at the first stages of development of new recruits. After 4 days, the dispersal bags were removed from both restored sites.

*Ex situ* recruitment consisted of acquiring a supply of zygotes and culturing settlers in the laboratory. Fertile apical branchlets (around 2-3 cm in length) from the donor populations (March 2011, Fornells Bay) were collected and placed in plastic bags without seawater and transported to the laboratory under cold and dark conditions. Once in the laboratory, the bags containing the fertile branchlets were stored in the fridge (at 4°C and in dark conditions) for 12 h to promote zygote liberation.

Concurrently, 16 natural flat schist stones with similar surface areas (approximately 0.04 m<sup>2</sup>) were placed at the bottom of ten 12-L tanks filled with filtered seawater, and fertile apical branchlets of *G. barbata* were placed on dispersal bags floating on the water surface of each tank for 4 days (Figure 2A-C). Moreover, some glass slides were placed on top of and between the stones to enable microscopically monitoring of zygote development during the first months (Figure 2F-J). For the first 4 days, the hydrodynamic conditions of the tank were kept as stable as possible to facilitate zygote settlement. Afterwards, zygotes were cultured in a closed-water circuit with a renovation rate of 2 L per day using natural seawater at 21°C and natural light conditions. Seawater temperature was controlled with refrigerators (Hailea Chiller HC 500 A of Hailea). After 3 months (June 2011), stones with *G. barbata* recruits were transported to the restoration sites and six stones were placed at a distance of 25 m from the *in situ* restored area at each site (Figure 2E). It was not necessary to fix the stones since the restoration areas were extremely sheltered and the stones were heavy enough to prevent any movement.

### Monitoring the restored and reference populations

After installing the *in situ* and *ex situ* recruitment set ups, both sites were visited monthly to ensure that the experiment was properly maintained. After five months, both *in situ* and *ex situ* recruits were large enough to allow visual density and height measurements. Then, the density (the total number of individuals per 0.04 m<sup>2</sup>) and the size structure distribution (the length of the main axis) of *G. barbata* individuals from each stone (approximately 20 × 20 cm) were monitored *in situ* twice in 2011 (August and November) and once during 2012, 2013, 2014, 2016 and 2017 (August) at each restored site and for each restoration technique.

At the beginning of the experiment, 3 natural *G. barbata* populations (Fornells Bay; Figure 1) were also selected as reference populations for comparison with the restored populations. The densities and size structure distributions of each reference population

were monitored in 20 randomly distributed, 20×20-cm quadrats at the beginning and end of the experiment (i.e. August 2011, 2016 and 2017).

### **Dispersal capacity of the *in situ* recruitment method**

At the same time, a new experiment was set up to explore the extension range of the *in situ* recruitment method. We studied the dispersion capacity of the *G. barbata* zygotes. For this purpose, we fixed a new pick (with 2 dispersal bags each) at each site, and six stones (approximately 0.04 m<sup>2</sup> each) were placed just below the dispersal bags (0 m) along with six at a distance of 2 m, and finally six at a distance of 4 m. The dispersal bags were removed after 4 days, and the number of recruits from each stone was counted in August 2011 (Figure 2D).

### **Data analysis**

#### *Comparison of techniques*

To compare the two restoration techniques, the mean densities and size distribution at both restored sites were evaluated. The mean density (number of individuals / 0.04 m<sup>2</sup>) over time was analysed using a generalized linear mixed model (GLMM) with technique (2 levels: *ex situ* vs. *in situ*), site (2 levels: site 1 and site 2) and time (7 levels) as fixed factors, and stone as a random factor. Descriptive statistics were also calculated for the size structure distribution (the skewness and kurtosis) of restored populations and compared among both techniques and sites. The significance of the skewness and kurtosis values was calculated according to Sokal and Rohlf (1995).

#### *Restoration success*

Restoration success was analysed by comparing the final densities and size structures between restored and reference populations. The final density (August 2017) of restored populations was compared with that of reference populations by means of a generalized linear model (GLM) with one fixed factor with two levels (restored vs. control). Changes in the size structure distributions of the restored and reference populations over time

were plotted using non-metric multidimensional scaling (MDS) to visualize their progression. The relative percentage of individuals in each size class (in 1-cm intervals) was the variable in the data matrix, and the Bray-Curtis distance (Bray and Curtis, 1957) with a dummy variable (= 1) was used to construct the similarity matrix.

### *Dispersal capacity*

Finally, the range in dispersal capacity obtained with the *in situ* method was analysed using GLM, with site (2 levels) and distance from the dispersal bag (3 levels) as fixed factors. Pair-wise comparisons were also performed between distances.

GLMs and GLMMs are suitable for this kind of data since GLMs can handle non-normal data (Bolker et al., 2009) and GLMMs combine the properties of GLMs and linear mixed models, which incorporate random effects and therefore can cope with repeated measures over time (Pinheiro and Bates, 2000). All analyses were performed using the lme4 package (Bates et al., 2015) for R software (R Core Team, 2019) and the statistical software Primer & Permanova v.6 (Clarke and Gorley, 2006).

### *Costs*

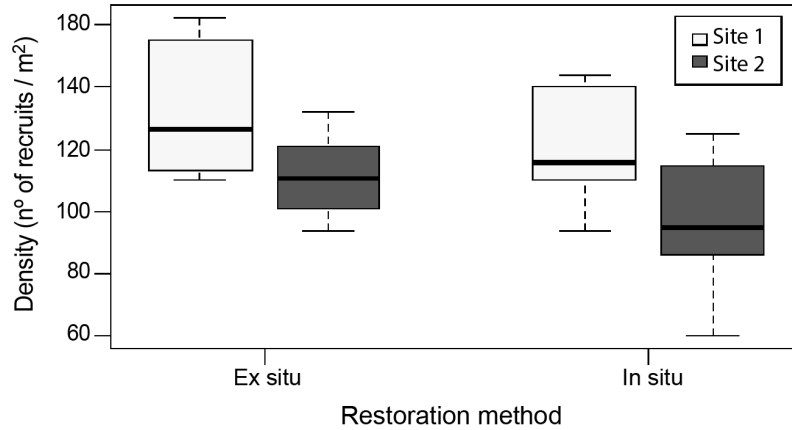
We compared the cost of restoring a population (25 m<sup>2</sup>) using the *ex situ* and *in situ* methods, considering the travel, transportation, personnel and material expenses (similarly to Carney et al., 2005). We did not consider the long-term monitoring costs since these costs are equivalent for the two techniques.

## RESULTS

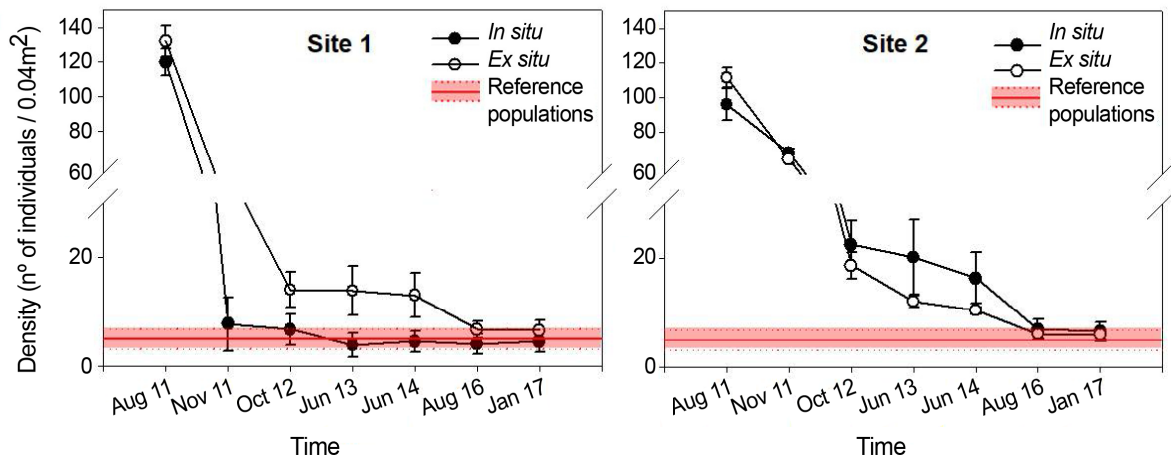
### Comparison of techniques

The density of recruits was similar between the two restoration techniques (Figure 3 and 4; Table 1). The mean initial densities ranged between  $120 \pm 7$  recruits / 0.04 m<sup>2</sup> (site 1) and  $96 \pm 9$  recruits / 0.04 m<sup>2</sup> (site 2) in the *in situ* experiment and between  $132 \pm 2$  recruits / 0.04 m<sup>2</sup> (site 1) and  $111 \pm 9$  recruits / 0.04 m<sup>2</sup> (site 2) in the *ex situ* experiment

(Figure 3). No recruits were observed outside of the free substrate (stones) with the *in situ* method. The densities of the two restored populations greatly decreased during the first year but remained more stable afterwards (Figure 4).



**Figure 3** | Boxplot of initial density (number of recruits / 0.04 m<sup>2</sup>) for each restoration technique and site. In the boxplot, the bold horizontal line indicates the median value (Q<sub>2</sub>); the box marks the interquartile distances, Q<sub>1</sub> and Q<sub>3</sub>; and the whiskers mark the values that are less than Q<sub>3</sub>+1.5\*IQR but greater than Q<sub>1</sub>-1.5\*IQR.



**Figure 4** | Mean density ( $\pm$  SE) through time for each restoration technique at each site. Reference population densities are represented in red, considering the mean and standard deviation values obtained from the reference populations in 2011, 2016 and 2017.

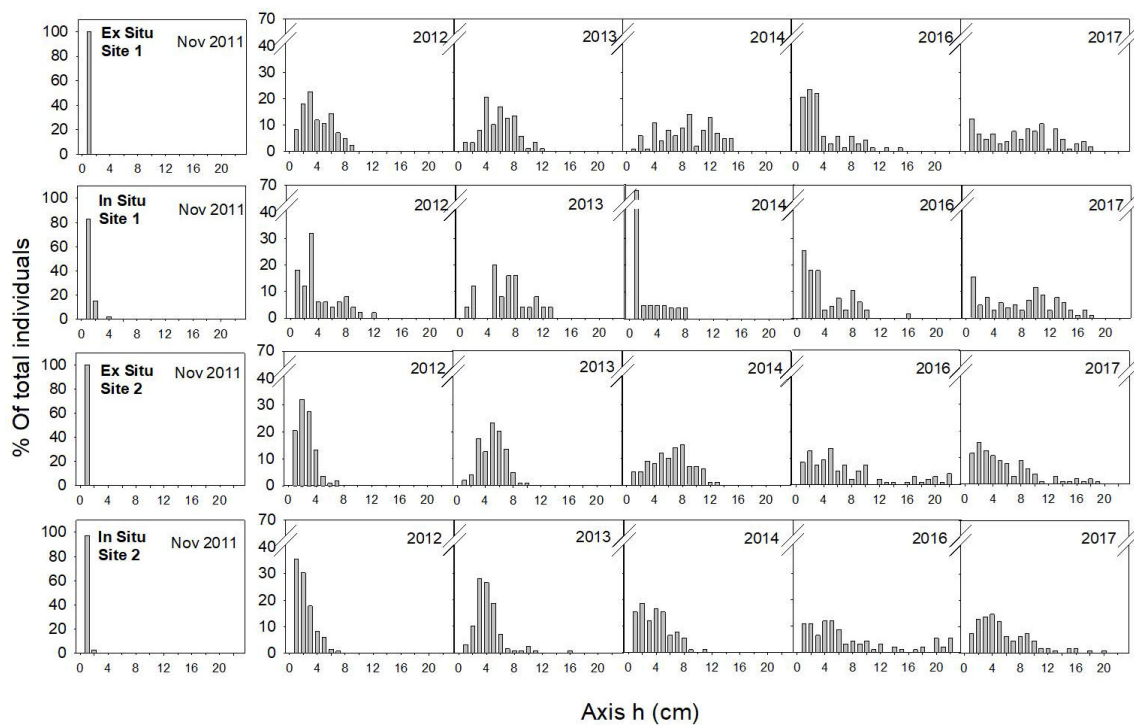
In November 2011, the main axes of almost all the individuals measured 1 cm, and one year later (August 2012), the skewness of the size-class structure was significantly



**Table 1** | Results of GLMM comparing the density (number of individuals/0.04 m<sup>2</sup>) through time in relation to the restoration techniques (*in situ* vs. *ex situ*). For each factor, we report the degrees of freedom and the F- and p-values. The significant values are highlighted in bold in the table.

Factor	df	F-value	p-value
Technique	1	0.11	0.43
Site	1	2.67	0.17
Time	6	796.26	<b>&lt;.0001</b>
Technique * Site	1	2.94	0.66
Technique * Time	6	0.48	<b>&lt;.0001</b>
Site * Time	6	42.14	<b>&lt;.0001</b>
Site * Technique * Time	6	21.25	<b>&lt;.0001</b>

positive, indicating the prevalence of small size-classes in the population. However, few individuals had reached axis lengths greater than 10 cm (Table 2; Figure 5). Two years later (2013), all populations were approximately bell shaped and symmetric, with a large proportion of individuals having axis lengths between 2 and 5 cm, although some fertile individuals reached axis lengths of 14-16 cm (Table 2, Figure 5).

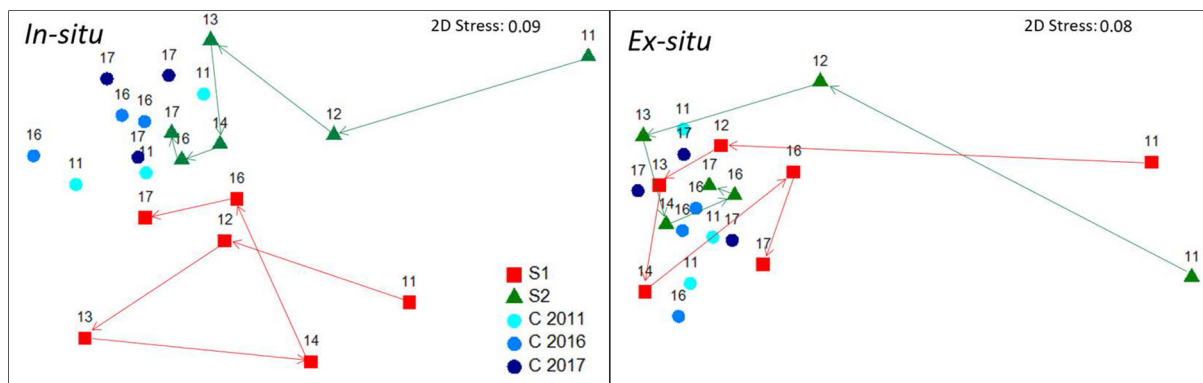


**Figure 5** | Size-class frequency distribution of the restored populations over time for each site and restoration technique. The X-axis represents the size-classes (length of the main axis) in 1-cm intervals, and the Y-axis represents the relative frequency of each size-class.

In 2014, the size-class structures of the populations were symmetric and bell shaped, and most individuals were of intermediate size (Table 2; Figure 5). One exception to this result was the population restored using the *in situ* method at site 1, where we found high mortality of large individuals but also high recruitment (Table 2; Figure 5). These recruits were the result of new settlement events resulting from the already fertile restored individuals from 2013.

### Restoration success

In 2017, six years after the restoration action, the size of each of the four restored *G. barbata* patches was roughly 25 m<sup>2</sup>. When comparing the final densities of restored populations with the densities of the reference populations (August 2017), no significant differences were observed ( $F = 0.08$ ,  $P = 0.49$ ; Figure 4). The evolution of the size-class distribution through time resulting from both techniques, sites and reference populations is illustrated in the MDS (Figure 6). The reference populations are displayed on the left side of the MDS (from 2011 to 2017), while the restored populations progressed from the right side in 2011 to the left side, ultimately moving closer to the reference populations. In 2014, the *in situ* restored population from site 1 returned to the right side of the MDS due to the mortality of large individuals and the high recruitment that was experienced (Figure 6). In 2016, all populations were located close to the reference populations, and they remained stable in 2017 (Figure 6).



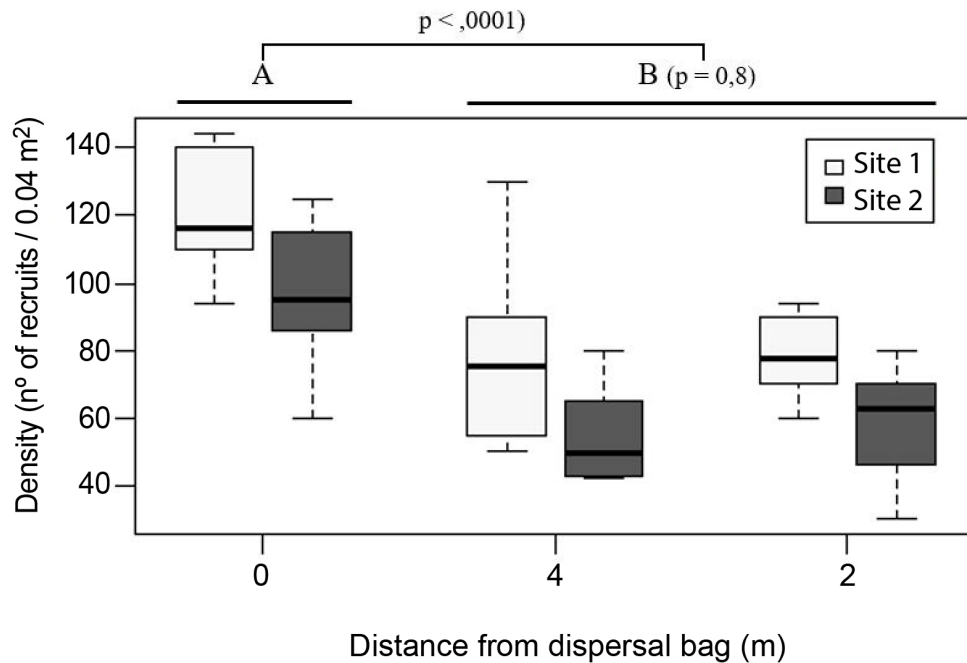
**Figure 6** | MDS ordination plot of the path followed by restored and natural populations over time for both *in situ* and *ex situ* techniques, according to the size-class data of each population. Numbers depicted over each point are years.

**Table 2** | Characteristics of restored *G. barbata* populations through time and in relation to the restoration technique and site (N: number of individuals; h: length of the main axis (cm); g<sub>1</sub>: skewness; g<sub>2</sub>: kurtosis; Sig: significance of skewness and kurtosis values). These parameters are considered significant if the absolute value of the coefficient/standard error (SE) is greater than 2; the significant values are highlighted in bold in the table.

Date	Method	site	N	mean h	max h	g <sub>1</sub>	SE g <sub>1</sub>	sig. g <sub>1</sub>	g <sub>2</sub>	SE g <sub>2</sub>	sig. g <sub>2</sub>
2011 Aug	<i>in situ</i>	1	720	0,5	0,5	-	-	-	-	-	-
		2	576	0,5	0,5	-	-	-	-	-	-
	<i>ex situ</i>	1	793	0,5	0,5	-	-	-	-	-	-
		2	669	0,5	0,5	-	-	-	-	-	-
2011 Nov	<i>in situ</i>	1	46	0,83	4	3,25	0,35	<b>9,28</b>	13,59	0,69	<b>19,76</b>
		2	406	0,6	1,5	2,25	0,12	<b>18,58</b>	4,44	0,24	<b>18,37</b>
	<i>ex situ</i>	1	214	0,5	0,5	-	-	-	-	-	-
		2	384	0,5	0,5	-	-	-	-	-	-
2012	<i>in situ</i>	1	40	3,73	12	1,09	0,37	<b>2,92</b>	0,54	0,73	0,74
		2	135	1,99	6,5	1,15	0,21	<b>5,51</b>	0,81	0,41	1,96
	<i>ex situ</i>	1	84	3,89	8,5	0,4	0,26	1,52	-0,78	0,52	-1,50
		2	112	2,34	6,5	0,96	0,23	<b>4,20</b>	1,42	0,45	<b>3,13</b>
2013	<i>in situ</i>	1	26	6,81	13	-0,09	0,46	-0,20	-0,31	0,89	-0,35
		2	128	3,95	10,5	1,46	0,21	<b>6,82</b>	3,5	0,42	<b>8,24</b>
	<i>ex situ</i>	1	88	5,68	12	0,19	0,26	0,74	-0,09	0,51	-0,18
		2	103	4,88	10	0,1	0,24	0,42	-0,01	0,47	-0,02
2014	<i>in situ</i>	1	22	1,98	8	1,49	0,49	<b>3,03</b>	0,98	0,95	1,03
		2	91	3,75	11	0,63	0,25	<b>2,49</b>	0,1	0,50	0,20
	<i>ex situ</i>	1	85	8,55	15	-0,15	0,26	-0,57	-0,93	0,52	-1,80
		2	81	6,27	13	-0,05	0,27	-0,19	-0,58	0,53	-1,10
2016	<i>in situ</i>	1	67	3,94	16	1,25	0,29	<b>4,27</b>	1,67	0,58	<b>2,89</b>
		2	92	7,72	22	1,09	0,25	<b>4,34</b>	-0,05	0,50	-0,10
	<i>ex situ</i>	1	68	3,92	15	1,47	0,29	<b>5,06</b>	1,62	0,57	<b>2,82</b>
		2	94	7,52	22	1,13	0,25	<b>4,54</b>	0,21	0,49	0,43
2017	<i>in situ</i>	1	103	7,7	17,5	0,007	0,24	0,03	-1,17	0,47	<b>-2,48</b>
		2	110	5,54	20	1,29	0,23	<b>5,60</b>	1,68	0,46	<b>3,68</b>
	<i>ex situ</i>	1	105	8,12	18	0,11	0,24	0,47	-1	0,47	<b>-2,14</b>
		2	103	5,72	19	1,24	0,24	<b>5,21</b>	1,04	0,47	<b>2,21</b>

### Dispersal capacity

At both sites, stones situated below the dispersal bags (distance of 0 m) showed higher densities of *G. barbata* recruits than did those situated at distances of 2 and 4 m ( $P < 0.0001$ ; Figure 7), while no differences were found between 2 and 4 m ( $P = 0.8$ ; Figure 7).



**Figure 7** | Boxplot of number of recruits on the stones placed *in situ* at increasing distances from the dispersal bags 5 months after their deployment. In the boxplot, the bold horizontal line indicates the median value (Q2); the box marks the interquartile distances, Q1 and Q3; and the whiskers mark the values that less than  $Q3+1.5 \cdot IQR$  but greater than  $Q1-1.5 \cdot IQR$ .

### Costs

The cost of restoring  $25 \text{ m}^2$  of *G. barbata* forest ranged between 1,092 € using the *in situ* seeding technique and 2,665 € using the *ex situ* seeding technique (Table 3). The higher cost ascribed to the *ex situ* technique is related to the required infrastructure and the greater number of hours needed for culture maintenance.

**Table 3** | Cost for the different concepts required to restore an area of 25 m<sup>2</sup> depending on the restoration technique used.

Concept	Rate	Cost	Total (€)
<i>Ex situ</i>			
Field time			
Collection	1 h/2pax	40 €/h*pax	80
Ex-plant	3 h/2pax	40 €/h*pax	240
Transport			
Car	200 km	0.40 €/km	80
Lab time			
Set up culture	4 h/2pax	40 €/h*pax	320
Culture maintenance	3 h/week*pax	40 €/h*pax	1440
Materials			
Tanks	10	25 €	250
Water pump	1	60 €	60
Silicon Tubes	5 m	2 €/m	10
Epoxy	2	70 €/kg	140
Aerator	3	15 €	45
<b>TOTAL</b>			<b>2665</b>
Concept	Rate	Cost	Total (€)
<i>In situ</i>			
Field time			
Collection	1 h/2pax	40 €/h*pax	80
Set up dispersal bags	4 h/2pax	40 €/h*pax	320
Set up free substrate	3 h/2pax	40 €/h*pax	240
Removal dispersal bags	1 h/2pax	40 €/h*pax	80
Materials			
Iron Stick	16	7 €/Pick	112
Epoxy	2	70 €/kg	140
Transport			
Car	300 km	0.40 €/km	120
<b>TOTAL</b>			<b>1092</b>

## DISCUSSION

The present study is the first example of active restoration for locally-extinct populations of habitat-forming fucoids using recruitment enhancement without adult transplantation of threatened populations, and these restored populations became self-sustaining, with densities and size-class structures comparable to those of the reference populations within five years. Active transplantation of adults or juveniles has been used as a mechanism to successfully restore habitat-forming species of fucoids (Susini et al., 2007; Campbell et al., 2014). The concept of recruitment enhancement has recently gained recognition as it applies to the restoration of threatened species (Yatsuya, 2010; Gianni et al., 2013; Falace et al., 2018). However, there have been only a few attempts at using this method, and most have been limited to the recruit stage with less than 1 year of monitoring (Stekoll and Deysher, 1996; Choi et al., 2000; Terawaki et al., 2003; Yu et al., 2012).

Here, we used recruitment enhancement methods to successfully restore a locally-extinct *G. barbata* population with only one restoration action in 2011. Because the locally-extinct population was unable to recover naturally, even thirty years after the primary stress had been ameliorated (Hoyo, 1981; Sales et al., 2011), we used seeding to overcome the limited natural dispersal rates that are typical of zygotes of the genus *Cystoseira s.l.* (Mangialajo et al., 2012), and we overrode the limited natural recruitment (Vadas et al., 1992; Capdevila et al., 2015) by cleaning the stones from organisms and sediment, providing free substrate to avoid competition. After six years, the sizes of each restored population was approximately 25 m<sup>2</sup>, which is comparable to the size-patches of natural *G. barbata* populations in Fornells Bay.

Recruitment was high and similar under both techniques, although a large proportion of recruits died during the first year. This sharp drop in density is common in natural populations due to the high natural sensitivity of the first fucoid life stages (Vadas et al., 1992; Irving et al., 2009;). Although the density of individuals was similar between

restored and reference sites in the second year following the restoration action, it took five years for the individuals of the restored populations to achieve comparable size-class structures to the reference ones. Thus, density is useful for monitoring success during the first period after a restoration action (recruits of settlers; here, 2 years), but after this stage, density should be complemented with other attributes, such as size structure, that will better describe the mature stage of the population.

Obtaining a *Cystoseira s.l.* population that reaches a well-represented and stable size distribution is the first goal for complete forest restoration. As for other structural species, the restoration success criteria should be linked to the recovery of the ecosystem function and services, and obtaining mature individuals that are able to self-sustain the new population is likely the first step for enhancing biodiversity and ecological processes. Complementary studies on the evolution of the associated community will probably elucidate whether the proposed indicators for population success may also be indicative of the overall recovery of ecosystem functions and services.

Both of the *in situ* and *ex situ* recruitment enhancement techniques applied here are probably suitable for other macroalgal species that produce large and fast-sinking zygotes with limited dispersion and that are poor competitors for space in their early stages (i.e. late-successional species). Thus, the techniques tested here could be used to restore other Mediterranean populations of *Cystoseira s.l.* spp., especially since the Council of Europe, specifically the Marine Strategy Framework Directive (United Nations Environment Programme/Mediterranean Action Plan [UNEP/MAP], 2013), pushes for active restoration to achieve a Good Environmental Status for a considerable number of habitats.

Knowledge of the biological traits of the target species will determine the choice between *in situ* and *ex situ* techniques. The *in situ* technique is especially recommended for species with high dispersal capacity, such as kelps, with a dispersal potential of hundreds of metres (Reed et al., 1988; Fredriksen et al., 1995). In contrast, the *ex situ*

technique is more appropriate for species with a low dispersal capacity, such as *C. amentacea*, whose zygotes are not able to disperse a distance of even 40 cm (Mangialajo et al., 2012). Another benefit of the *ex situ* technique is that it minimizes the high mortality rates experienced by recruits and juveniles as a result of disturbances, predation or competition (Benedetti-Cecchi and Cinelli, 1992; Capdevila et al., 2015). The more the culture is prolonged, the more likely the critical life stages will be left behind, which ultimately enhances success. In our case, however, sources of mortality seemed to be rather irrelevant since the *ex situ* and the *in situ* survival rates were very similar during the first year. The *ex situ* technique should reduce the unpredictability of natural events and maximize success, while the *in situ* technique requires less infrastructure and maintenance, making it a cheaper option.

In summary, we provide a promising cost-effective method (consisting of two different techniques) that can be used to address the increasing need for the restoration of threatened species, especially furoid forests. Moreover, we show that individual density is not a valid metric to assess the state of population recovery, and we propose the size distribution of the restored individuals as a suitable indicator of population maturity.

### **Acknowledgements**

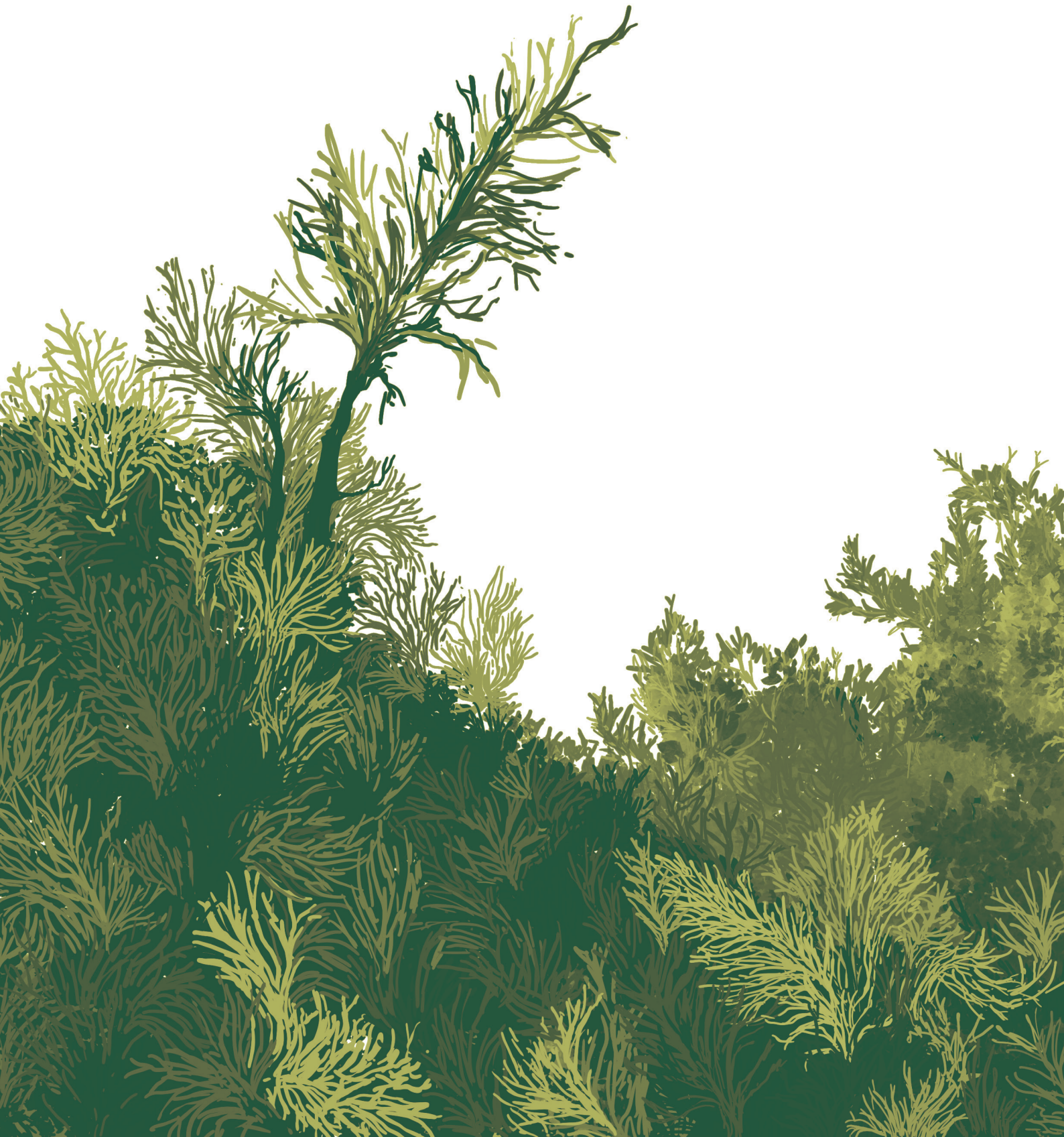
This project has received funding from the Horizon 2020 EU Research and Innovation Program under grant agreement No. 689518 (MERCES), the Fundación Biodiversidad under the framework of the project: “Conservación y restauración de poblaciones de especies amenazadas del género *Cystoseira*” and the Spanish Ministry Project ANIMA (CGL2016-76341-R, MINECO/FEDER, UE). This project has also been funded by Dirección General de Innovación e Investigación (Govern Illes Balears) and European Regional Development Fund (FEDER). JV has been funded by a IFUdG-2016 grant. The outputs presented here only reflect the views of the authors, and the EU cannot be held responsible for any use of the information contained therein. We acknowledge the contributions of Eva María Vidal, Fiona Tomas and María García in sampling and Jorge



Santamaría in data analysis. The authors also acknowledge the D.G. d'Espais Naturals i Biodiversitat, the D.G. de Pesca i Medi Marí, and the Servei en Recerca i Desenvolupament del Govern de les Illes Balears for providing permits and the Jaume Ferrer Marine Station (Instituto Español de Oceanografía) for technical and facility support. EC and JV are members of the Marine Conservation Research Group ([www.medrecover.org](http://www.medrecover.org)) from the Generalitat de Catalunya. American Journal Experts edited the English in this manuscript.



# General Discussion and Concluding Remarks





Chapters 1 and 2 of this thesis have shed some light on the effects of climate change on *Cystoseira s.l.* populations providing remarkable insights into the effects of temperature on this seaweed. They assess the influence of temperature at different life stages and how local factors and processes may modulate the response of *Cystoseira s.l.* populations to chronic gradual warming and acute marine heatwaves. Chapter 3 describes two non-invasive restoration techniques based on the enhancement of recruitment potential. Both techniques have proven to be cost-effective to recover *Cystoseira s.l.* populations after 6 years with only one restoration action, offering new and promising tools to locally restore these ecosystems. All the work has focused on shallow coastal *Cystoseira s.l.* species and populations, which constitute habitats considered as early warning systems for climate change impacts (Helmuth et al., 2006b).

Results have been discussed widely at the end of each corresponding chapter. In this general discussion I intend to combine the different outputs of each chapter to discuss the implications and the contribution of this thesis in a broader and easy-going way.

### **EFFECTS OF CLIMATE CHANGE ON *CYSTOSEIRA S.L.* AND LOCAL SCALE VARIABILITY**

Global warming, by means of gradual or abrupt temperature changes, is a major driver of species redistribution (Sunday et al., 2012; Poloczanska et al., 2016; Pecl et al., 2017). It is well known that temperature influences processes across all levels of biological organization, from genes, through populations, to ecosystems (Brierley and Kingsford, 2009). As temperature determines the performance of seaweeds (van den Hoek, 1982; Yarish et al., 1986; van den Hoek et al., 1990), prolonged periods with seawater temperatures above thermal optimum can cause direct negative impacts, including

damage of algal tissue, delay in development, reduction in growth rate and reproduction, decrease of resilience to disturbances and, ultimately, large mortality events (Eggert, 2012; Harley et al., 2012). However, despite global trends in climate change being defined over large geographic scales and mean temperature increasing at a global scale, organisms respond to the local environmental conditions in which they live (Hallet et al., 2004; Helmuth et al., 2006b). In this sense, seaweed responses to stressors are highly variable not only at regional but also at local scale, suggesting an influence of local drivers in the patterns of seaweed responses (Helmuth et al., 2006b, 2006a; Krumhansl et al., 2016).

Understanding and forecasting the species responses to climate change is critical to guide effective management and conservation strategies to mitigate the effects of climate change. In this thesis we have experimentally determined the direct effects of temperature for *Cystoseira s.l.* performance at the individual-level. Although it could seem an obvious step, it is challenging owing to the several life stages upon which temperature can act (Schiel and Foster, 2006; Matson and Edwards, 2007). Even for the best studied seaweeds, microscopic life stages are less well understood, while most are lacking information in this regard (Harley et al., 2012). In the case of *Cystoseira s.l.* species, except *C. compressa* (Perkol-Finkel and Airoidi, 2010; Savva et al., 2018), the effects of temperature were only known in early-life stages of a deep-water species (Capdevila et al., 2018a). In the first chapter, we have found that acute warming events, such as marine heat waves, can drastically impair the performance of the individuals of shallow *Cystoseira s.l.* species at different life-stages. The fertility and the survivorship of recruits resulted particularly susceptible to warming, which is of special concern since temperature impacts to the overall population may be magnified by the impacts on recruitment-related processes. As in other seaweeds, temperature effects vary among life-history stages, increasing the complexity of individual-level responses of *Cystoseira s.l.* to warming (Matson and Edwards, 2007; Harley et al., 2012). This finding provides new information on the thermal tolerance of *E. crinita* and, despite

we can establish no upper thermal limit, it is relevant to better understand the response of *Cystoseira s.l.* to thermal stress.

In parallel, understanding the variability of environmental factors at spatial and temporal scales relevant to populations could give us a better sense of how *Cystoseira s.l.* populations may respond to future climate change (Helmuth et al., 2006b, 2006a; Harley et al., 2012; Krumhansl et al., 2016). Thanks to a time-series of observational data, in summer 2015, we could register an important decline of adult individuals of a *Cystoseira s.l.* population at the Catalan coast. This population decline coincided with a MHW, which surprisingly hardly affected another nearby population (~50 km apart). Remarkably, the regional quantification of the MHW, described from satellite SST data, did not capture local-scale variation in the intensity of MHW, while the temperature loggers deployed *in-situ* at each population registered a markedly more intense MHW in the enclosed habitat when compared with the open cove. These results suggest that local-scale environmental heterogeneity, may modulate regional and global patterns and drive small-scale variability in thermal conditions, which may cause different responses at population-level. This variability, should be carefully considered under the general assumption of global patterns of climate change over large geographic scales, since often, small-scale and short-term environmental changes tend to be masked within large-scale patterns.

Furthermore, these findings reveal the importance of continuous monitoring programs that allow obtaining long-term and fine-scale data, which are often difficult to maintain. While determining the impacts of a stressor on organisms can be “more or less easily” addressed under laboratory experiments, finding evidence to identify a stressor as the cause of, or to correlate with, *in-situ* changes in the population, is only plausible through observational time series analyses. When establishing a long-term monitoring program, a study area, the sampling points, the periodicity and the variables to sample have to be defined. The occurrence of catastrophic events like MHWs cannot be predicted, which is somehow disappointing, in particular if the

interest lies in defining the *in-situ* effects of a concrete stressor (in this case temperature) on a population. Many of the studies reporting an ecosystem change linked to a given stressor that occurs stochastically in nature have been detected by chance, thanks to long-term monitoring programs. Anyway, only by gathering baseline time-series of fine ecological and environmental information at temporal and spatial scales relevant for organisms and populations we will be able to understand the natural dynamics of populations and therefore, their responses to changing conditions, specially to punctual and acute climatic events (e.g. MHWs but also storms) (Thibaut et al., 2015; Ricart et al., 2018).

Besides, populations can also show differential responses to environmental changes throughout the geographic distribution of the species, as a result of the interaction between local environmental conditions and biological processes. As proved for other seaweeds (Bennett et al., 2015; King et al., 2018) we have demonstrated the existence of geographic intraspecific variability in thermal tolerance across *E. crinita* distribution. This information is of special interest when combined with modelling approaches on the distribution of *E. crinita* and its response to warming. The model outputs evince the relevance of intraspecific variability when forecasting species responses under future warming trends since, if ignored, highly contrasting scenarios can be delivered. Specifically, more realistic predictions of future species vulnerability were obtained when considering such variability than when a homogeneous response was assumed, suggesting that failure to account for intraspecific variability may result in under or overestimation of future species vulnerability and, thus, a possible extinction risk. However, our models have some limitations. Despite knowing the different implications of temperature at the different life stages, it could not be tested for all the studied populations, and therefore, this variable was not considered in the model. In the case it would have been, a higher vulnerability of the species would be expected. Similarly, dispersal capacity (Assis et al., 2017) and the interaction of multiple factors (Crain et al., 2008; Côté et al., 2016) are also important factors defining the



vulnerability of populations. Obtaining empirical information is a challenging task and in addition, integrating all this knowledge requires more complex models (Rodríguez et al., 2019), but it is essential if we are to improve the predictions' accuracy to finally provide powerful complementary tools to guide management and conservation strategies.

Considering all the reported findings together, we show that the direct impacts of temperature will be ultimately determined by the relationship between i) the rate and magnitude of warming, which can be highly variable at local scales, ii) the life stage-specific responses and iii) the geographical intraspecific variability in thermal tolerance. Overall, our results contribute to the understanding of how *Cystoseira s.l.* populations may respond to warming impacts at local and regional scales. But also, as suggested by other authors (Helmuth et al., 2006b; Harley et al., 2012; Bates et al., 2018), they evidence the importance to link biodiversity shifts to changes in conditions at the scales of space and time that are relevant to organisms. Therefore, our results may help to guide local-scale and adaptive management and conservation strategies, as for example local-scale site prioritization, identification of local-scale climatic refugee or selection of thermal resistant populations, which have been identified as most important to support ecosystem resistance and resilience and thus buffer the incoming impacts (Strain et al., 2015; O'Leary et al., 2017; Bates et al., 2018).

## **TO WHAT EXTENT IS CLIMATE CHANGE A WORRYING THREAT TO *CYSTOSEIRA S.L.* FORESTS?**

After showing that ocean warming may represent a threat for *Cystoeira s.l.* populations, what is the real threat posed by climate change to *Cystoseira s.l.* forests?

During at least four decades (1970s to 2010s), *Cystoseira s.l.* forests have suffered dramatic and widespread regressions throughout the Mediterranean (Cormaci and Furnari, 1999; Thibaut et al., 2005; Serio et al., 2006). Mostly from 1987, a large

number of climate-driven impacts (especially warming impacts) have been reported on numerous Mediterranean marine species (Marbà et al., 2015; Garrabou et al., 2019), causing, among others, numerous mass mortality events of benthic invertebrates with important ecological consequences (Cerrano et al., 2000; Perez et al., 2000; Garrabou et al., 2009; Cebrian et al., 2011; Rivetti et al., 2014; Verdura et al., 2019). However, the widespread *Cystoseira s.l.* deforestation has been mainly related to habitat destruction (Thibaut et al., 2005, 2015; Sales and Ballesteros, 2010; Mariani et al., 2019), changes in water quality or pollution (Munda, 1982, 1993; Sales et al., 2011; Pinedo et al., 2013, 2015), overgrazing by native (Gros, 1978; Sala et al., 1998; Thibaut et al., 2005; Hereu, 2006) or invasive species (Sala et al., 2011; Bianchi et al., 2014) and out competition by other species like mussels (Bellan-Santini, 1965; Gros, 1978; Soltan et al., 2001; Thibaut et al., 2005) or turf algae (Pinedo et al., 2013). So far, warming has not been clearly related to *Cystoseira s. l.* rarefaction, although some authors blame climate change as one of the multiple factors that can cause widespread forest regression in some places (e.g. Cormaci and Furnari, 1999; Bianchi et al., 2014; Blanfuné et al., 2019). In fact, our most accurate modelling predicts relatively low risk of extinction. Thus, despite we show that *Cystoseira s.l.* species are vulnerable to warming, it does not seem to be currently posing a serious threat to *Cystoseira s.l.* populations. In this context and considering the projected rapid rates of warming and the increase of punctual extreme events in the Mediterranean Sea (Darmaraki et al., 2019; Oliver et al., 2019), climate change is likely to become another major threat for these ecosystems, especially for those populations whose resilience has been eroded by previous perturbations (e.g. small-size populations) and if climate change shows synergistic effects with other stressors acting at the same time and place.

Finally, although this thesis was only focused on the direct effects of warming, other stressors derived by climate change may also have an effect on the widespread regression of *Cystoseira s.l.* forests. For instance, the increase of storms severity and

frequency (Navarro et al., 2011), or indirect effects of warming such as disruption and modification of species interactions (Vergés et al., 2014; Pagès et al., 2018).

## **CAN WE COUNTERACT *CYSTOSEIRA S.L.* DEFORESTATION?**

Given the ecological consequences of the widespread disappearance of *Cystoseira s.l.* forests, management actions aiming to promote their reestablishment are increasing. In the north Adriatic Sea, the improvement of water quality and the reduction of herbivory pressure has led to a natural recover of *Cystoseira s.l.* populations (Iveša et al., 2016). However, populations are not usually able to recover naturally, even when the area switches back to pre-disturbed conditions (Soltan et al., 2001; Sales et al., 2011; Pinedo et al., 2013). In such cases, active restoration emerges as the only tool to assist the recovery of these degraded populations. In the last chapter of this thesis we show that active restoration based on recruitment enhancement may become a feasible tool to re-establish *Cystoseira s.l.* populations. In our study case, the lack of nearby populations hindered the natural recovery of the population even after the improvement of water quality probably dating from almost thirty years ago (Sales et al., 2011).

The knowledge of the previous presence of the species in the area and the recovery of the environmental conditions to pre-disturbance levels, allowed a successful restoration of *Gongolaria barbata*. Both requirements are seldom fulfilled in other candidate places to be considered for restoration of *Cystoseira s.l.* populations. There is almost no information on the past distribution of many macroalgal populations, which makes it difficult to select sites for restoration (but see Thibaut et al. 2005, 2015, 2017, Mariani et al. 2019). Regarding the second requirement, there are very few areas where anthropogenic pressures have been reversed, meaning that most coastal places are still under the pressures that caused the decline or even the local extinction of *Cystoseira s.l.* Additionally, integrative knowledge of the current distribution, ecological quality

and stressors affecting *Cystoseira s.l.* populations at a Mediterranean scale is rather limited (but see Fabbrizzi et al., 2020).

We contend that the conservation of macroalgal forests requires a broad spectrum of measures, ranging from mitigating human impacts to restoring the most degraded populations and habitats, together with additional efforts in those areas where there is little natural habitat left after being destroyed or modified. In my opinion, recovering *Cystoseira s.l.* populations must imply mitigating or removing the coastal perturbations with the final objective to promote their natural recovery and only in those places where natural recovery is not taking place, active restoration measures have to be implemented.

Taking advantage of the high reproductive potential (Guern, 1962; Mangialajo et al., 2012) of *Cystoseira s.l.* species, we provide two new restoration techniques based on recruitment enhancement. This is of special concern given the impaired ecological status of many *Cystoseira s.l.* species (UNEP/MAP, 2013), and therefore both techniques were designed on the premise of not harming the source populations. The described methodologies are innovative since the few trials to restore these species were based on the transplantation of adults or juveniles (Falace et al., 2006; Susini et al., 2007; Perkol-Finkel and Airoidi, 2010; Robvieux, 2013). Both designed techniques resulted cost effective, providing a sufficient supply of new recruits with just one restoration action. Thus, we have been able to reduce the impact in the source population and reduce the cost of the restoration action, which is usually high (Eger et al., 2020).

We also highlight the need of mid-term monitoring programs (6 years in the species used in our assays but probably longer in other long-lived species) as well as the tracking of multiple success indicators, since both strategies are essential to both assess the restoration success and to conclude the achievement of a mature and self-sustaining population. Thus, our results coincide with the growing general claim for

the need of longer restoration projects, since the majority of the work is conducted and assessed in less than 2 years (Basconi et al., 2020; Eger et al., 2020). We are aware that the main reason behind this is the insufficient and intermittent financial investments, derived from short-term and discontinuous projects, which can be extremely limiting to assess the recovery and the final outcome of the restoration (success or failure) (Eger et al., 2020).

It is commonly mentioned that restoration projects are often conducted over small spatial scales (<1 ha) (Eger et al., 2020). Although this is true, small areas can be of high ecological relevance if we are restoring populations which in natural conditions already occupy reduced areas, such as many sheltered shallow water *Cystoseira s.l.* species, and, in particular, the target species of this study. We have reported a restored area of about 100 m<sup>2</sup> 6 years after the beginning of the restoration action. Afterwards, Gran (2020) carried out an exhaustive cartography showing that the population covered about 763 m<sup>2</sup> 9 years after the beginning of the restoration action, which confirms its success. Indeed, re-establishing a mature and self-sustaining population of a structural species is the first step to finally restore the functions and the diversity of the whole forest, which is likely to take more time (Marzinelli et al., 2016). Despite we are not presenting results in this regard, complementary samplings of the associated biodiversity have been performed and are being processed. Future analyses of biodiversity and ecological functions will be run in the near future by our research team and we will determine the effects of the *Cystoseira s.l.* re-establishment on the associated biota and the recovery of the forest functionality in the sense of Ruiz-Jaen and Aide (2005) and Marzinelli et al. (2016).

Finally, it is worth mentioning that the success of the restoration action depends on a wide variety of factors like the selection of the methodology, the site, the season and the donor population. The causes of the restoration failure are not usually analysed, let alone reported in most studies. Under the increasing restoration efforts, a wide number of criteria and methods are expected to become available soon, while

refinement of restoration techniques is needed in order to ensure future successful restoration actions. Recently, it has been shown that some complementary techniques to the supply of recruits increase the success of restoration in areas where ecological interactions can compromise the survival of the early stages of life (e.g. sea urchins culling or herbivory exclusion cages) (Tamburello et al., 2019; Medrano et al., 2020a). Additionally, Wood et al. (2020) showed the chance of using genomics to inform choice of donor sites and design restoration programs aiming at increasing the genetic diversity of the restored populations. In this context, the collaborative development of best practice roadmaps and specific protocols (e.g. site or method selection guidelines) could be considered to help design restoration projects in view of being cost-effective and ensuring the long-term restoration success. If we are to scale up restoration actions, baseline information with an in-depth knowledge of the species ecology and of the areas to be restored is also needed (Tamburello et al., 2019).

My personal opinion is that we already have the tools and knowledge to counteract marine deforestation. But, are we ready? Instead, we should first stop deforestation, which is not actually happening. Indeed, restoration may sound like a paradox in such an impacted world. While habitat restoration is feasible in some areas and can be implemented, we should prioritize the mitigation of the anthropogenic stressors and locally manage the ecosystems in order to foster their resilience (O’Leary et al., 2017). However, while some stressors are relatively easy to be removed or minimised, others like climate change may need centuries or even millennia for being mitigated.

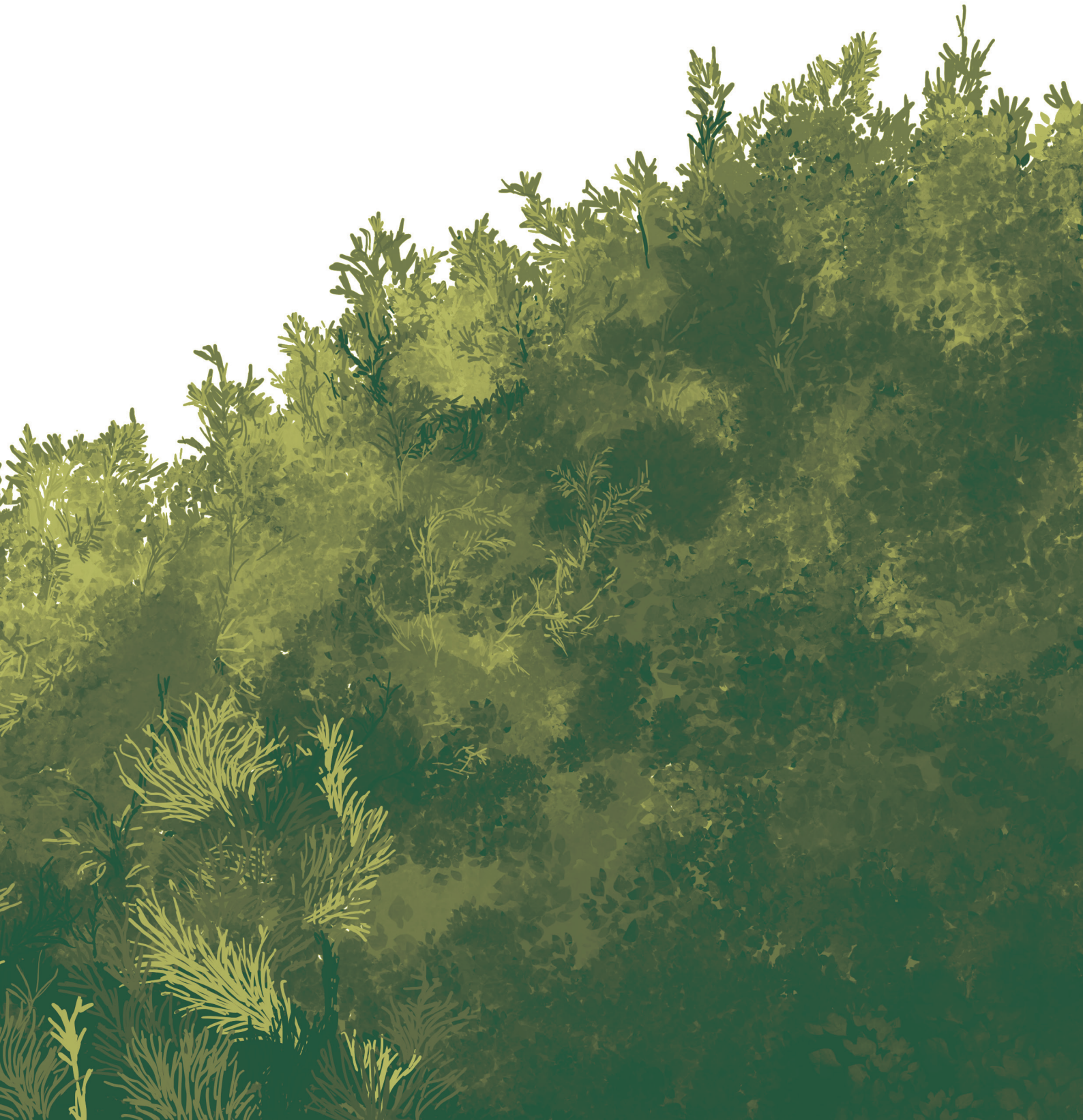
Even if an awareness of the necessity for urgently conserving climatic refuges is raising in the current times (Assis et al., 2016), can we expect restoration methods to be able to prevent or revert future climate change impacts? A promising but, as yet, untapped opportunity for enhancing the climate-resilience of restoration investments rests in the exploitation of natural genetic variability of key species (Prober et al., 2015). There is an emerging debate on enhancing “future-proof” populations to anticipated local conditions (Wood et al., 2019) as for example via restoring with purposely selected

donor populations with desirable traits in the context of climate change (Wood et al., 2020) or via “assisted gen flow” whereby more resistant individuals are introduced to more vulnerable populations (Aitken et al., 2008; Aitken and Whitlock, 2013). However, in these cases genetic risks should be first considered (Aitken and Whitlock, 2013). Nevertheless, while the capacity of plants to adapt to environmental change through plasticity, selection, or gene flow has been intensively explored (Prober et al., 2015); for marine habitats and species the available knowledge is still scarce. In addition, the impacts of climate change are highly variable geographically, and a place-based understanding of climate change threats to marine ecosystems is needed. Combined modelling approaches considering intrinsic adaptation of habitats and species, together with predictions of climate change trends and impacts, are essential to properly assess the fate that species and habitats will follow when restored.





# Conclusions





## Chapter 1

- \* Temperatures of 28 °C drastically affect the viability of *Ericaria crinita* individuals from northern Catalonia (NW Mediterranean), specially on zygotes settlement and early-life stages survival.
- \* Reduction of the photosynthetic efficiency is one the first sign of thermal stress on adult individuals, while longer exposures imply biomass losses and reduction of C:N ratio. Settlement of zygotes and survival of early-life stages drastically dropped after short exposures. Fertility and recruitment are the processes most severely impaired by thermal stress.
- \* Local-scale factors can lead between-site differences in thermal conditions, and thus mediate ecological responses to extreme climatic events.
- \* Local-scale variability in the magnitude of extreme climatic events can lead to local extinctions of already fragmented populations of habitat forming seaweeds, even towards the species' core range.
- \* Local-scale climatic refugia could be identified and managed to safeguard the persistence of canopy-forming seaweeds.

## Chapter 2

- \* *Ericaria crinita* shows an intraspecific variability on thermal tolerance thresholds across the geographic distribution range of the species, with populations from the coldest parts of the species' distribution showing lower thermo-tolerance.

## Conclusions

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- \* Intraspecific variability on thermal tolerance shapes the species' response to warming, and thus the future vulnerability and declines of its populations.
- \* Intraspecific variability may lead populations of *Ericaria crinita* to similar thermal vulnerability regardless of their geographical distribution.
- \* Disregarding intraspecific variability in forecasting species responses may result in an under or overestimation of species vulnerability to future warming.
- \* Fine scale ecological and environmental data is key to understand and forecast species responses to global warming.

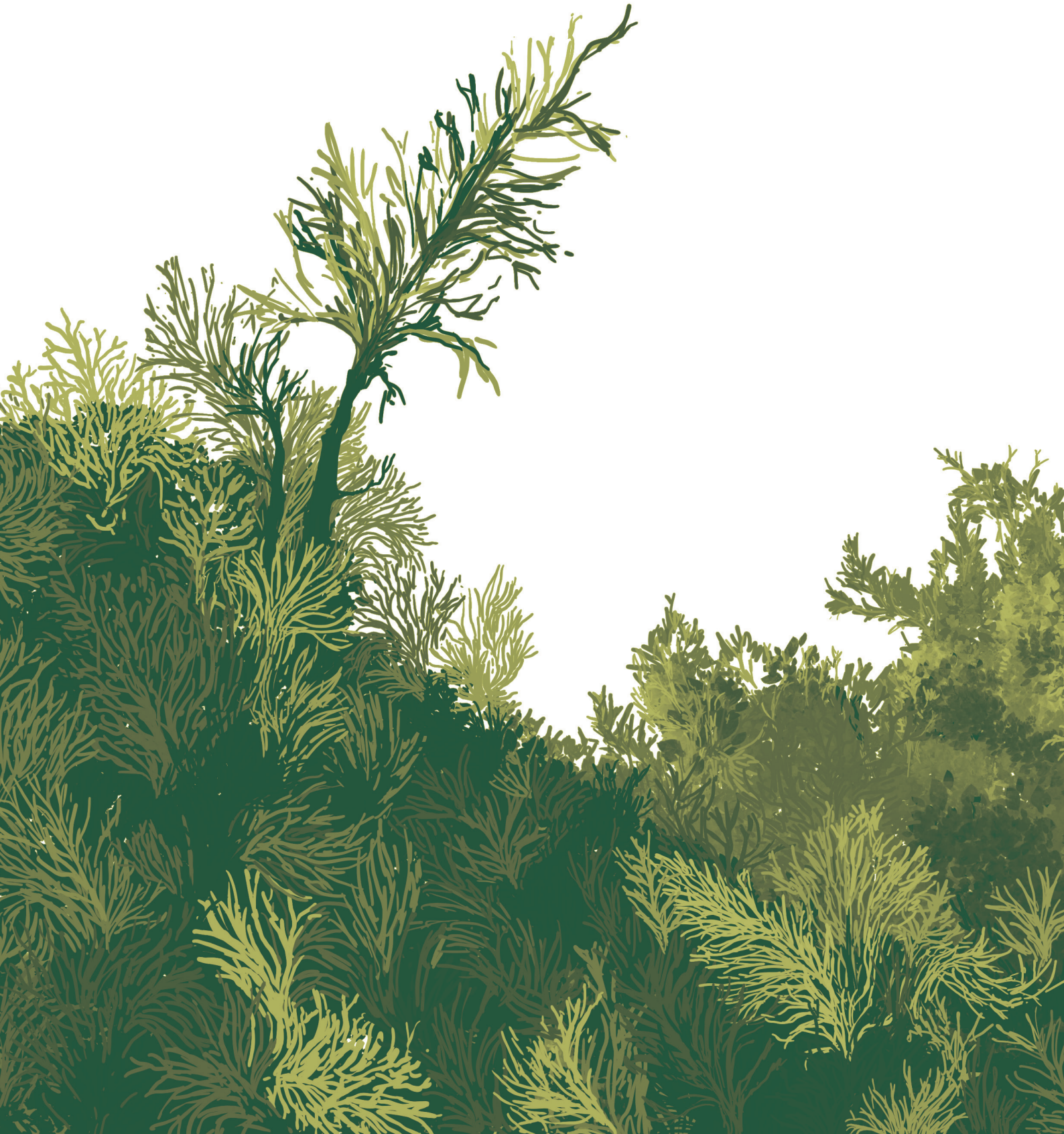
## Chapter 3

- \* A new restoration method of recruitment enhancement based on obtaining recruits from fertile branches of donor populations has proved to be successful for restoring *Cystoseira s.l.* populations
- \* Recruits can be obtained using the in-situ technique, by which they are directly settled into the field, or of the ex-situ one, by which they are grown under controlled conditions at the laboratory and afterwards transplanted to the field. Protocols and indications to apply and select the optimal technique are provided.
- \* Both, ex-situ and in-situ techniques of recruitment enhancement are cost-effective for the restoration of *Cystoseira s.l.* populations.
- \* The mitigation or removal of the stressor is the first and key step before applying restoration programs.

- \* The knowledge of the past existence of the species in a specific area, increases the probability of restoration success, as it ensures the ecological requirements of the species.
- \* Mid/long-term monitoring and multiple attributes of success are necessary to evaluate the success of the restoration.
- \* Density is a useful success attribute during the first period after a restoration action, but afterwards it should be complemented with other attributes, such as size structure, to better describe the mature stage of the population
- \* Density and size-structure are good attributes to be compared with reference populations in order to determine obtaining a mature and self-maintaining population.



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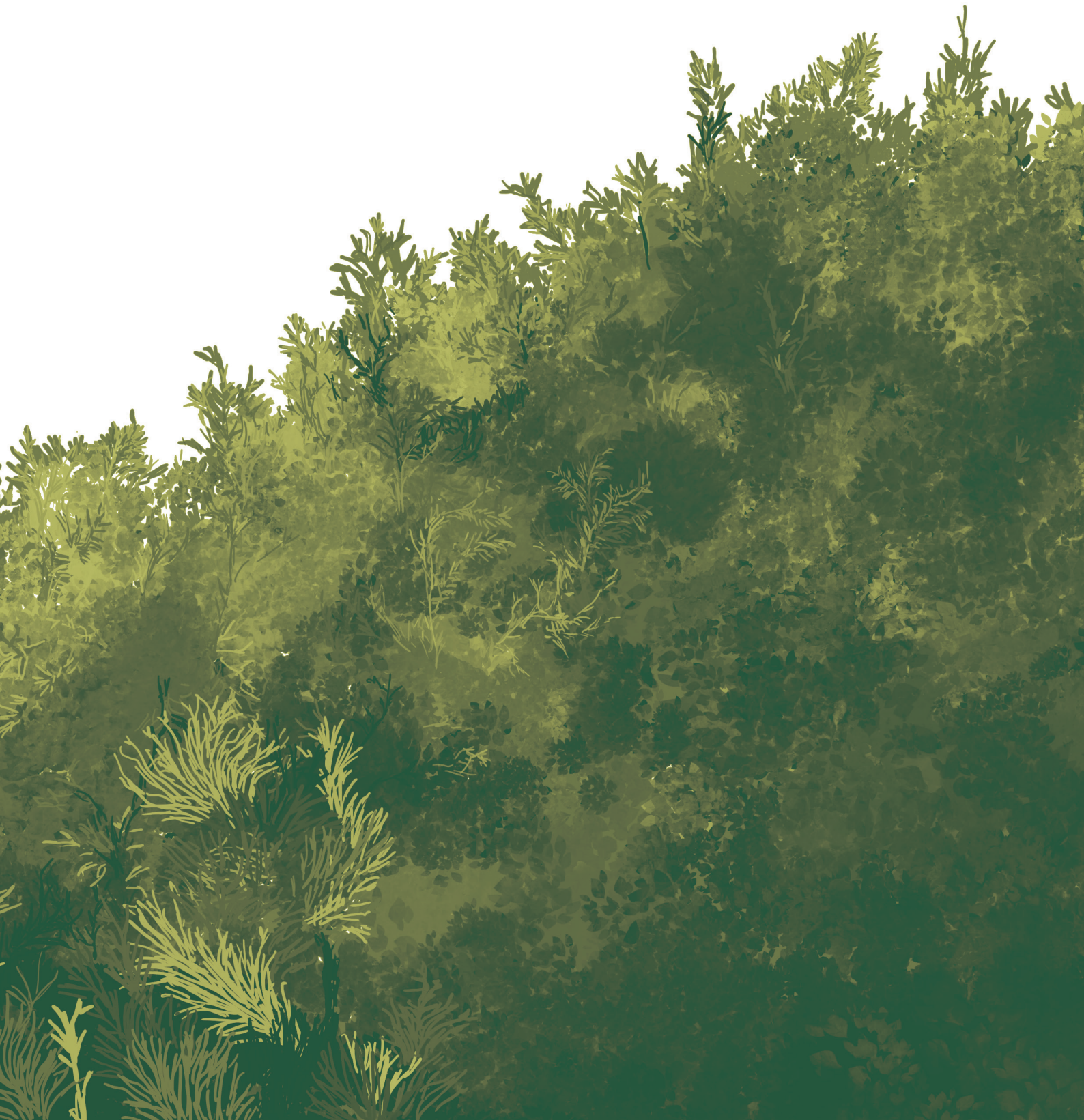
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# Supporting information







## CHAPTER 1 SUPPORTING INFORMATION

### Local-scale climatic refugia offer sanctuary for a habitat-forming species during a marine heatwave.

**Annex S1** | Details on the obtaining of data regarding *Ericaria crinita* distribution and sea surface temperature (SST) mapped in Fig. 1.

The approximate distribution of *E. crinita* is mapped in Fig. 1 in accordance with published observations from 1980 coinciding with the search in ISI Web of Science and Google Scholar using the key words: Mediterranean seaweeds, *Cystoseira crinita*, *Carpodesmia crinita* and *Ericaria crinita* (see reference list below); A. Badreddine, L. Bahbah, S. Beqiraj, M. Orlando, M. Sales and D. Serio personal communications and authors' own knowledge of field observations. Mediterranean mean SST during the period from 1982 to 2019 are also presented in Figure 1. Sea-surface temperature data was obtained from the National Oceanic and Atmospheric Administration (NOAA) High-Resolution SST dataset, developed using an optimum interpolation (OI) technique with a spatial grid resolution of 0.25 degree and temporal resolution of 1 day (OISST.V2) (Reynolds et al., 2007).

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**Table S2 | A)** Summary table of the results from analyses of deviance (Wald  $\chi^2$  test) for each fitted model testing the influence of the fixed factor (temperature) on adults' wet weight, Yield and C:N. For each response variable chi-square value ( $\chi^2$ ), degrees of freedom (Df) and  $p$  values are reported. **B)** Summary statistics from the Tukey test for the post-hoc pairwise comparisons between the different levels of the fixed factor. Note that in the case of the analysis of C:N, the fixed factor has 4 levels, corresponding to the three experimental temperatures and the individuals from the natural population (Np).

A) Effects of fixed factor				B) Tukey post-hoc test		
Response	$\chi^2$	Df	$p$	Pairwise	$z$ -value	$p$
Wet weight	76.922	2	0.021 *	21°C - 24°C	-0.245	0.967
				21°C - 28°C	-2.515	0.031 *
				24°C - 28°C	-2.270	0.060
Yield	97.858	2	0.008 **	21°C - 24°C	-0.155	0.987
				21°C - 28°C	-2.834	0.013 *
				24°C - 28°C	-2.687	0.020 *
C:N	19.012	3	< 0.001 ***	Np - 21°C	0.824	0.843
				Np- 24°C	1.674	0.338
				Np - 28°C	4.139	< 0.001 ***
				21°C - 24°C	-0.84	0.835
				21°C - 28°C	-3.276	0.006 **
				24°C - 28°C	-2.436	0.070

**Table S3** | Results of the Analyses of the Carbon and Nitrogen content. Values of Carbon and Nitrogen contents and their ratio are detailed for each sampled individual. Mean and standard deviation (SD) values for the different treatments are also detailed.

Individual	Treatment	Sampling time	Carbon	Nitrogen	C:N
1	NP	02/05/2017	23.93	0.94	25.46
2	NP	02/05/2017	30.04	1.03	29.17
3	NP	02/05/2017	22.2	0.69	32.17
4	NP	02/05/2017	29.59	1.24	23.86
5	NP	02/05/2017	29.48	0.97	30.39
			<b>Mean</b>	<b>0.97</b>	<b>28.21</b>
			<b>SD</b>	<b>0.20</b>	<b>3.45</b>
6	21 C°	11/06/2017	24.55	1.2	20.46
7	21 C°	11/06/2017	33.65	1.34	25.11
8	21 C°	11/06/2017	30.56	1.2	25.47
9	21 C°	11/06/2017	33.93	0.99	34.27
10	21 C°	11/06/2017	29.2	1.13	25.84
			<b>Mean</b>	<b>1.17</b>	<b>26.23</b>
			<b>SD</b>	<b>0.13</b>	<b>5.00</b>
11	24 C°	11/06/2017	35.37	1.26	28.07
12	24 C°	11/06/2017	35.29	1.58	22.33
13	24 C°	11/06/2017	32.99	1.24	26.60
14	24 C°	11/06/2017	33.62	1.33	25.28
15	24 C°	11/06/2017	26.68	1.41	18.92
			<b>Mean</b>	<b>1.36</b>	<b>24.24</b>
			<b>SD</b>	<b>0.14</b>	<b>3.65</b>
16	28 C°	11/06/2017	30.9	1.54	20.07
17	28 C°	11/06/2017	34.08	2.39	14.26
18	28 C°	11/06/2017	34.83	1.85	18.83
19	28 C°	11/06/2017	34.57	1.95	17.73
20	28 C°	11/06/2017	30.85	1.53	20.16
			<b>Mean</b>	<b>1.85</b>	<b>18.21</b>
			<b>SD</b>	<b>0.35</b>	<b>2.42</b>

**Table S4** | **A)** Summary table of the results from analyses of deviance (Wald  $\chi^2$  test) for each fitted model testing the influence of the fixed factor (temperature) on zygote settlement and recruits' survival. For each response variable chi-square value ( $\chi^2$ ), degrees of freedom (Df) and *p* values are reported. **B)**



Summary statistics from the Tukey test for the post-hoc pairwise comparisons between the different levels of the fixed factor.

A) Effects of fixed factor				B) Tukey post-hoc test		
Response	$\chi^2$	Df	<i>p</i>	Pairwise	<i>z</i> -value	<i>p</i>
Zygote settlement	24.706	2	< 0.001 ***	21°C - 24°C	-1.503	0.282
				21°C - 28°C	-4.97	< 0.001 ***
				24°C - 28°C	-3.958	< 0.001 ***
Recruits' survival	13.936	2	< 0.001 ***	21°C - 24°C	-0.976	0.592
				21°C - 28°C	-3.61	< 0.001 ***
				24°C - 28°C	-2.638	0.023 *

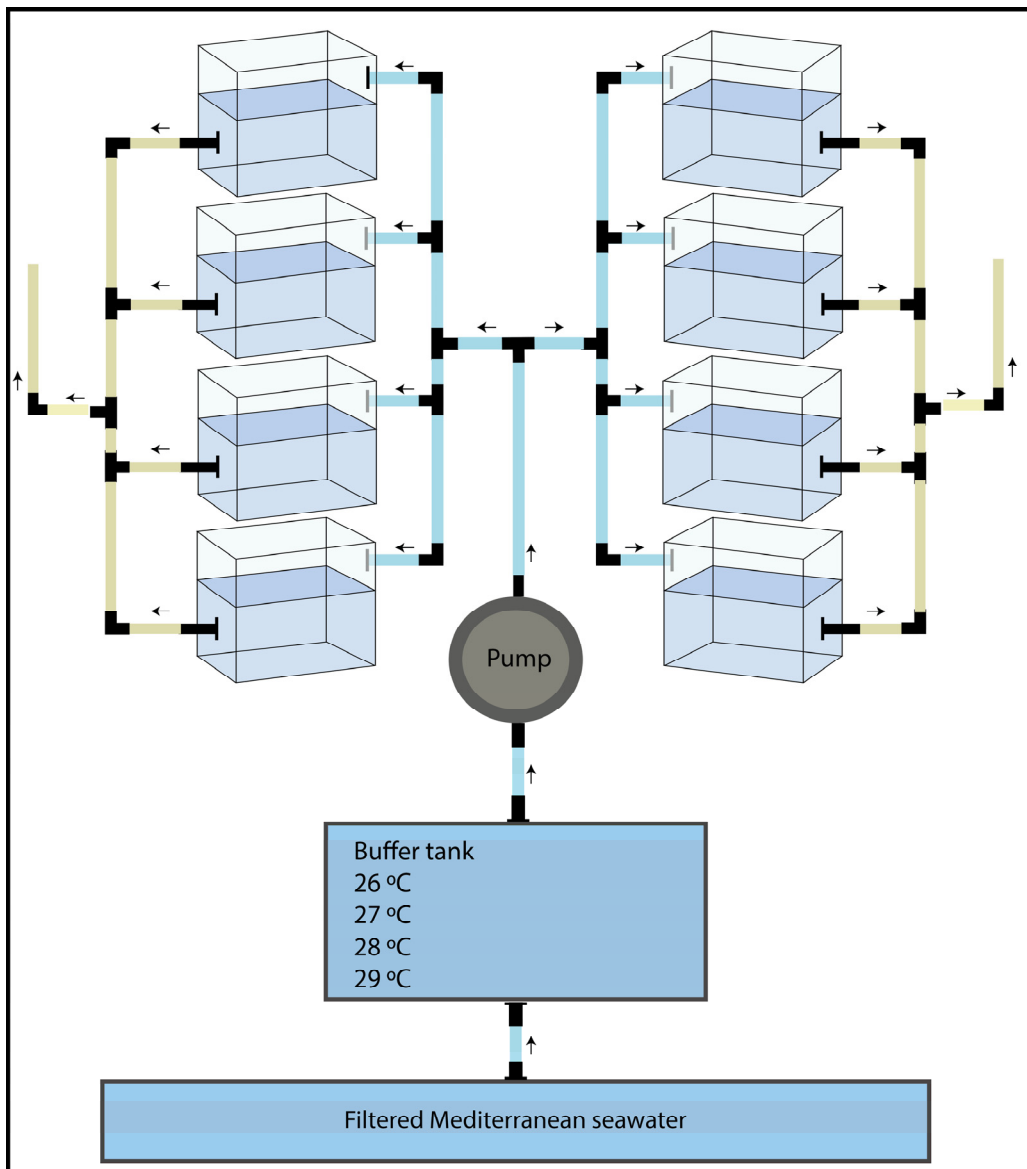


## CHAPTER 2 SUPPORTING INFORMATION

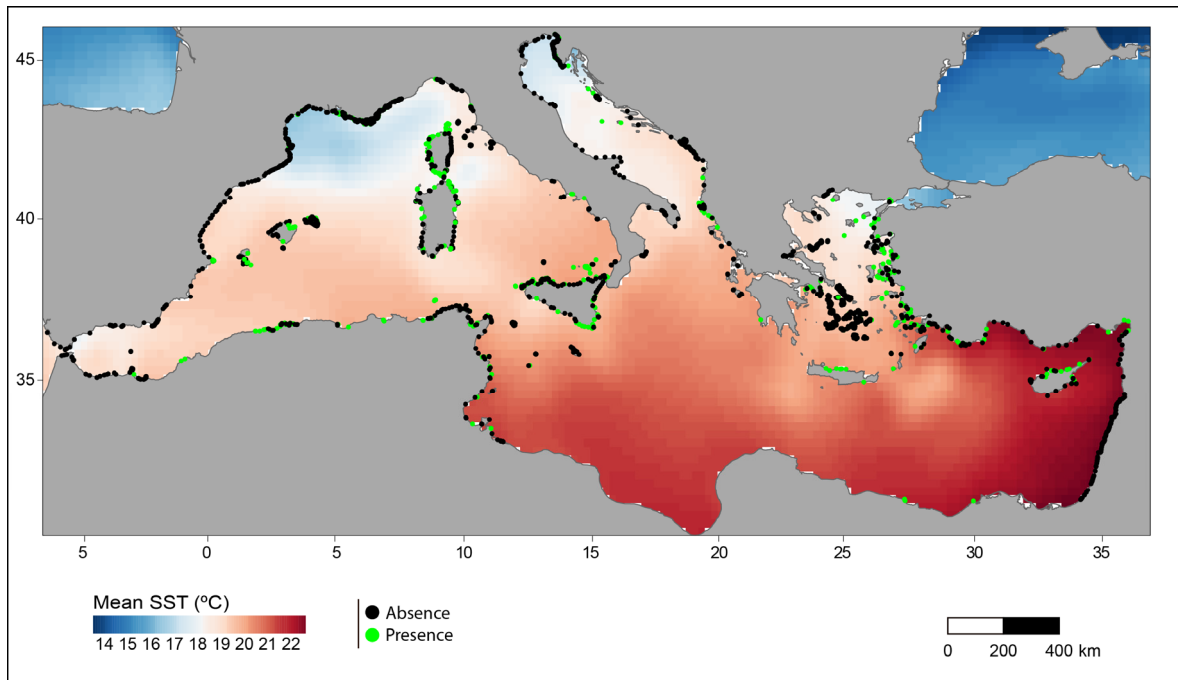
**Intraspecific variability in thermal tolerance shapes future declines in macroalgal forests**

**Table S1** | Geographical coordinates of the eight populations studied. Latitude and longitude are in decimal degrees

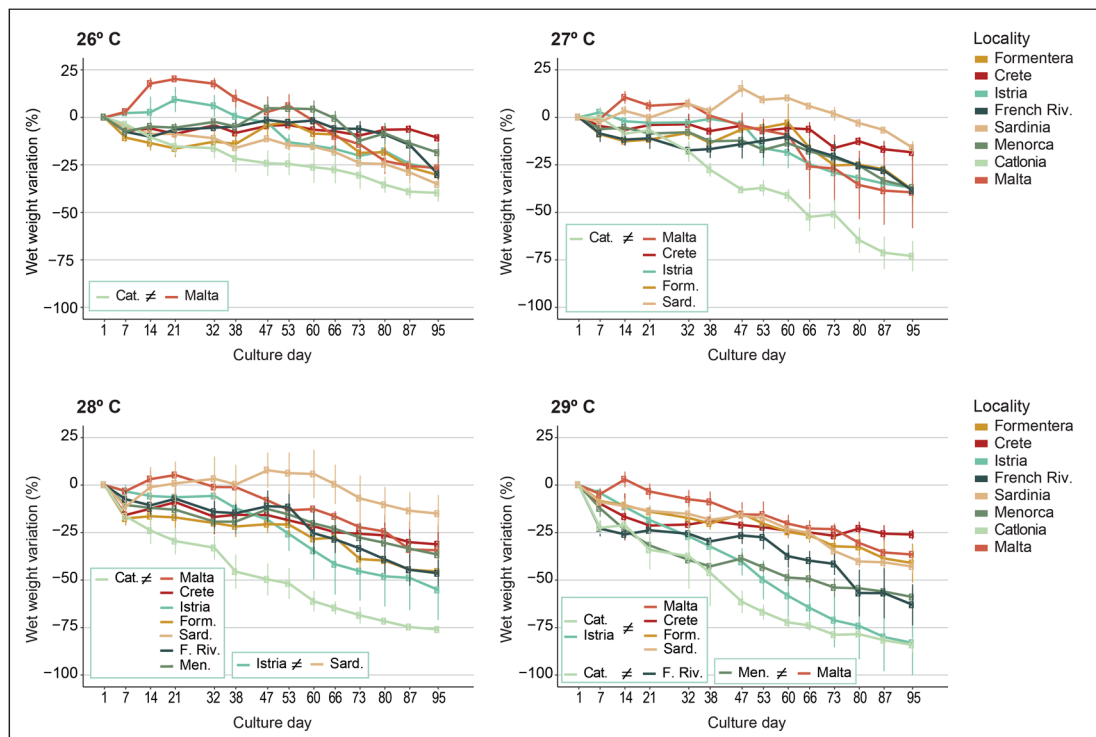
Population	Region	Country	Latitude (°N)	Longitude (°E)
Formentera	Balearic Islands	Spain	38.79690	1.42312
Heraklion	Crete	Greece	35.401694	25.033276
Rovinj	Istria	Croatia	45.14817	13.59396
Iles de Lérins	Alpes Maritimes (French Riviera)	France	43.50439	7.0453
Sardinia	Sardinia	Italy	39.91000	8.39657
Menorca	Balearic Islands	Spain	40.057072	4.066347
Palamós	Catalonia	Spain	41.86627	3.17552
Malta	Malta	Malta	35.9579130	14.419692



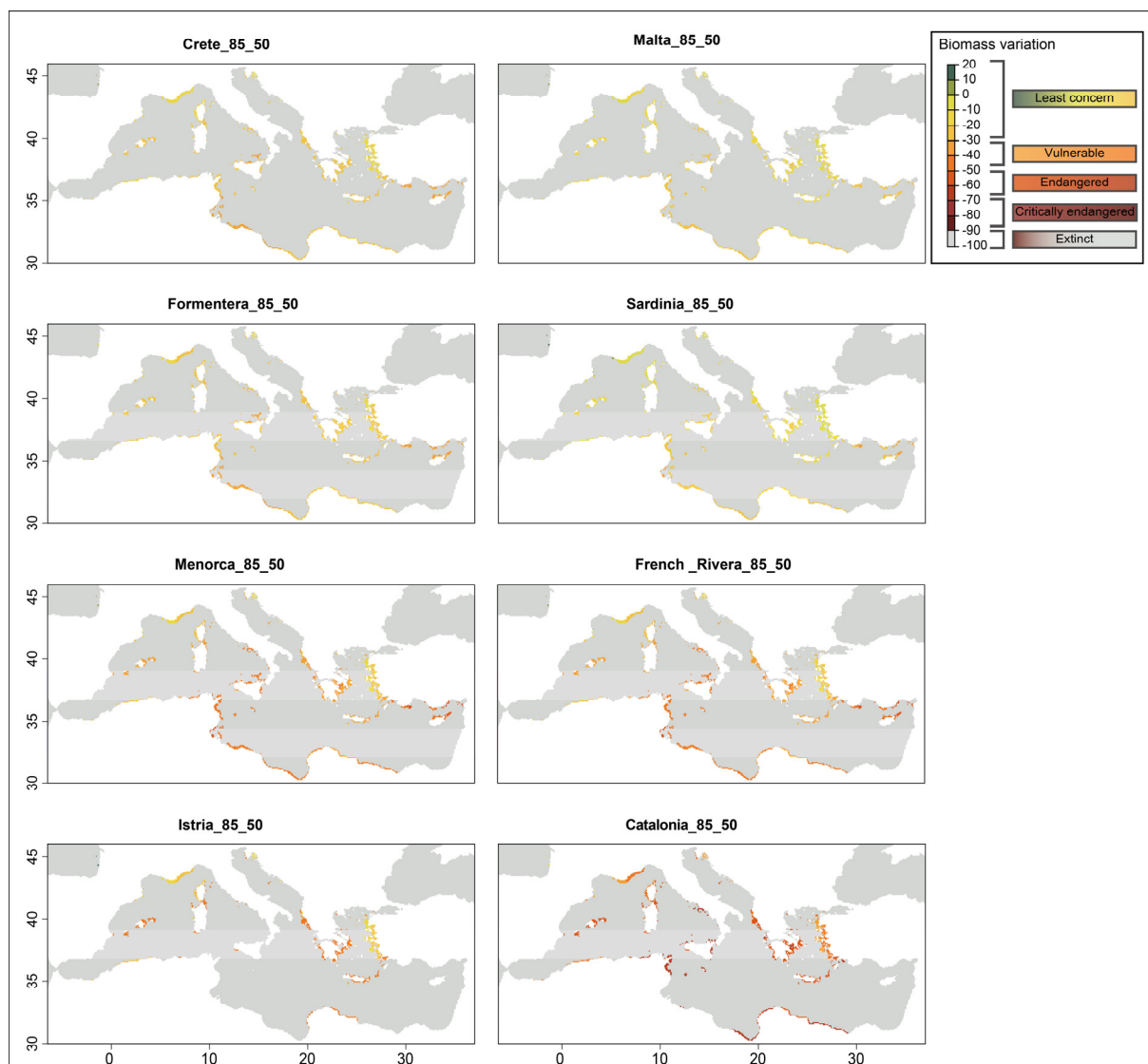
**Figure S1** | Experimental setting of the performed experiments. The aquarium set represented in the image corresponds to an aquarium set, used as a replicate. Each temperature condition comprised 3 independent sets of 8 L aquariums (3 replicates). One individual from each of the 8 populations was randomly placed in an individual aquarium in each set. Each set was connected to a large buffer tank, supplied with filtered Mediterranean seawater, from which water was pumped continuously into the experimental tanks. Each temperature condition was achieved by heating the seawater in their respective buffer tank.



**Figure S2** | Occurrence map of the presence and absence records of *E. crinita* across the Mediterranean Sea which were used in this study.



**Figure S3** | Temperature effects on the wet weight variation (mean  $\pm$  SE) among *E. crinita* individuals of the eight different populations studied over the 95 days of exposure to the different thermal experiment conditions. Significant differences between populations in each of the experimental conditions (p-values from Tukey's test with 95% confidence intervals) are indicated with letters for each graph.



**Figure S4** | Projections of the thermal vulnerability of *E. crinita*, considering the thermo-tolerance response of each of the populations as constant for the species throughout its entire distribution. The eight different thermal responses obtained in the experiment for each population studied were individually combined with future SSTs projected for 2050 under the RCP 8.5 scenario.

**Annex S1** | Reference list of the data source for *E. crinita* occurrences (presences and absences) in the Mediterranean Sea (from 1980 to 2019).

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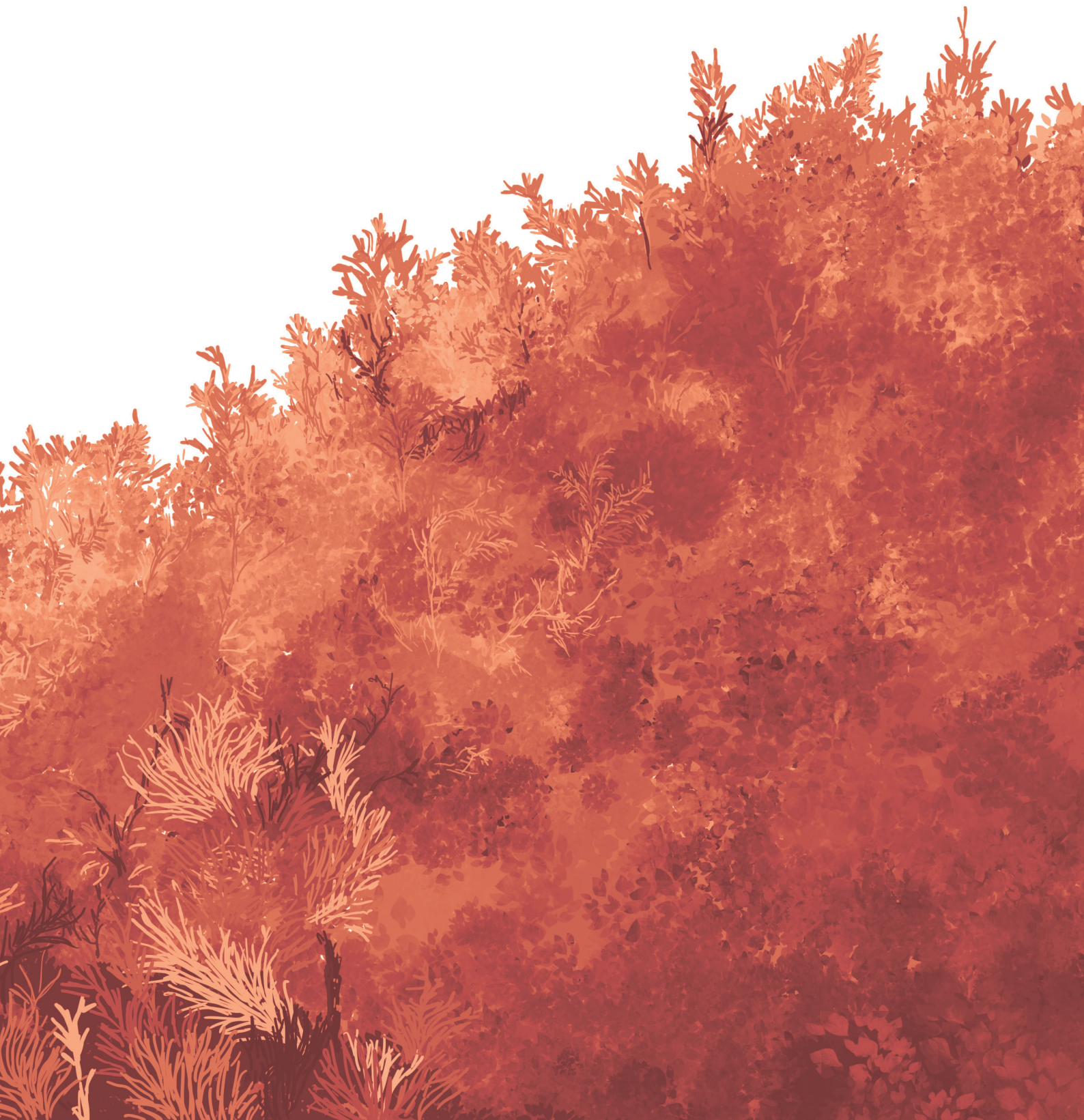
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# Published Work





# Local-scale climatic refugia offer sanctuary for a habitat-forming species during a marine heatwave

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## Funding information

Ministerio de Economía, Industria y Competitividad, Gobierno de España, Grant/Award Number: CGL2016-76341-R and FPI (BES-2017-079907); European Maritime and Fisheries Fund EMFF, Grant/Award Number: EASME/EMFF/2017/1.2.1.12/S4/01/SI2.789059; UK Research and Innovation, Grant/Award Number: MR/S032827/1; Horizon 2020 Framework Programme, Grant/Award Number: 689518; Generalitat de Catalunya, Grant/Award Number: 2017 SGR 1521; Universitat de Girona, Grant/Award Number: IFUdG-2016 and MPCUdG2016/097; European Regional Development Fund, Grant/Award Number: CGL2016-76341-R; "la Caixa" Foundation, Grant/Award Number: LCF/BQ/ES17/11600001

Handling Editor: Kathy Van Alstyne

## Abstract

1. Gradual climate change and discrete extreme climatic events have driven shifts in the structure of populations and the distribution of species in many marine ecosystems. The most profound impacts of recent warming trends have been generally observed at species' warm edges and on large conspicuous species. However, given that different species and populations exhibit different responses to warming, and that responses are highly variable at regional scales, there is a need to broaden the evidence to include less conspicuous species and to focus on both local- and regional-scale processes.
2. We examined the population dynamics of canopy-forming seaweed populations situated at the core range of their distribution during a regional marine heatwave (MHW) event that occurred in the Mediterranean Sea in 2015, to determine between-site variability in relation to the intensity of the MHW. We combined field observations with a thermo-tolerance experiment to elucidate mechanisms underlying observed responses.
3. Despite our study populations are located in the species core range, the MHW was concomitant with a high mortality and structural shifts in only one of the two surveyed populations, most likely due to differences in habitat characteristics between sites (e.g. degree of shelter and seawater transfer). The experiment showed high mortalities at temperatures of 28°C, having the most severe implications for early life stages and fertility, which is consistent with warming being the cause of population changes in the field. Crucially, the regional-scale quantification of the MHW (as described by satellite-derived SSTs) did not capture local-scale variation in MHW conditions at the study sites, which likely explained variation in population-level responses to warming.
4. *Synthesis.* Enclosed and semi-enclosed seas, such as the Mediterranean Sea, often highly impacted by human perturbations, are also global hotspots for ocean warming and are highly susceptible to future MHWs. Our findings highlight that local-scale variability in the magnitude of extreme climatic events can lead to local extinctions of already fragmented populations of habitat-forming seaweeds, even towards the species' core range. However, our results highlight the potential for

local-scale climatic refugia, which could be identified and managed to safeguard the persistence of canopy-forming seaweeds.

**KEYWORDS**

canopy-forming seaweeds, *Carpodesmia*, climate change, climatic refugia, coastal ecosystems, *Cystoseira*, *Ericaria*, marine heatwaves

**How to cite this article:** Verdura J, Santamaría J, Ballesteros E, et al. Local-scale climatic refugia offer sanctuary for a habitat-forming species during a marine heatwave.

*J Ecol.* 2021;00:1–16. <https://doi.org/10.1111/1365-2745.13599>





# Restoration of a Canopy-Forming Alga Based on Recruitment Enhancement: Methods and Long-Term Success Assessment

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## OPEN ACCESS

### Edited by:

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### Specialty section:

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

**Received:** 30 July 2018

**Accepted:** 26 November 2018

**Published:** 10 December 2018

### Citation:

Verdura J, Sales M, Ballesteros E,  
Cefali ME and Cebrian E (2018)  
Restoration of a Canopy-Forming  
Alga Based on Recruitment  
Enhancement: Methods  
and Long-Term Success Assessment.  
*Front. Plant Sci.* 9:1832.  
doi: 10.3389/fpls.2018.01832

Marine forests dominated by macroalgae have experienced noticeable regression along some temperate and subpolar rocky shores. Along continuously disturbed shores, where natural recovery is extremely difficult, these forests are often permanently replaced by less structured assemblages. Thus, implementation of an active restoration plan emerges as an option to ensure their conservation. To date, active transplantation of individuals from natural and healthy populations has been proposed as a prime vehicle for restoring habitat-forming species. However, given the threatened and critical conservation status of many populations, less invasive techniques are required. Some authors have experimentally explored the applicability of several non-destructive techniques based on recruitment enhancement for macroalgae restoration; however, these techniques have not been effectively applied to restore forest-forming furoids. Here, for the first time, we successfully restored four populations of *Cystoseira barbata* (i.e., they established self-maintaining populations of roughly 25 m<sup>2</sup>) in areas from which they had completely disappeared at least 50 years ago using recruitment-enhancement techniques. We compared the feasibility and costs of active macroalgal restoration by means of *in situ* (wild-collected zygotes and recruits) and *ex situ* (provisioning of lab-cultured recruits) techniques. Mid/long-term monitoring of the restored and reference populations allowed us to define the best indicators of success for the different restoration phases. After 6 years, the densities and size structure distributions of the restored populations were similar and comparable to those of the natural reference populations. However, the costs of the *in situ* recruitment technique were considerably lower than those of the *ex situ* technique. The restoration method, monitoring and success indicators proposed here may have applicability for other macroalgal species, especially those that produce rapidly sinking zygotes. Recruitment enhancement should become an essential tool for preserving *Cystoseira* forests and their associated biodiversity.

**Keywords:** conservation, cost-effective restoration, *Cystoseira*, Fucales, human impacts, marine forests, recruitment enhancement, seaweed restoration

## INTRODUCTION

Canopy-forming brown macroalgae, such as kelps (Laminariales) and fucoids (Fucales), are habitat-forming species in the intertidal and subtidal zones of most temperate and subpolar regions (Steneck et al., 2002; Schiel and Foster, 2006). These macroalgae create structurally complex communities that have several similarities with terrestrial forests (Dayton et al., 1984, 1992; Ballesteros et al., 2009; Reed and Foster, 2012; Gianni et al., 2013). In addition to playing a crucial role in coastal primary production and nutrient cycling, these marine forests increase the three-dimensional complexity and spatial heterogeneity of rocky bottoms, providing food, shelter, nurseries and habitat for many other species (e.g., fish, invertebrates and other algae); thus, they host high biodiversity (Mann, 1973; Seed and O'Connor, 1981; Dayton, 1985; Graham, 2004; Schiel and Foster, 2006).

Compared to many other structurally complex ecosystems around the world, marine forests are suffering from a small global decline on average, despite large regional variation in both the direction and magnitude of the changes, meaning that while global declines are small on average, local-scale declines can be severe (Krumhansl et al., 2016). In many areas, the cumulative impacts of different human pressures, such as habitat destruction, pollution, overgrazing, invasive species and ocean warming, have largely disturbed canopy-forming macroalgae in recent decades (Steneck et al., 2002; Thibaut et al., 2005; Airoldi and Beck, 2007; Connell et al., 2008; Ling et al., 2009; Vergés et al., 2014, 2016; Wernberg et al., 2016). As a result, vast underwater marine forests have gone missing from many coastal areas and are being replaced by simpler and less productive communities dominated by opportunistic taxa (such as turfs or barrens) (Benedetti-Cecchi et al., 2001; Thibaut et al., 2005; Connell et al., 2008; Mangialajo et al., 2008; Ling et al., 2009; Smale and Wernberg, 2013; Vergés et al., 2014; Valdazo et al., 2017). Although some giant kelp populations have been shown to recover quickly from local- to large-scale disturbances (Dayton et al., 1992; Edwards, 2004), this is not always the case for other giant kelp populations, not for other kelps (e.g., Dayton, 1973) or fucoids (Coleman et al., 2008; Sales et al., 2011; Smale and Wernberg, 2013). The low dispersal abilities of zygotes and/or spores have been blamed for the lack of furoid population recovery (Kendrick and Walker, 1991; Chapman, 1995; Dudgeon and Petraitis, 2001). In these cases, and when populations have become extinct, natural recovery is almost impossible, and active restoration emerges as the only tool to recover these missing forests (Stekoll and Deysher, 1996; Terawaki et al., 2003; Falace et al., 2006; Susini et al., 2007; Sales et al., 2011; Campbell et al., 2014).

The Mediterranean Sea, a marine biodiversity hotspot, has experienced large alterations in its ecosystems (Coll et al., 2010; Lotze et al., 2011). Marine forests dominated by species of the genus *Cystoseira* (Fucales) are widespread on well-preserved Mediterranean rocky bottoms (Giaccone, 1973; Ballesteros, 1988, 1990a,b; Ballesteros et al., 1998, 2009; Zabala and Ballesteros, 1989; Sales et al., 2012). Despite not reaching the size of kelp or some other fucoids, *Cystoseira* species produce a dense canopy (rarely > 1 m high) creating a “forest-like” assemblage, with

species growing in the understory that are not found without their presence. This is the reason we speak about *Cystoseira* forests.

Some *Cystoseira* forests have severely declined in recent decades (Cormaci and Furnari, 1999; Thibaut et al., 2005; Serio et al., 2006; Blanfuné et al., 2016). Since zygotes of *Cystoseira* species are very large (around 100–120  $\mu\text{m}$ ) and exhibit low dispersal abilities (Guern, 1962; Clayton, 1992), transplantation techniques have been used as a tool for environmental mitigation (Falace et al., 2006; Susini et al., 2007; Perkol-Finkel et al., 2012; Robvieux, 2013).

However, since most *Cystoseira* species are considered threatened or endangered by the Barcelona Convention (Annex II) (United Nations Environment Programme/Mediterranean Action Plan [UNEP/MAP], 2013), individual transplants from remaining populations are undesirable, and therefore, less invasive restoration actions are required (see Gianni et al., 2013 for a review). As a result, new recruits of certain furoid species have been artificially obtained and monitored for one year (Stekoll and Deysher, 1996; Terawaki et al., 2003; Yatsuya, 2010; Yu et al., 2012; Falace et al., 2018), introducing the possibility of recruitment enhancement as a new strategy for restoring *Cystoseira* populations.

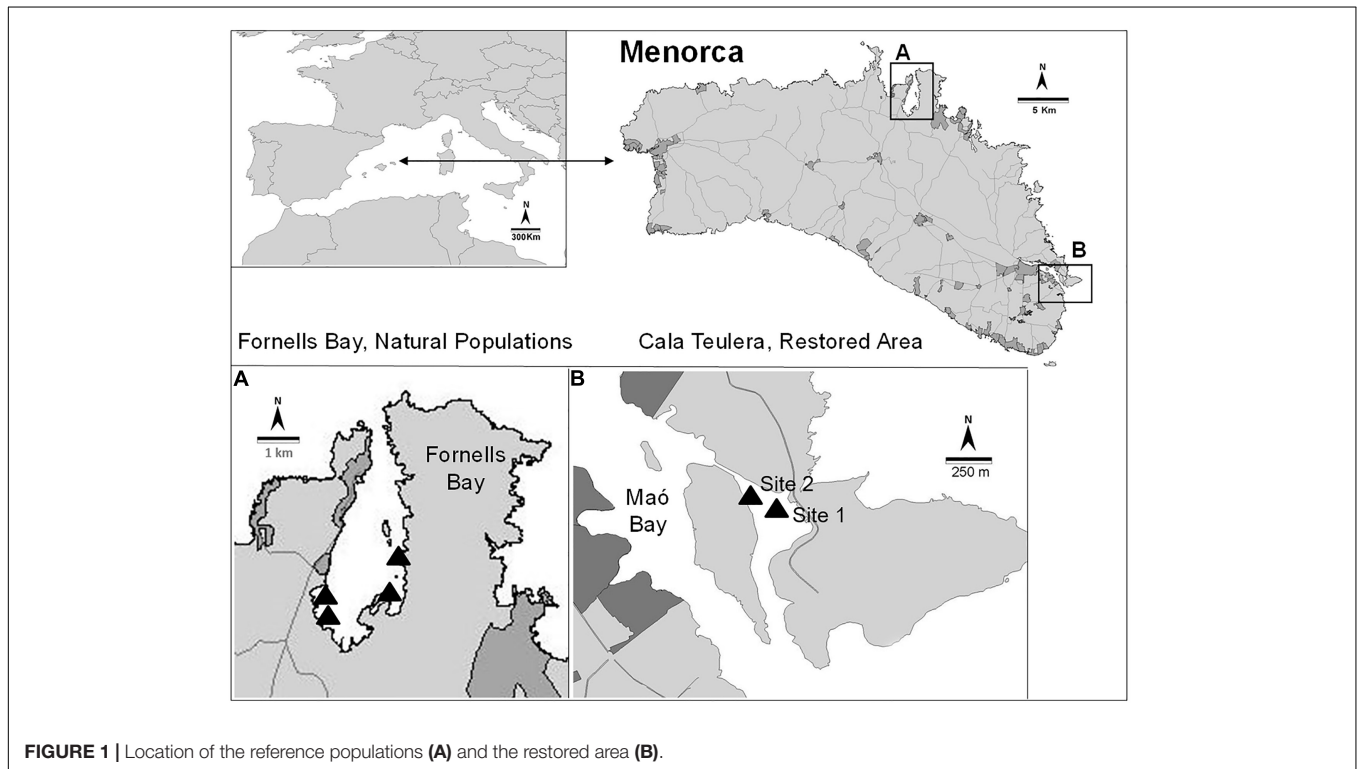
In this context, the general objective of this study is to provide and experimentally test non-destructive restoration methods that can lead to the establishment of self-sustaining *Cystoseira* populations and to describe the proper success indicators for the different restoration stages. Specifically, we describe two techniques using *in situ* and *ex situ* recruitment enhancement aimed at restoring populations of *C. barbata*, and the success of each is assessed by comparing restored and reference populations over six years. Moreover, because the success and broad-scale application of a restoration technique also depends on its cost feasibility, we also describe this key piece of information.

## MATERIALS AND METHODS

### Species and Study Site

This study focuses on the species *Cystoseira barbata* (Stackhouse) C. Agardh, which typically develops in shallow and sheltered environments (Sales and Ballesteros, 2009) across the Mediterranean Sea. The reduction in its range is strongly correlated with human development (Thibaut et al., 2005, 2015; Bologna and Sava, 2006), and the species is classified as threatened under the Barcelona Convention (United Nations Environment Programme/Mediterranean Action Plan [UNEP/MAP], 2013). These features make *C. barbata* a perfect target species for restoration in places from which it has disappeared.

This study was conducted in Menorca (Balearic Islands, NW Mediterranean), which has been a UNESCO Biosphere Reserve since 1993. Most coastal areas in Menorca are well preserved and have limited urbanization. The coastal water quality is high, so the extent and cover of habitats dominated by *Cystoseira* species is outstanding (Sales and Ballesteros, 2009). *Cystoseira barbata* naturally makes small patches (usually around a few square meters) in very sheltered and shallow environments.



**FIGURE 1** | Location of the reference populations (A) and the restored area (B).

This species is extremely rare not only in Menorca but also in other Mediterranean areas (Gómez-Garreta et al., 2002) because there are very few places matching its environmental requirements, with the exception of the northern Adriatic Sea. *Cystoseira barbata* is present in Fornells Bay (Menorca), one of the few places where the environmental conditions are suitable for its development. However, this species was reported from Cala Teulera ( $39^{\circ}52'40.64''$  N,  $4^{\circ}18'22.03'$  E; Bay of Maó, **Figure 1**) in the XVIII century (Rodríguez-Femenías, 1888), but it disappeared from this area due to direct dumping of urban and industrial sewage into the bay during the 1970s, leading to impaired water quality. A sewage outfall was built in 1980, and waste waters were diverted into the open sea (Hoyo, 1981). However, no recovery of the *C. barbata* populations was detected during the next 30 years (Sales et al., 2011). Nevertheless, Cala Teulera still shelters a reduced meadow of the seagrass *Cymodocea nodosa* and some stands of *Cystoseira compressa* var. *pustulata* and *Cystoseira foeniculacea* f. *tenuiramosa*. In contrast, Fornells Bay ( $40^{\circ}2'10.12''$  N,  $4^{\circ}7'43.24'$  E; **Figure 1**) continues to be characterized by low human influence and extensive sheltered seagrass meadows (e.g., *Posidonia oceanica*, *C. nodosa*, *Zostera noltii*) (Delgado et al., 1997) and healthy *Cystoseira* spp. forests, including the only preserved *C. barbata* populations from Menorca (Sales and Ballesteros, 2009). For this reason, the stands in Fornells Bay were selected as donor populations to restore two different sites in Cala Teulera (**Figure 1**).

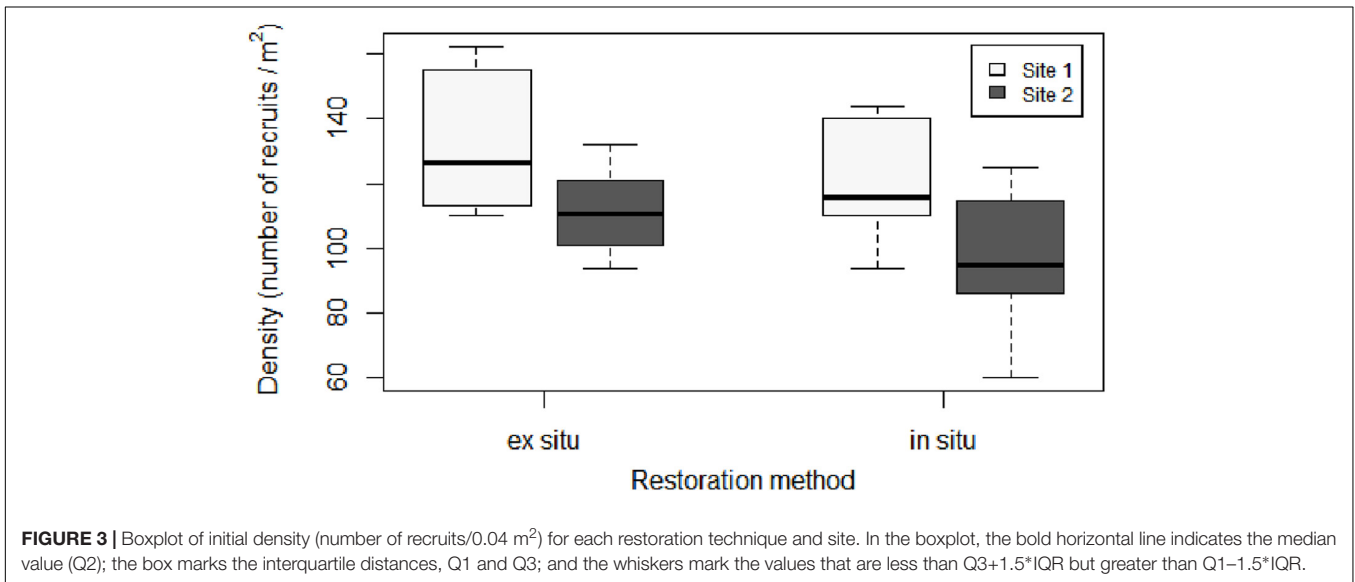
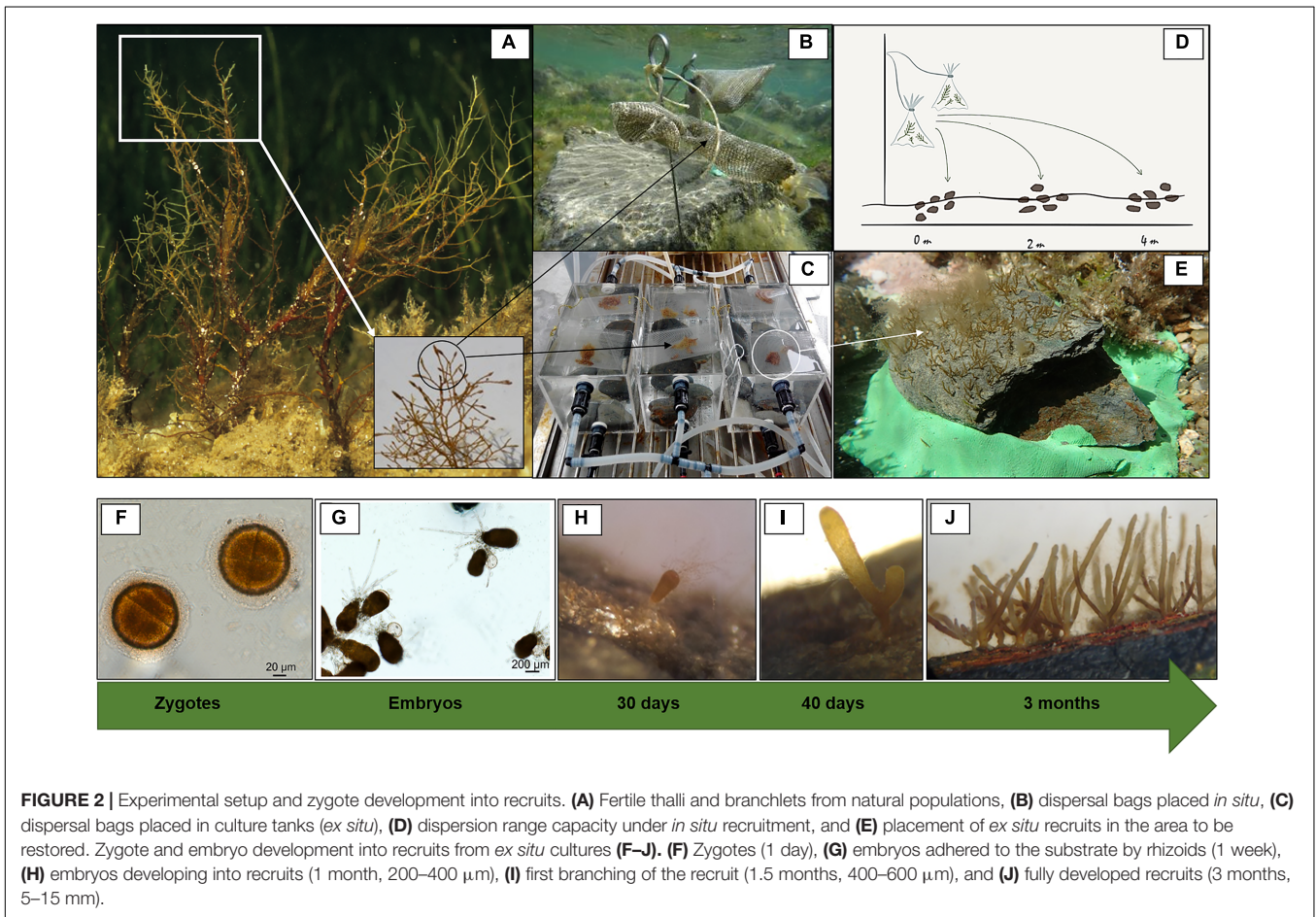
## Applied Restoration Techniques

Two different restoration techniques involving *in situ* and *ex situ* recruitment enhancement were experimentally tested to

promote *C. barbata* recovery. Both techniques are considered non-destructive since they only rely on harvesting a small proportion ( $< 5\%$ ) of reproductive fertile branchlets from wild individuals. Both donor and restored sites were situated between depths of 0.2 m and 1 m. *In situ* recruitment consisted of collecting fertile apical branchlets (March 2011) from the donor populations (Fornells Bay) that were then transported to the restoration sites and placed in dispersal bags that were 8 cm wide and 10 cm long (**Figures 2A,B**) and made of 36% fiberglass and 64% PVC with a mesh size of  $1.20 \times 1.28$  mm.

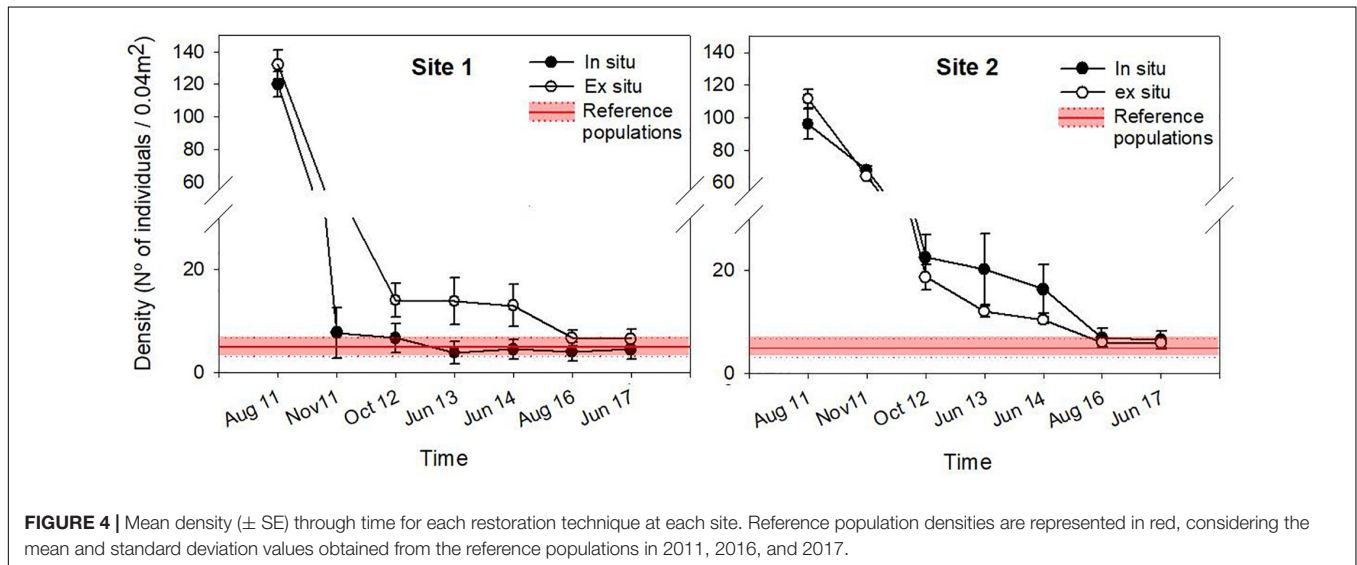
Bags were tied to a pick and directly fixed at a vertical distance of 0.25 cm from the bottom using a hammer (**Figure 2B**). Eight bags (two for each pick) containing approximately twenty fertile receptacles each were placed at each of the two selected restoration sites at distances of 2–3 m from each other. At both sites, six natural flat schist stones with similar surface areas (approximately  $0.04 \text{ m}^2$ ) were collected, cleaned of organisms and sediment and randomly placed in radii from 0.1 to 4 m around the dispersal bags to promote *C. barbata* settlement. We used stones adjacent to our study areas, and not from the same area, to avoid disturbing the study site when cleaning the stones from organisms and sediment. The stones were cleaned to provide free substrate and avoid competition at the first stages of development of new recruits. After 4 days, the dispersal bags were removed from both restored sites.

*Ex situ* recruitment consisted of acquiring a supply of zygotes and culturing settlers in the laboratory. Fertile apical branchlets (around 2–3 cm in length) from the donor populations (March 2011, Fornells Bay) were collected and placed in plastic bags without seawater and transported to the laboratory under cold



and dark conditions. Once in the laboratory, the bags containing the fertile branchlets were stored in the fridge (at 4°C and in dark conditions) for 12 h to promote zygote liberation. Concurrently, 16 natural flat schist stones with similar surface

areas (approximately 0.04 m<sup>2</sup>) were placed at the bottom of ten 12-L tanks filled with filtered seawater, and fertile apical branchlets of *C. barbata* were placed on dispersal bags floating on the water surface of each tank for 4 days (Figures 2A,C).



**FIGURE 4 |** Mean density ( $\pm$  SE) through time for each restoration technique at each site. Reference population densities are represented in red, considering the mean and standard deviation values obtained from the reference populations in 2011, 2016, and 2017.

Moreover, some glass slides were placed on top of and between the stones to enable microscopically monitoring of zygote development during the first months (Figures 2F–J). zygote development to be microscopically monitored during the first month. For the first 4 days, the hydrodynamic conditions of the tank were kept as stable as possible to facilitate zygote settlement. Afterward, zygotes were cultured in a closed-water circuit with a renovation rate of 2 L per day using natural seawater at 21°C and natural light conditions. Seawater temperature was controlled with refrigerators (Hailea Chiller HC 500 A of Hailea). After 3 months (June 2011), stones with *C. barbata* recruits were transported to the restoration sites and six stones were placed at a distance of 25 m from the *in situ* restored area at each site (Figure 2E). It was not necessary to fix the stones since the restoration areas were extremely sheltered and the stones were heavy enough to prevent any movement.

## Monitoring the Restored and Reference Populations

After installing the *in situ* and *ex situ* recruitment set ups, both sites were visited monthly to ensure that the experiment was

**TABLE 1 |** Results of GLMM comparing the density (number of individuals/0.04 m<sup>2</sup>) through time in relation to the restoration techniques (*in situ* vs. *ex situ*).

Factor	df	F-value	p-value
Technique	1	0.11	0.43
Site	1	2.67	0.17
Time	6	796.26	< 0.0001
Technique * Site	1	2.94	0.66
Technique * Time	6	0.48	< 0.0001
Site * Time	6	42.14	< 0.0001
Site * Technique * Time	6	21.25	< 0.0001

For each factor, we report the degrees of freedom and the F- and p-values. The significant values are highlighted in bold in the table.

properly maintained. After five months, both *in situ* and *ex situ* recruits were large enough to allow visual density and height measurements. Then, the density (the total number of individuals per 0.04 m<sup>2</sup>) and the size structure distribution (the length of the main axis) of *C. barbata* individuals from each stone (approximately 20 × 20 cm) were monitored *in situ* twice in 2011 (August and November) and once during 2012, 2013, 2014, 2016, and 2017 (August) at each restored site and for each restoration technique.

At the beginning of the experiment, 3 natural *C. barbata* populations (Fornells Bay; Figure 1) were also selected as reference populations for comparison with the restored populations. The densities and size structure distributions of each reference population were monitored in 20 randomly distributed, 20 × 20-cm quadrats at the beginning and end of the experiment (i.e., August 2011, 2016, and 2017).

## Dispersal Capacity of the *in situ* Recruitment Method

At the same time, a new experiment was set up to explore the extension range of the *in situ* recruitment method. We studied the dispersion capacity of the *C. barbata* zygotes. For this purpose, we fixed a new pick (with 2 dispersal bags each) at each site, and six stones (approximately 0.04 m<sup>2</sup> each) were placed just below the dispersal bags (0 m) along with six at a distance of 2 m, and finally six at a distance of 4 m. The dispersal bags were removed after 4 days, and the number of recruits from each stone was counted in August 2011 (Figure 2D).

## Data Analysis

### Comparison of Techniques

To compare the two restoration techniques, the mean densities and size distribution at both restored sites were evaluated. The mean density (number of individuals/0.04 m<sup>2</sup>) over time was analyzed using a generalized linear mixed model (GLMM) with technique (2 levels: *ex situ* vs. *in situ*), site (2 levels:

site 1 and site 2) and time (7 levels) as fixed factors, and stone as a random factor. Descriptive statistics were also calculated for the size structure distribution (the skewness and kurtosis) of restored populations and compared among both techniques and sites. The significance of the skewness and kurtosis values was calculated according to Sokal and Rohlf (1995).

### Restoration Success

Restoration success was analyzed by comparing the final densities and size structures between restored and reference populations. The final density (August 2017) of restored populations was compared with that of reference populations by means of a generalized linear model (GLM) with one fixed factor with two levels (restored vs. control). Changes in the size structure distributions of the restored and reference populations over time were plotted using non-metric multidimensional scaling (MDS) to visualize their progression. The relative percentage of individuals in each size class (in 1-cm intervals) was the variable in the data matrix, and the Bray-Curtis distance (Bray and Curtis, 1957) with a

dummy variable (= 1) was used to construct the similarity matrix.

### Dispersal Capacity

Finally, the range in dispersal capacity obtained with the *in situ* method was analyzed using GLM, with site (2 levels) and distance from the dispersal bag (3 levels) as fixed factors. Pair-wise comparisons were also performed between distances.

GLMs and GLMMs are suitable for this kind of data since GLMs can handle non-normal data (Bolker et al., 2009) and GLMMs combine the properties of GLMs and linear mixed models, which incorporate random effects and therefore can cope with repeated measures over time (Pinheiro and Bates, 2000). All analyses were performed using the lme4 package (Bates et al., 2015) (Bates et al., 2015) for R software (R Core Team, 2016) and the statistical software Primer & Permanova v.6 (Clarke and Gorley, 2006).

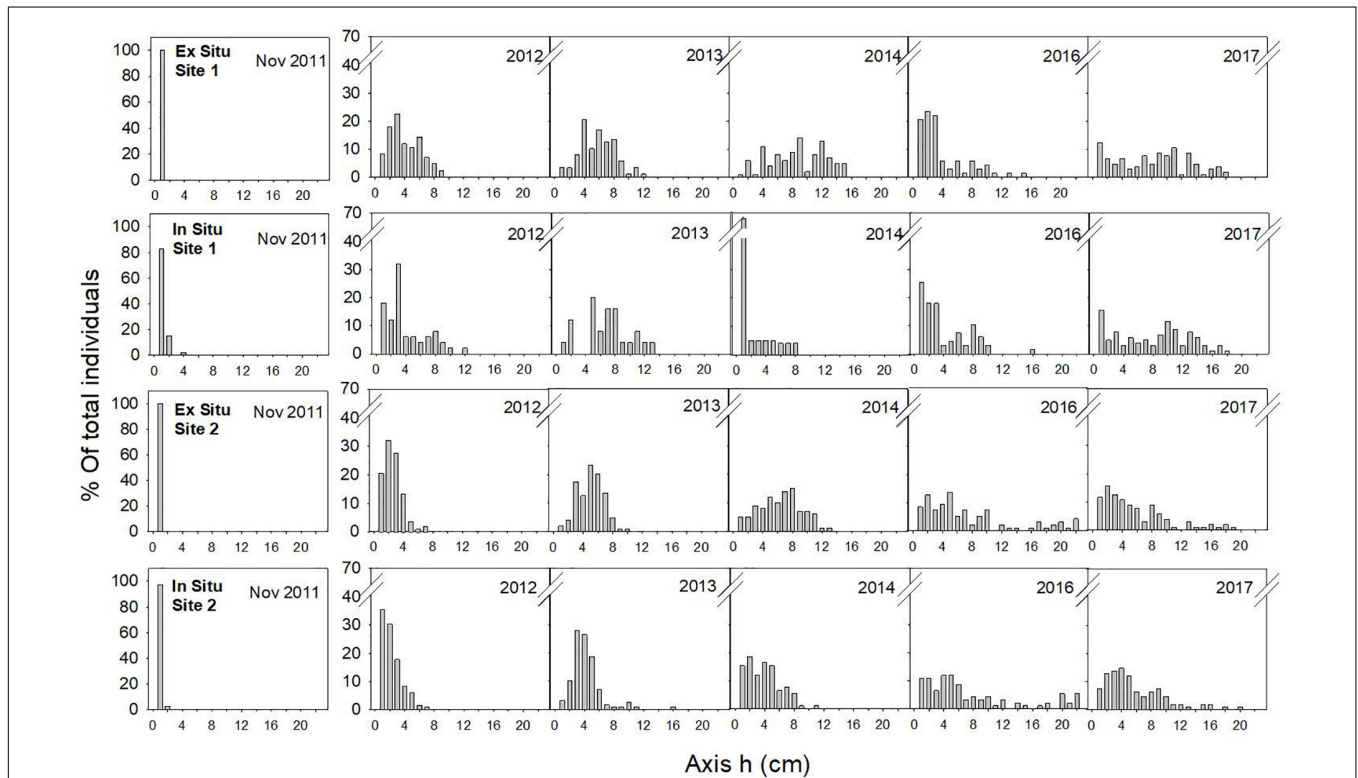
### Costs

We compared the cost of restoring a population (25 m<sup>2</sup>) using the *ex situ* and *in situ* methods, considering the travel, transportation,

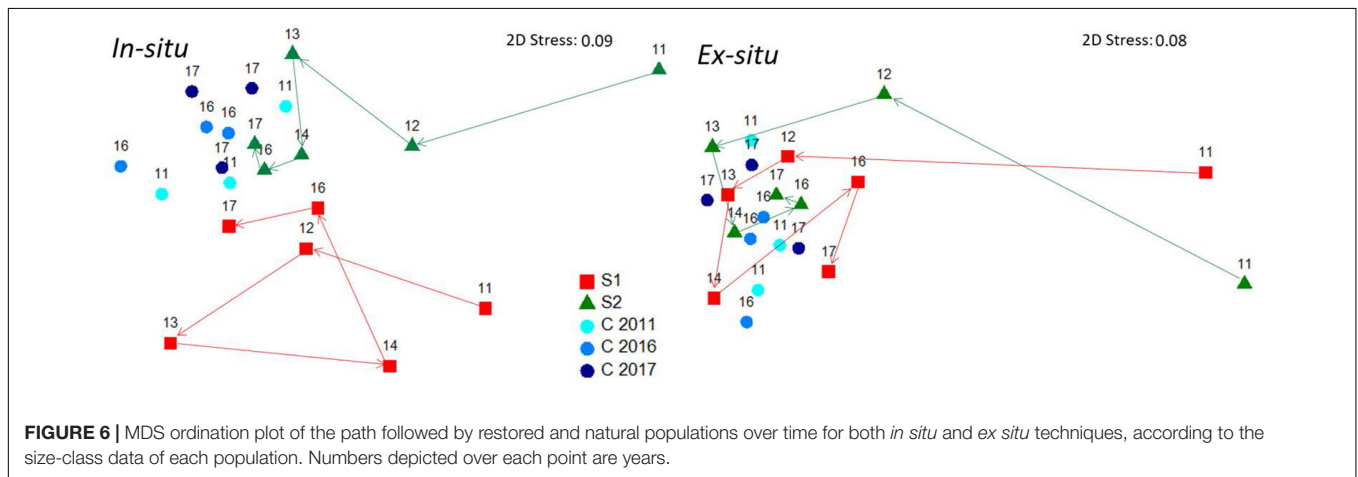
**TABLE 2** | Characteristics of restored *C. barbata* populations through time and in relation to the restoration technique and site (N: number of *Cystoseira* individuals; h: length of the main axis (cm); g1: skewness; g2: kurtosis; Sig: significance of skewness and kurtosis values).

Date	Method	site	N	mean h	max h	g <sub>1</sub>	SE g <sub>1</sub>	sig. g <sub>1</sub>	g <sub>2</sub>	SE g <sub>2</sub>	sig. g <sub>2</sub>
2011 Aug	<i>in situ</i>	1	720	0,5	0,5	–	–	–	–	–	–
		2	576	0,5	0,5	–	–	–	–	–	–
	<i>ex situ</i>	1	793	0,5	0,5	–	–	–	–	–	–
		2	669	0,5	0,5	–	–	–	–	–	–
2011 Nov	<i>in situ</i>	1	46	0,83	4	3,25	0,35	<b>9,28</b>	13,59	0,69	<b>19,76</b>
		2	406	0,6	1,5	2,25	0,12	<b>18,58</b>	4,44	0,24	<b>18,37</b>
	<i>ex situ</i>	1	214	0,5	0,5	–	–	–	–	–	–
		2	384	0,5	0,5	–	–	–	–	–	–
2012	<i>in situ</i>	1	40	3,73	12	1,09	0,37	<b>2,92</b>	0,54	0,73	0,74
		2	135	1,99	6,5	1,15	0,21	<b>5,51</b>	0,81	0,41	1,96
	<i>ex situ</i>	1	84	3,89	8,5	0,4	0,26	1,52	–0,78	0,52	–1,50
		2	112	2,34	6,5	0,96	0,23	<b>4,20</b>	1,42	0,45	<b>3,13</b>
2013	<i>in situ</i>	1	26	6,81	13	–0,09	0,46	–0,20	–0,31	0,89	–0,35
		2	128	3,95	10,5	1,46	0,21	<b>6,82</b>	3,5	0,42	<b>8,24</b>
	<i>ex situ</i>	1	88	5,68	12	0,19	0,26	0,74	–0,09	0,51	–0,18
		2	103	4,88	10	0,1	0,24	0,42	–0,01	0,47	–0,02
2014	<i>in situ</i>	1	22	1,98	8	1,49	0,49	<b>3,03</b>	0,98	0,95	1,03
		2	91	3,75	11	0,63	0,25	<b>2,49</b>	0,1	0,50	0,20
	<i>ex situ</i>	1	85	8,55	15	–0,15	0,26	–0,57	–0,93	0,52	–1,80
		2	81	6,27	13	–0,05	0,27	–0,19	–0,58	0,53	–1,10
2016	<i>in situ</i>	1	67	3,94	16	1,25	0,29	<b>4,27</b>	1,67	0,58	<b>2,89</b>
		2	92	7,72	22	1,09	0,25	<b>4,34</b>	–0,05	0,50	–0,10
	<i>ex situ</i>	1	68	3,92	15	1,47	0,29	<b>5,06</b>	1,62	0,57	<b>2,82</b>
		2	94	7,52	22	1,13	0,25	<b>4,54</b>	0,21	0,49	0,43
2017	<i>in situ</i>	1	103	7,7	17,5	0,007	0,24	0,03	–1,17	0,47	– <b>2,48</b>
		2	110	5,54	20	1,29	0,23	<b>5,60</b>	1,68	0,46	<b>3,68</b>
	<i>ex situ</i>	1	105	8,12	18	0,11	0,24	0,47	–1	0,47	– <b>2,14</b>
		2	103	5,72	19	1,24	0,24	<b>5,21</b>	1,04	0,47	<b>2,21</b>

These parameters are considered significant if the absolute value of the coefficient/standard error (SE) is greater than 2; the significant values are highlighted in bold in the table.



**FIGURE 5 |** Size-class frequency distribution of the restored populations over time for each site and restoration technique. The X-axis represents the size-classes (length of the main axis) in 1-cm intervals, and the Y-axis represents the relative frequency of each size-class.



**FIGURE 6 |** MDS ordination plot of the path followed by restored and natural populations over time for both *in situ* and *ex situ* techniques, according to the size-class data of each population. Numbers depicted over each point are years.

personnel and material expenses (similarly to Carney et al., 2005). We did not consider the long-term monitoring costs since these costs are equivalent for the two techniques.

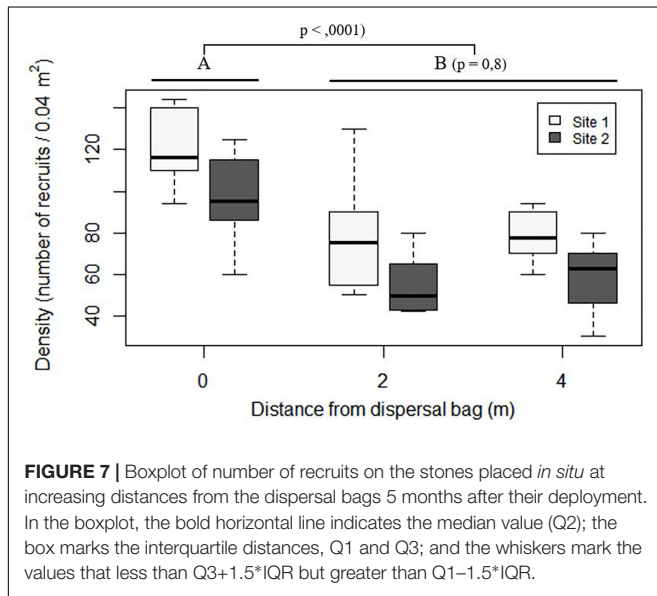
## RESULTS

### Comparison of Techniques

The density of recruits was similar between the two restoration techniques (Figures 3, 4 and Table 1). The mean initial densities

ranged between  $120 \pm 7$  recruits/0.04 m<sup>2</sup> (site 1) and  $96 \pm 9$  recruits/0.04 m<sup>2</sup> (site 2) in the *in situ* experiment and between  $132 \pm 2$  recruits/0.04 m<sup>2</sup> (site 1) and  $111 \pm 9$  recruits/0.04 m<sup>2</sup> (site 2) in the *ex situ* experiment (Figure 3). No recruits were observed outside of the free substrate (stones) with the *in situ* method. The densities of the two restored populations greatly decreased during the first year but remained more stable afterward (Figure 4).

In November 2011, the main axes of almost all the individuals measured 1 cm, and one year later (August 2012), the skewness



of the size-class structure was significantly positive, indicating the prevalence of small size-classes in the population. However, few individuals had reached axis lengths greater than 10 cm (Table 2 and Figure 5). Two years later (2013), all populations were approximately bell shaped and symmetric, with a large proportion of individuals having axis lengths between 2 and 5 cm, although some fertile individuals reached axis lengths of 14–16 cm (Table 2 and Figure 5).

In 2014, the size-class structures of the populations were symmetric and bell shaped, and most individuals were of intermediate size (Table 2 and Figure 5). One exception to this result was the population restored using the *in situ* method at site 1, where we found high mortality of large individuals but also high recruitment (Table 2 and Figure 5). These recruits were the result of new settlement events resulting from the already fertile restored individuals from 2013.

## Restoration Success

In 2017, six years after the restoration action, the size of each of the four restored *C. barbata* patches was roughly 25 m<sup>2</sup>. When comparing the final densities of restored populations with the densities of the reference populations (August 2017), no significant differences were observed ( $F = 0.08$ ,  $P = 0.49$ ; Figure 4). The evolution of the size-class distribution through time resulting from both techniques, sites and reference populations is illustrated in the MDS (Figure 6). The reference populations are displayed on the left side of the MDS (from 2011 to 2017), while the restored populations progressed from the right side in 2011 to the left side, ultimately moving closer to the reference populations. In 2014, the *in situ* restored population from site 1 returned to the right side of the MDS due to the mortality of large individuals and the high recruitment that was experienced (Figure 6). In 2016, all populations were located close to the reference populations, and they remained stable in 2017 (Figure 6).

**TABLE 3** | Cost for the different concepts required to restore an area of 25 m<sup>2</sup> depending on the restoration technique used.

Concept	Rate	Cost	Total (€)
<b>Ex situ</b>			
Field time			
Collection	1h/2pax	40€/h*pax	80
Ex-plant	3h/2pax	40€/h*pax	240
Transport			
Car	200 km	0.40 €/km	80
Lab time			
Set up culture	4 h/2pax	40€/h*pax	320
Culture maintenance	3 h/week*pax	40€/h*pax	1440
Materials			
Tanks	10	25 €	250
Water pump	1	60 €	60
Silicon Tubes	5 m	2 €/m	10
Epoxy	2	70 €/kg	140
Aerator	3	15 €	45
<b>TOTAL</b>			<b>2665</b>
<b>In situ</b>			
Field time			
Collection	1h/2pax	40€/h*pax	80
Set up dispersal bags	4h/2pax	40€/h*pax	320
Set up free substrate	3h/2pax	40€/h*pax	240
Removal dispersal bags	1h/2pax	40€/h*pax	80
Materials			
Iron Stick	16	7€/Pick	112
Epoxy	2	70 €/kg	140
Transport			
Car	300 km	0.40 €/km	120
<b>TOTAL</b>			<b>1092</b>

## Dispersal Capacity

At both sites, stones situated below the dispersal bags (distance of 0 m) showed higher densities of *C. barbata* recruits than did those situated at distances of 2 and 4 m ( $P < 0.0001$ ; Figure 7), while no differences were found between 2 and 4 m ( $P = 0.8$ ; Figure 7).

## Costs

The cost of restoring 25 m<sup>2</sup> of *C. barbata* forest ranged between 1,092 € using the *in situ* seeding technique and 2,665 € using the *ex situ* seeding technique (Table 3). The higher cost ascribed to the *ex situ* technique is related to the required infrastructure and the greater number of hours needed for culture maintenance.

## DISCUSSION

The present study is the first example of active restoration for locally extinct populations of habitat-forming fucoids using recruitment enhancement without adult transplantation of threatened populations, and these restored populations became self-sustaining, with densities and size-class structures comparable to those of the reference populations within five



years. Active transplantation of adults or juveniles has been used as a mechanism to successfully restore habitat-forming species of fucoids (Susini et al., 2007; Campbell et al., 2014). The concept of recruitment enhancement has recently gained recognition as it applies to the restoration of threatened species (Yatsuya, 2010; Gianni et al., 2013; Falace et al., 2018). However, there have been only a few attempts at using this method, and most have been limited to the recruit stage with less than 1 year of monitoring (Stekoll and Deysher, 1996; Choi et al., 2000; Terawaki et al., 2003; Yu et al., 2012).

Here, we used recruitment enhancement methods to successfully restore a locally extinct *C. barbata* population with only one restoration action in 2011. Because the locally extinct population was unable to recover naturally, even thirty years after the primary stress had been ameliorated (Hoyo, 1981; Sales et al., 2011), we used seeding to overcome the limited natural dispersal rates that are typical of zygotes of the genus *Cystoseira* (Mangialajo et al., 2012), and we overrode the limited natural recruitment (Vadas et al., 1992; Capdevila et al., 2015) by cleaning the stones from organisms and sediment, providing free substrate to avoid competition. After six years, the sizes of each restored population was approximately 25 m<sup>2</sup>, which is comparable to the size-patches of natural *C. barbata* populations in Fornells Bay.

Recruitment was high and similar under both techniques, although a large proportion of recruits died during the first year. This sharp drop in density is common in natural populations due to the high natural sensitivity of the first fucoid life stages (Vadas et al., 1992; Irving et al., 2009). Although the density of individuals was similar between restored and reference sites in the second year following the restoration action, it took five years for the individuals of the restored populations to achieve comparable size-class structures to the reference ones. Thus, density is useful for monitoring success during the first period after a restoration action (recruits of settlers; here, 2 years), but after this stage, density should be complemented with other attributes, such as size structure, that will better describe the mature stage of the population.

Obtaining a *Cystoseira* population that reaches a well-represented and stable size distribution is the first goal for complete forest restoration. As for other structural species, the restoration success criteria should be linked to the recovery of the ecosystem function and services, and obtaining mature individuals that are able to self-sustain the new population is likely the first step for enhancing biodiversity and ecological processes. Complementary studies on the evolution of the associated community will probably elucidate whether the proposed indicators for population success may also be indicative of the overall recovery of ecosystem functions and services.

Both of the *in situ* and *ex situ* recruitment enhancement techniques applied here are probably suitable for other macroalgal species that produce large and fast-sinking zygotes with limited dispersion and that are poor competitors for space in their early stages (i.e., late-successional species). Thus, the techniques tested here could be used to restore other Mediterranean populations of *Cystoseira* spp., especially

since the Council of Europe, specifically the Marine Strategy Framework Directive (United Nations Environment Programme/Mediterranean Action Plan [UNEP/MAP], 2013), pushes for active restoration to achieve a Good Environmental Status for a considerable number of habitats.

Knowledge of the biological traits of the target species will determine the choice between *in situ* and *ex situ* techniques. The *in situ* technique is especially recommended for species with high dispersal capacity, such as kelps, with a dispersal potential of hundreds of meters (Reed et al., 1988; Fredriksen et al., 1995). In contrast, the *ex situ* technique is more appropriate for species with a low dispersal capacity, such as *C. amentacea*, whose zygotes are not able to disperse a distance of even 40 cm (Mangialajo et al., 2012). Another benefit of the *ex situ* technique is that it minimizes the high mortality rates experienced by recruits and juveniles as a result of disturbances, predation or competition (Benedetti-Cecchi and Cinelli, 1992; Capdevila et al., 2015). The more the culture is prolonged, the more likely the critical life stages will be left behind, which ultimately enhances success. In our case, however, sources of mortality seemed to be rather irrelevant since the *ex situ* and the *in situ* survival rates were very similar during the first year. The *ex situ* technique should reduce the unpredictability of natural events and maximize success, while the *in situ* technique requires less infrastructure and maintenance, making it a cheaper option.

In summary, we provide a promising cost-effective method (consisting of two different techniques) that can be used to address the increasing need for the restoration of threatened species, especially fucoid forests. Moreover, we show that individual density is not a valid metric to assess the state of population recovery, and we propose the size distribution of the restored individuals as a suitable indicator of population maturity.

## AUTHOR CONTRIBUTIONS

EC and MS conceived the ideas, designed the methodology, and established the restoration action. All authors were involved in collecting data during the monitored period. JV, MS, and EC wrote the manuscript, and all authors contributed critically to the drafts and gave their final approval for publication.

## FUNDING

This project has received funding from the Horizon 2020 EU Research and Innovation Program under grant agreement No. 689518 (MERCES), the Fundación Biodiversidad under the framework of the project: “Conservación y restauración de poblaciones de especies amenazadas del género *Cystoseira*” and the Spanish Ministry Project ANIMA (CGL2016-76341-R, MINECO/FEDER, UE). This project has also been funded by Dirección General de Innovación e Investigación (Govern Illes Balears) and European Regional Development Fund (FEDER). JV has been funded by a IFuG-2016 grant. The outputs presented here only reflect the views of the authors, and

the EU cannot be held responsible for any use of the information contained therein.

## ACKNOWLEDGMENTS

We acknowledge the contributions of Eva María Vidal, Fiona Tomas, and María García in sampling and Jorge Santamaria in data analysis. We also acknowledge the

D.G. d'Espais Naturals i Biodiversitat, the D.G. de Pesca i Medi Marí, and the Servei en Recerca i Desenvolupament del Govern de les Illes Balears for providing permits and the Jaume Ferrer Marine Station (Instituto Español de Oceanografía) for technical and facility support. EC and JV are members of the Marine Conservation Research Group ([www.medrecover.org](http://www.medrecover.org)) from the Generalitat de Catalunya. American Journal Experts edited the English in this manuscript.

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- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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