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Tropicalisation effects on temperate
habitat-forming seaweeds

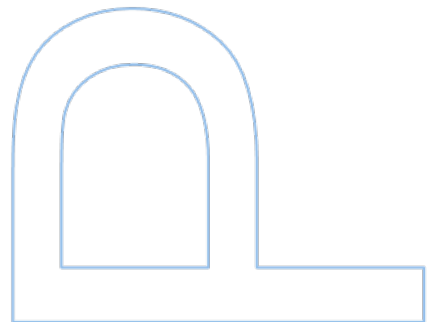
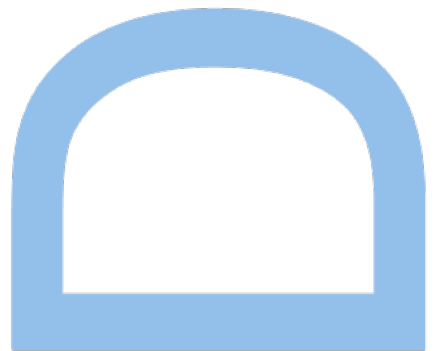
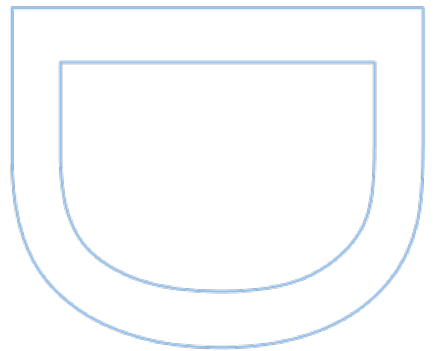
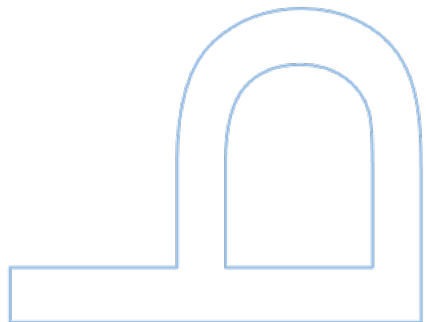
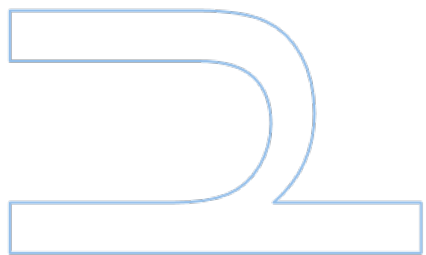
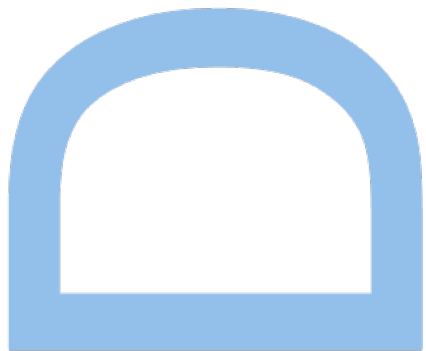
Jonas de Azevedo

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Tropicalisation effects on temperate habitat-forming seaweeds

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2026





Tropicalisation effects on temperate habitat- forming seaweeds

Jonas de Azevedo

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Orientador

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Dedicado ao planeta Terra

Dentro da baleia a vida é tão mais fácil
Nada incomoda o silêncio e a paz de Jonas
Quando o tempo é mal, a tempestade fica de fora
A baleia é mais segura que um grande navio

E ele diz que se chama Jonas
E ele diz que é um santo Homem
E ele diz que mora dentro da baleia por vontade própria

E ele diz que está comprometido
E ele diz que assinou papel
Que vai mantê-lo dentro da baleia
Até o fim da vida
Até o fim da vida
Até subir pro céu

(Sá, Rodrix e Guarabyra, 1973)

Agradecimentos

Antes de iniciar o doutoramento, em plena pandemia, recebi o apoio de muitas pessoas que tornaram possível chegar até aqui. Com um plano de investigação que abrangia toda a costa portuguesa, percebi que seria preciso adaptar meu estilo de vida para acompanhar o trabalho de campo em diferentes regiões. Inspirado por grandes exemplos: ao casal Sylwia e David Mateus, que já viviam em autocaravana, e o meu orientador Francisco Arenas (Paco), que também já teve essa experiência — decidi embarcar nessa aventura e fazer o doutoramento vivendo sobre rodas.

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Resumo

O aquecimento global está a impulsionar transformações biogeográficas profundas, caracterizadas pela retração de espécies de afinidade fria, frequentemente acompanhada pela expansão de espécies de águas quentes. Estas mudanças alteram o cenário funcional, promovendo a substituição de habitats complexos, como as florestas de algas formadoras de habitat (canopias), por sistemas simplificados dominados por espécies de pequeno porte (*turf*). Esta tese de Doutoramento investiga os efeitos da tropicalização sobre macroalgas formadoras de habitat na costa portuguesa, uma região de transição que representa o limite sul de distribuição (*trailing edge*) para diversas espécies boreais no Atlântico Nordeste. Esta região é particularmente singular, pois a sua resposta ao aquecimento global é modulada pelo sistema de *upwelling* ibérico, que pode atuar como um refúgio térmico ou intensificar o stress biológico durante períodos de relaxamento do afloramento. O objetivo central foi quantificar o ritmo de mudança na estrutura das comunidades bentónicas e identificar como a interação entre fatores climáticos e biológicos modula a transformação das florestas marinhas, considerando o papel mediador do *upwelling* ibérico.

O **Capítulo I** apresenta a introdução geral da tese, enquadrando os principais conceitos, hipóteses e abordagens desenvolvidas nos capítulos subsequentes.

No **Capítulo II**, analisou-se como a composição das comunidades de macroalgas em recifes subtidais ao longo da costa portuguesa se modificou ao longo dos últimos anos e em que medida essas mudanças refletem o processo de tropicalização. Os resultados evidenciaram alterações estruturais e funcionais significativas num período relativamente curto de seis anos, incluindo o aumento de espécies de afinidade térmica quente e da abundância de algas de pequeno porte do tipo *turf*. A riqueza de espécies, a diversidade e a biomassa total aumentaram, acompanhadas por um incremento do Índice de Temperatura da Comunidade (ITC). Estes resultados indicam uma rápida reorganização das comunidades subtidais nesta região de transição, constituindo forte evidência dos efeitos das alterações climáticas antropogénicas sobre os ecossistemas costeiros.

No **Capítulo III**, investigou-se a dinâmica de tropicalização em poças de maré intertidais, utilizando uma abordagem conceitualmente comparável à aplicada no subtidal (Capítulo II), com o objetivo de avaliar se habitats termicamente mais dinâmicos exibem respostas distintas ao aquecimento oceânico. Os resultados revelaram uma marcada heterogeneidade espacial, com trajetórias contrastantes de mudança estrutural entre regiões, variando entre colapsos funcionais abruptos e processos graduais de

homogeneização. Estes padrões demonstram que a variação microambiental característica do intertidal desempenha um papel central na modulação da taxa e da natureza das respostas ecológicas às alterações climáticas ao longo da costa.

O **Capítulo IV** demonstrou que a herbivoria exerce um papel central na amplificação dos impactos das alterações climáticas sobre as florestas marinhas. A expansão latitudinal e o aumento da atividade do peixe herbívoro mediterrânico *Sarpa salpa* estiveram associados ao aquecimento oceânico. A sua preferência alimentar por macroalgas castanhas, incluindo espécies formadoras de habitat, intensifica a pressão biológica de consumo sobre estes habitats estruturantes. A correlação positiva entre a Temperatura da Superfície do Mar (SST, do inglês *Sea Surface Temperature*) e a presença de *S. salpa* nas regiões do Norte identifica esta herbivoria crescente como uma ameaça emergente para os refúgios boreais ao longo da costa portuguesa.

Com base nestes resultados, o **Capítulo V** testou experimentalmente os mecanismos funcionais associados à pressão herbívora exercida por *S. salpa*. Os ensaios demonstraram que a seletividade alimentar entre macroalgas formadoras de habitat está significativamente associada à qualidade nutricional, nomeadamente a baixos rácios Carbono:Azoto (C:N), traduzindo-se numa maior assimilação de azoto. Este padrão sugere que, em regiões onde ocorre aumento da temperatura da água do mar e consequentemente tropicalização, espécies estruturalmente importantes e nutricionalmente mais valiosas podem tornar-se particularmente vulneráveis nas margens quentes da sua distribuição.

Por fim, o **Capítulo VI** apresenta uma síntese integrativa dos resultados obtidos ao longo da tese, articulando padrões de tropicalização, trajetórias de mudança estrutural e mecanismos de pressão biológica identificados. O capítulo destaca as principais conclusões relativas à vulnerabilidade diferencial das macroalgas formadoras de habitat ao aquecimento oceânico e discute implicações para a conservação, gestão e restauro destes habitats sob cenários futuros de alterações climáticas.

Em conclusão, esta tese demonstra que o declínio das florestas marinhas temperadas resulta da interação sinérgica entre o stresse térmico induzido pelo aquecimento oceânico e o aumento da pressão biológica. Os resultados reforçam a urgência de integrar a gestão da pressão biótica e a proteção de refúgios boreais nas estratégias de conservação marinha, particularmente em regiões de transição climática.

PLAVRAS-CHAVE

Tropicalização, Deborealização, *Sarpa salpa*,
Herbivoria, CTI, Mudança Funcional, Afloramento,
Refúgios Boreais

Abstract

Global warming is driving profound biogeographic shifts, characterised by the contraction of cold-affinity species frequently accompanied by the expansion of warm-water species. These changes alter the functional landscape, promoting the replacement of complex habitats, such as canopy-forming algal forests, by simplified systems dominated by small-sized turf-forming species. This Doctoral thesis investigates the effects of tropicalisation on habitat-forming macroalgae along the Portuguese coast, a transition region representing a critical southern distribution limit (trailing edge) for several boreal species in the Northeast Atlantic. This region is particularly unique as its response to global warming is modulated by the Iberian upwelling system, which can act as a thermal refugium or intensify biological stress during periods of upwelling relaxation. The central objective was to quantify the rate of change in benthic community structure and to identify how the interaction between climatic and biological factors modulates the transformation of marine forests, considering the mediating role of the Iberian upwelling.

Chapter I provides a general introduction to the thesis, framing the key concepts, hypotheses, and approaches developed in subsequent chapters.

In **Chapter II**, we analysed how the composition of macroalgal communities in subtidal reefs along the Portuguese coast has changed in recent years and the extent to which these shifts reflect the process of tropicalisation. The results revealed significant structural and functional alterations over a relatively short six-year period, including an increase in warm-affinity species and the abundance of small-sized turf algae. Species richness, diversity, and total biomass increased, accompanied by a rise in the Community Temperature Index (CTI). These results indicate a rapid reorganisation of subtidal communities in this transition region, providing strong evidence of the effects of anthropogenic climate change on coastal ecosystems.

In **Chapter III**, the dynamics of tropicalisation in intertidal rockpools were investigated using a conceptually comparable approach to that applied in the subtidal (**Chapter II**), aiming to evaluate whether thermally more dynamic habitats exhibit distinct responses to ocean warming. The results revealed marked spatial heterogeneity, with contrasting trajectories of structural change between regions, ranging from abrupt functional collapses to gradual homogenisation processes. These patterns demonstrate that the microenvironmental variation characteristic of the intertidal zone plays a central role in modulating the rate and nature of ecological responses to climate change along the coast.

Chapter IV demonstrated that herbivory plays a central role in amplifying the impacts of climate change on marine forests. The latitudinal expansion and increased activity of the Mediterranean herbivorous fish *Sarpa salpa* were associated with ocean warming. Its feeding preference for brown macroalgae, including habitat-forming species, intensifies the biotic pressure on these structural habitats. The positive correlation between Sea Surface Temperature (SST) and the presence of *S. salpa* in northern regions identifies this increasing herbivory as an emerging threat to boreal refugia along the Portuguese coast.

Based on these results, **Chapter V** experimentally tested the functional mechanisms associated with the herbivorous pressure exerted by *S. salpa*. The trials demonstrated that dietary selectivity among habitat-forming macroalgae is significantly associated with nutritional quality, namely low Carbon:Nitrogen (C:N) ratios, resulting in higher nitrogen assimilation. This pattern suggests that in regions experiencing sea temperature rise and subsequent tropicalisation, structurally important and nutritionally valuable species may become particularly vulnerable at the warm margins of their distribution.

Finally, **Chapter VI** presents an integrative synthesis of the results obtained throughout the thesis, articulating tropicalisation patterns, structural change trajectories, and the identified mechanisms of biotic pressure. The chapter highlights key conclusions regarding the differential vulnerability of habitat-forming macroalgae to ocean warming and discusses implications for the conservation, management, and restoration of these habitats under future climate change scenarios.

In conclusion, this thesis demonstrates that the decline of temperate marine forests results from the synergistic interaction between thermal stress induced by ocean warming and increased biotic pressure. The results reinforce the urgency of integrating the management of biotic interactions and the protection of boreal refugia into marine conservation strategies, particularly in regions of climatic transition.

KEYWORDS

Tropicalisation, Deborealisation, *Sarpa salpa*,
Herbivory, CTI, Functional Shift, Upwelling,
Trailing Edge

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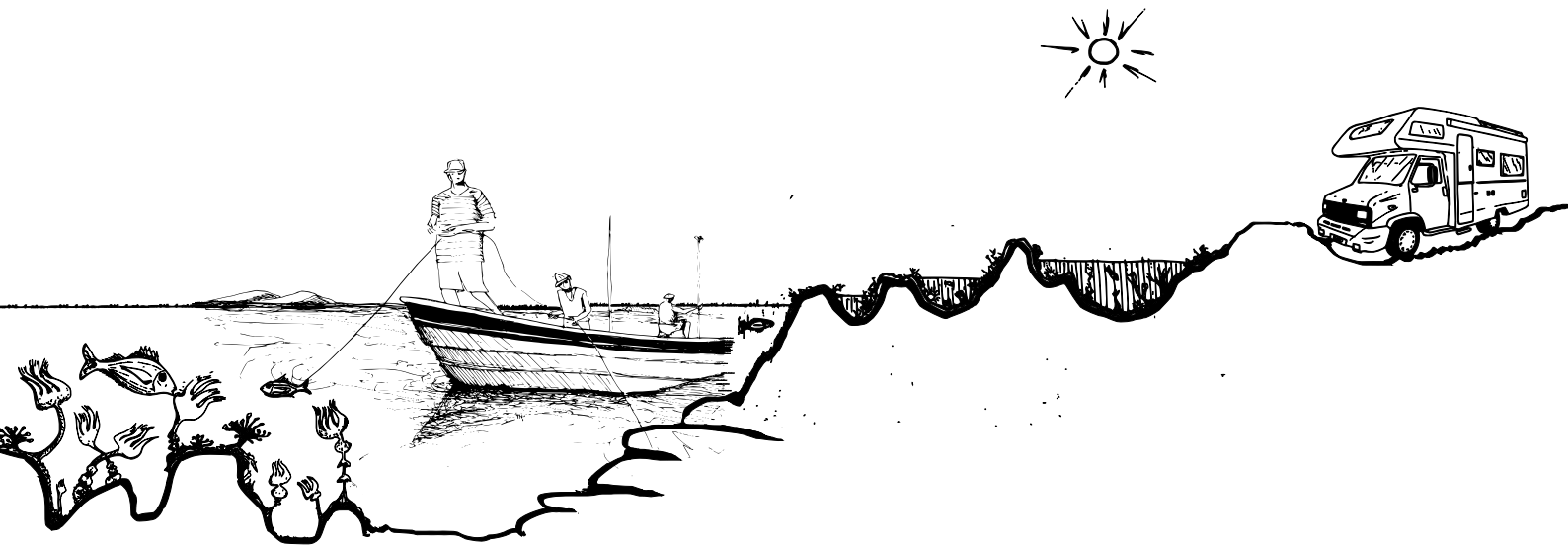
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List of Abbreviations

CPUE	CATCH PER UNIT EFFORT
CTI	COMMUNITY TEMPERATURE INDEX
DGES	DIREÇÃO GERAL DO ENSINO SUPERIOR
FCT	FUNDAÇÃO PARA A CIÊNCIA E A TECNOLOGIA
FCUP	FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO
UP	UNIVERSIDADE DO PORTO
<i>s.l.</i>	SENSU LATO
SST	SEA SURFACE TEMPERATURE
STI	SPECIES TEMPERATURE INDEX

Chapter I.

General Introduction



1.1 Habitat-forming seaweeds, their ecological importance and global distribution

Marine ecosystems are largely structured around habitat-forming species, organisms that provide physical structure and essential ecological services to entire communities. Among these, canopy-forming brown seaweeds, including kelps (Order Laminariales, Figure I-1a, b; and related Tilopteridales, Figure I-1c) and fucoids (Order Fucales; Figure I-1d, c), are recognized as foundation species of temperate reefs (Dayton, 1985; Bruno & Bertness, 2000; Schiel & Foster, 2006; Bolton, 2010; Pessarrodona et al., 2021). Through their large size and complex morphology, these large brown algae create three-dimensional habitats that provide food, shelter, and nursery grounds for a wide array of organisms, thereby enhancing biodiversity and sustaining key ecological functions (Filbee-Dexter & Scheibling 2014, Duarte et al. 2021, Eger et al. 2023, UNEP 2023).

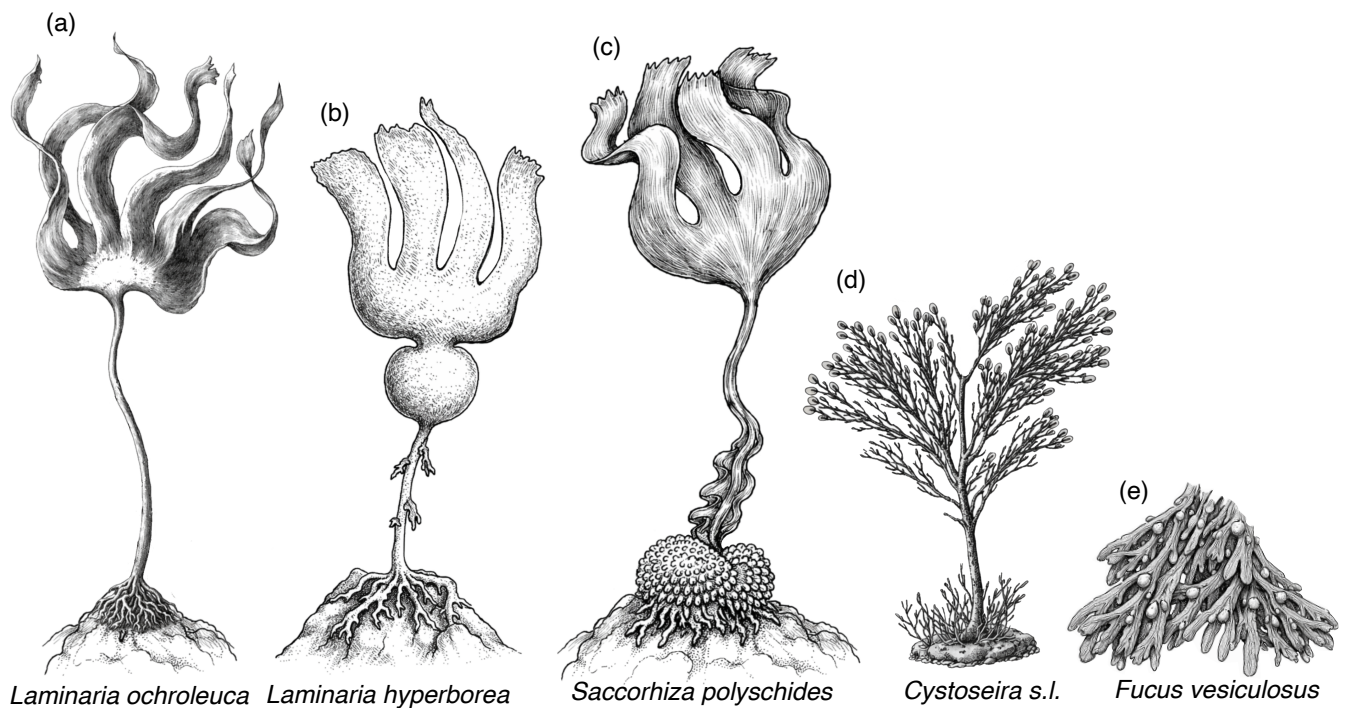


Figure I-1 Morphological diversity of canopy-forming brown seaweeds (Phaeophyceae). Kelps are represented by (a) *Laminaria ochroleuca*, (b) *Laminaria hyperborea* (Order Laminariales), and (c) *Saccorhiza polyschides* (Order Tilopteridales). Fucoids are represented by (d) *Cystoseira sensu latu (s.l.)* and (e) *Fucus vesiculosus* (Order Fucales). The illustration highlights the distinct architectural complexities and structural components, such as blades, stipes, and holdfasts, that characterise these foundation species. Illustrated by Heron Silva and Jonas de Azevedo.

In addition to their role in structuring communities, habitat-forming seaweeds contribute substantially to ecosystem functioning and services at regional and global scales. Through high primary productivity, nutrient cycling, and carbon sequestration, marine forests support coastal productivity, fisheries, and climate regulation (Krause-Jensen & Duarte, 2016; UNEP, 2023). As a result, these systems rank among the most productive and valuable ecosystems on Earth, making their persistence a critical conservation priority (Arenas et al., 2006; Martins et al., 2016; Smale et al., 2019).

Kelps are typically cold-affinity species that dominate shallow subtidal rocky habitats (generally <30 m depth) in temperate and high-latitude coastal areas worldwide (Dayton, 1985; Lüning, 1990). Under exceptionally clear water conditions, some species can occur at much greater depths; Graham et al. (2007) developed a predictive model suggesting that tropical kelp habitats could potentially extend from 30 to 200 m depth, a range supported by records of deep-water species such as *Laminaria abyssalis* off the Brazilian coast (Joly & Oliveira Filho, 1967; Graham et al., 2007). Globally, kelp forests are dominated by five genera (*Laminaria*, *Ecklonia*, *Lessonia*, *Nereocystis*, and *Macrocystis*) with distinct geographic patterns: *Laminaria* predominates in the Atlantic and western Pacific, *Ecklonia* in Australasia and South Africa, *Nereocystis* along the Pacific coast of North America, and *Macrocystis* along the Pacific coasts of the Americas, southern Australia, and New Zealand (Lüning, 1990; Mann, 2000; Guiry & Guiry, 2017).

Fucoids, by contrast, generally display broader thermal tolerances and occur across temperate, subtropical, and tropical regions. In temperate reefs, fucoids often coexist with kelps, contributing substantially to the structural complexity of marine forests. In the Mediterranean Sea, canopy-forming fucoids are dominated by species belonging to *Cystoseira* sensu lato (including *Cystoseira*, *Gongolaria*, and *Ericaria*) as well as *Sargassum* spp. (Molinari Novoa & Guiry, 2020). These perennial macroalgae form dense canopies in photophilic infralittoral and upper circalittoral communities, supporting high biodiversity, productivity, and key ecosystem functions (Rilov et al., 2018; Bermejo et al., 2018). Consequently, canopy-forming fucoids are widely regarded as indicators of ecosystem health and resilience in temperate marine forests.

Together, kelps and fucoids engineer complex marine forests that rank among the most productive and valuable ecosystems on Earth, making their persistence a critical conservation priority (Arenas et al., 2006; Martins et al., 2016; Smale et al., 2019; UNEP, 2023). The strong dependence of these systems on habitat-forming species and their associated ecological functions also makes marine forests particularly sensitive to environmental change, an issue explored in the following sections.

1.2 Climate change and the process of tropicalisation

Climate change represents one of the most pervasive global threats to biodiversity, driving profound transformations across terrestrial and marine ecosystems (Alsuwaiyan et al., 2021; McLean et al., 2021; Osland et al., 2021; Bañón et al., 2024). In the ocean, anthropogenically driven warming has pushed environmental conditions beyond historical baselines, with sea surface temperatures increasing at an accelerating rate over recent decades (IPCC, 2023). This warming is compounded by additional stressors, including ocean acidification, increased frequency and intensity of marine heatwaves, and widespread habitat degradation, which together destabilise marine ecosystems worldwide (Wernberg et al., 2013; García-Molinos et al., 2016; Blowes et al., 2019; Burrows et al., 2020).

Marine organisms are particularly vulnerable to ocean warming because most are ectothermic, with physiological processes tightly constrained by ambient temperatures (Pörtner & Farrell, 2008; Somero, 2012). Moreover, the velocity of climate change in marine systems often exceeds that on land (Burrows et al., 2011), limiting the capacity of many species to adapt *in situ*. As a consequence, species increasingly track their thermal niches in space and time, leading to widespread poleward range shifts and pronounced reorganization of community composition, especially in temperate regions (Filbee-Dexter & Wernberg, 2018; Smale et al., 2019).

One of the most conspicuous ecological manifestations of these changes is tropicalisation, defined as the poleward expansion and establishment of warm-affinity species into temperate ecosystems. The concurrent contraction or loss of cold-affinity species is often referred to as deborealisation (Vergés et al., 2014a, 2016; McLean et al., 2021). Together, these processes fundamentally reshape the structure, diversity, and functioning of recipient communities.

A widely used metric to quantify this reorganization is the Community Temperature Index (CTI), which represents the average thermal affinity of species within a community, weighted by their relative abundance (Sunday et al., 2015; Burrows et al., 2019; de Azevedo et al., 2023). Increases in CTI provide robust evidence of tropicalisation, reflecting a growing dominance of warm-water species and/or the loss of cold-affinity taxa.

1.3 Drivers of tropicalisation and functional collapse at trailing edges of temperate marine forests

Temperate marine forests are at the frontline of climate-driven change due to their ecological importance and biogeographic position at the interface between temperate and subtropical realms. These systems frequently occur at the trailing edges of cold-water species distributions, where populations are particularly sensitive to environmental change (Poloczanska et al., 2013; Wernberg et al., 2019). In such contexts, canopy-forming seaweeds act as foundation species, exerting a disproportionate influence on community structure and ecosystem functioning (Filbee-Dexter & Wernberg, 2018; Duarte et al., 2021; Eger et al., 2023; UNEP, 2023). Ocean climate change threatens these ecosystems through tropicalisation, driven by a combination of direct and indirect drivers:

- **Direct climatic stress:** Rising temperatures, particularly during marine heatwaves, directly compromise the physiological performance, growth, and survival of cold-affinity canopy formers (Assis et al., 2017; Franco et al., 2018; Smale et al., 2019).

- **Indirect biological pressures:** The incursion of warm-affinity species alters key ecological interactions, amplifying the impacts of ocean warming:

- (1) **Herbivory:** The arrival and increased abundance of tropical and subtropical herbivores (e.g., fish, sea urchins) can intensify grazing pressure, leading to overgrazing and the rapid loss of canopy cover (Vergés et al., 2014a,b, 2016; Franco et al., 2015; Barrientos et al., 2022; Reis et al., 2024).
- (2) **Competition:** A critical threat arises from competition with fast-growing, opportunistic species facilitated by disturbance. Turf-forming algae (“turfs”) play a key role in this dynamic. Unlike the complex three-dimensional habitat provided by canopy-forming seaweeds, turfs form low-structure, dense mats of fine filaments that trap sediments, alter local chemical conditions, and simplify the benthic environment (Airoldi, 1998, 2007; Connell et al., 2014). These assemblages are typically more tolerant to environmental stress and can rapidly dominate space following the decline of kelps or fucoids.

Together, these interacting drivers underpin the rapid transitions from canopy-dominated forests to turf-dominated states observed in many temperate systems, including those examined in this thesis.

This shift from canopy to turf dominance represents a severe loss of functional diversity, with profound consequences for ecosystem resilience. Functional diversity (the range and distribution of ecological traits within a community) is a key determinant of ecosystem processes and stability (Díaz & Cabido, 2001; Mouillot et al., 2013; Filbee-Dexter & Wernberg, 2018). Canopy-forming seaweeds possess a unique suite of traits (e.g., large size, complex morphology, high productivity) that create habitat, modify environmental conditions, and sustain diverse food webs (Littler & Littler, 1980; Arenas et al., 2006). High functional diversity, including a balance of morpho-functional groups, buffers ecosystems against disturbance (Bertocci et al., 2015; Pessarrodona et al., 2021). Conversely, the homogenization of communities into low-structure turf assemblages drastically reduces functional diversity, simplifying habitat structure, altering trophic interactions, and eroding ecosystem resilience (Filbee-Dexter & Wernberg, 2018). This functional homogenization represents a core mechanism through which tropicalisation degrades temperate marine ecosystems.

Consequently, habitat-forming seaweeds with cold-water affinities are especially vulnerable in temperate regions. However, other habitat-forming seaweed species with broader thermal tolerances are also exposed to the same synergistic pressures of increased herbivory and competition from turf-forming and invasive species, all operating under the overarching stress of rising temperatures. The interplay between direct thermal stress and indirect biotic interactions sets the stage for rapid and often dramatic functional and compositional shifts. Understanding these synergies is therefore essential to predict the fate of these critical ecosystems and to inform management strategies aimed at enhancing their resilience.

1.4 Study region: Coast of Portugal, Iberian Peninsula

The Iberian Peninsula lies within the Lusitanian province (South European Atlantic Shelf ecoregion), which is characterised by broadly similar ecosystem types but marked local heterogeneity in oceanographic and biological conditions (Spalding et al., 2007; Lemos & Pires, 2004; Gómez-Gesteira et al., 2008). This heterogeneity is particularly pronounced along the Portuguese continental coast, which occupies a critical biogeographic transition zone between temperate and subtropical regions.

Along the Portuguese coast, this transition is expressed through a strong latitudinal gradient in key environmental drivers, most notably mean seawater temperature, which decreases from south to north and increases from west to east (Lemos & Pires, 2004; Tuya et al., 2012; Pinho et al., 2016). A dominant feature shaping this gradient is the Iberian upwelling system, characterised by the seasonal influx of cold, nutrient-rich waters into the coastal zone (Sousa et al., 2020; de Azevedo et al., 2023). The intensity of this upwelling follows a marked latitudinal gradient, being strongest and most persistent along the northwestern coasts of Portugal and Spain (Figure I-2). This process creates a regional “boreal refuge” in northern Portugal, promoting the persistence of cold-affinity species at relatively low latitudes and supporting highly productive and diverse kelp assemblages (Tuya et al., 2012). It therefore acts as a critical buffer against ocean warming, helping to maintain suitable environmental conditions for cold-affinity species near their southern distributional limits (Lima et al., 2007; Lourenço et al., 2016; de Bettignies et al., 2021; Varela et al., 2022).

Conversely, toward the southern portion of the Portuguese coast, the influence of upwelling progressively weakens under increasing Mediterranean influence, resulting in warmer and less nutrient-rich waters (Peliz et al., 2005; Tuya et al., 2012; de Azevedo et al., 2023). This pronounced environmental contrast along a relatively short latitudinal range creates a natural laboratory for studying climate-driven ecological change.

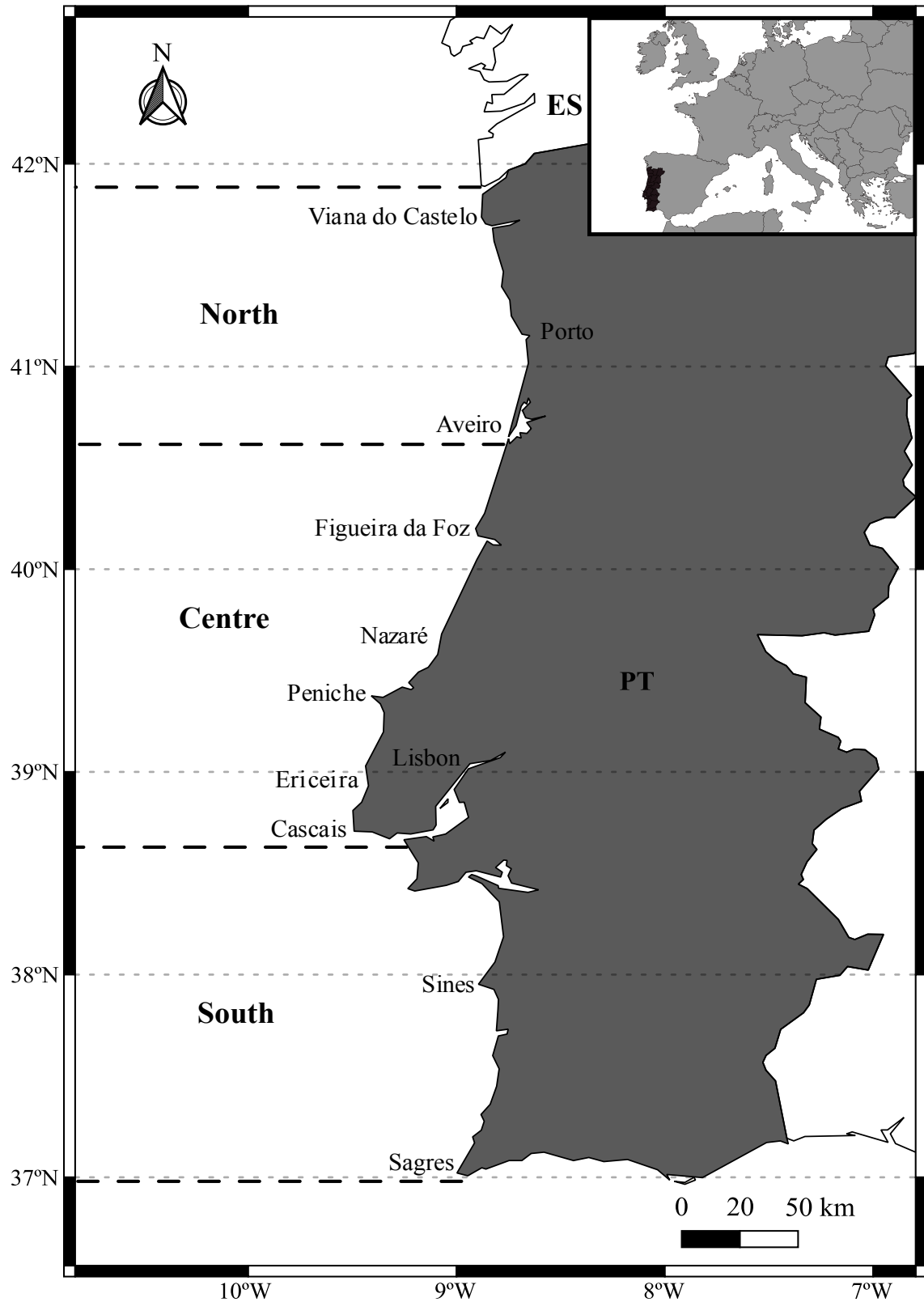


Figure I-2 Study area overview along the Portuguese coast. The map identifies the North, Centre, and South sectors across a latitudinal gradient from 37°N to 42°N. Dashed lines represent regional boundaries. Inset: location of Portugal in Europe.

This gradient structures clear shifts in the composition of habitat-forming seaweeds, making the Portuguese coast a model system for studying tropicalisation processes. Northern shores, under strong upwelling influence, host populations of cold-temperate fucoids (e.g., *Ascophyllum nodosum*, *Fucus* spp.) and kelps (e.g., *Laminaria hyperborea*, *Saccharina latissima*, and *Saccorhiza polyschides*), many of which occur near their southern distribution limits in Europe (Araújo et al., 2009; Franco et al., 2015). The presence of *Laminaria hyperborea* and *Saccharina latissima* specifically characterises the southernmost North Atlantic kelp forests along this coast (de Azevedo et al., 2023), reinforcing the role of this region as a boreal refuge (Lima et al., 2007; Lourenço et al., 2016). In contrast, southern shores, characterised by warmer conditions, show a greater prevalence of warm-affinity species, including canopy-forming fucoids such as *Cystoseira* sensu lato and *Sargassum* spp. (Lima et al., 2007; Pinho et al., 2016).

Critically, this unique system is dynamic and increasingly threatened. Climate change projections suggest a potential future weakening of the Iberian upwelling system due to enhanced surface heating and ocean stratification (Sydeman et al., 2014; Sousa et al., 2020). Such changes are expected to result not only in seawater warming but also in reduced nutrient inputs, exacerbating stress on cold-affinity species. There is already growing evidence of distributional shifts in this region, with some cold-affinity species retracting their southern ranges or persisting only at reduced densities within localised refugia (Viejo et al., 2011; Lima et al., 2007; Díez et al., 2012; Casado-Amezúa et al., 2019). Because upwelling dynamics are a fundamental driver of marine forest structure and functioning, the Portuguese coast represents a sentinel system for understanding how ongoing and future environmental stressors affect temperate coastal assemblages undergoing tropicalisation.

This PhD project was conducted across three regions of the Portuguese continental coast (Figure I-2), explicitly leveraging this pronounced environmental and biological gradient. Observational surveys and manipulative experiments were performed across all regions or within selected subsets, depending on the specific hypotheses addressed in each chapter.

1.6 General aim and thesis structure

Given the ecological and socio-economic importance of habitat-forming seaweeds, it is essential to assess how these complex and dynamic systems are changing over time and how they respond to the direct and indirect effects of climate change. The overarching aim of this PhD thesis is to characterise recent changes in the structure and functioning of temperate marine forests along the Portuguese coast and to evaluate the synergistic roles of climatic and biological drivers in promoting tropicalisation and ecosystem degradation.

To address this aim, the thesis is structured around two major research tasks, each subdivided into specific objectives that integrate observational and experimental approaches:

Task 1 Climate-driven community shifts in habitat-forming seaweeds

This task focuses on identifying and quantifying recent temporal and spatial changes in habitat-forming seaweed communities along the Portuguese coast, and on assessing whether these changes are consistent with tropicalisation processes. Particular emphasis is placed on contrasting responses across habitats with different environmental dynamics.

- **Task 1.1** Assessment of long-term changes in **subtidal** seaweed communities, focusing on structural, functional, and thermal shifts indicative of tropicalisation.
- **Task 1.2** Assessment of long-term changes in **intertidal** seaweed communities, evaluating whether thermally dynamic habitats (e.g., rockpools) exhibit distinct trajectories of change relative to subtidal systems.

Task 2 Biological amplification of climate impacts: the role of herbivory

This task investigates how biological interactions, particularly herbivory, interact with ocean warming to amplify impacts on habitat-forming seaweeds and accelerate ecosystem change.

- **Task 2.1** Evaluation of spatial and temporal trends in the abundance and distribution of herbivorous grazers along the Portuguese coast, with a focus on the thermophilic fish *Sarpa salpa*.
- **Task 2.2** Examination of grazing pressure and feeding preferences of *Sarpa salpa* on habitat-forming seaweeds, combining:
 - **Task 2.2.1** Stomach content analyses to characterise in situ dietary composition across regions.
 - **Task 2.2.2** Controlled feeding experiments to test selectivity patterns and explore underlying functional mechanisms, including the role of nutritional traits in driving preference.

The outcomes of these tasks are presented across the following chapters:

- **Chapter II:** Rapid tropicalisation evidence of subtidal seaweed assemblages along a coastal transitional zone
- **Chapter III:** Decadal shifts in intertidal rockpool seaweed assemblages: Deborealisation and structural collapse along the Portuguese coast
- **Chapter IV:** Rising Herbivory Pressure of *Sarpa salpa* and the Emerging Threat to Southern European Marine Forests
- **Chapter V:** Grazing preferences of *Sarpa salpa* on temperate habitat-forming macroalgae
- **Chapter VI:** Conclusions and future remarks, synthesising the main findings and discussing their implications for the conservation, management, and restoration of temperate marine forests under ongoing climate change

1.7 Research Outputs

The research conducted under this thesis has resulted in several peer-reviewed publications and manuscripts:

Peer-Reviewed Publications

Chapter II: de Azevedo, J., Franco, J.N., Vale, C.G., Lemos, M., Arenas, F. (2023). Rapid tropicalization evidence of subtidal seaweed assemblages along a coastal transitional zone. *Scientific Reports*, 13(1): 11419. <https://doi.org/10.1038/s41598-023-38514-x>

Chapter IV: de Azevedo, J., Franco, J.N., Dolbeth, M., Meyer, H.S., Barreiro, A., Salas-Leitón, E.A., Arenas, F. (2026). Rising Herbivory Pressure of *Sarpa salpa* and the Emerging Threat to Southern European Marine Forests. *Marine Environmental Research*, 216: 107884. <https://doi.org/10.1016/j.marenvres.2026.107884>

Chapter V: de Azevedo, J., Arenas, F., Barreiro, A., Franco, J.N. (2026). Grazing preferences of *Sarpa salpa* on temperate habitat-forming macroalgae. *Aquatic Botany*, 204: 103994. <https://doi.org/10.1016/j.aquabot.2026.103994>

Manuscripts in Preparation

Chapter III: de Azevedo, J., Franco, J.N., Lansu, N., Matias, M., Arenas, F. (2026). Decadal shifts in intertidal rockpool seaweed assemblages: Deborealisation and structural collapse along the Portuguese coast. (to be submitted).

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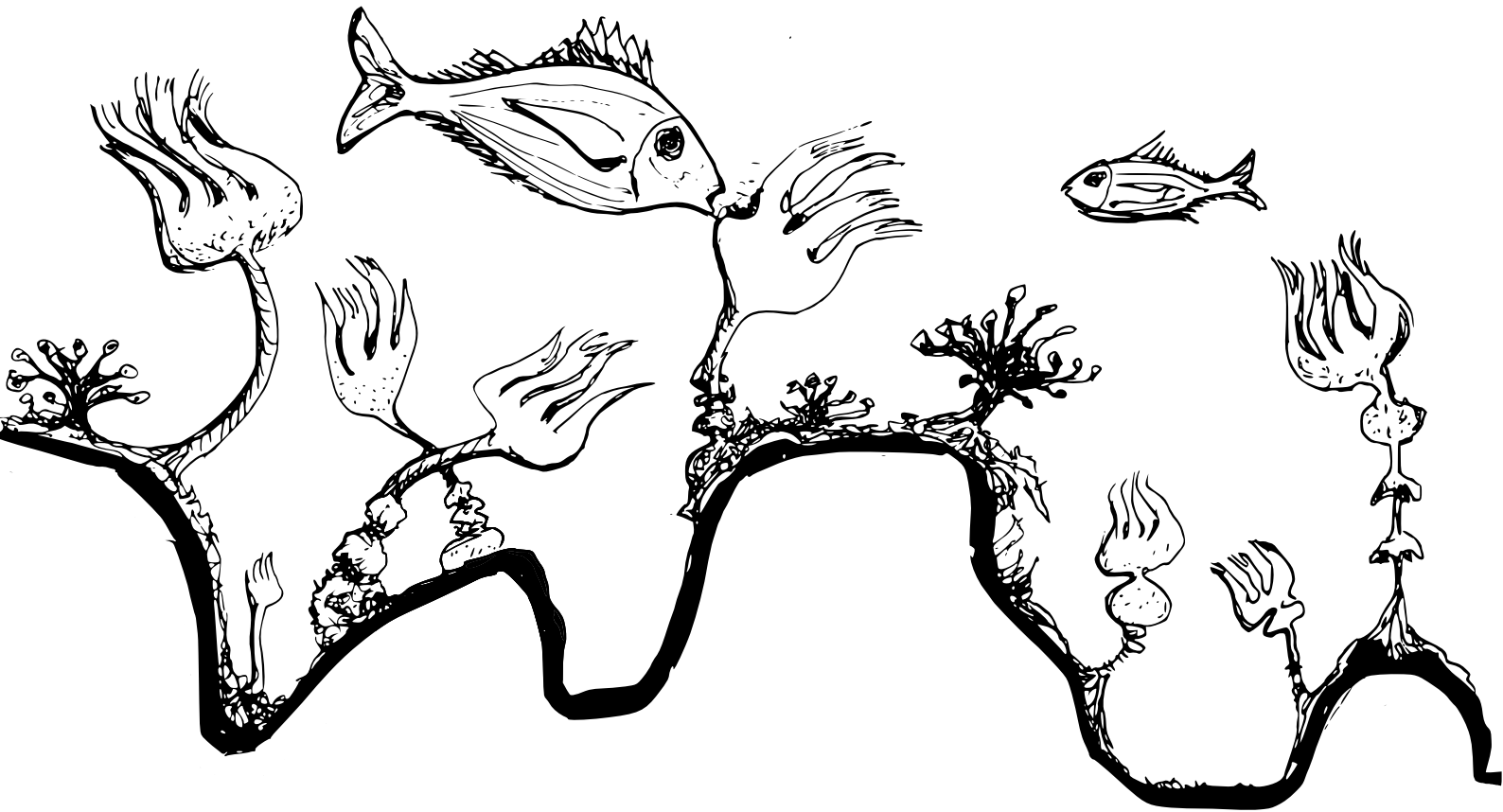
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Chapter II.



Rapid tropicalization evidence of subtidal seaweed assemblages along a coastal transitional zone

Publication Note

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2.1 Abstract

Anthropogenic climate change, particularly seawater warming, is expected to drive quick shifts in marine species distribution transforming coastal communities. These shifts in distribution will be particularly noticeable in biogeographical transition zones. The continental Portuguese coast stretches from north to south along 900 Km. Despite this short spatial scale, the strong physical gradient intensified by the Iberian upwelling creates a transition zone where seaweed species from boreal and Lusitanian-Mediterranean origin coexist. On the northern coast, kelp marine forests thrive in the cold, nutrient-rich oceanic waters. In the south, communities resemble Mediterranean-type seaweed assemblages and are dominated by turfs. Recent evidence suggests that in these coastal areas, marine intertidal species are shifting their distribution edges as a result of rising seawater temperatures. Taking advantage of previous abundance data collected in 2012 from subtidal seaweed communities, a new sampling program was carried out in the same regions in 2018 to assess recent changes. The results confirmed the latitudinal gradient in macroalgal assemblages. More importantly we found significant structural and functional changes in a short period of six years, with regional increases of abundance of warm-affinity species, small seaweeds like turfs. Species richness, diversity, and biomass increase, all accompanied by an increase of community temperature index (CTI). Our findings suggest that subtidal seaweed communities in this transitional area have undergone major changes within a few years. Evidence of “fast tropicalization” of the subtidal communities of the Portuguese coast are strong indication of the effects of anthropic climate change over coastal assemblages.

Keywords

Climate change, Seaweeds, Kelps, Turf, CTI – Community temperature index

2.2 Introduction

Change is a characteristic of our Earth through its history but current anthropogenically driven climate change has pushed the oceans well outside their historical limits (Hoegh-Guldberg & Bruno, 2010). Current ocean temperature exceeds the values recorded over the past 400 000 years (see IPCC report in Arias et al., 2021), increasing at a rate of 0.88 °C per decade, in the last 40 years (Arias et al., 2021). The sea water temperature is recognized as the main driver of key physiological processes in marine ectotherms. Species are often adapted to optimum temperature ranges, occupying restricted thermal niches along latitudinal temperature gradients (Boyce, 1978; Pörtner & Farrell, 2008). In marine ecosystems, where high velocities of climate-change exceed those in the terrestrial ecosystems, marine isotherms move very fast (ca. 21.7 km/decade, Burrows et al., 2011). Thus, to avoid local extinction, marine species are forced either to adapt to new conditions or to track their climatic niches in space or time, i.e. spatial and phenological shifts (García-Molinos et al., 2016), triggering fast compositional changes (Blowes et al., 2019). Globally, the observed trends are poleward range shifts, resulting in increase of species at higher latitudes (Blowes et al., 2019). In temperate ecosystems, the outcome is a rising proportion of species with tropical affinity, a process named ‘tropicalization’ in marine habitats (Wernberg et al., 2013; García-Molinos et al., 2016). Tropicalization phenomenon is profusely reported in marine literature (Vergés et al. 2014; Sunday et al., 2015). This phenomenon is often described by using the community temperature index (CTI), which is the average of species’ thermal affinities weighted by their abundance in the community (Burrows et al., 2019; 2020). CTI changes reflects differences in the community composition and species dominance. This measure allows tracking shifts in communities due to thermal changes by the relative contribution of each species with a given thermal affinity (Burrows et al., 2020).

The Portuguese continental coast is a biogeographic transition zone, harbouring fauna and flora with cold and warm-water temperature affinities (Boaventura et al., 2002; Pereira et al., 2006; Lima et al., 2007; Tuya et al., 2012). The Iberian upwelling system (active from 37° N to 43° N) affects the coastal area, having large effects on its oceanographic conditions (Bode et al., 2011; Varela et al., 2018). The upwelling system creates unique conditions for the persistence of species with cold-water affinity (Lourenço et al., 2016; García et al., 2021). The intensity of the upwelling follows a latitudinal pattern, being stronger on the North coast of Portugal and Spain which results in a highly productive ecosystem with low sea surface temperature and nutrient-rich seawater nearshore, even in summer (de Bettignies et al., 2021; Varela et al., 2022). In

the southern continental coastal area of Portugal, seawater is warm, poorer in nutrients, and has a strong Mediterranean influence (Peliz et al., 2005). Contrary to other eastern boundary upwelling systems, the intensity of the Iberian upwelling is predicted to decrease in the future (Bode et al., 2011; Sydeman et al., 2014; Varela et al., 2022). This upwelling weakening may result in an increase in the sea surface temperatures along the Portuguese continental coast (Lima et al., 2006; Santos et al., 2012) and a reduction in the surface input of nutrients in the nearshore (Sousa et al., 2020).

Regardless of the intensity and direction of the ocean climate changes in the Atlantic seashores of the Iberian Peninsula, there is growing evidence that coastal communities are undergoing rapid transformations. While these community changes may involve the replacement of certain species by others that perform similar roles and functions, allowing for functional redundancy (Rosenfeld, 2002), we contend that this is not the situation along the Portuguese continental coast. For instance, there is a documented increase in the abundance of marine species with an affinity for warm seawater (Lima et al., 2006; Duarte et al., 2013; Piñeiro-Corbeira et al., 2016; Ramos et al., 2020), along the Iberian coast. Conversely, certain habitat forming seaweed species, such as kelps from the genus *Laminariales* and *Tilopteridales*, are experiencing a decline (Fernández, 2011; Duarte et al., 2013; Franco et al., 2015; Assis et al., 2017; Casado-Amezúa et al., 2019). Kelps are vital components of subtidal and intertidal communities in temperate reefs. Functioning as “engineering species”, they create marine forests with a complex three-dimensional structure that significantly influences the physical, chemical, and biological dynamics of the ecosystem. These forests provide shelter and nursery habitats for multiple species (Smale et al. 2013). Moreover, kelp forests support artisanal fisheries (Bertocci et al. 2015) and contribute to carbon sequestration (Krause-Jensen & Duarte 2016; Krause-Jensen et al., 2018).

However, most kelp species thrive in cold nutrient-rich seawaters (Dayton, 1985; Schiel & Foster, 2006), and are highly vulnerable to warming oceans, particularly in regions near their southern limits, such as in Portugal (Franco et al., 2018). The decline in habitat-forming species often creates the opportunity for proliferation of more opportunistic assemblages, like turf species, which tend to increase in abundance and dominate seaweed communities (Filbee-Dexter & Wernberg, 2018). However, turf species create a smaller and less complex three-dimensional structure, resulting in a more homogeneous ecosystem with reduced available resources and functional diversity (Airoldi, 1998; Arenas et al., 2006; Miller et al., 2009; Matias et al., 2015; Pessarrodona et al., 2021).

The general objective of this work is to understand the magnitude and direction of recent changes on the subtidal seaweed communities along the Portuguese coast while examining evidence of tropicalization or other climate change-linked shifts. Although there is ample research on intertidal species in the Portuguese coastal area (Pereira et al., 2006; Lima et al., 2007), limited information is available on subtidal coastal assemblages, particularly regarding seaweed communities (but see Tuya et al., 2012 and Pinho et al., 2016). Leveraging a previous sampling survey, we conducted an assessment of changes occurring in the subtidal seaweeds assemblages over a period of six years (2012-2018) across different areas along the latitudinal gradient of the continental Portuguese coastline. We aimed to answer the following questions: Are there temporal differences in the structure of seaweed communities across this latitudinal gradient? If changes in the communities' composition are observed, what is their nature? And finally, are changes linked with the species thermal affinities? Given the current scenario of fast seawater warming and the accelerated pace of changes (Burrows et al. 2011), we hypothesize that alterations in the structure of the algal assemblages are detectable even within this relatively time frame. Furthermore, we anticipate shifts in the communities' thermal affinities, including an increase in the abundance of warm-water affinity species, and a potential reduction of cold- water affinity species. Finally, we expect that these changes will lead to measurable functional changes in the communities, including an enhanced abundance of turf species and a reduction in the abundance of canopy species.

2.3 Material and Methods

Study area. Following the survey design of Tuya et al. (2012), three regions were surveyed along the continental coast of Portugal: Viana do Castelo - 41° N, (VIA), Peniche - 39.2° N (PEN), and Sines - 37.8° N (SIN) (Figure II-1). The coast of Portugal continental has a north-to-south orientation and the selected regions have a similar wave exposure to the dominant NW and W swells. This coastline is characterized by extensive sandy beaches interspersed with limestone, sandstone, shale, or granitic reefs in both the intertidal and the shallow subtidal (Tuya et al., 2012). At each region, five different subtidal reefs were sampled using a protocol similar to Pinho et al. (2015) and detailed below.

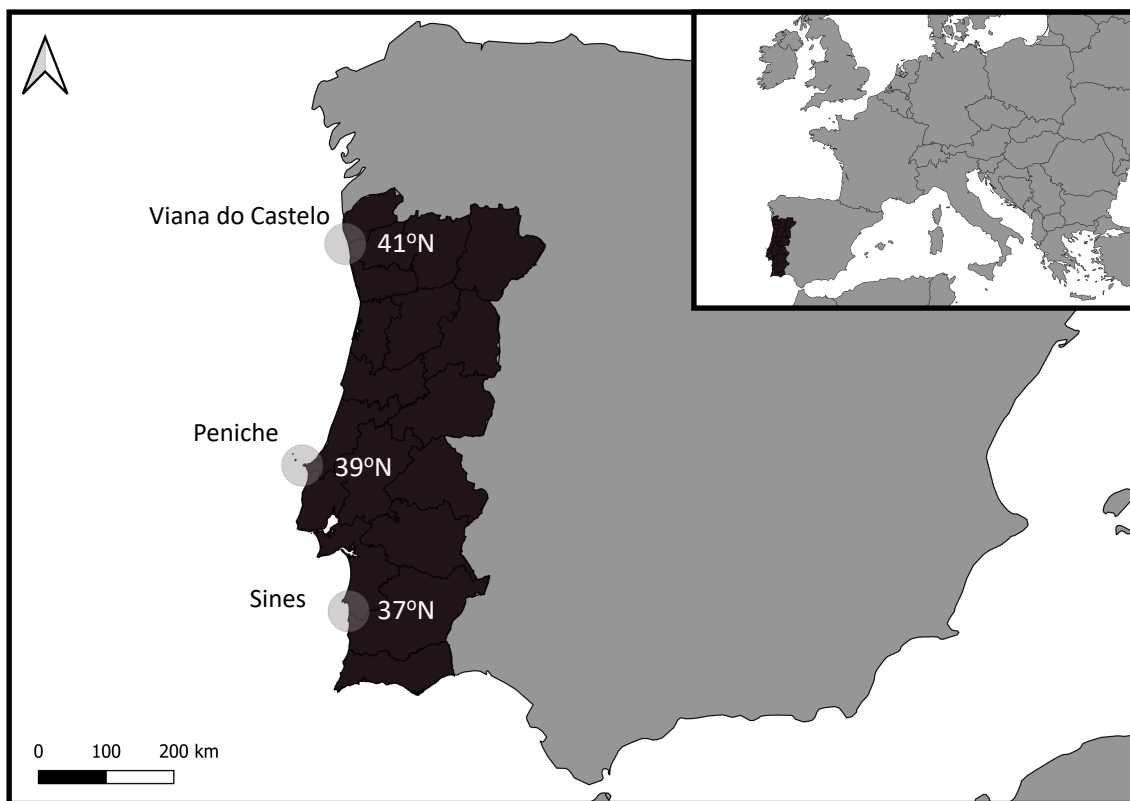


Figure II-1 Map of Portugal continental showing the location of the three study regions: Viana do Castelo (41° N), Peniche (39° N) and Sines (37° N) (QGIS 3.28.3-Firenze, shapefile-<https://gisco-services.ec.europa.eu/distribution/v2/countries/download/ref-countries-2020-01m.shp.zip>).

Collection and data analyses. Surveys were conducted during six diving campaigns carried out in the summers of 2012 and 2018. For each survey campaign and within each region (Viana do Castelo, Peniche and Sines), five rocky reef areas located one to five km apart and at depths between 6 and 12 m were randomly selected. Scientific diving techniques were utilized to sample the selected areas. Within each reef, six quadrats of 50×50 cm (0.25 m²) were haphazardly placed on the top of horizontal reefs bottom. All macrophytes present were carefully scraped and collected in textile bags. The samples were appropriately labelled and transported to the laboratory in cool boxes and frozen

until processing. Seaweeds within each sample were identified to the lowest feasible taxonomic level, mostly species, and their weight was determined after excess water was removed through shaking. Species richness was determined as the total count of distinct species in the samples, while the Shannon H' diversity index, incorporated both species richness and evenness to assess the diversity and distribution of the species within the community (Arenas et al., 2006). Finally, to perform a morpho-functional analysis of the samples, the seaweed species were categorized into four morpho-functional groups: 'encrusting', 'turf-forming', 'subcanopy', and 'canopy', following the algal morpho-functional classifications described by Arenas et al. (2006).

A detailed quantitative assessment of the thermal shifts was done using the community temperature index (CTI), which has been used to assess the thermal shift of communities and as an indicator of climate change (Bowler & Böhning-Gaese, 2017; Cheung et al., 2013). The CTI is the weighted mean of species' thermal preferences calculated for a community (Burrows et al., 2019). The temperature preference of species is given by the species temperature index (STI), which is the 50th percentile (median) of temperatures experienced throughout the species' area of occupancy (Burrows et al., 2019). To assess each species' probable area of occupancy, and the respective profile of the temperature experienced, ecological niche models were implemented (detailed description in Supplementary Material). Only taxa identified at the species level were used in this analysis. Presence data were retrieved from OBIS (<https://obis.org/>) and GBIF (<https://www.gbif.org/>) public databases, and pruned to remove misidentifications, possible introductions as well as remove duplications, and data clustering (details in Attachments Chapter II- Supplementary Material). Uncorrelated environmental variables (Table II-S1) were retrieved from Bio-Oracle (<https://www.bio-oracle.org/>) at 5 arcmin of resolution (c. 9.2 km at the equator). Models were built using the Maximum Entropy approach (Phillips et al., 2006), with default parameters, 5 replicates, and 25% of the data for testing. To assess the global area of occupancy of each species, continuous models were converted into binary maps using a threshold that maximizes both numbers of presences and absences correctly classified by the model (Vale et al., 2014). To define the thermal "niche" of each species, the maps of the area of occupancy were used to extract the quantiles of the sea surface temperature, for each species (Burrows et al., 2019). The median (50th percentile) of temperatures in each species occupied areas was used as the STI (Burrows et al., 2019). The Community Temperature Index (CTI) was calculated for each locality, rocky reefs, and time (all diving campaigns) as the abundance-weighted mean of all STIs (Burrows et al., 2020). Finally, the four processes promoting changes in CTI were assessed

following the methodology of McLean et al. (2021), where (i) *tropicalization* is an increase in the abundance of warm-affinity species; (ii) *borealization* is an increase in abundance of cold-affinity species; (iii) *detropicalization* is a decrease in the abundance of warm-affinity species; and (iv) *deborealization* is a decrease in the abundance of cold-affinity species). Here, warm-affinity species exhibit STIs higher than the mean of the community, while cold-affinity species have STIs lower than the mean (McLean et al., 2021).

Statistical analysis. Data were analysed using R software (R Core Team, 2019) and plots were produced using the package ggplot2 (Wickham, 2016). As the survey design followed Tuya et al. (2012), we replicated the analytical procedure but incorporated time as a factor to compare 2012 and 2018 data. Community level analyses were conducted using the vegan library (Oksanen et al., 2019) and Primer v6 (Clarke Gorley, 2006). Non-metric multi-dimensional scaling (NMDS) plots were used to visualize differences in algal assemblage composition among regions and times. Those differences were tested using a three-way permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) based on Bray-Curtis dissimilarities calculated from square-root transformed data. The PERMANOVA model included three factors: Time (two levels, fixed), Region (three levels, fixed and crossed with Time), and Reef (five levels, random and nested within both Time and Region). When tests were significant and for those relevant effects, post-hoc comparison tests were carried out to identify the nature of differences. To test for homogeneity of dispersion among our multivariate samples we ran a PERMADISP test using Primer.

Analyses of variance (ANOVA) on univariate community descriptors and CTIs were carried out using the GAD library (Sandrini-Neto & Camargo, 2022) and the same design used in Permanova. Before performing each ANOVA, the assumption of homogeneity of variances was checked using Cochran's C test (Underwood, 1997). When the assumption was not achieved, data were transformed to avoid heterogeneous variances. ANOVAs on the abundance of functional groups were not homogeneous in all the analyses, however balanced ANOVAs are quite robust to departures of homoscedasticity (Underwood, 1997) and significant p values were smaller than 0.01, thus type I errors were improbable. When relevant, Student- Newman-Keuls (SNK) tests were used for a posteriori comparison of means.

2.4 Results

In 2012 and 2018, a total of 105 taxa of macroalgae were identified from the 180 samples, including 95 species, 9 genera, and one taxon at family-level (complete list of observed macroalgae available in Attachments Chapter II- Table II-S2). In the first survey (2012), 79 taxa were recorded, including 71 species and 8 genera. In 2018, we found a slightly larger number of taxa, with 84 taxa, including 77 species, 6 genera and one taxa at family-level. Among the reefs in the northernmost region (Viana do Castelo), *Laminaria hyperborea*, *Saccorhiza polyschides* and *Dilsea carnosa* were the most abundant species. In the central region of Peniche, the dominant species were the red seaweed *Plocamium cartilagineum* and two introduced seaweeds, *Asparagopsis armata* and *Symphyclocladia marchantioides*, with the later only recorded in the 2018 survey. Finally,

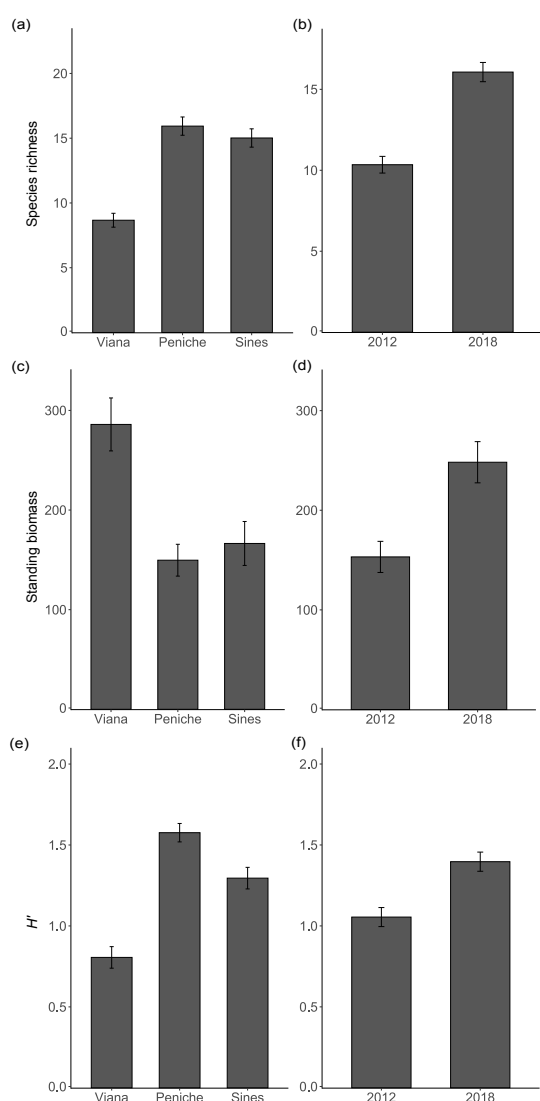


Figure II-2 Bar plots to illustrate significant effects of region and time for (a, b) Species richness, (c, d) Algal standing biomass (wet weight g 0.25 m²) and (e, f) Shannon H' diversity index. Bars are earned mean values $\pm$ SE, n=6.

in the reefs of the South region, the most abundant species in the samples were *Asparagopsis armata*, *Sphaerococcus coronopifolius*, and *Codium adhaerens*. Spatial and temporal differences among assemblages were observed through the diversity of descriptors (Figure II-2). Seaweed species richness, assemblages' biomass, and Shannon diversity indexes significantly increased between 2012 and 2018 (Table II-1, Figure II-2a, d, f). No significant changes were observed in evenness between the two years. In terms of regional differences, Viana sites exhibited lower species richness and Shannon diversity indexes compared to Sines and Peniche (Table II-1, Figure II-2a, e). Evenness values were higher in Peniche than in Viana, and median evenness in Sines (Table II-1). Regional differences were also observed in standing crop biomass, with Viana reefs showing higher biomass than Sines and Peniche (Table II-1, Figure II-2d). Finally, significant differences were found in all descriptors at the local scale (among reefs; Table II-1).

Table II-1 ANOVA examining the effects of time, region and reef on the richness of algal taxa, total algal biomass, the Shannon H' diversity index and the Pielou's evenness index. * p < 0.05, ** p < 0.01, *** p < 0.001.

Source of variation	df	Taxa richness		Algal biomass		Shannon H' index		Pielou's evenness index	
		MS	F	MS	F	MS	F	MS	F
Time (T)	1	1584.2	25.970***	17.13	10.515**	6.93	10.266**	0.17	2.173
Region (R)	2	946.12	15.510***	12.12	7.438**	8.28	12.255***	0.52	6.402**
T x R	2	129.12	2.116	4.85	2.980	0.03	0.045	0.03	0.481
Reef (T x R)	24	61	6.168***	1.62	3.3141***	0.68	4.637***	0.08	4.278***
Residual	150	9.89		0.49		0.15		0.01	
Cochran C' test		C = 0.104, P = 0.19		C = 0.085, P = 0.66		C = 0.118, P = 0.073		C = 0.12117, P = 0.059	
Transformation		None		Log(x)		None		None	
SNK test		Time	Region	Time	Region	Time	Region	Region	
		2012<2018	VIA<PEN=SIN	2012<2018	PEN=SIN<VIA	2012<2018	VIA<PEN=SIN	VIA=SIN<PEN	

When considering the complete assemblages' dataset, i.e. using multivariate analyses of species abundance through Permanova, patterns were similar to those detected using univariate descriptors (Table II-2). However, the interaction Time x Region was found to be significant (Pseudo- $F_{1,24}:33.57$, $P(\text{perm}) < 0.001$, Table II-2). Pairwise comparisons were all significant, preventing the identification of the "true" nature of the interaction. When exploring reefs variability using the multivariate Permadisp procedure for the centroids of each factor (see ND`MS in Figure II-3), we found temporal trends in the variability were significant at Peniche where the deviation of the centroids increased in 2018 regarding 2012 (Permadisp $F_{1,174}: 6.0$, $P(\text{perm}): 0.001$).

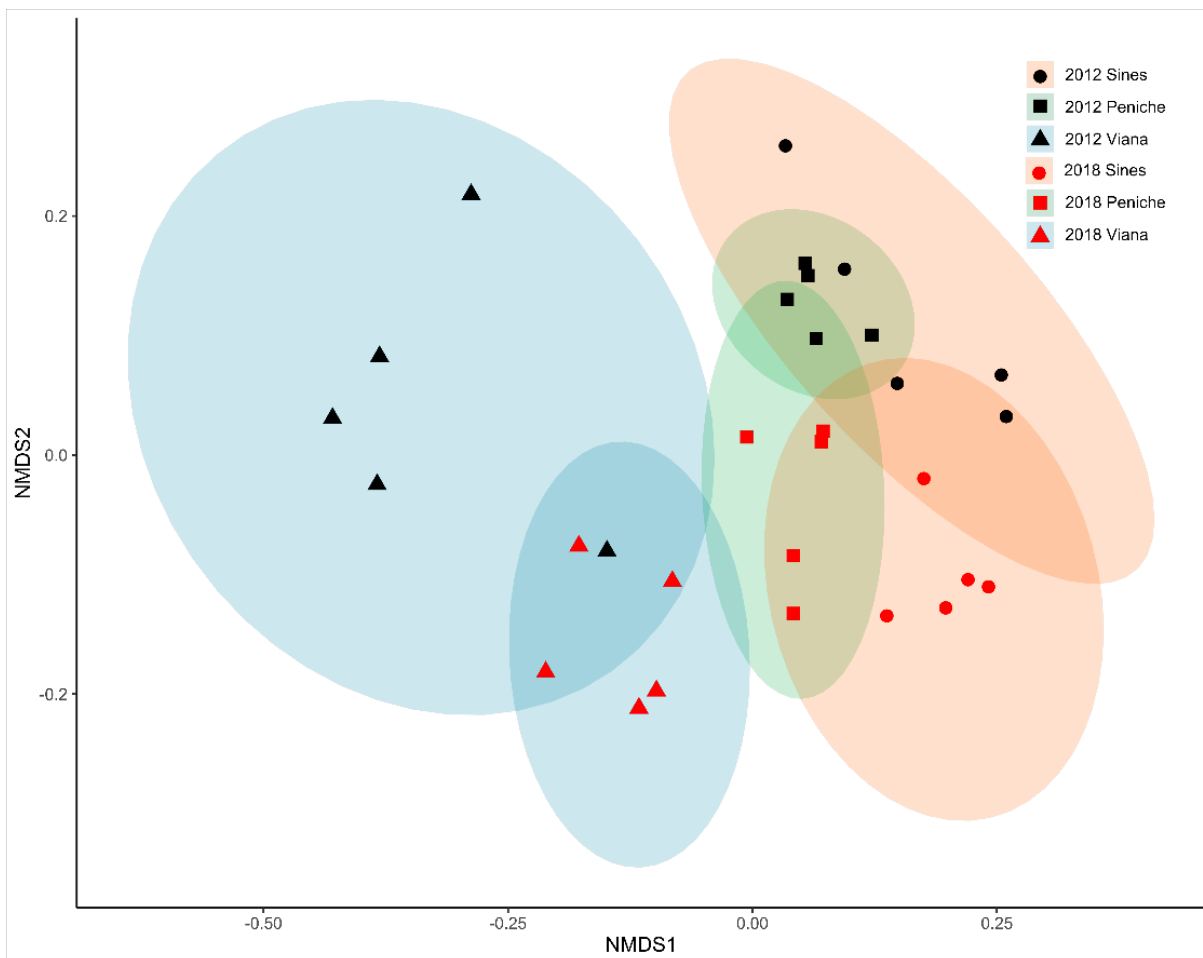


Figure II-3 Two-dimensional non-metric multidimensional scaling plot showing similarities in the structure of algal assemblages between reefs within each region over the two sampling years. NMDS - Non-metric multidimensional scaling.

Table II-2 Permutational multivariate analysis of variance (PERMANOVA) and pairwise comparisons examining the effects of time, region and reef on whole macroalgal assemblages. Significant effects are reported in bold.

Source of variation	df	MS	Pseudo- <i>F</i>	<i>p</i>	Denominator
Time (T)	1	5	54.036	0.001	Reef (T x R)
Region (R)	2	6.98	75.498	0.001	T x R
T x R	2	3.1	33.578	0.001	Reef (T x R)
Reef (T x R)	24	0.76	8.281	0.001	Residual
Residual	150	0.09	0.24157		

Pairwise comparisons		T x R				
	F.Model	2012			2018	
		R ²	p.value	F.Model	R ²	p.value
Viana x Peniche	22.96	0.283	0.001	14.79	0.203	0.001
Viana x Sines	14.32	0.198	0.001	14.00	0.194	0.001
Peniche x Sines	8.99	0.134	0.01	9.67	0.142	0.001

Community temperature index (CTI). Overall, the community temperature index (CTI) significantly rose from $13.70 \pm 0.15^{\circ}\text{C}$ (mean $\pm$ SE) in 2012 to $14.73 \pm 0.10^{\circ}\text{C}$ in 2018 (Table II-3). Algae communities of Sines (14.12 ± 0.18) and Peniche ($14.48^{\circ}\text{C} \pm 0.13$) hold significantly higher CTIs than Viana's assemblages ($12.49 \pm 0.26^{\circ}\text{C}$) in 2012 (Table II-3, Figure II-4a). In 2018, there were no significant differences among communities (Figure II-4a). At the regional level, CTIs increased significantly from 2012 to 2018, in Sines and Viana (Table II-3, Figure II-4b). The abundance of warm-affinity species (with STIs higher than mean CTIs) increased in all regions, suggesting that tropicalization is the main driver of changes in the CTIs of each region (Figure II-5). The abundance of cold-affinity species increases in Sines and Peniche, contributing to a moderate increase of the CTIs compared with communities in Viana. The processes behind the significant increase of CTIs in Viana were tropicalization and deborealization (decrease in abundance of cold-affinity species, Figure II-5).

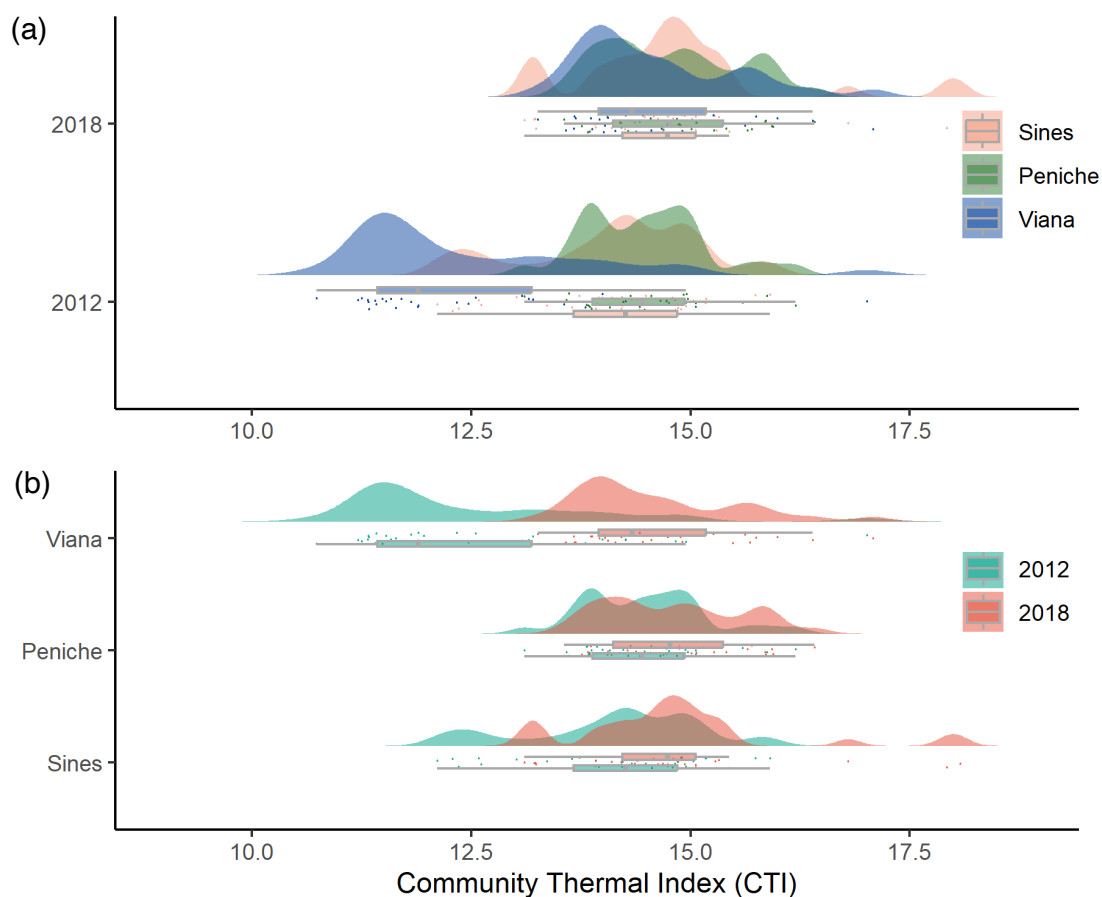


Figure II-4 Visual demonstration of the significant effects of interaction between time and region in the community temperature index (CTI): (a) differences among regions in each year and (b) differences between years in each region on values of CTI (community temperature index). Points correspond to each reef.

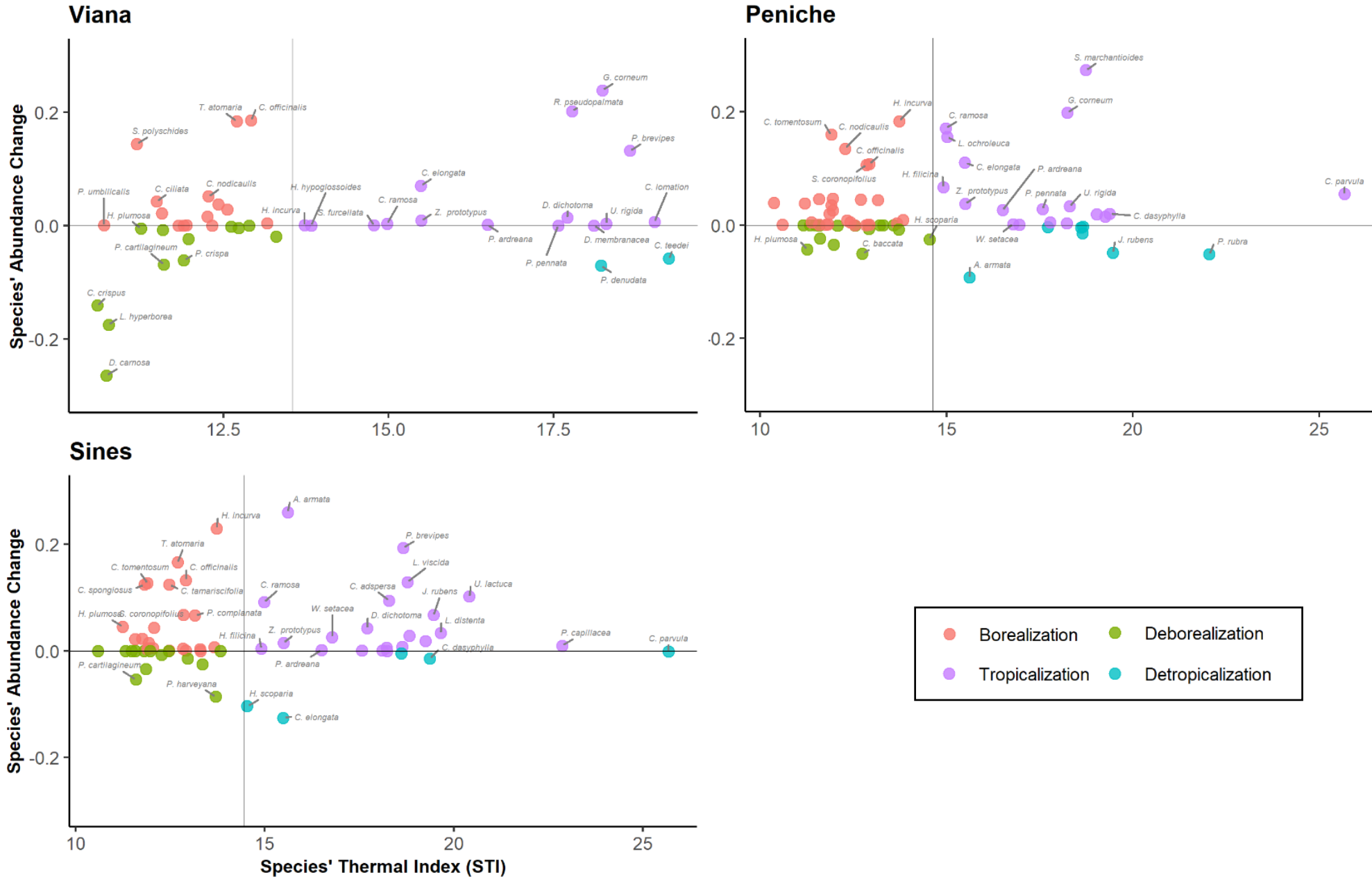


Figure II-5 Processes behind the processes of community temperature index changes in each region. Thin horizontal line indicates no change in species abundance and thin vertical line is the mean CTI for each region.

Table II-3 ANOVA examining the effects of time, region and reef on the community temperature indexes (CTIs). Computed least-squares means for significant factors and factor combinations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant values in bold. Time x Region a posteriori contrasts only include those combinations with significant differences.

Source of variation	df	MS	F value
Time (T)	1	48.29	90.39***
Region (R)	2	20.53	38.43***
T x R	2	13.58	25.42***
Reef (T x R)	30	0.53	0.46
Residual	144	1.18	
Cochran C' test	C = 0.12	p-value = 0.06	
Contrast	t-ratio		p-value
2012-2018	-6.4		< 0.0001
PEN - SIN	0.85		0.67
PEN - VIA	5.49		< 0.0001
SIN - VIA	4.63		< 0.0001
PEN12 - VIA12	7.09		< 0.0001
SIN12 - VIA12	5.83		< 0.0001
VIA12 - VIA18	-7.54		< 0.0001

Morpho-functional groups abundance. Regional differences were found for most of the morpho-functional groups (Figure II-6, Table II-4), except for subcanopy species which did not show any latitudinal effect (Figure II-6b, Table II-4). Turf species were more abundant in the centre and south regions (Figure II-6c, Table II-4) while encrusting species were more abundant in the south (Figure II-6d, Table II-4). The north region (Viana) holds a larger amount of canopy algae than the other regions (SNK $p < 0.001$, Figure II-6a, Table II-4). Regarding the temporal evolution of the functional groups, only turf species showed a significant change, with an overall increase in biomass from 2012 to 2018 (SNK $p < 0.01$, Table II-4).

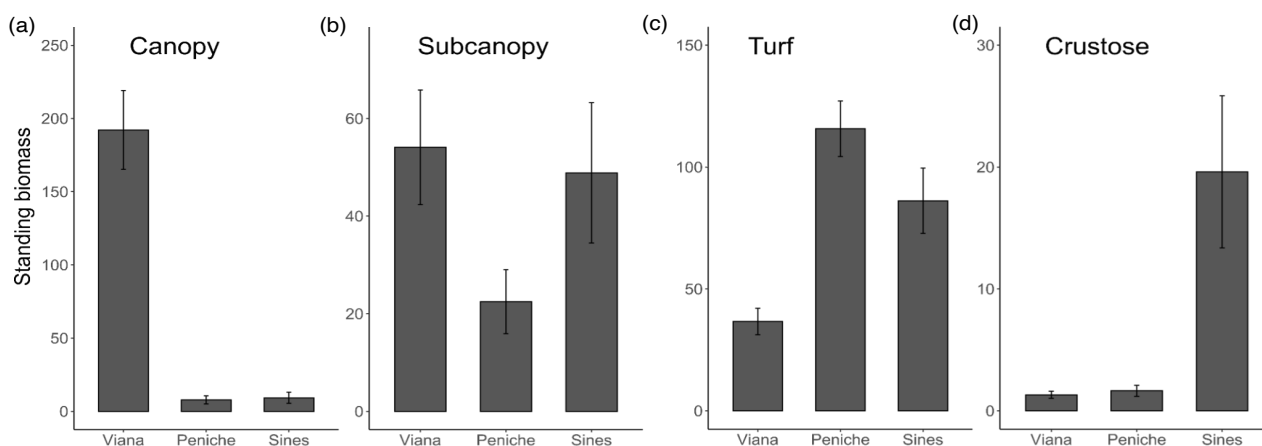


Figure II-6 Bar plots to illustrate the significant effects of region on the standing biomass (wet weight g 0.25 m²) for the four morpho-functional groups for (a) Canopy, (b) Subcanopy, (c) Turf and (d) Crustose. Bars are mean values for the two surveys (2012-2018) $\pm$ SE, n=6.

Table II-4 ANOVA examining the effects of time, region, and reef on the mean of encrusting species, turf species, subcanopy species and canopy species. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Source of variation	df	Encrusting MS	<i>F</i>	Turf MS	<i>F</i>	Subcanopy MS	<i>F</i>	Canopy MS	<i>F</i>
Time (T)	1	124	0.108	412.68	11.6932**	57221	2.676	2089	0.038
Region (R)	2	6577.5	5.752**	364.43	10.326***	17225	0.805	674246	12.451***
T x R	2	139.8	0.122	28.52	0.8081	10074	0.471	3837	0.07
Reef (T x R)	24	1143.4	1.547	35.29	3.5149***	21383	4.121***	54149	6.075***
Residual	150	738.8		10.04		5188		8912	
Cochran C' test		C = 0.4159, $P < 0.001$		C = 0.11291, $P = 0.107$		C = 0.34024, $P < 0.001$		C = 0.23942, $P < 0.001$	
Transformation		None		Sqrt(x)		None		None	
SNK test		Region		Time	Region			Region	
		VIA=PEN<SIN		2012<2018	VIA<PEN=SIN			PEN=SIN<VIA	

2.5 Discussion

Our surveys conducted between 2012 and 2018 provided valuable insights into the changes occurring in the subtidal seaweed communities along the Portuguese continental coast. The approach goes beyond simply assessing the magnitude of structural changes that occurred. It also aims to shed light on the underlying processes that drive these changes. Our results provide evidence that the macroalgae assemblages are undergoing a fast tropicalization process, which poses a threat to the persistence of Europe's southernmost kelp forests.

One of the most notable findings from our subtidal surveys was the rapid rate at which these assemblages underwent changes within a relatively short time period. Most of the analysed descriptors revealed significant differences between 2012 and 2018, indicating a high rate of the response to climate change in coastal communities (Poloczanska et al., 2013). Both, structural and functional changes were detected in the assemblages. The overall species richness and diversity, as indicated by descriptors like the Shannon H' index, increased over the 6-year period. This increase in species number can be attributed, in part, to the progressive colonization of the Portuguese coast by warm-water affinity species, including non-native species, which inflated local species diversity (Sax & Gaines, 2003). Ocean temperature is the key driver controlling geographic distribution of subtidal seaweed species. It is predicted that warmer conditions will result in shifts in species range. A comprehensive modelling projection by García-Molinos et al. (2016) suggested that increased regional diversities due to the prevalence of range shift expansions outweighing range contractions, are a common phenomenon under future environmental scenarios. Local-regional signals of increasing species richness in marine systems have been reported as a general trend in a meta-analyses study (Dornelas et al., 2014). Indeed, we found evidence of range expansions, such as the red seaweed *Liagora distenta*, a warm-affinity species Mediterranean specie that was not found in 2012. Also, the arrival and proliferation of non-native species, such as *Symphycladia marchantioides*. This species from subtropical origin may be favoured by ocean warming because of its higher thermal lethal limits (van den Hoek & Breeman, 1990). Since its first record appearance near Lisboa in 2007 (Herbarium MACOI, specimen no 3564, University of Coimbra, PT), *Symphycladia marchantioides*, has spread and become abundant in Portugal. Future ecological impacts of this introduction are unknown but because of its creeping growth habit, it may locally dominate the substrate probably preventing the settlement and growth of other seaweeds (authors pers. obs.).

The structure of subtidal seaweed communities along the Portuguese coast follows the latitudinal gradient of temperature, consistent with previous observations (Tuya et al., 2012). Significant differences among regions were found for most of the community descriptors examined, with the only exception of the biomass of subcanopy function group. As expected, assemblages in the North were dominated by large kelp species (*Laminaria* spp. and *Saccorhiza polyschides*), which exhibited higher biomass but lower species richness. In contrast, the central and southern communities showed high species richness, with the turfs being the dominant functional group. Indeed, there is a north-south latitudinal gradient, with a large biogeographical disruption between northern communities and those present in the central and southern regions. This pattern follows the existing latitudinal thermal gradient (Lima et al., 2006; Tuya et al., 2012). Temperature, known as the most relevant physical driver controlling the geographic boundaries of seaweed species (Lüning, 1990; van den Hoek & Breeman, 1990; Bartsch et al., 2012), decreases with increasing latitude, following a recognized large-scale pattern in physical oceanography. The impact of seawater temperature gradient on the composition of these communities is also evident in the community temperature index (CTI) results.

However, the latitudinal thermal gradient may not be the only cause driving the large differences among regions. The Iberian upwelling system creates unique conditions for the persistence of cold-water species, reinforcing the disruption between north and central-south communities. Reefs from the northern region are subject to the highest intensity of the Iberian upwelling in Portugal (Ferreira Cordeiro et al., 2018). Under upwelling favourable winds (i.e. northerly winds) surface coastal waters are transported offshore and replaced by subsurface cold and nutrient rich seawaters. On the Portuguese coast, the seawater summer temperatures are eased off by persistent upwelling events (from June to September), providing thermal refugia to cold-affinity species like kelps (Alvarez et al., 2011; Seabra et al., 2019). In fact, in recent years, kelp species have disappeared at higher latitudes of the Iberian Peninsula (the Cantabrian Sea; Fernández, 2011; Voerman et al., 2013; Casado-Amezúa et al., 2019), where the upwelling influence is less intense (Alvarez et al. 2011). There has been some debate regarding the future trends of the Iberian upwelling system, with some early publications predicting increases in its intensity (Alves & Miranda, 2013; Miranda et al., 2013) while others suggesting reductions (Pérez et al., 2010; Sydeman et al., 2014). Recent evidence suggests that even under increased upwelling favourable winds, the strengthening of stratification due to ocean warming will counteract the intensified upwelling, resulting in an overall decrease in the upwelling intensity (Sousa et al., 2020).

Whether this scenario is already happening and its consequences for coastal assemblages requires further attention.

The thermal signature of the subtidal seaweed communities measured using the community temperature index (CTI) increased from 2012 to 2018. As expected, seaweed communities of Sines and Peniche were more similar, holding significantly higher CTIs than Viana do Castelo assemblages in 2012. In 2018, there was a significant increase of CTIs in Viana do Castelo, resulting in the absence of differences among regions. This result suggested a trend to biotic homogenization of subtidal communities along the coast of Portugal, a process recognized in many other European seaweeds assemblages (Mineur et al., 2015). During the period of the study, there was an increase in the abundance of species with warm-water affinity along the coast of Portugal. For instance, the abundance of *Gelidium corneum* in Peniche and Viana do Castelo and *Cryptopleura ramosa* in all regions. This increase in the abundance of warm-affinity species suggests that the observed changes are primarily driven by tropicalization. Similar tropicalization of temperate marine ecosystems has been widely reported in coastal assemblages (Vergés et al., 2014; 2016), including along the Portuguese coast for fish communities (Horta et al., 2014). In addition to tropicalization, changes in Viana subtidal communities are also associated with a process of deborealization, characterized by the decrease in abundance of cold-affinity species. Despite being less discussed, deborealization often acts synergistically with tropicalization and has profound implications on marine communities (McLean et al., 2021). If cold-affinity species are unable to find thermal refuge like that provided by the upwelling, deborealization might become the main process driving changes in subtidal seaweed communities on the northern Portuguese coast.

The last remarkable finding was the rapid functional changes detected in the seaweed assemblages over a six-year period, Turf-forming species significantly increased their biomass in the algal assemblages. This morpho-functional group has been linked to anthropogenic climate change (Airoldi, 1998; Filbee-Dexter et al., 2016). According to Airoldi (1998), turfs are small, fast-growing, opportunistic species with high coverage and turnover rates that can be highly stress tolerant compared to other larger functional groups. Turfs transform deeply marine ecosystems by replacing structurally diverse seaweed canopies with simplified habitats dominated by filamentous algae, which provide limited resources for ecosystem maintenance (Moy & Christie, 2012; Connell & Irving, 2008; Davoult et al., 2011; Fernández, 2011; Filbee-Dexter et al., 2016; Wernberg et al., 2016). Besides being a bio-indicator of environmental stress, an

increase in turf abundance can damage the maintenance of biodiversity of well-structured ecosystems. Turfs provide small three-dimensional structures that increase the rate of sediment build-up in its structure and may inhibit the recruitment of larger algae that require a firm substrate, limiting the recovery of the canopy population (Airoldi, 2003; Schmidt & Scheibling, 2007; Connell & Russell, 2010). This accumulation of sediment was observed particularly in our samples containing the invasive species of turf *Symphyocladia marchantioides*, which grows close to the substrate forming a mat. Under these conditions, it is easy to speculate that turf expansion might imperil the persistence of canopy macroalgae, i.e. marine forest like kelps, even though this study did not show any significant decrease in this functional group.

2.6 Conclusion

In conclusion, the coast of north Portugal hosts the southernmost North Atlantic seaweed forests of several cold-adapted species like *Laminaria hyperborea* and *Saccharina latissima*. Iberian upwelling is probably key in maintaining those northerly communities at these latitudes. However, we confirmed that subtidal macroalgal communities are experiencing major changes at very fast pace. This novel evidence of “fast tropicalization” of the subtidal communities on the Portuguese coast is likely a strong sign of the effects of anthropic climate change over these unique assemblages. The loss of these marine forests would lead to the loss of their key ecological and economic role. A comprehensive understanding of the mechanisms responsible for the observed changes is urgently required for an effective management and protection of these relictual populations, as well for the implementation of successful restoration actions. Simultaneously, monitoring programs like the one presented in this study should provide information about the evolution of these meridional marine assemblages.

2.7 Acknowledgements

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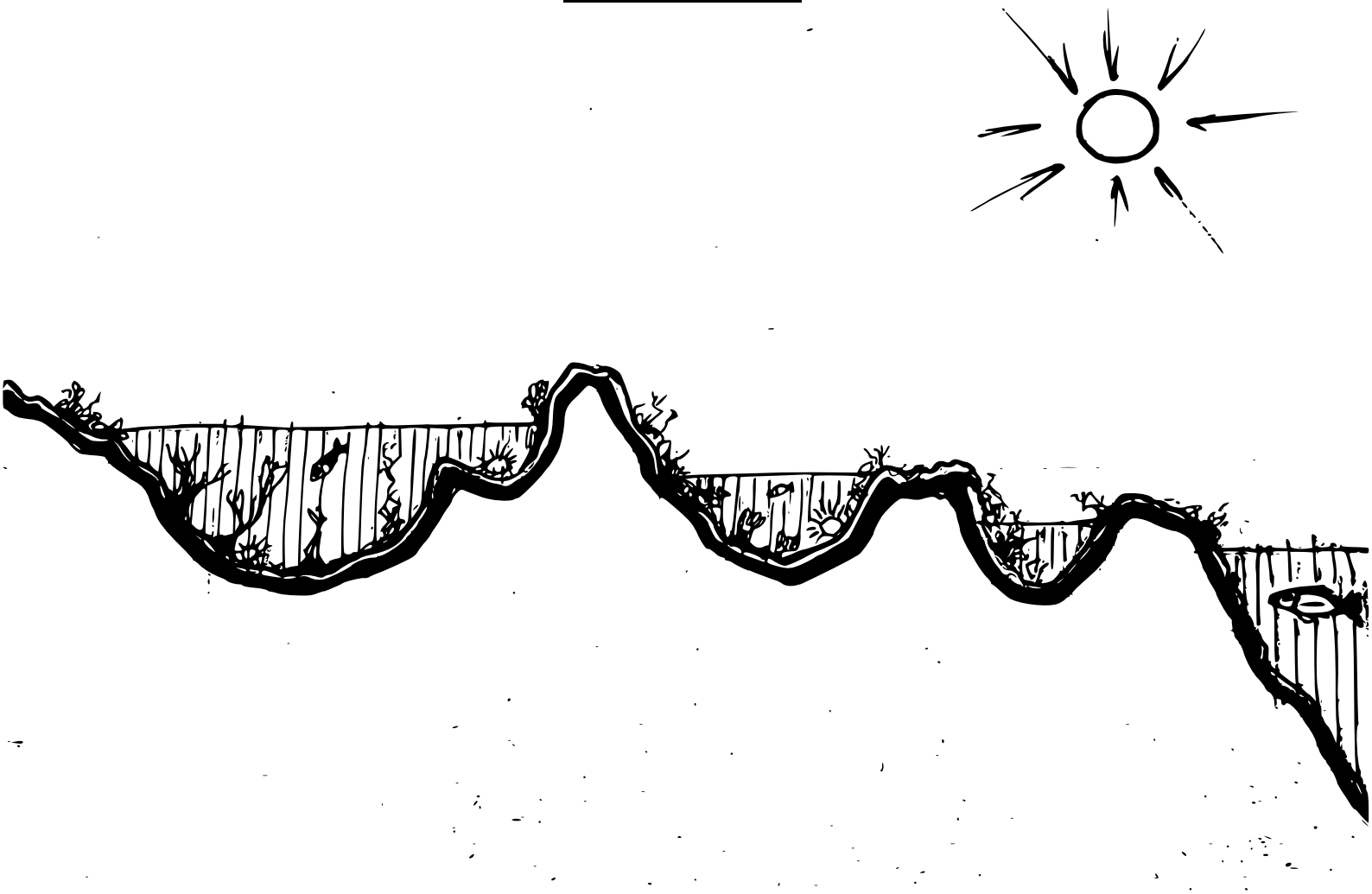
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Chapter III.



Decadal shifts in intertidal rockpool seaweed assemblages: Deborealisation and structural collapse along the Portuguese coast

Publication Note

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3.1 Abstract

Intertidal rockpools represent highly heterogeneous and thermally variable habitats that can mediate the impacts of climate change on coastal communities, yet their long-term responses to ocean warming remain poorly resolved. Here, we examined decadal changes in seaweed community composition and functional structure in intertidal rockpools along the Portuguese coast, comparing two biogeographically distinct regions (North and Centre) between 2006 and 2021. Our analyses revealed strong regional divergence in community trajectories. Species richness declined consistently in both regions; however, a marked collapse in total standing biomass occurred exclusively in the North. This decline was associated with a pronounced transition from canopy-forming macroalgae to turf-dominated assemblages and a significant increase in the Community Temperature Index (CTI), primarily driven by deborealisation through the selective loss of cold-affinity species. In contrast, rockpools in the Centre region displayed greater structural and thermal stability, supported by functional compensation within the canopy layer and the persistence of the clonal brown alga *Bifurcaria bifurcata*. Despite these differences, both regions showed an increase in turf-forming invasive algae, indicating a pervasive reduction in vertical complexity. Together, these results demonstrate that intertidal rockpool communities do not respond uniformly to warming but instead follow distinct degradation pathways (rapid functional collapse in the North versus gradual homogenisation in the Centre) highlighting the critical role of local environmental context in shaping ecosystem responses to climate change.

Keywords: Climate change, CTI - Community Temperature Index, Deborealisation, Habitat-forming loss, Turf

3.2 Introduction

Change has always been a defining feature of Earth's climate system, but the current pace and scale of anthropogenically driven climate change have pushed the oceans beyond their historical limits (Harley et al., 2006; Arias et al., 2021). Over the past decades, ocean temperatures have been rising at an alarming and accelerating pace, intensifying climate-related stressors such as ocean acidification and habitat loss (IPCC, 2019; Arias et al., 2021). This thermal shift has profound implications for marine life, as most aquatic organisms are ectothermic and rely on external temperatures to regulate their physiological processes (Pörtner & Farrell, 2008; Somero, 2012). In marine ecosystems, where the velocity of climate change often surpasses that of terrestrial environments, rapidly shifting isotherms are compelling species to either adapt to novel conditions or track their thermal niches across space and time (Burrows et al., 2011; Lenoir et al. 2020).

Globally, these responses have driven broad-scale shifts in species distributions and community composition, with a rise in species richness at higher latitudes due to the poleward expansion of warm-affinity species, ultimately leading to the tropicalisation of temperate ecosystems (Filbee-Dexter & Wernberg, 2018; Smale et al., 2019; de Azevedo et al., 2023; Bañón et al., 2024;). Tropicalisation refers to the increasing dominance of warm-affinity species in areas historically inhabited by cold-affinity species (Bianchi & Morri, 2003; Vergés et al., 2014), and is commonly assessed using the Community Temperature Index (CTI, Devictor et al., 2008), a metric that captures the average thermal affinities of species in a community, weighted by their relative abundance, serving as a useful indicator of climate-driven ecological shifts (Vergés et al., 2014; Sunday et al., 2015; Burrows et al., 2019; de Azevedo et al., 2023). These shifts are particularly critical for marine forests, which are highly vulnerable to CC-related drivers such as increased herbivory, thermal stress, and competition for space with opportunistic species that exhibit broader thermal tolerance and distribution ranges. (Smale et al., 2013; Bertocci et al., 2015; Barrientos et al., 2022; de Azevedo et al., 2023).

In intertidal environments, rockpools act as critical refugia, harboring a high diversity of macroalgae that might otherwise be unable to survive the desiccating conditions of the emergent rock (Arenas et al., 2006). These habitats are characterised by high spatial and seasonal heterogeneity, often dominated by diverse assemblages of Corallinaceae and canopy-forming brown algae that function as ecosystem engineers (Araújo et al., 2006). Unlike the more stable subtidal environment, rockpools function as

semi-closed systems with complex food webs (Mendonça et al., 2018). However, the unique position of rockpools at the land-sea interface subjects them to extreme thermal fluctuations, making the persistence of habitat-forming species particularly sensitive to climatic shifts and the proliferation of opportunistic or invasive taxa (e.g., *Sargassum muticum* in temperate shores; Bertocci et al., 2012; Cacabelos et al., 2013).

The Portuguese coast, where the present study was conducted, represents a biogeographic transition zone supporting both cold- and warm-affinity species due to its unique oceanographic conditions (Pinho et al., 2016; Tuya et al., 2012; de Azevedo et al., 2023). This region constitutes the southernmost limit for many important cold-water habitat-forming seaweeds, which structure rockpool communities that sustain rich biodiversity and complex habitats (Araújo et al., 2006; Mendonça et al., 2018). The persistence of these cold-water species is closely linked to the Iberian upwelling system, characterised by the seasonal influx of cold, nutrient-rich waters into the coastal zone (Sousa et al., 2020; de Azevedo et al., 2023). Upwelling intensity follows a latitudinal gradient, being strongest along the northern coasts of Portugal and Spain, which helps buffer sea surface warming and supports the survival of cold-affinity species at their southern distributional limit (de Bettignies et al., 2021; Varela et al., 2022). Conversely, toward the southern portion of the Portuguese coast, the upwelling influence progressively weakens under increasing Mediterranean influence, resulting in warmer and less nutrient-rich waters (Peliz et al., 2005; Tuya et al., 2012; de Azevedo et al., 2023).

Climate change projections suggest a potential weakening of the Iberian upwelling in the future, which may exacerbate sea surface warming and further challenge the persistence of cold-affinity species along the Portuguese coastline (Sydeman et al., 2014; Sousa et al., 2020; Varela et al., 2022; de Azevedo et al., 2023;). While subtidal responses and temporal shifts in the distribution of seaweeds have been relatively well-documented, the intertidal zone of this region (particularly in thermally dynamic habitats such as intertidal rockpools) remains understudied in the context of climate change, with limited information on how seaweed community composition and distribution are shifting (Olabarria et al., 2012, Chabrierie & Arenas, 2024). Recent evidence highlights that these shifts are not uniform across the coastline, with divergent responses in community stability and resilience being observed between northern and central regions (Martins et al., 2026).

Rockpools within the intertidal zone experience extreme thermal fluctuations due to their cyclical exposure and submersion driven by tidal regimes (Ganning, 1971;

Huggett & Griffiths, 1986; Wilson et al., 2015). These environments often exhibit much higher daily and seasonal temperature variability than subtidal habitats, exposing resident organisms to acute and chronic thermal stress (Ganning, 1971; Huggett & Griffiths, 1986; Alvarez et al., 2011; Seuront et al., 2019). Furthermore, during low tide, rockpool temperatures can decouple from the surrounding sea surface temperature (SST), creating thermal bottlenecks. In these environments, intertidal species often live close to their physiological tolerance limits, exhibiting differential population sensitivity to heat stress, which makes them potentially more sensitive (or in some cases more resilient) to ongoing ocean warming compared to their subtidal counterparts (Wilson et al., 2015; Pereira et al., 2025). This vulnerability, particularly among habitat-forming species, favours their replacement by stress-tolerant turf-forming algae (Arenas et al., 2006; Bertocci et al., 2012). Such shifts can be further exacerbated by intensified biological pressures, as rising temperatures have been shown to drive kelp recruits into "hiding" due to increased herbivory, hindering the recovery of canopy-forming populations (Franco et al., 2015). Ultimately, this transition from complex canopies to simplified assemblages is increasingly recognized as a signature of the reorganization of temperate reefs under climate pressure (Martins et al., 2026).

This study aims to investigate recent changes in the composition and functional structure of seaweed communities inhabiting intertidal rockpools along the Portuguese coast, with a particular focus on detecting potential signals of tropicalisation and other shifts linked to climate change. While the effects of ocean warming on subtidal macroalgal assemblages have been increasingly recognized in this region (Tuya et al., 2012; Pinho et al., 2016; de Azevedo et al., 2023), far less is known about how intertidal systems (especially the thermally variable habitats of rockpools) are responding to the same pressures (but see Lima et al., 2007). Understanding whether the ecological shifts documented in the more thermally stable subtidal zone are also occurring in the environmentally dynamic intertidal rockpools is key to evaluating broader patterns of change across coastal marine ecosystems. Here, we compare data collected 15 years apart (2006 and 2021) across northern and central regions of the Portuguese mainland coast, capturing a latitudinal gradient influenced by distinct oceanographic conditions. Our goal is to determine whether seaweed communities in intertidal rockpools have undergone compositional and functional shifts over time, and whether such changes are associated with species' thermal affinities. We expect to find an increase in warm-affinity and turf-forming species, accompanied by a reduction in cold-affinity and canopy-forming taxa, reflecting broader patterns of ecosystem change in the context of rapid ocean warming.

3.3 Material and Methods

Study Area. The study was conducted in two regions along the Portuguese coast—North and Centre—each encompassing three rocky shore beaches. North: MO – Moledo beach (41° 84' N, 8° 87' W), AM - Amorosa beach (41° 64' N, 8° 82' W) and AG – Aguda beach (41° 04' N, 8° 65' W). Centre: RI – Ericeira Ribeira d'Ilhas beach (38° 98' N, 9° 42' W), SS – Ericeira São Sebastião beach (38° 97' N, 9° 42' W) and SB - Ericeira Sul beach (38° 95' N, 9° 41' W) (Figure III-1). The coast of Mainland Portugal has a predominantly north-to-south orientation, and the selected rocky shores are similarly exposed to dominant NW and W swells. Substrate composition varies between regions: northern shores are primarily composed of granite, while central shores consist mainly of limestone and sandy limestone, featuring smooth, flattened to irregular surfaces. The tidal regime is semidiurnal, with an extreme tidal range of approximately 3.5 to 4 meters during spring tides. Sea surface temperature (SST) exhibits marked seasonality along the coast, with slightly higher temperatures in the south, largely influenced by warmer oceanic currents (Tuya et al., 2012).

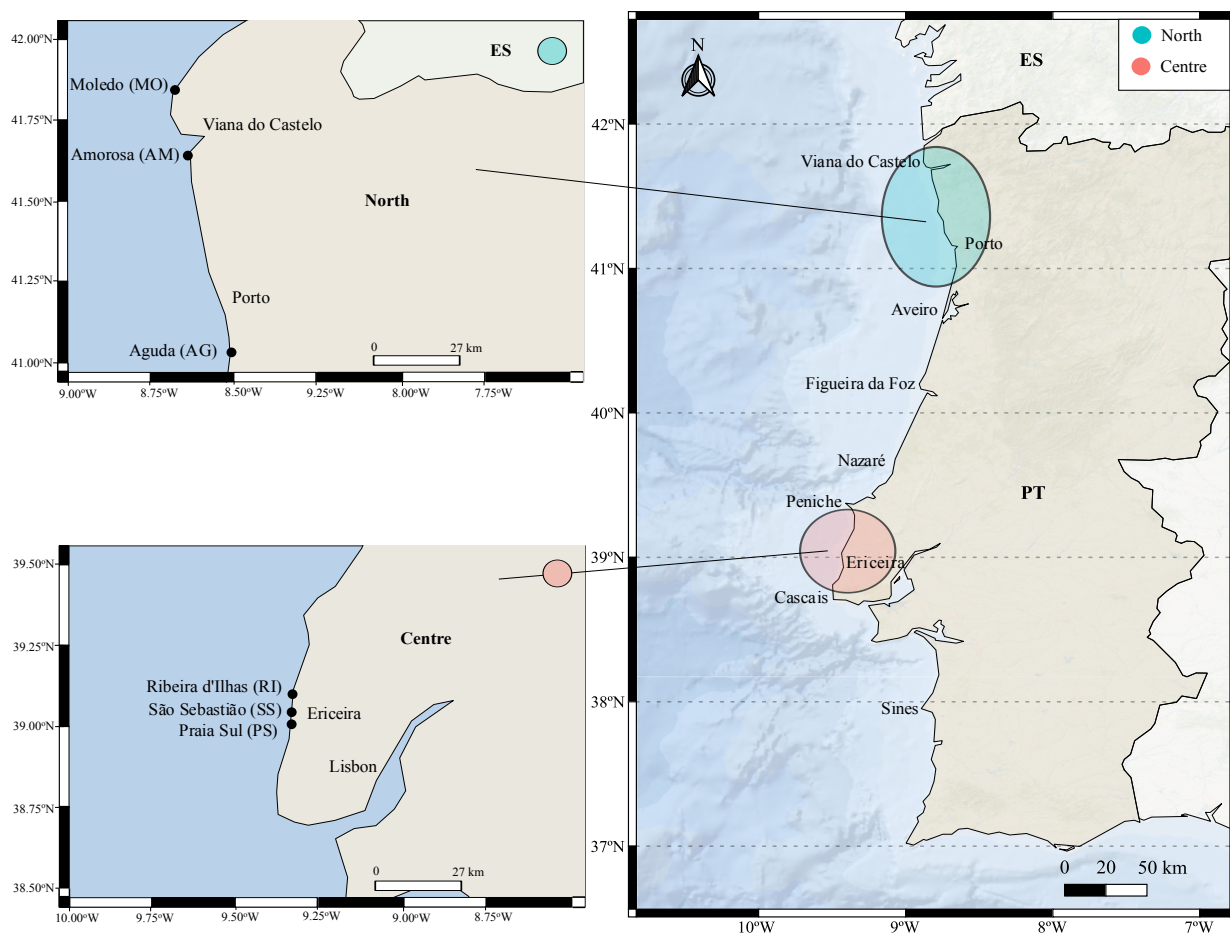


Figure III-1 Map of continental Portugal showing the two study regions (North and Centre). Insets detail the sampled beaches within each region: Moledo (MO), Amorosa (AM) and Aguda (AG) in the North; and Ribeira d'Ilhas (RI), São Sebastião (SS) and Praia Sul (PS) in the Centre.

Data Collection. Surveys were conducted during twelve field campaigns carried out at low tide between May and June of 2006 and 2021. In each region (North and Centre), three rocky shore beaches were selected, with a minimum spacing of one km to ensure statistical independence and minimise environmental autocorrelation. Selected beaches consisted of natural rocky shores with well-developed intertidal rockpools and extended continuously for at least 500 m, providing sufficient sampling area and ecological representativeness. Each beach was divided into three transects (Sites), each approximately 100 m long and spaced 100 m apart. Within each transect, four intertidal rockpools of similar surface area ($\sim 8 \text{ m}^2$) and depth ($\sim 20 \text{ cm}$) were haphazardly selected to minimise environmental variation, totalling twelve rockpools per beach. In each selected rockpool, three quadrats measuring $30 \times 30 \text{ cm}$ (0.09 m^2) were haphazardly placed on the horizontal bottom, below the pool water level. With the exception of encrusting algae, all macrophyte biomass present within each quadrat was carefully scraped and collected in plastic bags. In total, 216 quadrats were sampled per year, covering a total surface area of approximately 19.44 m^2 . Samples were appropriately labelled, transported to the laboratory in cool boxes, and frozen until processing. Seaweeds from each sample were identified to the lowest feasible taxonomic level, mostly to species, and their biomass was determined after drying in an oven at 50°C for a minimum of two days. Species with biomass too small to be processed were identified and recorded as present in the sample and were assigned the lowest measured biomass value (0.01 g) for analysis purposes. For the community descriptors, species richness was determined as the total count of distinct species in the samples, while the Shannon H' diversity index, incorporated both species richness and evenness to assess the diversity and distribution of the species within the community (Arenas et al., 2006).

Community functional characterization. To investigate the functional characteristics of seaweed communities, all identified species were classified according to two complementary types of functional traits: categorical (morpho-functional) and continuous (thermal affinity). Hence, regarding morpho-functional traits, species were assigned into three groups: 'canopy', 'sub-canopy', and 'turf-forming', reflecting their structural complexity and ecological roles (following Arenas et al., 2006; Althaus et al., 2015).

In addition, each taxon was characterised by its thermal affinity, expressed as the Species Temperature Index (STI). Species Temperature Indices were derived using Species Distribution Models (SDM) implemented in biomod2 (Thuiller et al., 2023). Species distribution data were downloaded from OBIS (<https://obis.org/>) and GBIF (<https://www.gbif.org/>) public databases. For each species with ≥ 20 occurrence records,

we modelled habitat suitability using Maximum Entropy (MAXENT; Phillips et al., 2006) against six environmental predictors from Bio-ORACLE v2.0 (SST max, SST min, nitrate, PAR max, pH, salinity; Assis et al., 2018). Models were evaluated using 5-fold cross-validation with five pseudo-absence replicates (1,000 pseudo-absences each). The top 10 models (TSS ≥ 0.7) were combined into ensemble predictions using weighted mean averaging (Marmion et al., 2009). The STI for each species was defined as the mean SST at the 90th percentile of habitat suitability (T_{90}), representing the average temperature experienced within the most suitable habitat of the species. This approach differs from simple range-based methods by weighting temperature values by habitat suitability, providing a more ecologically meaningful estimate of species' thermal preferences (Bowler & Böhning-Gaese, 2017). More details see Attachments. Chapter III – S1.

Community-level composition for both categorical and continuous traits was then quantified using Community-Weighted Means (CWM) (Devictor et al., 2008; de Bello et al., 2021), calculated as:

$$CWM = \frac{\sum_{i=1}^N T_i \omega_i}{\sum \omega_i}$$

where ω_i is the abundance (biomass) of each species i , T_i is its trait value (either the categorical group or the STI), and N is the number of species in the community. For morpho-functional groups, CWM values represent the relative biomass contribution (%) of each category to the total community. For thermal affinity, the CWM corresponds to the Community Temperature Index (CTI) (Devictor et al., 2008; Burrows et al., 2019).

Finally, temporal shifts in biomass were assigned to four thermal processes following McLean et al. (2021): (i) *tropicalisation* (increase in warm-affinity species), (ii) *borealisation* (increase in cold-affinity species), (iii) *detropicalisation* (decline in warm-affinity species), and (iv) *deborealisation* (decline in cold-affinity species). Species were classified as warm- or cold-affinity depending on whether their STI was above or below the community mean (CTI) at each time point. Together, these trait-based metrics provided an integrative framework to assess the thermal and functional identity of seaweed assemblages and their potential responses to environmental change.

Statistical analyses. Spatial and temporal differences in macroalgal assemblage structure were assessed using a three-way Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2017) based on Bray-Curtis dissimilarities, implemented in the *vegan* package (Oksanen et al., 2022). Biomass data were square-root transformed prior to the analysis to balance the contribution of dominant and rare species. The experimental design included Time (two levels, fixed) and Region (two levels, fixed) as crossed factors, and Beach (three levels, random) nested within Region. Patterns in community composition were visualized through Principal Coordinates Analysis (PCoA). To identify the species driving these multivariate patterns, a vector-fitting analysis (*envfit*) was performed, with the significance of species vectors (r^2) assessed through 999 permutations. The homogeneity of multivariate dispersions was tested using PERMDISP to ensure that significant PERMANOVA results reflected shifts in community location rather than differences in dispersion. When significant interactions were detected, pairwise comparisons were performed as post-hoc tests using the *vegan* package.

To evaluate shifts in community descriptors (species richness, total standing biomass, Shannon diversity, and Pielou's evenness), morpho-functional groups, and the Community Temperature Index (CTI), we employed a Generalized Linear Mixed Models (GLMM) and Linear Mixed Models (LMM) framework. This approach accounted for the hierarchical sampling design by including Beach, Site (nested within Beach), and Pool (nested within Site) as random effects. Response variables were modelled according to their underlying distributions: species richness was fitted with a Negative Binomial distribution using the *glmmTMB* package (Brooks et al., 2017); total standing biomass (aggregated per quadrat) followed a Gamma distribution with a log-link function; and CTI and diversity indices were analysed using Gaussian LMMs via the *lme4* package (Bates et al., 2015). Changes in CTI were further explored graphically by decomposing the relative contribution of species' thermal affinities into four ecological processes (tropicalisation, deborealisation, borealisation, and detropicalisation), following the framework of McLean et al. (2021).

For functional traits expressed as proportions (morphofunctional groups), we utilized Zero-Inflated Beta (ZIB) mixed models (*glmmTMB*) to handle the continuous 0–1 range and the high frequency of zeros. Model assumptions were rigorously verified through the graphical inspection of residuals and simulated residuals via the *DHARMA* package (Hartig, 2022), following the protocol of Zuur et al. (2010). The significance of fixed effects—Time, Region, and their interaction ($T \times R$)—was assessed using Type III

Wald chi-square tests (*car* package) for GLMMs and ZIB models, and *F*-tests with Satterthwaite's approximation for degrees of freedom in LMMs (*lmerTest* package). Post-hoc comparisons of Estimated Marginal Means (EMM) were conducted using the *emmeans* package (Lenth, 2023) with Tukey adjustments. All statistical analyses were performed in R (v. 4.3.1; R Core Team, 2023) and plots were produced using *ggplot2* (Wickham, 2016).

3.4 Results

The analysis of 216 samples revealed a total of 103 macroalgal taxa, comprising 97 species, 5 identified to genus level, and 1 to order level (see Attachments. Chapter III- Table III-S1). Pronounced regional differences were observed in community dominance and temporal stability. In the North, the 2006 assemblages were characterised by the dominance of the non-indigenous canopy-forming brown alga *Sargassum muticum*, alongside *Ulva sp.* and the red seaweed *Chondrus crispus*. By 2021, a major shift occurred as *S. muticum* was largely replaced by *Ulva spp.*, the kelp *Saccorhiza polyschides*, and the invasive red alga *Grateloupia turuturu*. In contrast, the Centre region exhibited higher temporal stability. The brown alga *Bifurcaria bifurcata* remained the primary dominant species across both surveys. In 2006, it was mainly accompanied by *Ulva sp.* and the native canopy-forming *Treptacantha baccata*, while in 2021, the community was defined by the persistence of *B. bifurcata* alongside the introduced red seaweed *Symphyocladia marchantioides* and the native *Ericaria selaginoides*.

The structural and diversity indices of the macroalgal communities exhibited distinct spatio-temporal patterns (Figure III-2; Table III-1). Species richness significantly declined over time ($c^2 = 19.46$, $p < 0.001$), with consistent losses in both regions. Standing biomass was affected by a significant Time (T) $\times$ Region (R) interaction ($c^2 = 6.04$, $p = 0.014$), driven by a sharp decline in the North while remaining stable in the Centre. Regarding diversity, Shannon (H') was higher in the Centre ($F = 11.27$, $p = 0.028$) but showed no temporal shifts. Conversely, Pielou's evenness (J) significantly increased by 2021 ($F = 8.39$, $p = 0.007$), suggesting a more uniform distribution among the remaining species after the richness decline.

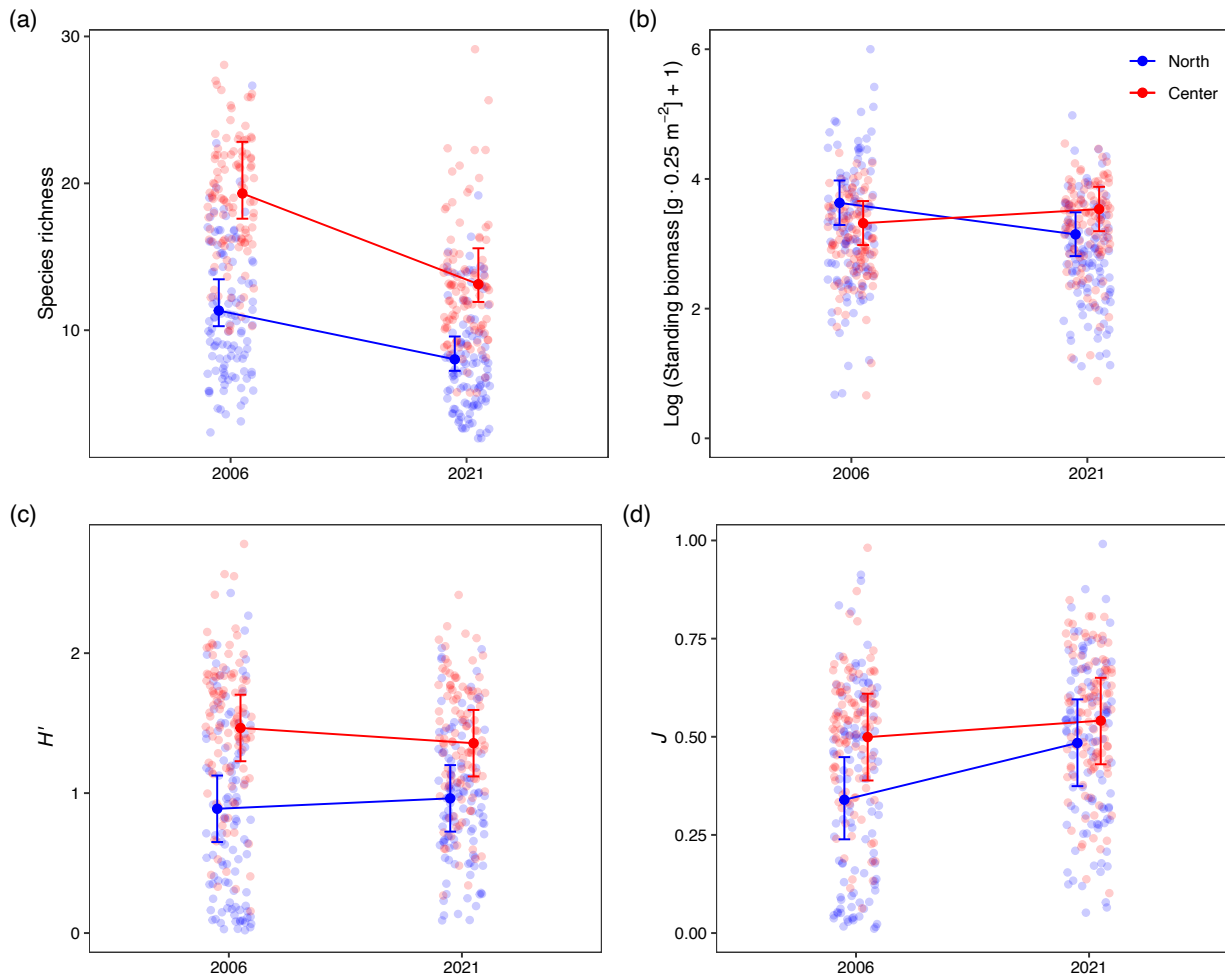


Figure III-2 Effects detected for intertidal biological descriptors. Main points and error bars values represent model-predicted means $\pm$ 95% CI (EMMs) derived from: (a) NB GLMM for species richness; (b) Gamma GLMM for algal biomass; (c) LMM for Shannon diversity (H') and (d) Pielou's evenness (J). Background jittered points represent the raw observations. Significant interactions and main effects are detailed in Table 1.

Table III-1 Summary of mixed-effects model results for community biological descriptors: Species richness, total Standing biomass, Shannon diversity (H'), and Pielou's evenness (J). Wald χ^2 values are reported for richness (GLMM, Negative Binomial distribution) and biomass (GLMM, Gamma distribution); F -values are reported for Shannon and Evenness (LMM). Significant values are in bold (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Estimated variances (Var) for random effects (Beach, Site, and Pool) represent the hierarchical structure of the study. Post-hoc comparisons (lower section) summarise significant spatio-temporal shifts based on estimated marginal means.

		Species richness	Biomass	Shannon H'	Pielou J
SOV	df	χ^2	χ^2	F	F
Time	1	19.46***	5.77*	0.04	8.39**
Region	1	35.32***	1.62	11.27*	2.25
T x R	1	0.13	6.04*	1.01	2.6
Random Effects		Var	Var	Var	Var
Beach (R)		0.0042	0.0312	0.019	0.0064
Site (R x B)		0.0174	0.1413	0.0521	0.0058
Pool (R x B x S)		0.0207	0.0902	0.0297	0.0038
Residual		-	0.382	0.1814	0.0352
Comparison					
Time		06 > 21	(see below)	n.s.	06 > 21
Region		C > N	n.s.	C > N	n.s.
T x R			N: 06 > 21	n.s.	n.s.
			C: 06 = 21		

When considering the complete assemblage dataset using multivariate analyses of species abundance (PERMANOVA), patterns were consistent with those detected using univariate descriptors (Table III-2). Significant effects of Time ($R^2 = 2.6\%$, $F = 15.51$, $p = 0.001$), Region ($R^2 = 8.9\%$, $F = 52.58$, $p = 0.001$), and their interaction (T x R: $R^2 = 3.7\%$, $F = 21.51$, $p = 0.001$; Table III-2) were detected. Significant variation was also observed at the local scale, with Beach nested within Region ($R^2 = 7.9\%$, $F = 11.63$, $p = 0.001$) and for the interaction Beach (T x R) ($R^2 = 5.2\%$, $F = 7.63$, $p = 0.001$; Table III-2). All pairwise comparisons were significant, indicating marked spatial and temporal variability in community composition. These patterns were reflected in the PCoA ordination, which showed a clear separation between Northern and Central assemblages in both years (Figure III-3). Temporal shifts were evident in both regions, with a stronger separation between 2006 and 2021 samples in the North. Vector fitting analysis identified *Bifurcaria bifurcata* as the species most strongly associated with the ordination structure ($r^2 = 0.63$, $p < 0.001$), particularly along the axis separating central assemblages. In contrast, northern assemblages were driven by higher abundances of *Saccorhiza polyschides* ($r^2 = 0.13$, $p < 0.001$), *Asparagopsis armata* ($r^2 = 0.10$, $p < 0.001$), and *Symphyclocladia marchantioides* ($r^2 = 0.16$, $p < 0.001$).

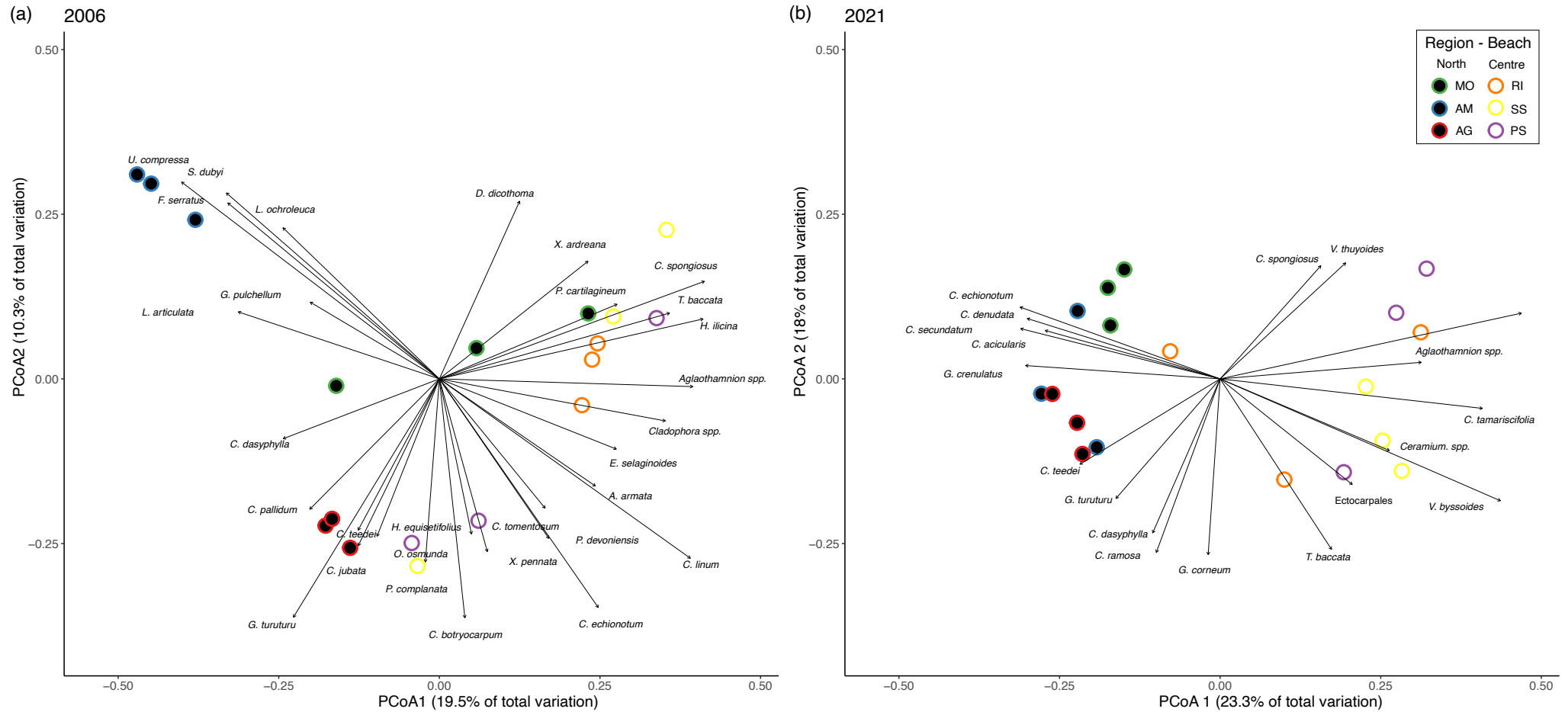


Figure III-3 Principal Coordinate Analysis (PCoA) ordination of macroalgal community composition in intertidal rockpools for (a) 2006 (ordination, $R^2 = 29.8\%$) and (b) 2021 (ordination, $R^2 = 41.3\%$). Each data point represents the averaged community composition for a single beach transect. Beach codes: North - MO (Moledo), AM (Amorosa), AG (Aguda); Centre - RI (Ribeira d'Ilhas), SS (São Sebastião), PS (Praia Sul).

Table III-2 PERMANOVA and pairwise comparisons examining the effects of Time, Region and Beach on whole intertidal rockpool macroalgae assemblages' composition. Partitioning of variance was calculated using Bray-Curtis dissimilarity on square-root transformed data. The dispersion of assemblages at the local scale was tested using PERMDISP. Significant *p*-values and associated Pseudo-*F* statistics are reported in bold.

Source of variance	df	MS	Pseudo- <i>F</i>	<i>p</i>
Time	1	4.434	15.511	0.001
Region	1	15.03	52.583	0.001
T x R	1	6.149	21.514	0.001
Beach (R)	4	3.326	11.634	0.001
Beach (T x R)	4	2.181	7.63	0.001
Residual	420	0.286		
PERMDISP (Beach)	5	0.026	2.0062	0.069
Pairwise comparisons				
pairs	F-Model	R ²	p.value	
2006:N vs 2006:C	31.598	0.129	0.001	
2006:N vs 2021:N	15.715	0.068	0.001	
2006:N vs 2021:C	31.149	0.127	0.001	
2006:C vs 2021:N	27.269	0.113	0.001	
2006:C vs 2021:C	16.211	0.07	0.001	
2021:N vs 2021:C	32.183	0.131	0.001	

Community temperature index (CTI). The Community Temperature Index (CTI) showed a significant Time × Region interaction (Table III-3, Figure III-4). The LMM revealed a highly significant interaction between time and region ($F = 16.83$, $p < 0.001$; Table III-3). At the regional level, CTI values in the Centre region remained stable between 2006 (14.38 ± 0.30 °C; mean ± SE) and 2021 (13.92 ± 0.30 °C; $t = -1.50$, $p = 0.14$; Table III-3). In contrast, the North region exhibited a significant increase in CTI, rising from 14.06 ± 0.34 °C in 2006 to 15.40 ± 0.34 °C in 2021 ($t = 4.10$, $p < 0.001$; Table III-3, Figure III-4). While no significant differences in CTI were found between regions in 2006 ($t = 0.69$, $p = 0.49$), by 2021 the CTI in the North was significantly higher than in the Centre ($t = 3.39$, $p = 0.002$; Table III-3).

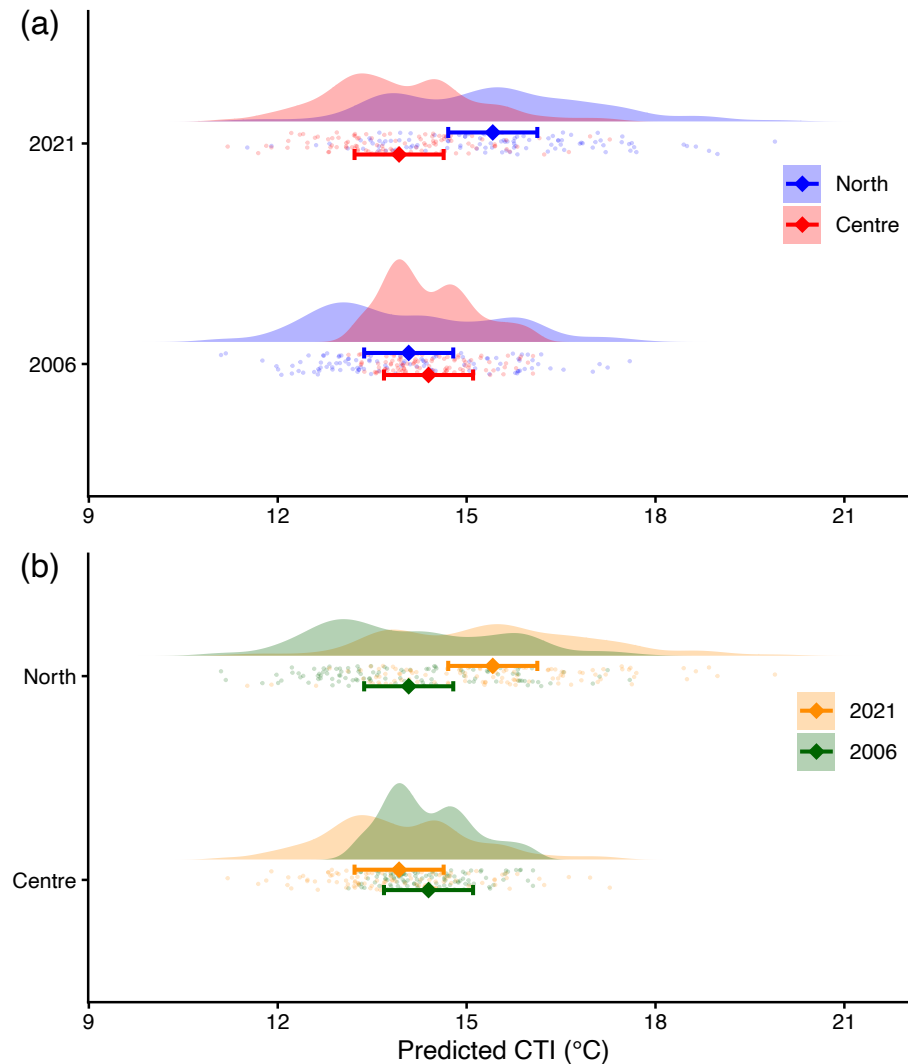


Figure III-4 Visual demonstration of the significant interaction effects between Time and Region on the Community Temperature Index (CTI): (a) differences among regions within each sampling year and (b) temporal differences between years within each region. Background jittered points correspond to individual rockpools.

Table III-3 Results of LMM for community temperature index (CTI). Significant values are in bold (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Estimated variances (Var) for random effects (Beach, Site, and Pool) represent the hierarchical structure of the study. Post-hoc comparisons summarize significant spatio-temporal shifts based on estimated marginal means.

SOV	df	CTI (F)
Time (T)	1	3.9
Region (R)	1	2.58
T x R	1	16.82***
Random Effects		Var
Beach (R)		0.128
Site (R x B)		0.2505
Pool (R x B x S)		0.4573
Residual		0.8378
Comparison		
Time		(see below)
Region		n.s.
T x R		N: 21 > 06 C: 21 = 06

Decomposition of CTI changes into species-level thermal affinity shifts revealed that different processes contributed to CTI variation among regions (Figure III-5). In the North, CTI increases were mainly associated with deborealisation, characterised by declines in the abundance of cold-affinity species, whereas changes linked to tropicalisation were comparatively limited. In the Centre, CTI variation reflected a combination of tropicalisation and detropicalisation processes, with increases and decreases in warm-affinity species partially offsetting each other. Overall, borealisation processes were weak or absent across regions, indicating that CTI increases were primarily associated with shifts in warm-affinity species and/or losses of cold-affinity taxa (Figure III-5).

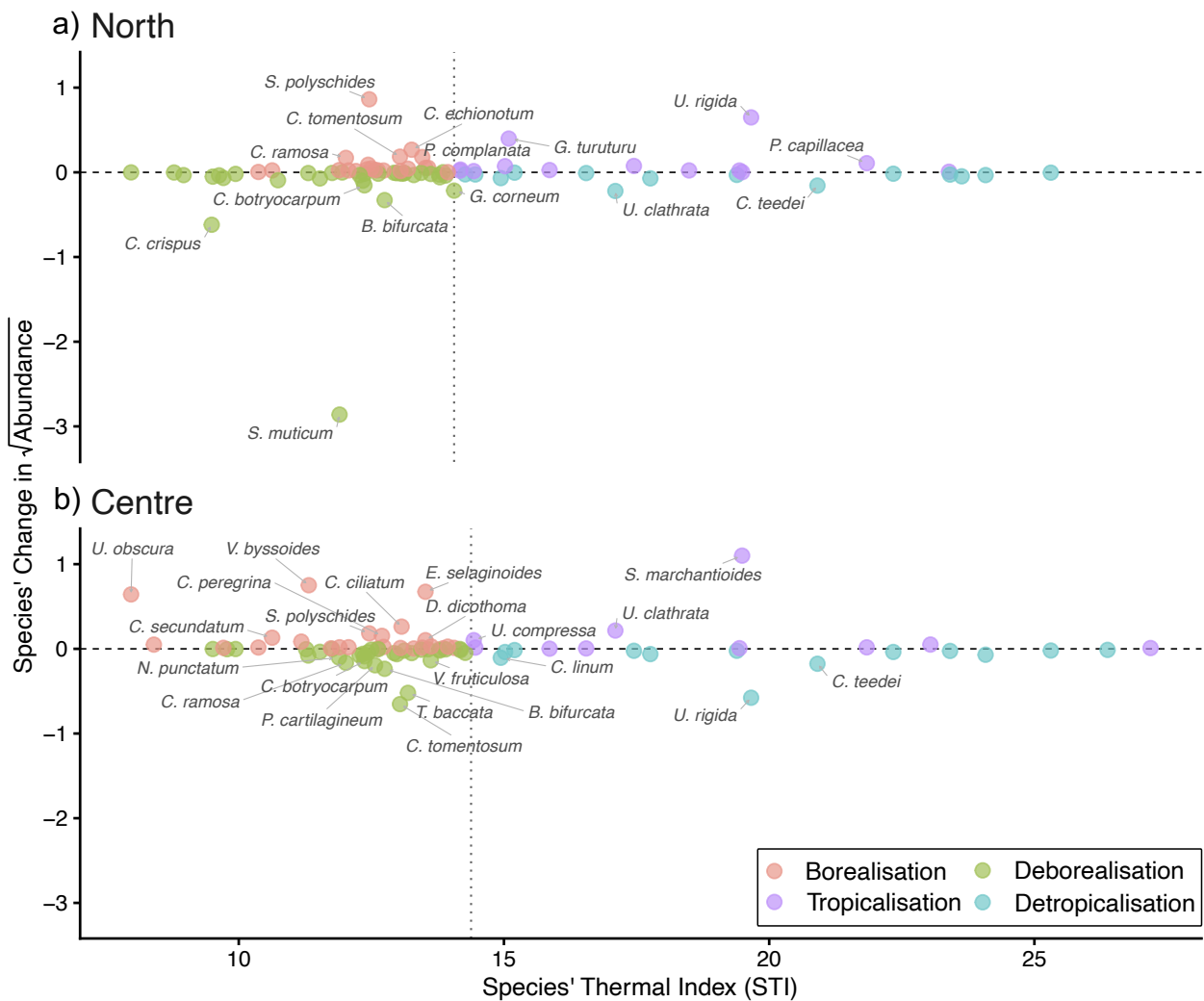


Figure III-5 Processes driving the changes in the Community Temperature Index (CTI) for each region: (a) North and (b) Centre. The plots illustrate the contribution of individual taxa to the observed CTI shifts. The thin horizontal line indicates no change in species abundance, while the thin vertical line represents the mean CTI for each region.

Morpho-functional groups structure. The community composition of rocky intertidal rockpools, expressed as morpho-functional groups, varied significantly between regions and over time (Figure III-6; Table III-4). For the canopy stratum, the conditional model revealed a significant interaction between time and region ($\chi^2 = 9.66$, $p = 0.0019$; Table III-4). In the North region, predicted canopy cover declined from approximately 42% in 2006 to 22% in 2021, whereas no significant temporal change was detected in the Centre region (Figure III-6a, b). The zero-inflation component showed a significant effect of region ($\chi^2 = 34.25$, $p < 0.001$; Table III-4), with a significantly lower probability of complete canopy absence in the Centre compared to the North. For the subcanopy stratum, a significant time $\times$ region interaction was also detected in the conditional model ($\chi^2 = 4.11$, $p = 0.043$; Table III-4). Predicted relative abundance decreased in the Centre region between 2006 and 2021, while no significant temporal change was detected in the North region (Figure III-6). The zero-inflation model showed no significant effects of time or region on the total absence of this stratum ($p > 0.05$; Table III-4). In contrast, turf-forming algae exhibited a significant main effect of time ($\chi^2 = 5.59$, $p = 0.018$; Table III-4), with predicted cover increasing from 2006 to 2021 in both regions. No significant time $\times$ region interaction was detected ($\chi^2 = 0.59$, $p = 0.44$; Table III-4). By 2021, turf-forming algae accounted for the highest predicted cover in the North region (Figure III-6).

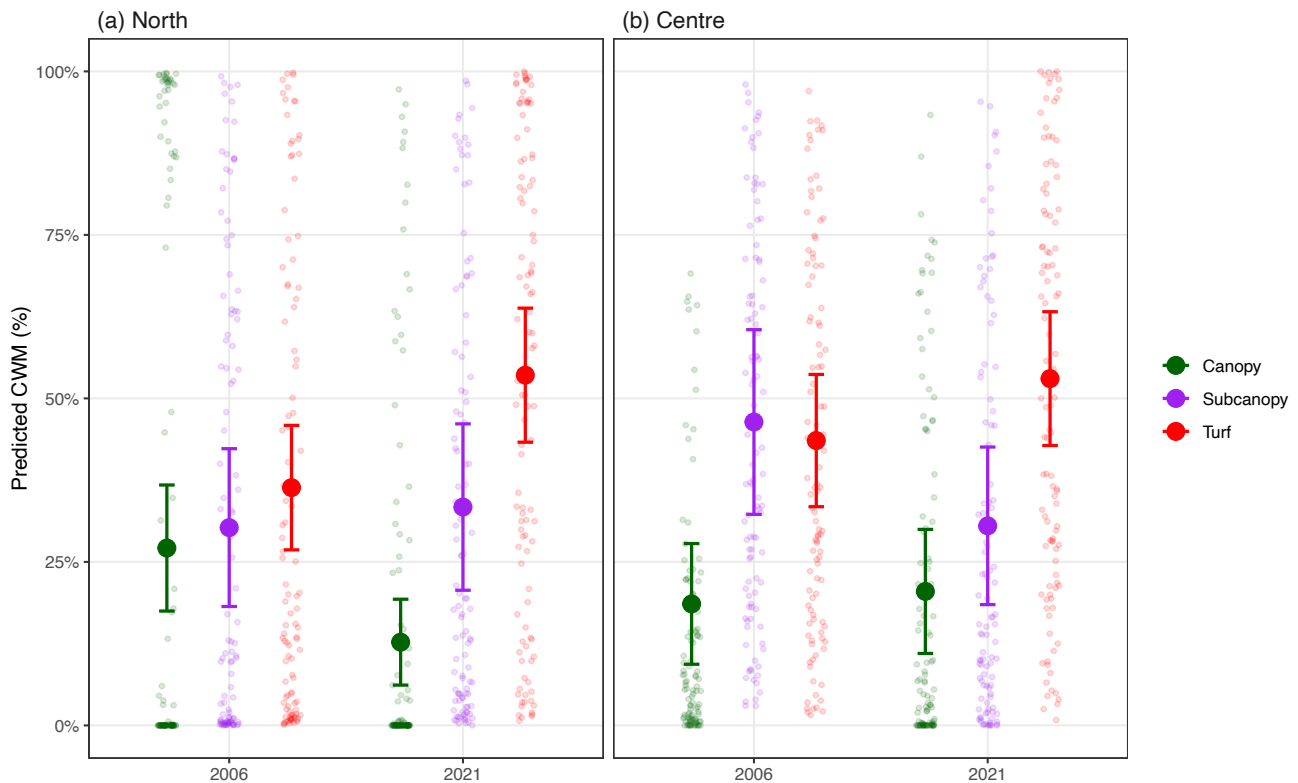


Figure III-6 Predicted community-weighted mean (CWM) cover of macroalgal morphofunctional groups (Canopy, Subcanopy, and Turf) between 2006 and 2021. Values represent model-predicted means $\pm$ 95% CI for North (a) and Centre (b) regions. Background jittered points represent the raw observations.

Table III-4 Analysis of Deviance (Type III Wald X^2 tests) for macroalgal functional groups cover. Results show conditional and zero-inflation (ZI) model components, including main effects and interactions. Random effects variance and dispersion parameters represent the hierarchical structure and precision of the Beta distribution. Trends summarised in the 'Contrasts' section reflect significant spatio-temporal shifts. Significant values are in bold (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

SOV	df	Canopy	Subcanopy	Turf
		Conditional model (X^2)	Conditional model (X^2)	Conditional model (X^2)
Time (T)	1	9.82**	0.29	5.59*
Region (R)	1	11.17***	2.72	1.03
T x R	1	9.66**	4.11*	0.59
Random Effects		Var	Var	Var
Beach (R)		0.198	0.172	< 0.001
Site (R x B)		0.257	0.281	0.342
Pool (R x B x S)		0.193	-	-
Dispersion		2.52	2.17	1.9
Contrasts		Trend	Trend	Trend
Time (T)		21 < 06	-	06 < 21
Region (R)		C < N	-	-
T x R		N: 21 < 06	C: 21 < 06	-
ZI model		X^2	X^2	
Time (T)		0.9	0	-
Region (R)		34.25***	0	-
T x R		1.73	0	-

3.5 Discussion

The results of this study provide compelling evidence that intertidal rockpool macroalgal communities along the Portuguese coast have undergone pronounced spatio-temporal restructuring over the past 15 years. While some descriptors such as species richness decreased consistently across regions (Figure III-2a; Table III-1), indicating a widespread loss of biodiversity, other community descriptors exhibited region-specific dynamics. In the North, the sharp decrease in total standing biomass (Figure III-2b) and Shannon diversity (Figure III-2c) highlights a severe degradation of the ecosystem's standing stock and niche complexity, following the collapse of perennial forests. Conversely, the Centre region (Ericeira) showed relative stability in biomass despite the loss of species richness, suggesting a functional compensation that maintained the overall bulk of the community. Importantly, the Community Temperature Index (CTI) analysis revealed divergent thermal trajectories between regions (Figure III-4), indicating that macroalgal assemblages are reorganizing in response to changes in species' thermal affinities. These findings align with emerging evidence from subtidal systems showing rapid community responses to warming and compositional turnover in marine macroalgae, consistent with the phenomenon of tropicalisation documented along the Portuguese coast and other transitional marine zones (Vergés et al., 2014; Wernberg et al., 2016; de Azevedo et al., 2023).

CTI changes — tropicalisation and allied processes. The significant increase in CTI in the North region, contrasted with the stability in the Centre (Ericeira), highlights the importance of disentangling the multiple processes driving community reorganization (Figure III-4; Table III-3). Our results reveal that deborealisation was the overriding driver in the North, largely dictated by the collapse of cold-affinity canopy-forming species (Figure III-5, 6). This selective loss of cold-water elements without a proportional gain in warm-affinity taxa contrasts with the signal of direct tropicalisation often reported for subtidal systems (de Azevedo et al., 2023). This pattern is consistent with a global trend where mean thermal affinity increases are frequently driven by the disappearance of cold-affinity species (McLean et al., 2021). The vulnerability of these Northern assemblages is linked to the weakening of the NW Iberian upwelling as a thermal buffer (Lemos & Pires, 2004), leading to a significant contraction of functional space as communities reach their physiological tipping points under combined thermal and nutritional (Chabrierie & Arenas, 2024; Martins et al., 2026).

Structural consequences — canopy loss, turf expansion, and ecological implications. The morpho-functional analysis demonstrates that the observed

compositional changes were accompanied by marked structural shifts (Figure III-6; Table III-4). In the North, the decline of complex perennial canopy-forming algae, most notably the collapse of the previously dominant *Sargassum muticum*, was associated with a marked 'canopy-to-turf' transition (Wernberg et al., 2016; Filbee-Dexter & Wernberg, 2018). This profound community reorganization is clearly reflected in the multivariate ordination, where Northern samples from 2021 show a significant separation from the 2006 baseline (Figure 3), indicating a shift toward a simplified alternative state. This simplification is further evidenced by the sharp decrease in total standing biomass, species richness, and Shannon diversity (H') in the North region (Figure III-2; Table III-1), confirming that the loss of structural biomass from habitat-formers was not compensated for by the incoming opportunistic taxa. Space in these pools was reclaimed by ruderal taxa such as *Ulva* spp. and *Ceramium* spp., alongside a significant expansion of opportunistic and invasive species. Consistent with the findings of Martins et al. (2026), we observed a substantial increase in the invasive red algae *Grateloupia turuturu* and the turf-forming *Asparagopsis armata* in the North. Although *G. turuturu* can reach sub-canopy sizes, its opportunistic nature and the turf-forming habit of *A. armata* fail to replace the perennial structural stability of the lost brown algal forests (de Azevedo et al., 2023; Martins et al., 2026). Even the increase of the kelp *Saccorhiza polyschides* in the North indicates a shift toward 'annualisation', as this fast-growing annual lacks the long-term persistence of the species it replaces.

In contrast, the Centre region (Ericeira) maintained a stable canopy cover (Figure III-6b), underpinned by a subtle internal species turnover: the expansion of *Ericaria selaginoides* (STI = 13.52 °C) functionally compensated for the loss of the colder-affinity *Treptacantha baccata* (STI = 13.19 °C). While this shift reflects a move towards a slightly warmer-affinity component, the relative stability of total biomass and Pielou's evenness (J) in the Centre (Figure III-2) suggests a higher degree of structural resilience compared to the North. Interestingly, despite an overall decline in the subcanopy functional group, driven by the reduction of species such as *Codium tomentosum*, *Bifurcaria bifurcata* remains the key structural driver in the Centre, as confirmed by our vector analysis ($r^2 = 0.63$, $p < 0.001$). As a clonal species with a rhizomatous base, *B. bifurcata* is able to keep the occupied substrate, forming dense and persistent prairies that are highly resilient in terms of cover. This biological trait, combined with its non-significant change in the PERMANOVA results, allows it to maintain a baseline of structural stability that contrasts with the more vulnerable recruitment-dependent species in the North.

However, this decline of *B. bifurcata* in our pools relative to its historical abundance contrasts with the persistence reported by (Martins et al., 2026) for Northern open shores, suggesting local-scale vulnerabilities specific to rockpool habitats. The 'thinning' of this sub-canopy layer and the significant expansion of the invasive turf-forming *Symphyclocladia marchantioides* ($r^2 = 0.16$, $p < 0.001$; Figure III-3) indicate an incipient degradation of vertical stratification. This trajectory suggests a process of homogenisation and miniaturisation of habitat structure (Pessarrodona et al., 2021), where the loss of multi-layered perennial forests leads to a flatter, less complex seascape. As documented by Airoidi (1998, 2003), sediment accumulation and space monopolisation by such turf-forming growth habits can significantly impair the recruitment of larger macroalgae, potentially threatening the long-term stability of these structural assemblages. This indicates that structural degradation may follow distinct pathways: the abrupt loss of canopy in the North versus the gradual encroachment of turf-forming invasives in the Centre. Similar trajectories have been reported in nearby subtidal systems (Wernberg et al., 2016; Filbee-Dexter & Wernberg, 2018; Pessarrodona et al., 2021; de Azevedo et al., 2023) and may be further exacerbated by intensifying herbivory in the region (Reis et al., 2024; de Azevedo et al., 2026).

Mechanistic links with climate and environmental gradients. The contrast between Northern 'deborealisation' and Central 'stability' is likely linked to the erosion of local thermal refugia. Historically, the upwelling system provided cold, nutrient-rich waters that buffered coastal assemblages (Lemos & Pires, 2004). However, recent experimental evidence suggests that the combined effect of rising temperatures and nutrient depletion negatively impacts the growth and metabolism of these assemblages (Chabrierie & Arenas, 2024). Furthermore, the isolation of rockpools during low tide exposes these communities to thermal extremes that exceed physiological limits (Araújo et al., 2006). This suggests that intertidal systems may reach critical tipping points faster than subtidal ones, leading to the biotic homogenization observed along the Portuguese latitudinal gradient (Brodie et al., 2014; Burrows et al., 2020).

Limitations and future directions. While this study provides a robust decadal perspective on rockpool reorganisation, certain limitations should be considered. First, our 15-year comparison relies on two discrete sampling periods (2006 and 2021). Although these years capture significant long-term shifts, they do not account for inter-annual variability or the potential influence of extreme episodic events, such as marine heatwaves, which may have accelerated the observed transitions. Future research incorporating continuous monitoring would be essential to disentangle gradual climate-

driven trends from pulse disturbances. Furthermore, other localised stressors not specifically addressed in this study, such as coastal development, increased sedimentation, and pollution, alongside natural physical disturbances like intensified storm frequency, may have exerted additional pressure on these communities and potentially interacted with thermal stress. Additionally, while CTI and morpho-functional cover provide proxies for community health, they do not directly measure changes in ecosystem functioning. Future studies should integrate faunal surveys and metabolic measurements to assess how the transition from perennial forests to opportunistic turfs and 'annualised' kelp beds, such as the expansion of *Saccorhiza polyschides*, affects energy flow. Crucially, the role of biotic interactions must be addressed; recent evidence suggests that intensified herbivory by fish like *Sarpa salpa* can significantly pressure habitat-forming macroalgae on the Portuguese coast (Reis et al., 2024; de Azevedo et al., 2026). Investigating whether warming and nutrient availability act synergistically with grazing pressure will be vital to refine our mechanistic understanding of the tipping points identified. Finally, expanding surveys with further time points and additional regions will help determine if the 'thinning' of sub-canopy layers in the Centre is a consistent precursor to the abrupt regime shifts observed in the North.

3.6 Conclusion

In conclusion, the 15-year reorganization of Portuguese rockpool communities reveals two distinct trajectories of ecological change along a latitudinal gradient. The North has undergone a rapid 'canopy-to-turf' regime shift and 'annualisation', driven primarily by deborealisation, the collapse of perennial, cold-adapted structural species like *Sargassum muticum*, and the subsequent rise of opportunistic and invasive taxa such as *Grateloupia turuturu* and *Asparagopsis armata*. In contrast, the Centre (Ericeira) has maintained structural resilience through canopy-level species turnover (e.g., the expansion of *Ericaria selaginoides*). Crucial to this stability was the persistence of the clonal species *Bifurcaria bifurcata*, which remained a dominant structural driver despite a generalized decline in other sub-canopy elements. Our findings reinforce the role of intertidal rockpools as early-warning systems for climate-driven shifts. The erosion of thermal refugia, compounded by the weakening of the Iberian upwelling, is pushing these ecosystems toward simplified functional states. The differing pathways of degradation, abrupt canopy loss in the North versus gradual invasive encroachment and vertical 'thinning' in the Centre, highlight that even structurally stable regions are undergoing a process of homogenization and miniaturization. Conservation strategies must account for these regional nuances, as the loss of perennial vertical complexity will likely impair the long-term persistence and biodiversity of macroalgal forests in the Northeast Atlantic, potentially exacerbated by intensifying herbivory pressures.

3.7 References

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Chapter IV.



Rising herbivory pressure of *Sarpa salpa* and
the emerging threat to southern European
marine forests

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4.1 Abstract

Climate change (CC) is driving shifts in marine ecosystems, particularly affecting temperate and subtropical marine forests, which are critical for biodiversity and ecosystem stability. Dominated by canopy-forming seaweeds, these forests are sensitive to CC-induced stressors, such as rising temperatures and tropicalisation, which favour turf-forming algae and increase the abundance of herbivorous fish, threatening ecosystem structure and function. Along the western coast of Portugal in Southern Europe, the herbivorous fish *Sarpa salpa* plays a key role in tropicalisation, potentially exacerbating the decline of cold-water seaweeds, whilst the Iberian upwelling system may offer localised protection through cooler conditions. This study combines stomach content analysis, subtidal surveys, functional trait analyses, fish landing records, and sea surface temperature (SST) data across the North, Centre, and South regions to assess the ecological role of *S. salpa*. Results confirmed a preference for brown algae, including habitat-forming species, with clear regional variation: kelps dominated diets in the North, whilst *Cystoseira* s.l. and turf-forming species prevailed in the Centre and South. Fish landing analyses revealed region-specific dynamics: landings in the North increased with higher SST, those in the Centre were unaffected by temperature, and those in the South were negatively correlated with SST. These findings highlight the potential threat of *S. salpa* to temperate marine forests under CC.

Keywords: Habitat-forming, Herbivory, Herbivorous fish, Tropicalisation, Iberian upwelling

4.2 Introduction

Climate change (CC) is a critical global threat that causes profound transformations in both terrestrial and marine ecosystems (Alsuwaiyan et al., 2021; McLean et al., 2021; Osland et al., 2021; Bañón et al., 2024). Rising global temperatures are leading to changes in species interactions and shifts in distributions, threatening biodiversity and destabilising marine ecosystems worldwide (Wernberg et al., 2012; García-Molinos et al., 2016; Blowes et al., 2019; Burrows et al., 2020). Over the past 40 years, sea surface temperature (SST) has risen at an alarming rate of 0.88°C per decade, intensifying multiple climate-driven stressors, including physiological thermal stress, shifts in species distributions, increased frequency of marine heatwaves, ocean acidification and habitat loss (IPCC, 2023). Seawater temperature is a key driver of physiological performance in marine ectotherms, which are typically adapted to narrow thermal optima and occupy restricted thermal niches along latitudinal gradients (Blowes et al., 2019; Burrows et al., 2020). As a result, warming oceans are driving widespread ecological changes across marine ecosystems (Wernberg et al., 2016). These changes impact a wide range of ecosystems and habitats, including the marine forests of temperate and subtropical zones, which are considered particularly vulnerable to CC-related drivers (Wernberg et al., 2016; Assis et al., 2017; de Azevedo et al., 2023).

Marine forests are vital habitats dominated by large, habitat-forming brown seaweeds, such as kelps (order Laminariales) and fucoids (order Fucales) (Dayton, 1985; Bruno & Bertness, 2001; Schiel & Foster, 2006; Bolton, 2010; Pessarrodona et al., 2021). These foundational species create complex three-dimensional habitats that support biodiversity, enhance coastal productivity, and play a critical role in the structure and function of reef ecosystems (Filbee-Dexter & Scheibling, 2014; Duarte et al., 2022; Eger et al., 2023; UNEP, 2023). However, as in other marine habitats, multiple CC-related stressors (e.g., warming, altered nutrient regimes, and increased herbivory) directly affect the performance and persistence of habitat-forming seaweeds, often causing population declines (Lamela-Silvarrey et al., 2012; Franco et al., 2015; Pinho et al., 2016; Filbee-Dexter & Wernberg, 2018; Smale et al., 2019). The loss or weakening of these foundational species reduces habitat complexity and resistance to invasion, thereby facilitating tropicalisation—the poleward expansion of warm-water species—which reshapes ecosystem structure and functionality (Vergés et al., 2016; Filbee-Dexter & Wernberg, 2018; Smale et al., 2019; de Azevedo et al., 2023). For example, extreme climatic events, such as heatwaves, storms and hurricanes, negatively impact the distribution, diversity, and abundance of habitat-forming seaweeds (Kroeker et al., 2010; Ceccherini et al., 2017; Smale et al., 2019). Moreover, rising seawater temperature can

directly cause physiological stress in the seaweeds, which alter metabolic rates, growth, and reproductive success of seaweeds, consequently shifting their abundance and distribution patterns (Franco et al., 2018; Piñeiro-Corbeira et al., 2018; Smale et al., 2019).

Rising seawater temperatures also have indirect effects, facilitating the colonization of temperate reefs by tropical and subtropical species, including turf-forming seaweeds and herbivorous fish (Filbee-Dexter & Wernberg, 2018; Sudo et al., 2019). In the case of turf, this morpho-functional group forms simple, three-dimensional structures that outcompete canopy-forming seaweeds, leading to habitats with reduced availability of resources and limited functional diversity (Airoldi, 1998; Arenas et al., 2006; Miller et al., 2009; Matias et al., 2015; Pessarrodona et al., 2021; de Azevedo et al., 2023). In parallel, the herbivorous fish species, with tropical and sub-tropical affinities, are responsible for amplifying the impacts of CC, causing profound changes in the recipient communities (Vergés et al., 2009; Filbee-Dexter & Scheibling, 2014; Barrientos et al., 2022). Salema (*Sarpa salpa*) is the only obligate herbivorous fish reported to date in continental Portugal (Franco et al., 2015). This species, with a marked shoaling behaviour, is an ecologically important macro-grazer in the Mediterranean Sea (Vergés et al., 2009). In Galicia, an Atlantic region located in the north-western corner of Iberian Peninsula, the activity of Salema has recently been reported as having dramatic impact, resulting in a severe decline of the subtidal marine forests (Barrientos et al., 2022). In the neighbouring Portugal, it is also usual to find herbivory marks by this species in dominant seaweeds (Franco et al., 2015; Reis et al., 2024).

The Portuguese coast, located in Southern Europe, represents a critical biogeographical transition zone for marine ecosystems. Along this coastline, the northern region constitutes the southernmost west Atlantic limit for many cold-water seaweeds, such as the kelp *Laminaria hyperborea* and the rockweed *Ascophyllum nodosum* (Franco et al., 2015; de Azevedo et al., 2023; 2025). These species form marine forests, which are crucial for establishing highly productive environments characterised by marked structural complexity and rich associated biodiversity (Arenas et al., 2006; Miller et al., 2009; Matias et al., 2015; Pessarrodona et al., 2021; de Azevedo et al., 2023). The persistence of these cold-water species along the Portuguese coast is strongly influenced by the Iberian upwelling system, a phenomenon characterised by the seasonal influx of nutrient-rich and colder waters to coastal zones (Tuya et al., 2012; Pérez-Matus et al., 2017; Sousa et al., 2020; de Azevedo et al., 2023; Chabrierie & Arenas, 2024). The upwelling intensity follows a clear latitudinal gradient along the

Portuguese coast, being strongest in the northern regions, moderating in the central coast, and weakening toward the southern coast, thereby supporting varying levels of productivity and buffering against rising sea surface temperatures across this transitional region (de Bettignies et al., 2021; Varela et al., 2022). This process not only mitigates the effects of increasing sea surface temperatures but also creates distinct ecological conditions from north to south, supporting the persistence of cold-water species particularly in northern and central Portugal (Tuya et al., 2012; Pérez-Matus et al., 2017; de Azevedo et al., 2023). This ecological value, combined with the biogeographical significance of the Portuguese coast as a transitional zone in Southern Europe, makes the marine forests along this coastline—with particular emphasis on the northern and central regions—priority habitats for studies that can promote their long-term sustainability.

The ongoing tropicalisation of northern Portugal's coastal habitats (de Azevedo et al., 2023), demands a thorough investigation of the ensuing ecological changes, including the effect of subtropical fish species such as Salema (*S. salpa*). This study aims to enhance knowledge of *S. salpa* within the context of tropicalisation, focusing on its role as a key factor threatening marine forests in northern Portugal. Additionally, it seeks to provide evidence of a potential increase in *S. salpa* populations over recent decades in response to rising temperatures. To better understand the potential ecological impact of this herbivore, we adopted a trait-based approach to characterise its feeding preferences. Rather than focusing solely on individual algal species, this framework groups taxa according to shared functional traits, offering a broader understanding of whether *S. salpa* targets specific morpho-functional groups. This perspective provides key insights into how its feeding behaviour may influence the structure, diversity, and functioning of benthic algal communities (Verlaque, 1990; Vergés et al., 2009, 2014b; Pessarrodona et al., 2021).

To achieve these objectives, we analysed the subtidal community composition and *S. salpa* stomach contents across geographic regions with distinct macroalgal assemblages to confirm their dietary preferences and assess whether their feeding behaviour poses a threat to local algal communities. Furthermore, we examined temporal trends in *S. salpa* landings alongside temperature records to infer population dynamics. We hypothesise that *S. salpa* may pose a potential threat to marine forests, although the Iberian upwelling system could provide localised protection by maintaining cooler thermal conditions that may limit its expansion.

4.3 Material and Methods

Study area. The study was conducted along the Portuguese continental coast, considering three regions: North, Centre, and South (Figure IV-1). These regions reflect a marked thermal gradient driven by the coastline's north-south orientation across a biogeographic transition zone (Tuya et al., 2012; Bueno-Pardo et al., 2020; de Azevedo et al., 2023; Franco et al., 2025). The upwelling system in the northern region contributes to cooler waters in summer, while temperatures gradually increase toward the southern region (Bañón et al., 2024; Sousa et al., 2020). This thermal gradient also underpins a biological gradient across these regions, where northern areas harbour more extensive marine forests (Tuya et al., 2012; de Azevedo et al., 2023). A detailed description of the oceanographic features of this transitional system, including regional differences in

upwelling intensity and associated circulation patterns, is provided in the Introduction.

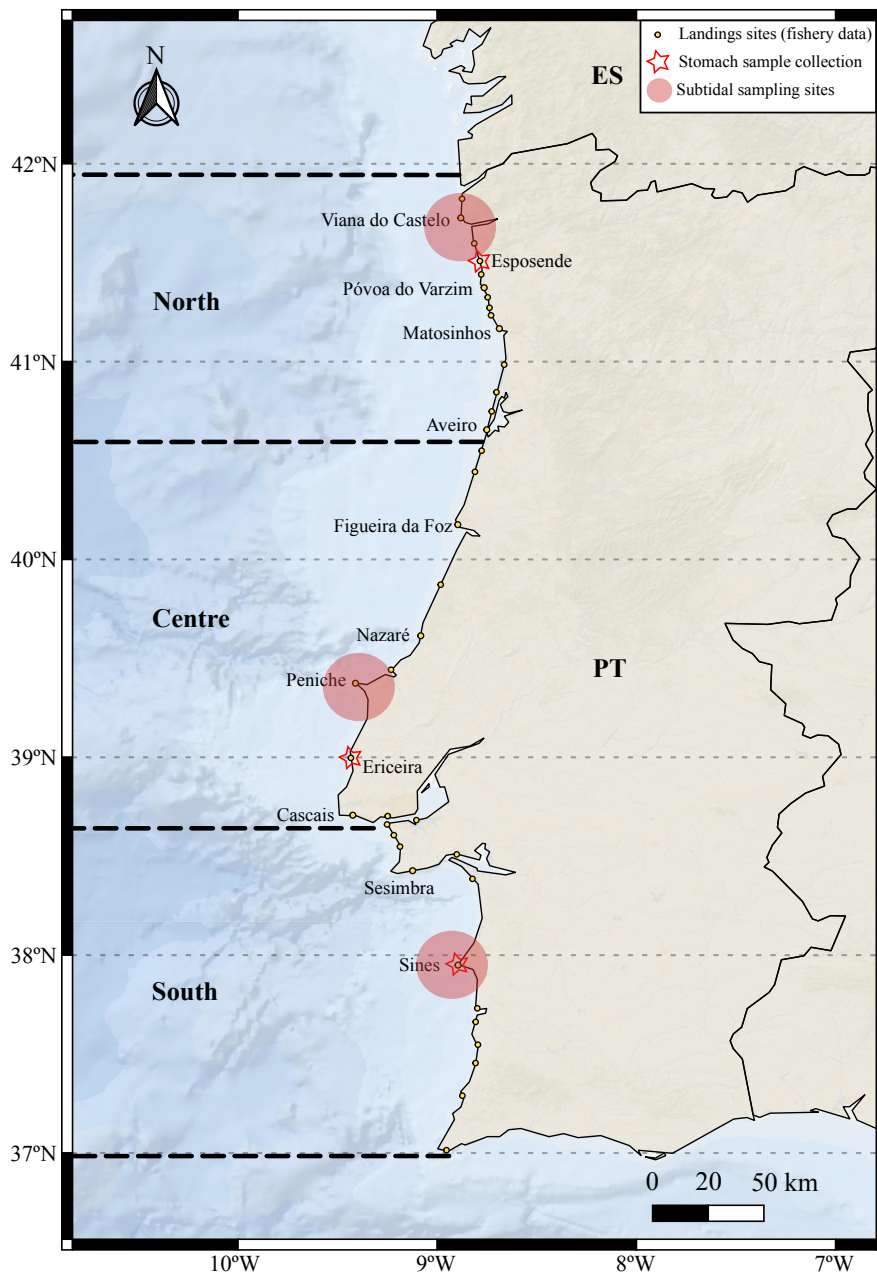


Figure IV-1 Map of continental Portugal showing the locations of the three study regions: North, Centre, and South. Symbols indicate major fishing ports, minor fishing ports, fish sampling sites where specimens were collected for stomach content analysis, and subtidal sampling sites for seaweed surveys.

Subtidal survey on macroalgal communities. Subtidal macroalgal communities were surveyed across the three regions (Figure IV-1), replicating the methodology of de Azevedo et al. (2023). Surveys were performed during six diving campaigns conducted in the summer of 2022. In each region, five rocky reef sites were haphazardly selected, located 1–5 km apart at depths of 6–12 m. At each site, scientific diving sampling techniques were employed to collect samples. Six 50 × 50 cm (0.25 m²) quadrats were haphazardly placed on horizontal rocky substrates within each reef. All seaweeds within the quadrats were carefully scraped and collected into textile bags, which were then labelled and transported to the laboratory in cool boxes. Samples were frozen upon arrival for subsequent analysis. In the laboratory, seaweeds from each sample were identified to the lowest feasible taxonomic level, primarily to species and their weight was determined after excess water was removed through shaking.

Stomach content analysis of captured *Salema porgies*. A total of 66 fish with lengths 33±3.9cm were considered for the analyses of stomach content and diet characterisation. They were caught during the summer of 2021 by artisanal fishers near the harbours of Esposende, Ericeira, and Sines (22 individuals per region), representing the North, Centre, and South regions, respectively (see Figure IV-1 for locations). The fish were carried to the laboratory on ice, where their lengths and weights were measured before being dissected. The respective stomachs were extracted, weighed, and preserved by freezing until analyses. The whole stomach content was examined for all the fishes. The algae found in the stomachs were identified to the lowest taxonomic level possible. *Saccorhiza polyschides* and *Laminaria* spp. were collectively referred to as “kelps,” while their associated epiphytes were grouped as “epiphytes,” since these could not be distinguished in the stomach contents. Following the methodology adapted from Santamaría (2021), each algae species, or group, was separated, wrapped in aluminium foil, and placed in an oven at 60 °C for 48 hours to calculate the consequent biomass as dry weight.

Functional traits of macroalgae. All identified algal taxa from the subtidal surveys and stomach contents were categorized on the following functional categorical traits: pigments, vertical use, texture, and functional form (Table IV-S1). This categorization allowed us to analyse dietary preferences in terms of trait-functional groups rather than taxonomic species, thereby linking algal identity to ecological function (Martins et al., 2026). Pigment type (red, brown, or green) was assigned based on the dominant photosynthetic pigments of each taxon, reflecting differences in light absorption, productivity, and carbon assimilation potential. Vertical use describes the typical vertical

position of each taxon within benthic algal assemblages and was classified into canopy-forming, subcanopy-forming, or turf-forming categories following a three-level scheme adapted from Arenas et al. (2006). In this region, canopy-forming taxa typically exhibit thalli higher than 60–80 cm in length, subcanopy-forming taxa around ~20 cm, and turf-forming taxa below 5 cm, with these values provided as indicative references rather than strict thresholds for classification. Texture was assigned following the functional-form model of Littler and Littler (1980), reflecting thallus mechanical properties (e.g., soft, fleshy–wiry, or rubbery), which influence susceptibility to herbivory and physical disturbance, with softer forms (soft palatable tissue) expected to be more vulnerable and tougher forms (hard structural tissue) more resistant. Functional form represents the dominant morpho-functional group of each taxon (e.g., thick blade, thick branched, sheet, filamentous, or branched), integrating thallus architecture and growth strategy. Trait category assignments were based on published literature (e.g., Martins et al. 2026 and references therein) and field observations and applied consistently across all taxa included in the analyses.

Sea surface temperature (SST) data. Sea surface temperature (SST) data were obtained from the Gulf of Biscay dataset, part of the Coupled Model Intercomparison Project Phase 6 (CMIP6) (Kristiansen et al., 2024). From this dataset, which provides daily potential temperature values at multiple depths, we extracted the mean, maximum, and minimum temperatures at a depth of 5 meters. The data were filtered by date and geographic location to focus on the coastal marine areas adjacent to the studied fisheries ports along mainland Portugal. For each port, values were sourced from the nearest oceanic grid cells (horizontal resolution of $1/12^\circ \times 1/12^\circ$, approximately 8–9 km) representing the immediate offshore waters, ensuring the measurements reflected local thermal conditions relevant to *Sarpa salpa* habitat. Further details on R packages and functions used for downloading, filtering, and extraction, are provided in the Attachments. Chapter IV- Supplementary Material.

Fisheries landing official data. To examine long-term temporal patterns in the presence of *Sarpa salpa* along the Portuguese coast, we used fishery landings as a proxy for fish abundance (Pauly et al., 2013). Official monthly records on *S. salpa* landings from 2002 to 2022 were obtained from DOCAPESCA (n = 23,782 landings). The dataset includes the total biomass (kg) landed per vessel per month (for vessels ≤ 10 m in length) operating in small-scale coastal fisheries. Port-level data were assigned to one of three coastal regions studied (Figure 1). The data from small vessels are especially pertinent, given that their operational bathymetry (depths < 50 m) aligns with

that of seaweed forest habitats, the key environments where *S. salpa* grazing occurs. The monthly resolution of the data enabled the analysis of 1) seasonal patterns in landings potentially linked to temperature-driven changes in fish availability or activity, and 2) the spatial–temporal overlap between fish landings and marine regions, particularly in relation to the presence of vulnerable benthic habitats.

Data analyses. All statistical analyses were conducted in R (v. 4.3.1; R Core Team, 2025), and all packages used are listed and properly referenced in the Supplementary Material. Analyses were structured to address two main objectives: (1) to assess spatial variation in the taxonomic and functional composition of macroalgal communities and *S. salpa* diets across regions, in order to evaluate potential links between local prey availability and consumption patterns; and (2) to explore long-term trends in the distribution of *S. salpa* along the Portuguese coast, using fishery landings data as a proxy for population abundance (Pauly et al., 2013), and relating these patterns to SST, a pivotal environmental driver. Together, these analyses aimed to understand spatial and temporal patterns in herbivore distribution, feeding behaviour, and resource availability, in order to evaluate potential impacts on the structure and resilience of marine forests.

Community composition: subtidal availability and diet structure. To assess spatial variation in macroalgal communities and diet composition, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) using the *adonis* function from the *vegan* package (Anderson, 2017; Oksanen et al., 2020). The analysis was applied to both field survey data (subtidal availability) and stomach content analysis (diet structure) as a function of the factor region (1-way PERMANOVA, fixed factor). All tests were based on Bray-Curtis similarity matrices derived from square root-transformed relative biomass data. Multivariate patterns were visualized using Principal Coordinates Analysis (PCoA).

Dietary diversity and niche breadth. To estimate the trophic niche breadth of *Salema porgy* in each region, the Shannon–Wiener diversity index (H') and Pielou's evenness index (J) were calculated based on the dry biomass of prey items, (i.e. field collected seaweeds; Marshall & Elliott, 1997; Clarke et al., 2014). These indices respectively reflect the diversity and distributional equity of dietary items. Differences in diversity and evenness across regions were tested using one-way ANOVA, followed by Tukey HSD for post hoc comparisons. The assumptions of ANOVA were verified graphically, following Zuur et al. (2010). To quantify dietary overlap among regions, we calculated the Morisita–Horn similarity index based on prey dry biomass. This index provides an abundance-weighted measure of similarity that is robust to differences in sample size

and prey availability, and ranges from 0 (no overlap) to 1 (complete overlap) (Morisita, 1959; Horn, 1966).

Prey importance and feeding strategy. The relative importance of each algal taxon in the diet was evaluated using two complementary metrics: frequency of occurrence (I_o , %) and gravimetric contribution (I_G , %). These metrics were calculated both across the full dataset and separately by region. Feeding strategy was assessed using Costello plots (Costello, 1990), where the position of each taxon within the I_G vs. I_o space identifies it as a dominant (consistently consumed), specialist (rarely consumed but in large amounts), or generalist (frequently consumed in small quantities) dietary component.

Community-weighted means (CWM) of functional traits. Functional trait composition of macroalgal communities and *Sarpa salpa* diets was analysed using community-weighted means (CWM), which is the abundance weighted value for each trait and allows examination of which are the dominant traits in the community, for our study in the diet (de Bello et al., 2021).

The CWM for a given trait T was calculated as:

$$CWM_T = \sum_{i=1}^n p_i \cdot T_i$$

Where p_i is the proportion of biomass of species i in the community (expressed as the relative biomass), T_i is the trait value of species i , n is the total number of species in the community. Given that traits in this study were categorical, the resulting CWM values represent the relative contribution of each trait category to the community biomass and are expressed as percentages.

Detailed methods on CWM calculation and R packages used can be found in Attachments. Chapter IV- Supplementary Methods.

Modelling fisheries landings and SST. The response variable in this analysis was the catch per unit effort (CPUE), calculated as the total kilograms of *Sarpa salpa* landed divided by the number of boats. When necessary, the CPUE values were log-transformed to meet model assumptions. The potential effect of boat size was discarded after analysing with a GAM model (Figure IV-S1). Temporal trends in CPUE were analysed using autoregressive integrated moving average models (ARIMA). To evaluate the influence of SST on CPUE, ARIMAX models (ARIMA with exogenous covariates) were applied, including temperature as a predictor. Separate models were fitted for each

region to account for spatial differences in temporal dynamics. Series stationarity and seasonal structure were evaluated using standard time-series diagnostics (including differencing where required). Model selection and diagnostics followed standard procedures based on residual analysis and information criteria (AIC). Further details on model specifications, diagnostics, and selection criteria are provided in the Supplementary Material.

4.4 Results

Subtidal macroalgal availability. A total of 80 taxa were identified across all subtidal samples (complete list of recorded seaweeds available in Table IV-S2). Significant regional differences in subtidal community composition were observed when considering the entire dataset (PERMANOVA, $P_{\text{perm}} < 0.05$, Table IV-1; ordination, $R^2 = 42.9\%$, Figure IV-2a). Pairwise comparisons were all significant, indicating that each region differed from the others in terms of community composition and structure (Table IV-1). The taxa that most significantly contributed to regional differentiation were *Saccorhiza polyschides*, associated to the North, *Laminaria ochroleuca* and *Metacallophyllis laciniata* to the Centre, *Sphaerococcus coronopifolius* and *Asparagopsis armata* to the South (Figure IV-2a). The South region displayed the lowest dispersion, reflecting high internal homogeneity and a distinct community (Figure IV-2a). In contrast, the Centre exhibited the highest dispersion, indicating greater heterogeneity and substantial overlap with both the North and South (Figure IV-2a). The North showed moderate homogeneity and closer affinity to the Centre than to the South, confirming a clear regional structure in community composition (Figure IV-2a).

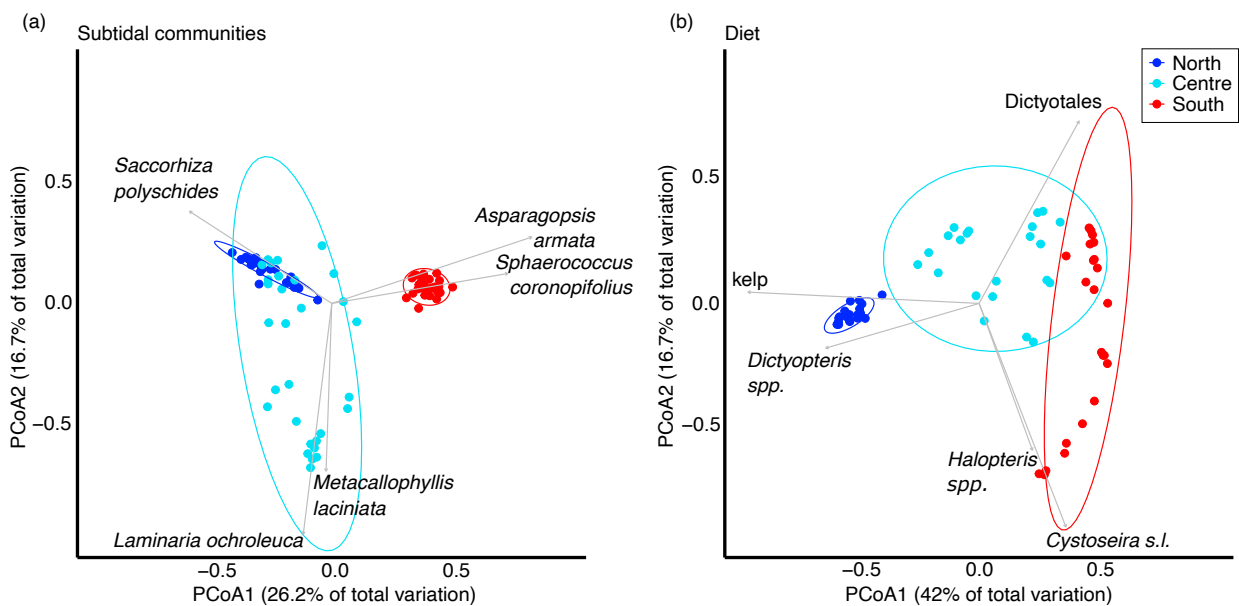


Figure IV-2 Principal Coordinates Analysis (PCoA) with confidence ellipses illustrating regional groupings of (a) subtidal seaweed community data (ordination, $R^2 = 42.9\%$) and (b) stomach content data (diet) (ordination, $R^2 = 58.7\%$).

Table IV-1 Permutational multivariate analysis of variance (PERMANOVA) and pairwise comparisons examining the effects of region on whole subtidal seaweeds community and *S. salpa* diet. Significant effects are reported in bold. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Subtidal	df	PERMANOVA			North x Centre			North x South			Centre x South		
		SS	R2	F	SS	R2	F	SS	R2	F	SS	R2	F
Model	2	10	0.3	17.13***	2.61	0.1	7.76***	6.39	0.3	22.77***	6.05	0.28	23.11***
Residual	87	25.5	0.7		19.5	0.9		16.3	0.7		15.2	0.72	
Total	89	35.5	1		22.1	1		22.7	1		21.2	1	
Diet	df												
		SS	R2	F	SS	R2	F	SS	R2	F	SS	R2	F
Model	2	10.6	0.5	31.06***	3.82	0.3	16.05***	6.05	0.4	33.166***	2.16	0.15	7.8***
Residual	64	10.9	0.5		10	0.7		7.85	0.6		11.9	0.85	
Total	66	21.5	1		13.8	1		13.9	1		14.1	1	

Diet composition and niche breadth of *S. salpa*. Diet composition varied significantly among regions (PERMANOVA, $P_{\text{perm}} < 0.05$; Table IV-1). The North region was characterised by the lowest dispersion and highest internal similarity, with a diet dominated by kelps species and *Dictyopteris spp.* (ordination, $R^2 = 58.7\%$; Figure IV-2b; $H' \sim 0.4$; $J \sim 0.3$, Table 2; Figure IV-3a, b). The Centre region showed the highest dispersion of stomach contents (Figure IV-2b), corresponding to its significantly greater diversity and evenness ($H' = 1.2 \pm 0.4$; $J = 0.6 \pm 0.2$, Table IV-2; Figure IV-3a, b). In contrast, the North and South regions showed no significant difference in terms of diversity and shared the lowest diversity and evenness values ($H' \sim 0.4$; $J \sim 0.3$, Table IV-2; Figure IV-3a, b), consistent with their low PCoA dispersion (Figure IV-2b). Accordingly, dietary overlap estimated using the Morisita–Horn index was moderate between Centre and South (0.60), lower between North and Centre (0.38), and nearly absent between North and South (0.003), confirming strong regional structuring in diet composition. In general, dietary evenness was low ($J < 0.6$), indicating a strong feeding preference for a few dominant taxa. This was particularly pronounced in the North, driven by the consistent occurrence and high gravimetric contribution of kelps ($I_o > 100\%$, $I_G > 85\%$) across all individuals. Similarly, low evenness characterised the South, where the diet was dominated by Dictyotales ($I_o > 85\%$; $I_G > 70\%$) and *Cystoseira s.l.* ($I_o > 65\%$, $I_G > 50\%$; Table S3). Epiphytes showed strong regional variation in their dietary importance, with the highest contribution in the Centre ($I_G \sim 27\%$, $I_o = 100\%$), a moderate contribution in the North ($I_G \sim 4\%$, $I_o \sim 72\%$), and a minimal contribution in the South ($I_G \sim 0.5\%$, $I_o \sim 8\%$; Table S3). Notably, while Dictyotales showed the highest I_o , kelps had the highest

I_G (71.1%; Table IV-S3), indicating a tendency toward dietary specialization and preference when present, as illustrated by I_G vs. I_O relationship (Figure IV-4).

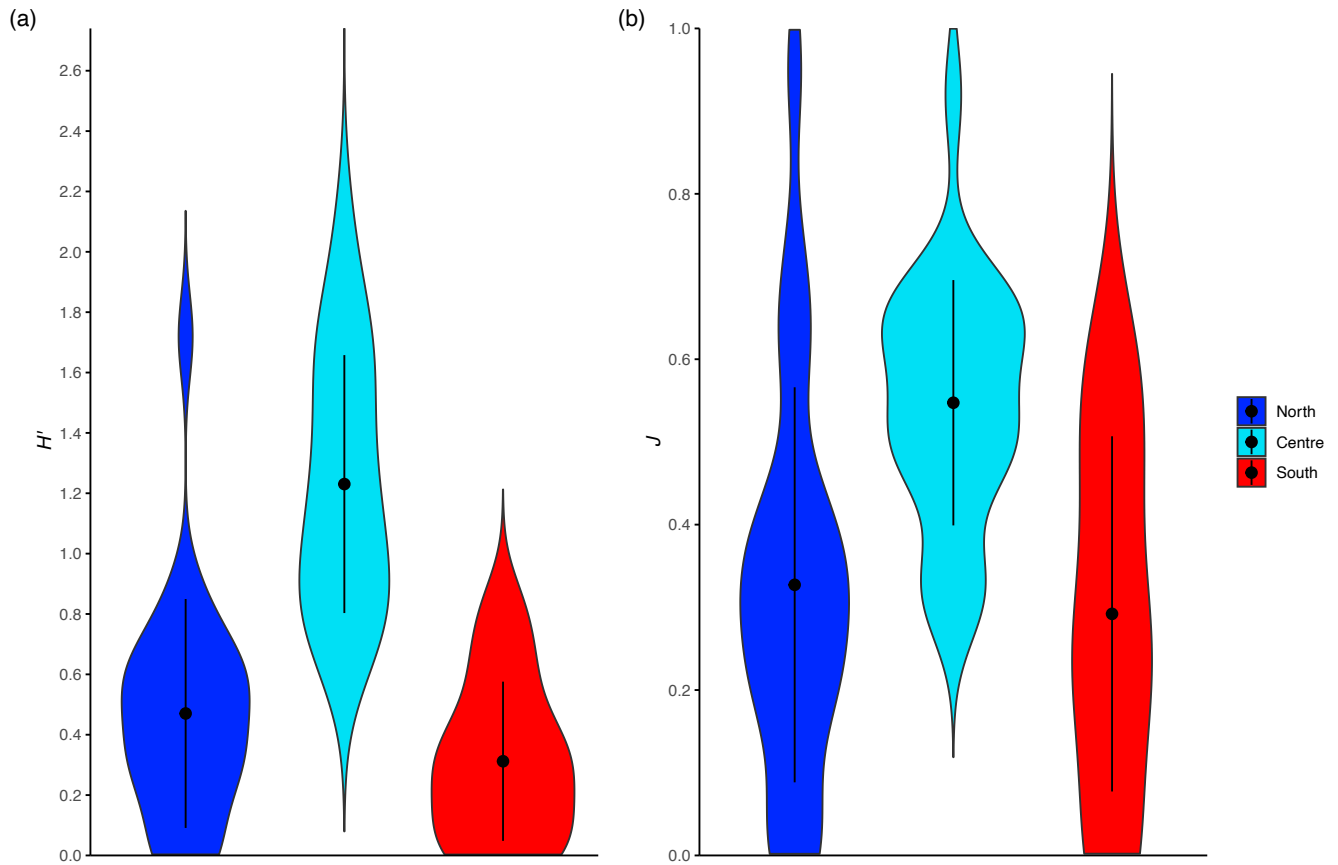


Figure IV-3 Niche breadth, mean $\pm$ S.D. values for diet diversity for (a) Shannon-Winner diversity (H') and (b) Pielou's evenness (J).

Table IV-2 ANOVA examining Shannon and evenness and species richness in stomach content (Niche breadth) from different regions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

SOV	df	Shannon H'		J Pielou evenness	
		MS	F	MS	F
Region	2	5.366	40.96***	0.4258	10.19***
Residuals	64	0.131		0.0418	
Levene's	2	$F = 2.429$ $p > 0.05$		$F = 1.603$ $p > 0.05$	
<i>TukeyHSD</i>		Centre > North = South		Centre > North = South	

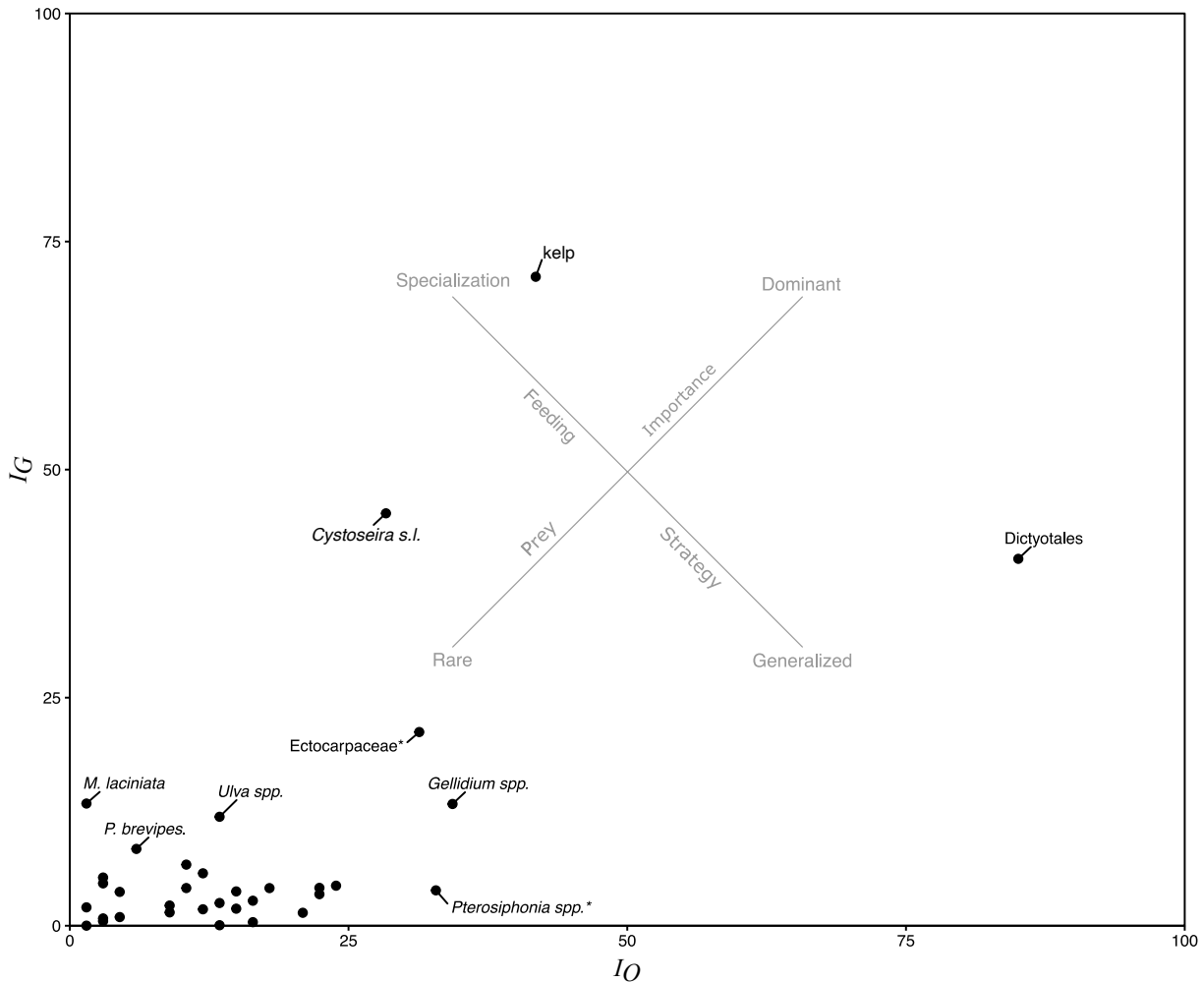


Figure IV-4 Relationship between I_G = gravimetric contribution (% dry weight) and I_O = frequency of occurrence (%) of prey items in the diet of *S. salpa*. The plot follows the graphical method of Costello (1990), where prey positioning reflects their dietary importance and the species' feeding behaviour. Epiphytes are indicated by (*).

Trait-based diet-environment comparisons. Pronounced disparities between dietary preferences and environmental food availability (i.e., subtidal seaweed communities) were found across regions in *S. salpa* when analysed in terms of functional traits (Figure IV-5). Brown seaweeds dominated the stomach contents across all regions, comprising nearly 100% of the diet in both the North and South despite their relatively low environmental availability (~38% and ~19%, respectively). In contrast, a closer match between diet composition and environmental availability was observed in the Centre (~68% in the diet vs ~78% in the environment). Regional patterns in the functional composition of the diet emerged for other traits: canopy species prevailed in the North (>85%), contrasting with subcanopy dominated the diet in the South (>62%) and Centre (>41%). Likewise, thick-blade dominated the diet in the North (>85%), while sheet-like forms dominated in the South (>62%), and texture preferences shifted from predominantly rubbery algae in the North (>85%) to soft algae in the South (>65%). Despite these regional differences, overarching trends showed consistent preferences for canopy (>45%) and subcanopy (>40%) species, thick-blade and sheet forms (>30%),

and rubbery or soft textures (>34%). However, these dietary preferences did not match the regional availability of algal traits in the subtidal seaweed communities (Figure IV-5).

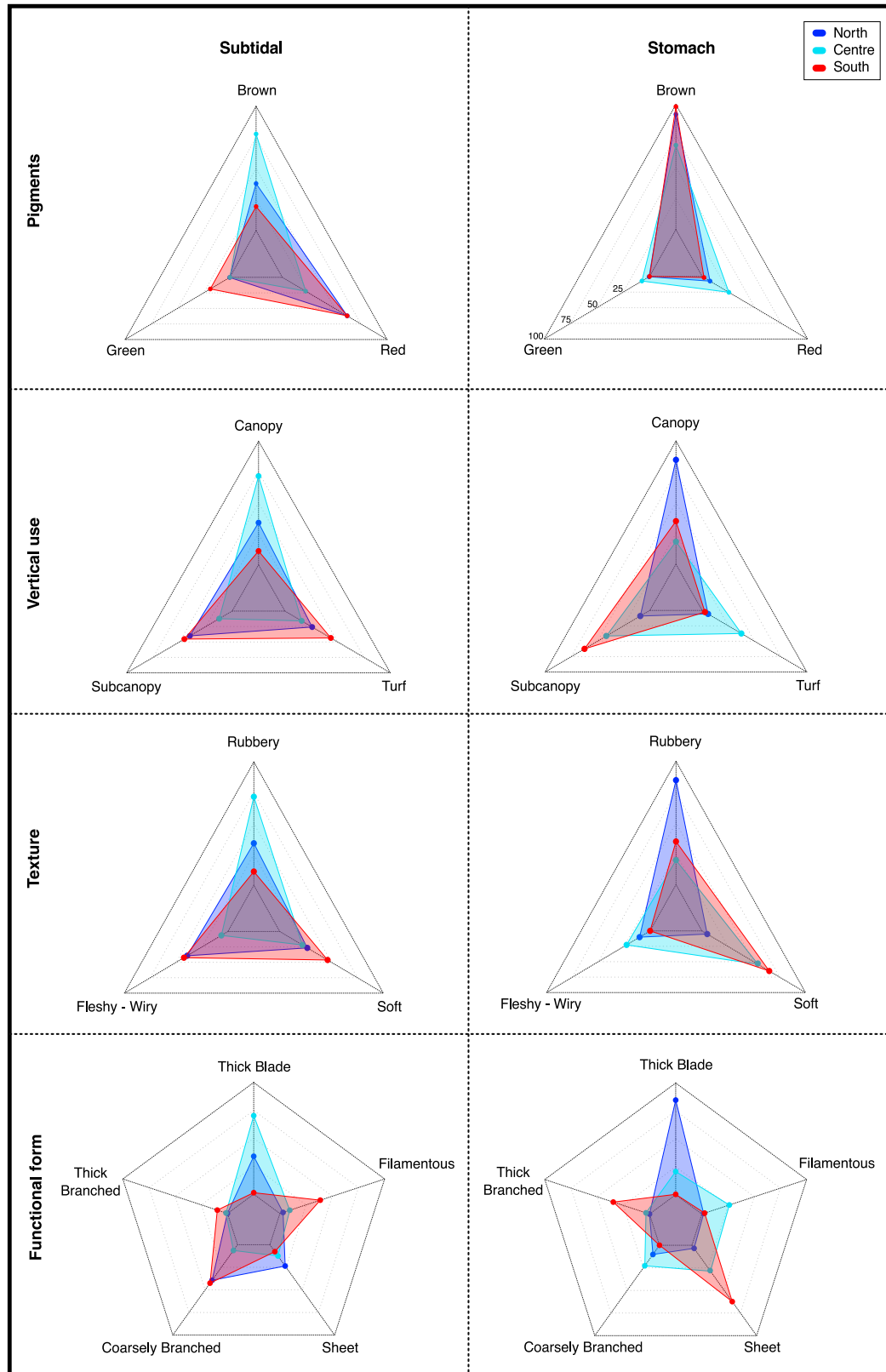


Figure IV-5 Radar chart of community weighted mean (CWM) for traits: Pigments, Vertical Use, Texture and Functional form.

Trends from the time and temperature on fish landings. *S. salpa* landings across the studied regions showed no consistent evidence of a strong unidirectional trend over the study period (Figure IV-6). Instead, dynamics varied regionally. In the North, landings were positively associated with temperature (coefficient = 18.24, $p < 0.01$; Table IV-3; Figure IV-7a) and displayed a complex seasonal autoregressive-moving average structure (ARIMA; Table IV-3; Figure IV-6a), indicating an SST warming trend paired with increased catches in this region. In the Centre, landings showed high autocorrelation and a pronounced seasonal cycle with limited short-term persistence, for which temperature did not significantly improve the model (ARIMA; Table IV-3; Figure IV-6b, IV-7b). In the South, landings exhibited a negative correlation with temperature (coefficient = -7.42, $p < 0.05$) and positive short-term autocorrelation (ARIMA; Table IV-3; Figure IV-6c, IV-7c). Overall, these results highlight region-specific temporal dynamics in *S. salpa* landings, with no overarching secular trend detected.

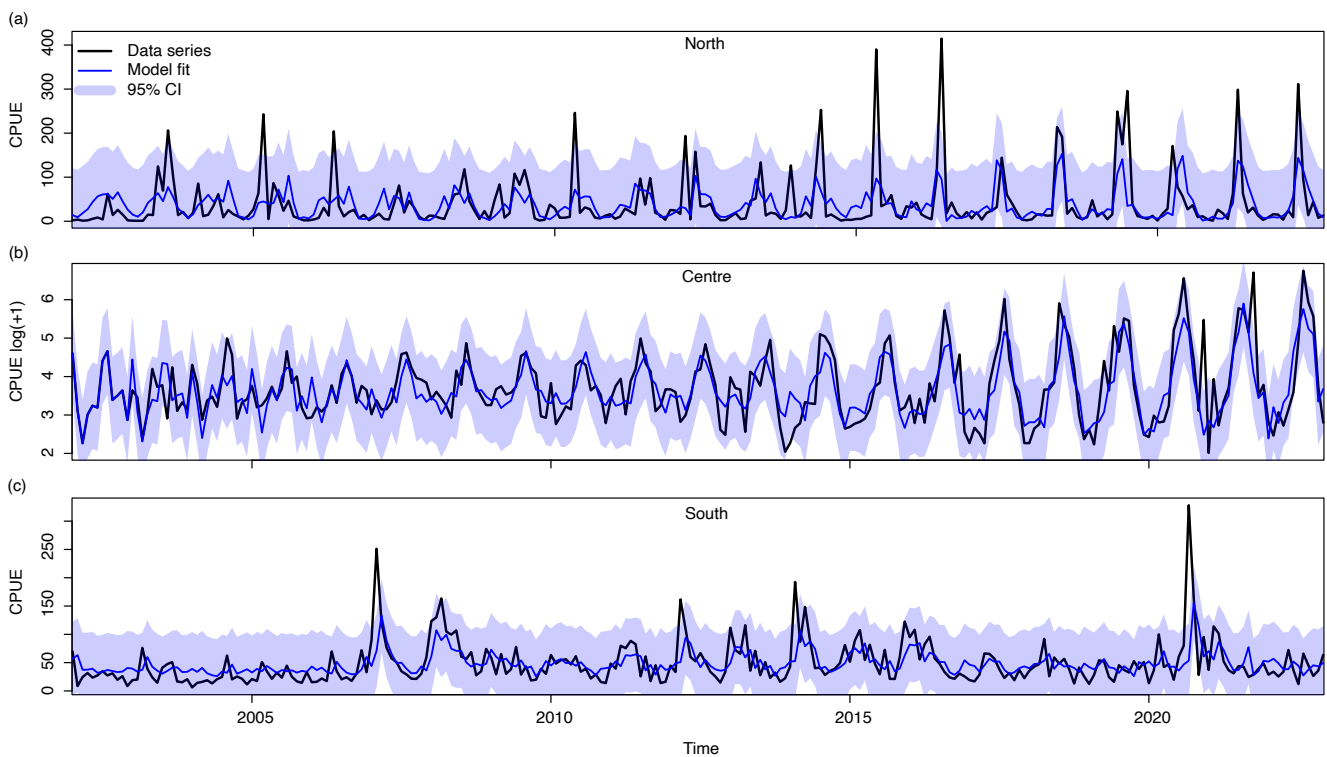


Figure IV-6 Observed and fitted values of CPUE time series of *Sarpa salpa* landings in the (a) North, (b) Centre, and (c) South regions of mainland Portugal. Black lines represent observed values and blue lines the fitted values from ARIMA/ARIMAX models.

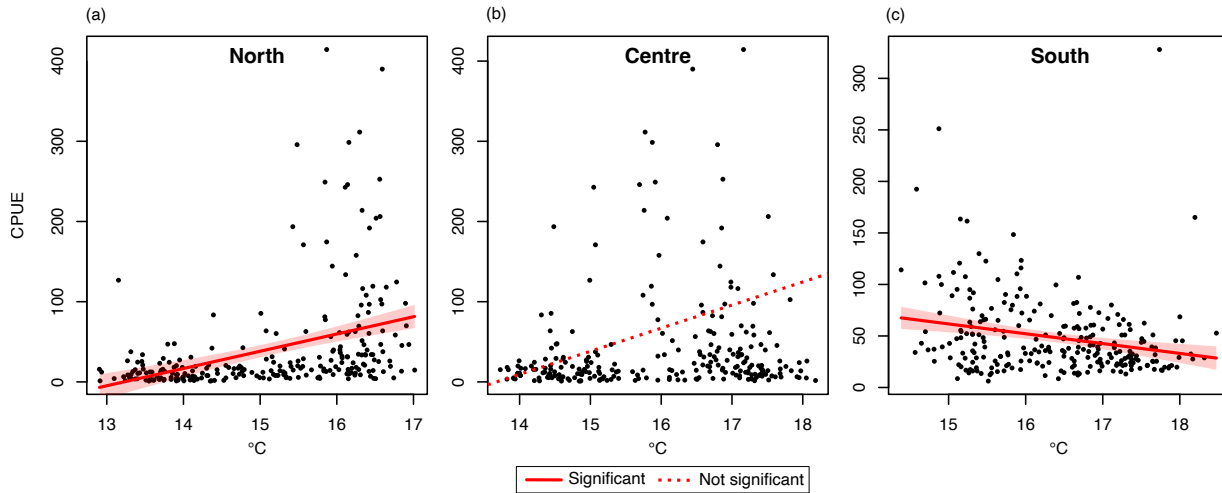


Figure IV-7 Relationship between SST (°C) and CPUE of *Sarpa salpa* landings in (a) the North, (b) the Centre, and (c) the South regions of mainland Portugal. Each point represents monthly CPUE values. Red solid line indicates the fitted relationship between SST and CPUE (significant at $p < 0.05$), dashed line represents non-significant fits, and shaded areas correspond to the 95% confidence intervals of the fitted models.

Table IV-3 ARIMA of CPUE and SST (°C) Model parameters are presented as $(p, d, q) \times (P, D, Q)$, where p = non-seasonal autoregressive order, d = non-seasonal differencing, q = non-seasonal moving average order, P = seasonal autoregressive order, D = seasonal differencing, Q = seasonal moving average order, and $[s]$ = length of the seasonal period, being 12 monthly. Model parameters are reported as AR (autoregressive), MA (moving average), and their seasonal counterparts (SAR, SMA). Subscripts (e.g., AR1, MA1, SMA2) indicate the order of the term, with seasonal terms referring to lags equal to the seasonal period (12 months).

Region	ARIMA (p,d,q) (P,D,Q)	SST Coeff $\pm$ SE	Parameters $\pm$ SE
North	(0,0,1) (1,0,2) [12]	18.24 $\pm$ 5.66	ma1= -0.0368 $\pm$ 0.06 sar1= 0.9281 $\pm$ 0.04 sma1= -0.8516 $\pm$ 0.08 sma2= 0.1176 $\pm$ 0.07
Centre	(1,0,0) (0,1,1) [12]	NA	sma1= -0.6438 $\pm$ 0.06 ar1= 0.0991 $\pm$ 0.07
South	(1,1,1) (0,0,1) [12]	-7.32 $\pm$ 3.37	ar1= 0.5441 $\pm$ 0.13 ma1= -0.1459 $\pm$ 0.15 sma1= 0.125 $\pm$ 0.06

4.5 Discussion

Understanding the impacts of CC on ecosystems is complex, as environmental drivers and ecological processes interact across spatial and temporal scales, shaping community structure and function (Menge, 2000; IPCC, 2023). In this context, the present study provides an integrated view of *Sarpa salpa* feeding ecology along the Portuguese coast, combining subtidal algal availability, diet composition, and long-term fisheries and temperature data. By linking these dimensions, we aim to understand how spatial variability, functional traits, and thermal patterns shape the trophic role and potential ecological impacts of this herbivorous fish on temperate marine forests.

Algae availability and dietary preferences along the latitudinal gradient. Subtidal macroalgal assemblages exhibited pronounced spatial heterogeneity across regions, following a clear latitudinal gradient. The southern region displayed a more tropical character, dominated by subcanopy-forming algae and red turfs, with scattered stands of *Cystoseira s.l.* forests. In contrast, the cooler waters of the North and Centre, under the stronger influence of the Iberian upwelling system, supported extensive kelp forests (*Laminaria hyperborea*, *L. ochroleuca* and *Saccorhiza polyschides*; (Tuya et al., 2012; de Bettignies et al., 2021; Varela et al., 2022; de Azevedo et al., 2023). The Centre, acting as a transitional zone, shared taxa from both extremes, resulting in higher diversity and the co-occurrence of kelps and *Cystoseira s.l.* forests. Members of Dictyotales, particularly *Dictyota dichotoma*, were consistently available for grazing across all regions. This pattern corroborates the existence of a well-defined physical and biological gradient along the Portuguese coast, as previously reported (Tuya et al., 2012; de Azevedo et al., 2023) and provides an essential ecological baseline for interpreting regional differences in the feeding ecology of *Sarpa salpa*.

Although subtidal surveys in the Centre region showed considerable canopy presence, forest occurrences are spatially fragmented, with concentrated stands interspersed with areas lacking forests. In contrast, the North region exhibits greater continuity of kelp forests. Such spatial patterns may partly explain regional differences in *S. salpa* diets, as the spatial configuration of forests can influence accessibility and foraging decisions (Tuya et al., 2012; de Azevedo et al., 2023). Overall, the stomach content analysis revealed a clear preference for brown pigmented algae across all regions, despite the broader availability of other algal groups in subtidal communities. Among brown taxa, the diet was consistently dominated by Dictyotales, kelps (i.e. *Laminaria* spp. and *Saccorhiza polyschides*), and *Cystoseira s.l.* However, subtidal availability serves only as a proxy, as *S. salpa* is a highly mobile species capable of

foraging beyond the sampled reefs, likely contributing to the observed inter-individual variability. The low dietary evenness ($J \approx 0.3$; Figure IV-3) and niche breadth values indicate a relatively specialised feeding niche for *S. salpa* in the regions and period sampled. However, given the absence of seasonal coverage and the moderate sample size, these results should be interpreted as evidence of specialised feeding under the surveyed conditions rather than as a definitive description of the species' full trophic niche. In the Centre region, epiphytes were present in nearly all samples, increasing the apparent dietary diversity; however, their frequent occurrence likely reflects incidental ingestion (Verlaque, 1990; Stergiou & Karpouzi, 2002).

This specialisation is, however, tactically flexible. The population-level diet reveals a dual strategy: a generalist component, characterised by the frequent but low-biomass consumption of abundant and accessible taxa like Dictyotales, and a specialist component, represented by the selective, high-biomass consumption of structurally dominant species such as kelps and *Cystoseira* s.l. Regional differences illustrate this pattern: in the North, diets were dominated almost exclusively by kelps, indicating a strong preference and feeding specialization when these large species were available. Although kelps—particularly *S. polyschides*—were also the dominant species in local assemblages (Barrientos et al., 2022), their disproportionate contribution to the diet highlights an active preference beyond mere availability. In the Centre and South, *Cystoseira* s.l. replaced kelps as the main structural prey, while Dictyotales remained a recurrent but lower-biomass food source across all regions—especially in the South, where kelps are rare, and these algae dominate the subtidal. This regional shift in the balance between specialist and generalist components reinforces that *S. salpa*'s foraging behaviour adapts to local resource availability while remaining constrained by a clear preference for brown macroalgae. Overall, this pattern suggests that *S. salpa* can alternate between generalist and specialist strategies depending on prey availability and environmental context, while remaining a highly selective herbivore capable of influencing algal community composition (Verlaque, 1990; Vergés et al., 2009). Consistent with these patterns, trophic overlap estimated using the Morisita–Horn index showed that diet similarity was highest between Centre and South, lower between North and Centre, and almost absent between North and South. This indicates that regional variation in resource use reflects genuine shifts in dietary composition rather than stochastic differences among samples.

The observed selectivity appears to be driven less by taxonomic identity than by functional traits shared among preferred brown algae. When analysed from a trait-based perspective, the preferentially consumed kelps and *Cystoseira* s.l. share a suite of

functional attributes—brown pigmentation, canopy-forming habit, rubbery textures, and complex morphologies. This trait-based selectivity reveals a hierarchical feeding pattern: while *S. salpa* has a generalist diet across the brown algal order Dictyotales, its overall feeding is still confined by a strong preference for brown algae over those with different pigmentation. Such patterns are consistent with previous reports describing an ontogenetic dietary shift in *S. salpa*, where adults progressively specialize on brown algae, particularly Fucales and Dictyotales (Verlaque, 1990). This shift likely reflects a trade-off between nutritional and ecological factors: protein-rich red algae may sustain rapid juvenile growth, whereas energy-dense brown algae are more suitable for maintaining larger adult body sizes (Christensen, 1978; Holdt & Kraan, 2011; Pereira, 2011). Increased jaw efficiency and vertical mobility with growth may also expand the range of accessible resources (Christensen, 1978; Verlaque, 1990).

Ecological Consequences of the Herbivory Impacts. This selective foraging behaviour can have significant ecological consequences. Intense herbivory by *S. salpa* may profoundly reshape community structure in marine forests. In areas with reduced vegetation cover, shoals have been observed to overgraze preferred taxa down to their holdfasts (Barrientos et al., 2022), promoting the dominance of encrusting and filamentous forms while leaving only avoided taxa, such as calcified algae, to persist (Verlaque, 1990). Similarly, Franco et al. (2015) demonstrated that intense grazing pressure in warmer regions can severely limit kelp recruitment, forcing juveniles to persist only within crevices that act as refuges from herbivory, thereby constraining forest recovery and expansion. Moreover, recent evidence suggests that under climate warming, protection from fishing can exacerbate herbivore impacts—*S. salpa* populations may thrive within marine protected areas, intensifying grazing pressure and further inhibiting macroalgal recovery (Reis et al., 2024). Together, these findings highlight the potential for warming and management interventions to interact synergistically, reinforcing herbivory-driven shifts from canopy-dominated to turf-dominated systems. Furthermore, the preferential consumption of structurally and reproductively important parts of habitat-forming species, as observed in other studies (Vergés et al., 2010; Gianni et al., 2017), may hinder the recovery and regeneration of these forests. These patterns are consistent with the expansion of the trophic spectrum in response to habitat degradation—a phenomenon commonly observed in voracious herbivores (Ogden & Lobel, 1978; Verlaque, 1990; Vergés et al., 2014a)—ultimately reflecting an increase in niche breadth. Consequently, sustained and spatially extensive herbivory by *S. salpa* may further prevent the recovery of marine forests and promote shifts toward less structurally complex assemblages, consolidating its role as a driver of

ecosystem change (Franco et al., 2015; Gianni et al., 2017; Barrientos et al., 2022; Reis et al., 2024).

Temperature-Driven Range Expansion of *Sarpa salpa* and its implications. In parallel with its ecological implications, the responses of *S. salpa* landings to temperature further support the idea that warming may modulate the species' ecological impact along the Portuguese coast. Although no clear long-term trend was detected, landings displayed marked regional contrasts in their relationship with temperature. In the cooler northern region—home to the most extensive marine forests—landings increased markedly during warmer periods, with the highest CPUE values clustering around 15–17 °C. This range aligns with the species' known thermal preference ($\approx 15.3\text{--}24$ °C) (Marras et al., 2015; FishBase, 2025) and suggests that episodic warming may facilitate temporary expansions of *S. salpa* into historically cooler reefs, thereby intensifying grazing pressure on habitat-forming macroalgae. This pattern highlights the potential role of low temperatures as a barrier that periodically protects northern marine forests, whereas prolonged warm periods may favour the arrival of this herbivore, intensifying grazing pressure on these vulnerable ecosystems (Vergés et al., 2014a,b, 2016). Given its affinity for warmer waters, continued ocean warming may enable the permanent establishment of *S. salpa* in historically cooler regions, granting it greater access to temperate marine forests and potentially leading to sustained herbivory pressure on these ecosystems. In the Centre region, landings followed a strong seasonal pattern, but temperature did not significantly influence fluctuations, implying that other seasonal factors, not captured in the data, may drive variability. In contrast, in the South region - where temperatures are higher - landings remained relatively stable and exhibited only a weak negative correlation with temperature, compared with the strong positive relationship observed in the North. Overall, these patterns reveal a clear latitudinal gradient in thermal sensitivity: southern populations remain relatively stable despite seasonal fluctuations, whereas northern populations are far more responsive to temperature anomalies. This pronounced sensitivity, combined with ongoing ocean warming, may facilitate the northward expansion of *S. salpa* into historically cooler regions, where the species has greater access to extensive marine forests—a trend consistent with observations of its range shifts in other Atlantic and Mediterranean areas (Vergés et al., 2014a; Gianni et al., 2017; Bañón et al., 2024).

While landings do not directly represent population size and should be therefore interpreted with caution (Pauly et al., 2013), *S. salpa* is a low-value species often taken as bycatch, and its capture records can nonetheless provide useful insights into population trends along the coast (Gianni et al., 2017). However, specific long-term

datasets remain scarce, making it difficult to accurately assess the scale and extent of this potential trend. Fluctuations in marine organism densities, driven by both natural and human-induced factors, are common but often go unnoticed, particularly for non-target species like *Sarpa salpa*. Historical observations at the extreme southern coast of Portugal (Faro) reported rising *S. salpa* densities during the 1980s (Ribeiro et al., 2008), suggesting that warming may have long supported population growth in warmer regions. Comparable dynamics have been documented across the Mediterranean and nearby Atlantic coasts: in NW Spain, high herbivore pressure by *S. salpa* has caused kelp forest collapse (Barrientos et al., 2022), while in the Portofino Marine Protected Area, Italy, increased *S. salpa* densities were identified as a potential driver behind the decline of large brown algal forests during the late 1990s (Parravicini et al., 2013). Additional studies highlight the potential threat posed by herbivorous fishes to subtidal *Cystoseira* s.l. forests (Hereu, 2006; Vergés et al., 2009, 2014a; Gianni et al., 2017). On a global scale, ocean warming enhances the metabolic rates and feeding activity of herbivorous fishes (Clarke, 2003; Doney et al., 2012), often triggering ecosystem phase shifts, such as those observed in Tosa Bay, Japan, where rising temperatures led to the replacement of kelp forests by coral-dominated reefs (Mezaki & Kubota, 2012). In the area of study of the present study, the Iberian upwelling system plays a key role in maintaining and protecting northern marine forests (Chabrierie & Arenas, 2024). While recent records indicate occasional periods of upwelling intensification, future projections suggest a decline in its effectiveness due to increased thermal stratification, which will reduce the supply of cooler, deeper waters to the surface (Sousa et al., 2020). Such a reduction in thermal buffering may increase the vulnerability of these forests to grazing by expanding populations of sub-tropical herbivores, including *S. salpa*.

Limitations of the study. While this study provides an integrated view of the feeding ecology and potential ecological role of *Sarpa salpa* along the Portuguese coast, some limitations should be acknowledged. Stomach content data were obtained from a moderate sample size and represent a single seasonal window, meaning that neither inter-annual nor seasonal variability in diet could be fully assessed. As such, the low dietary evenness and niche-breadth values reported here should be interpreted as evidence of feeding specialisation under the surveyed conditions, rather than as a definitive description of the species' full trophic niche across space and time. In addition, subtidal algal surveys provide only a spatial proxy of resource availability and may not fully capture the range of habitats used by this highly mobile herbivore. The fisheries data, although informative, are subject to reporting variability, reflect landings rather than abundance, and only include a fraction of the fishery sectors operating along the coast.

Together, these constraints highlight the need for future studies incorporating year-round sampling, broader spatial coverage, and independent abundance estimates to more completely characterise the trophic ecology, population dynamics, and ecological impacts of *S. salpa* under ongoing ocean warming.

4.6 Conclusion

Understanding the impacts of climate change on ecosystems is complex, as environmental drivers and ecological processes interact across spatial and temporal scales. In this context, the present study explored herbivory by *S. salpa* on benthic macroalgae and examined its potential range shifts along a tropicalized latitudinal gradient on the Portuguese coast. Our results suggest that *S. salpa* may play a role as an influential herbivore along this coast, particularly under ongoing ocean warming and the tropicalisation of temperate marine ecosystems. Dietary analyses and fisheries data indicate that the species exhibits thermal sensitivity, selective herbivory on structurally complex brown algae, and signs of seasonal, and possibly longer-term, expansions into cooler northern regions. Together, these patterns suggest that herbivory pressure on temperate marine forests could intensify in the future. Declines of key habitat-forming species, such as kelps and *Cystoseira* s.l., observed in other studies, may potentially be exacerbated by sustained foraging by *S. salpa*, especially under scenarios of weakened upwelling and increased frequency of marine heatwaves. As such, *S. salpa* may not only reflect broader climatic shifts but also act as a potential contributor to ecosystem restructuring, with possible cascading effects on biodiversity, carbon cycling, and the resilience of coastal systems. Continued monitoring and integrative studies combining environmental, functional, and fisheries data will be critical to anticipate and better understand the long-term impacts of CC on these temperate reef ecosystems.

4.7 Acknowledgments

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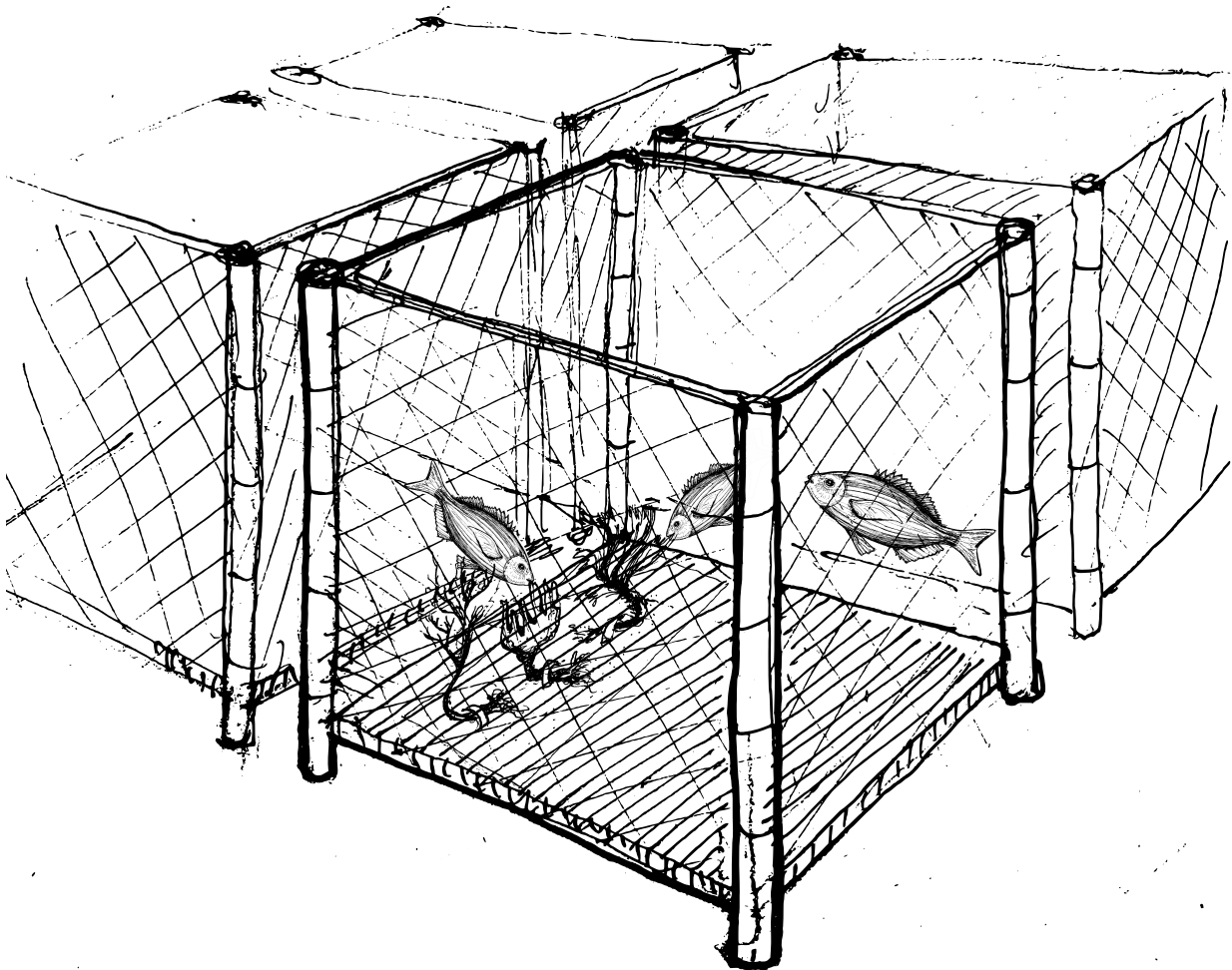
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Chapter V.



Grazing preferences of *Sarpa salpa* on
temperate habitat-forming macroalgae

Publication Note

The work presented in this chapter has been published as a research article in the international peer-reviewed journal **Aquatic Botany**:

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5.1 Abstract

Herbivory is intensifying on many temperate reefs under ocean warming and tropicalisation, raising concerns for canopy-forming seaweeds. Along the Portuguese coast, the obligate herbivore *Sarpa salpa* is abundant and frequently observed grazing on kelps and fucoids. We tested whether wild *S. salpa* shows consistent feeding preferences among four habitat-forming brown macroalgae—*Laminaria ochroleuca*, *Fucus spiralis*, *Sargassum muticum* and *Cystoseira humilis*—and whether simple stoichiometric signals (C and N) align with observed choices. Using outdoor, submerged mesocosms at Sines region (SW Portugal), we ran four sequential no-choice trials conducted over a 12-day period (with 48h intervals between session) followed by choice assays (days 5–6). Consumption was recorded as 24-h wet-mass loss, standardised to grazer biomass ($\text{g} \cdot \text{kg}^{-1}$), and elemental C and N were measured in algal tissues and fish faeces to infer assimilation. Fish readily consumed all species in no-choice trials, indicating no gross non-palatability. Under simultaneous availability, however, *S. salpa* consistently consumed more *F. spiralis*, *L. ochroleuca* and *S. muticum* than *C. humilis*, revealing non-uniform selection. Tissue composition differed among species: *L. ochroleuca* had the highest N and lowest C:N ratio, which aligned with the highest apparent N assimilation by the fish. This clear link between simple stoichiometry and assimilation suggests that species-specific nutritional payoffs are a key mechanistic driver of preference. Given the warming and tropicalising context of NE Atlantic reefs, this trait-based selectivity is significant: it may amplify canopy vulnerability (particularly of high-quality species) and favour turf-dominated states at warm range margins. We recommend integrating herbivory metrics (selectivity, bite rates, grazer biomass) into monitoring, conservation and restoration to safeguard Portuguese marine forests.

Keywords: herbivore selectivity; macroalgal forests; plant-herbivore interactions; stoichiometry (C:N); NE Atlantic reefs

5.2 Introduction

Herbivory is a fundamental ecological process that shapes the structure and dynamics of biological communities across scales (Schmitz et al., 2004) and can restructure diversity, physical habitat and ecosystem productivity (Stachowicz et al., 2007; Poore et al., 2012; Bennett et al., 2015). In aquatic ecosystems, grazing rates can exceed those on land by up to three-fold, and strong consumer-resource interactions frequently induce trophic cascades that cascade down to primary producers. (Cyr and Pace 1993; Shurin et al., 2002; Buñuel et al., 2020).

On temperate coasts, warming is expected to intensify herbivory via higher ectotherm metabolic rates that elevate consumption (Brown et al., 2004), poleward expansion of tropical–subtropical herbivores (Vergés et al., 2014, 2016), and grazer outbreaks linked to predator declines from fishing and heat stress (Christie et al., 2019; Carnell and Keough, 2019; Nowicki et al., 2021; Santana-Garcón et al., 2023). Such pressures can precipitate local overgrazing and cause phase shifts from kelp and fucoid forests to turf-dominated states (Bennett et al., 2015; Pérez-Matus et al., 2017; Vergés et al., 2014)

Kelp and fucoid forests underpin the structure and functioning of temperate reefs, providing three-dimensional habitat, nursery areas and refugia, and supporting high productivity (Arenas et al., 2006; Wernberg et al. 2019; Pessarrodona et al., 2022; UNEP, 2023). Along the western Iberian coast, which functions as a transition zone and the warm-range limit for many cold-water seaweeds, the persistence of marine forest is tightly coupled to upwelling (Araújo et al., 2009; Pinho et al., 2016; Des et al., 2020; Vale et al., 2021). However, these systems are now threatened by the combined effects of ocean warming, (Sousa et al., 2020) leading to a coastal community tropicalisation (Azevedo et al., 2023), and the increase of herbivory from fishes and invertebrates (Franco et al., 2015; Barrientos et al., 2022; Reis et al., 2024). This is contributing to local declines and reduced resilience of kelp and fucoid forests: intense herbivory under warm conditions forces recruits into cryptic refuges, constraining replenishment (Franco et al., 2015), while in some regions herbivorous fishes can drive reef-scale collapses of adult kelp stands through sustained grazing across all size classes (Barrientos et al., 2022), and protection-driven increases in fish herbivores inside MPAs may further amplify kelp biomass loss, particularly at depth (Reis et al., 2024). On rapidly tropicalising coasts such as western Iberia, these processes alter both herbivore pressure and the composition of macroalgal assemblages, bringing native and range-expanding species into increasing

contact. In this context, understanding the *mechanisms* that drive herbivore choice, beyond mere consumption, is critical to predicting which species are most vulnerable.

Across this climatic gradient, northern Portugal still supports patches of cold-temperate kelp forests dominated by *Laminaria hyperborea*, whereas the central coast marks the southern European distribution limit of *L. ochroleuca*, resulting in a mixture of cold- and warm-affinity macroalgal species. Further south, including the Sines region where our experiment was conducted—canopy-forming assemblages are dominated by warm-affinity fucoids (*Cystoseira s.l.*), *Sargassum spp.*, and turf-forming species, with the only kelp present being *Phyllariopsis brevipes* (Tuya et al., 2012; de Azevedo et al., 2023). This pattern reflects a progressive shift towards warm-affinity macroalgae in the southern regions. These habitat-forming canopies compete for limited rocky substrate, and recent environmental change has promoted an increase in turf-forming assemblages, which show a growing tendency to replace canopy-forming seaweed forests across central and southern Portugal (Tuya et al., 2012; de Azevedo et al., 2023).

In this tropicalisation context, *Sarpa salpa* (Salema), the only obligate herbivorous fish on western Iberia, forms large shoals and exerts strong grazing where abundant (Franco et al., 2015; Barrientos et al., 2022; Buñuel et al., 2020). Beyond the NE Atlantic, tropicalisation has strengthened consumer pressure on habitat-forming seaweeds, with range-extending tropical herbivores capable of producing order-of-magnitude increases in kelp consumption and canopy loss and accelerating macrophyte-to-detritus fluxes on temperate reefs (Zarco-Perelló et al., 2017; Zarco-Perelló et al., 2019).

Herbivory feeding outcomes reflect acceptability, electivity and preference, with preference inferred when items are consumed disproportionately under simultaneous availability (Jackson and Underwood, 2007; Singer, 2000; Underwood and Clarke, 2005). Algal traits shaping such choices include nutritional quality and stoichiometry (e.g., C:N), chemical deterrents such as phlorotannins in Fucales, and tissue biomechanics. For example, recent work highlights that phlorotannin production in *Fucus spiralis* is highly variable, with consequences for palatability (Pinteus et al., 2022; Catarino et al., 2022), and comparable intra- and interpopulation variation has been documented in other *Fucus* species (Koivikko et al., 2008). In pelagic *Sargassum*, tissue composition and stoichiometry can influence consumer selectivity (Changeux et al., 2023). Moreover, nutrient-driven shifts in physiology and microbiome composition (Theirllynck et al., 2025) and large-scale environmental drivers (Podlejski et al., 2024) further modulate the quality of *Sargassum* as a food resource.

Given the ecological setting, where potential warming intensifies herbivory and reshapes algal communities, we tested whether *S. salpa* exhibits consistent feeding preferences among four habitat-forming brown macroalgae common on the Portuguese coast—*Laminaria ochroleuca*, *Fucus spiralis*, *Sargassum muticum* and *Cystoseira humilis*—and whether basic stoichiometric signals (C and N) align with observed patterns. These four macroalgae are long-established habitat-formers on the Iberian coast, and their contrasting ecological traits make them ideal for assessing which temperate canopies may be most vulnerable to increasing herbivory under tropicalisation. We used sequential no-choice and simultaneous-choice mesocosm assays, quantifying 24-h consumption standardised to grazer biomass and measuring C and N in algal tissues and faeces to infer assimilation. We hypothesised that *S. salpa* would not graze uniformly but show preferences linked to species-specific traits (nutritional quality, toughness and deterrents), with implications for canopy vulnerability under warming and tropicalisation. Crucially, our combined two-stage assay and stoichiometric analysis provides a mechanistic understanding by testing if the observed preference aligns with species-specific nutritional payoffs (C:N ratio and N assimilation).

5.4 Material and Methods

Experimental setup. The feeding trial was conducted in December at the Recreational Marina of Sines (south-central Portugal) using four submerged outdoor mesocosms. Each mesocosm consisted of a cage ($2 \times 2 \times 2$ m; 8 m^3), constructed from PCV pipe and plastic mesh, and moored to the sheltered side of a pier, away from vessel traffic (Figure V-S1 A). The mesh floor of each mesocosm was fitted with a centrally mounted screw-cap bottle for quantitative faecal collection and a tarpaulin was placed beneath the mesh to funnel waste towards the collector (Figure V-S1 B). Temperature was recorded once daily at the mesocosm location and at an adjacent open-coast site outside the marina where the focal algae and *Sarpa salpa* occur naturally; no thermal differences were detected between sites during the experiment.

Food sources – Algae. Four habitat-forming brown macroalgae common to the Portuguese west coast were offered in the experiment: *Laminaria ochroleuca*, *Sargassum muticum*, *Fucus spiralis* and *Cystoseira humilis*. The experiment was conducted in Sines due to its sheltered conditions, which are ideal for mesocosm studies. While *L. ochroleuca* is virtually absent from this region, it is a dominant habitat-former further north and was included due to its ecological importance. Fresh thalli were therefore collected from intertidal reefs in northern Portugal and transported to the marina within 6h. The remaining species were all collected from intertidal reefs in Sines during winter, when lower water temperatures allow canopy-forming species to thrive in shallower depths. This intertidal zone is also an area where *S. salpa* is frequently observed grazing. Algae were collected on multiple dates throughout the experiment, and each batch was used in the corresponding trial. After collection, algal material was immediately held alive in mesh cages suspended beside the pier for no longer than 48 hours before use.

Herbivores. Twelve wild *Sarpa salpa* (mean total mass $\pm$ SD = 534.2 ± 47.4 g; total length = 32.9 ± 1.63 cm) were captured on the rocky shore of Sines region with hook-and-line gear using *F. spiralis* as bait, following local fishers' practice. Between catches, fish were temporarily held in portable mesh cages in sheltered seawater to minimise stress. They were then transferred to the marina in an aerated 100 L polypropylene tank. Upon arrival, fish were distributed into four experimental cages ($n=3$ individuals per cage) for acclimation. To standardise hunger levels, all individuals were fasted for four days prior to the experiment (Underwood et al., 2004; Jackson and Underwood, 2007).

Experimental design. The experimental design comprised two consecutive phases, each consisting of 24-hour trials where algae were introduced at approximately 9am.

Each experimental cage (3 fish per cage) served as an independent unit throughout all trials. A standard protocol was followed where, for every trial, the initial and final wet weight (blotted to remove excess of water) of each seaweed was recorded to quantify consumption, with biomass loss attributed to herbivory. (i) No-choice phase (four trials): During each trial, a single macroalgal species was offered, with each of the four cages receiving a different species. To control for potential carryover effects where prior consumption of one species could influence grazing on the next, the sequence of algal species offered was systematically rotated across cages over the four trials (Table V-S2). This design ensured that each cage received the four species in a unique order. (ii) Choice phase (two consecutive trials): All four algal species were presented simultaneously in each cage.

Across all trials in both phases, algae were offered *ad libitum* in similar initial wet masses (pooled mean $\pm$ SD = 145.2 $\pm$ 22.4 g), which far exceeded daily consumption. They were fixed to polyethylene racks on the cage floor using wooden clothes pegs, which held the thalli in place while still allowing unrestricted grazing (Figure V-S1). A 48-hour fasting period was implemented between all subsequent trials to standardise hunger levels and ensure gut clearance (Underwood et al., 2004; Jackson and Underwood, 2007; Booth et al., 2008; Goldenberg and Erzini, 2014). This fasting interval exceeds the reported gut transit time of *S. salpa* feeding on similar plant material (mean $\approx$ 17–20h at $\sim$ 17 °C; Goldenberg and Erzini, 2014) and was therefore considered sufficient to minimise cross-contamination of faecal material between trials.

Consumption data were handled following Underwood et al. (2004) and Grason and Miner (2012). To allow comparisons among fish of different sizes, consumption was measured at the cage level and then standardised to the total biomass of the three grazers in each cage. For each 24-h trial, consumption was expressed as grams of seaweed eaten per kilogram of fish ($\text{g kg}^{-1} 24 \text{ h}^{-1}$). In the no-choice phase, we calculated a relative consumption ratio for each algal species. To obtain a population-level estimate of relative palatability, we pooled the consumption data across all cages within a treatment. Specifically, this was done by summing the total mass of a given species consumed by all cages on a given day and dividing it by the total biomass of all algae consumed that day. This resulting ratio represents the species' relative palatability when offered as the sole option. In the choice phase, proportions were computed within cages by dividing the mass of each species eaten by the total consumption over 24 h. This two-stage design provided independent estimates of baseline consumption rates (no-choice) and active feeding preferences (choice). We treated the cage as the experimental unit in

all analyses ($n = 4$). Given the short duration of trials and calm sea conditions, autogenic changes in algal mass were assumed negligible, and observed wet-mass loss was attributed to herbivory (Franco et al., 2015).

Carbon and nitrogen content. Elemental composition of both the offered algae and the *egesta* was quantified to support stoichiometric analyses. Six independent subsamples of each algal species were reserved at the start. Faecal material for C:N analysis was collected specifically during the no-choice phase, as this design allowed the *egesta* to be unequivocally linked to a single algal species. Collection occurred at the end of each 48-hour fasting interval, immediately before starting the next feeding trial. All samples were dried in a forced-air oven at 60 °C for 48 h to constant mass, weighed to the nearest 0.1 mg, and then milled to a homogeneous powder in a Mixer Mill MM 400 (Retsch GmbH). Total carbon (C) and nitrogen (N) were determined on 2–3 mg aliquots using a Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific). Results are expressed as atomic C:N ratios.

Statistical analyses. To determine food preferences, we employed a two-factor ANOVA, with prey type (algae species) and choice (choice vs. no-choice) as fixed factors. This analysis followed Grason *et al.*, (2012) and Cardoso *et al.*, (2020). The interaction between those two fixed factors tests whether the proportion consumed differed between phases. To address potential non-independence, we generated 10 000 simulated datasets using a binomial probability distribution assuming no inherent preference (i.e., equal probability among algae species), yielding a null distribution of F-ratios ($\alpha = 0.05$). We computed the observed F-value from the empirical data and compared it to the null distribution. To mitigate pairing biases between stages, we conducted 1000 random bin pairings (for the no-choice data) and ran 1000 corresponding ANOVAs; the proportion of significant p-values was used to assess the strength of the interaction term.

Two additional one-way ANOVAs were performed in order to test the effect of the fixed factor prey type (algal species) on total consumption in separate experiments with no choice and choice of prey. In these one-way ANOVAs, post-hoc were performed with the Tukey test.

All statistical analyses were conducted using the R software (v. 4.3.; R Core Team, 2023), using the packages “plotrix” (Lemon, 2006), “lsmeans” (Lenth, 2016), “car” (Fox et al., 2023), “stringr” (Wickham, 2019) and “multcompView” (Graves et al., 2015). Detailed instructions for executing the randomization protocols, including the R script and example data, are provided in the Supplementary Material (Appendix V). The

complete analysis code and raw datasets are available from the author upon reasonable request.

ETHICAL NOTE. This study did not involve invasive procedures or cause harm to the fish. All individuals were maintained in conditions closely resembling their natural habitat, without restriction of their natural diet, and were released at the end of the experiment. According to EU Directive 2010/63 and Portuguese law (Decreto-Lei n.º 113/2013), this work does not constitute an animal experiment requiring formal authorisation.

5.5 Results

Preference experiments. Under captive condition, wild *S. salpa* readily consumed all four seaweeds *in* the no-choice trials, with no evidence of species-specific differences in rate (ANOVA species effect: $p = 0.979$) indicating no gross non-palatability when each seaweed species was offered alone.

In the choice stage, fish consistently ate more *F. spiralis*, *L. ochroleuca* and *S. muticum* over *C. humilis*, revealing reduced selection for *C. humilis* (Figure V-1a). Across 1000 random bin pairings, 20.8% of the simulated ANOVAs yielded a significant species x choice interaction ($p < 0.05$, Figure V-S2), providing evidence of non-uniform selection when all seaweed species were simultaneously available (Figure V-1a). Although no significant species-specific differences were detected in absolute consumption rates, the highest single-day maximum consumption was recorded for *L. ochroleuca* ($\sim 80 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$; Table V-S1; $\uparrow$ Figure V-1b), followed by *F. spiralis* ($\sim 35 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$; Table X-S1; Figure V-1b), *S. muticum* ($\sim 23 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$; Table V-S1; Figure V-1b), and *C. humilis* ($\sim 20 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$; Table V-S1; Figure V-1b). These values represent maximum cage-level consumption events (normalised by total fish biomass per cage; three individuals per cage). Mean consumption rates ($\pm \text{SE}$) for both no-choice and choice assays are reported in the Table V-S3, providing a more direct basis for species comparisons.

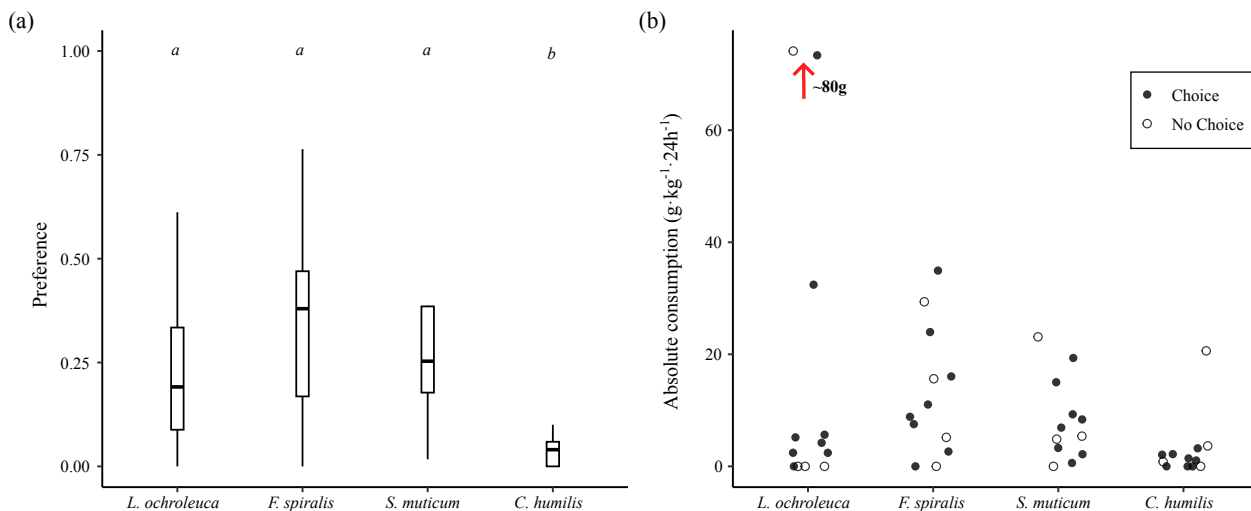


Figure V-1 *Sarpa salpa* preference for habitat-forming seaweed species: (a) Degree of preference (mean $\pm$ SE) in the “Choice” experiment; (b) daily absolute consumption expressed as grams of seaweed consumed per kilogram of total fish biomass per cage per 24 h ($\text{g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$), represented by each dot. Different letters denote groups that differ significantly under the simulation framework (20.8% of $p < 0.005$). ($\uparrow$) Indicates the highest consumption observed within 24 h.

Carbon and Nitrogen Composition of Seaweed Species and Assimilation by *S. salpa*. Nutritional metrics differed among species for all parameters (Figure V-2a, b, c, Table V-1). Carbon (% dry mass) was highest in *C. humilis* and lowest in *L. ochroleuca* (intermediate values in *F. spiralis* and *S. muticum*; $F = 6.164$, $p = 0.02$; Figure V-2b; Table V-1). Nitrogen (% dry mass) peaked in *L. ochroleuca* followed by *F. spiralis* and was lowest in *S. muticum* and *C. humilis* ($F = 9.414$, $p = 0.01$; Figure V-2b; Table V-1), indicating relatively greater nitrogen availability in *L. ochroleuca*. Consistently, C:N ratios differed significantly among species ($F = 18.15$, $p = 0.01$), with *L. ochroleuca* showing the lowest values, reflecting its comparatively higher nutritional quality (Figure V-2c; Table V-1).

Regarding assimilation (Δ = algae – faeces) in the no choice trials, carbon assimilation was highest for *F. spiralis* (followed by *S. muticum*, *C. humilis*, and *L. ochroleuca* ($F = 2.958$, $p = 0.04$; Figure V-2d; Table V-1), whereas nitrogen assimilation was highest in *L. ochroleuca* and *F. spiralis*, and lowest for *S. muticum* and *C. humilis* ($F = 4.477$, $p = 0.01$; Figure V-2e; Table V-1). Assumption checks indicated homogeneous variances (Levene tests, $p > 0.19$).

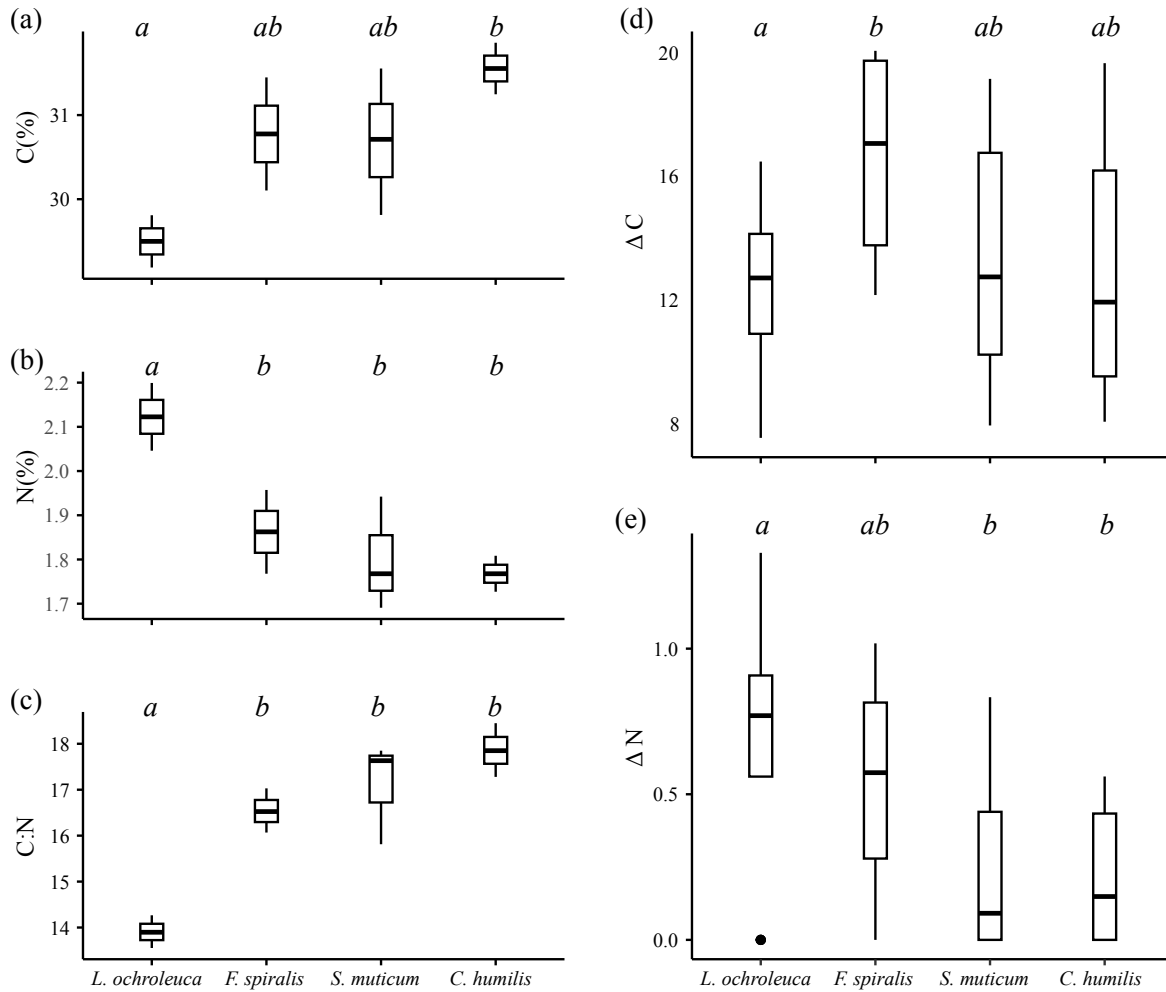


Figure V-2 Nutritional composition and assimilation capacity (mean $\pm$ SD) across species: (a) Percentage of carbon in dry mass; (b) percentage of nitrogen in dry mass; (c) carbon-to-nitrogen ratio; (d, e) carbon and nitrogen assimilation (Δ = algae – faeces) by *S. salpa* over 24 h in the "No-Choice" experiment. Different lower-case letters indicate post-hoc differences (SNK after ANOVA).

Table V-1 Nutritional composition and assimilation capacity (mean $\pm$ SD) of four seaweed species tested, along with ANOVA results comparing species for each parameter: carbon (%C), nitrogen (%N), C:N ratio, and assimilation (Δ = algae % – faeces %). Levene's tests confirmed homogeneity of variances.

Seaweed species, sources of variation	Carbon (% dry mass)		Nitrogen (% dry mass)		C:N in mass		Δ C		Δ N	
<i>L. ochroleuca</i>	29.5 $\pm$ 0.3		2.1 $\pm$ 0.07		13.9 $\pm$ 0.4		12.3 $\pm$ 2.9		0.7 $\pm$ 0.5	
<i>F. spiralis</i>	30.8 $\pm$ 0.7		1.9 $\pm$ 0.09		16.5 $\pm$ 0.5		16.6 $\pm$ 3.2		0.5 $\pm$ 0.4	
<i>S. muticum</i>	30.7 $\pm$ 0.9		1.8 $\pm$ 0.13		17.1 $\pm$ 1.1		13.5 $\pm$ 4.2		0.3 $\pm$ 0.3	
<i>C. humilis</i>	31.6 $\pm$ 0.3		1.8 $\pm$ 0.04		17.9 $\pm$ 0.6		13.1 $\pm$ 4.5		0.2 $\pm$ 0.2	
ANOVA	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Species (df = 3)	6.165	0.02	9.414	0.01	18.15	0.00	2.958	0.04	4.477	0.01
Levene test	0.889	0.48	0.511	0.69	0.36	0.78	1.633	0.19	0.673	0.57

5.6 Discussion

This study aimed to evaluate the feeding preferences of *Sarpa salpa* for habitat-forming brown macroalgae of western Iberia and to examine whether simple nutritional signals (C, N, C:N) align with observed consumption patterns. Across assays, fish consumed all four species when offered singly and showed clear avoidance of *Cystoseira humilis* under simultaneous availability. Among the remaining taxa, differences in consumption were weak or non-significant, yet nutritional patterns were more distinct: *Laminaria ochroleuca* showed the highest tissue N and lowest C:N ratio, which aligned with its highest apparent N assimilation. Together, these results suggest that while strong avoidance is restricted to *C. humilis*, preferences among the other canopy species are more subtle and may be influenced, at least in part, by nutritional quality.

Feeding patterns and mechanistic drivers of preference. Our assays show that *S. salpa* readily consumes all four canopy-forming brown algae when offered singly, indicating all are acceptable diets. However, the subsequent clear avoidance of *C. humilis* under simultaneous availability is a strong finding, consistent with active preference rather than simple palatability (Singer, 2000; Underwood and Clarke, 2005; Jackson and Underwood, 2007). This robust distinction, enabled by our two-stage experimental design, provides confidence that the observed feeding patterns reflect a genuine selective choice. These four macroalgae are long-established habitat-formers on the Iberian coast, and their contrasting ecological traits make them ideal for assessing which temperate seaweed canopies may be most vulnerable to increasing herbivory under tropicalisation. Secondary chemistry and tissue traits can also modulate palatability, as phlorotannins and tissue biomechanics influence bite profitability. These palatability-related traits can vary, and in some cases covary, with nutritional quality, as seen in *Fucus spiralis* phlorotannin levels and *Sargassum* tissue/stoichiometry variation (Pinteus et al., 2022; Changeux et al., 2023; Corbin and Oxenford, 2023).

In the Portuguese coastal assemblages context, these mechanisms play out in an ecologically realistic way: *Laminaria ochroleuca* sits near its warm range limit on the west coast, while fucoids remain widespread intertidally and *Sargassum muticum* is a common introduced species in subtidal fringe habitats. Because our results show that *S. salpa* consistently prefers *L. ochroleuca*, *S. muticum* and *F. spiralis*, and largely avoids *C. humilis*, this spatial configuration aligns closely with the species-specific payoffs identified in our assays. Such trait-based selectivity means that grazing pressure will not be evenly distributed across canopy taxa, potentially reducing the dominance of

preferred species and indirectly favouring the persistence of less-preferred but structurally important canopies like *C. humilis*. This creates multi-diet scenarios where trait-based selectivity by *S. salpa* can scale to community effects (Araújo et al., 2009; Pinho et al., 2016; Filbee-Dexter and Wernberg, 2018; Smale et al., 2020).

Ecological implications under warming and tropicalisation. Selective grazing by abundant shoals of *S. salpa* can amplify canopy vulnerability at warm range margins. This is particularly relevant if consumers repeatedly target higher-quality diets such as *L. ochroleuca*, *S. muticum* and *F. spiralis*, thereby exacerbating the impacts of warming/tropicalisation, which act as the main drivers of the ecological shift. In Portugal, herbivory under warm conditions forces kelp recruits into cryptic refuges, constraining replenishment (Franco et al., 2015). As temperate reefs tropicalise, range-extending herbivorous fishes can impose order-of-magnitude increases in kelp consumption, hastening canopy loss, and re-route energy flow by accelerating macrophyte-to-detritus fluxes (Zarco-Perelló et al., 2017; Zarco-Perelló et al., 2019). Once canopies collapse, reefs can flip to persistent turf states maintained by warming-mediated feedback and strengthened consumer control (Wernberg et al., 2016; Filbee-Dexter and Wernberg, 2018). This framing helps explain why seemingly modest differences in diet quality (C:N, defences) could tip local patches toward kelp/fucoid retention or turf dominance (Tuya et al., 2012; de Azevedo et al., 2023).

For the west Iberian margin, where forest persistence is tightly coupled to upwelling dynamics, the interaction between heat events and consumer pressure may be especially consequential: warming/tropicalisation increase grazing windows, whereas cool upwelling pulses may provide short-lived refuges for canopy recovery (Araújo et al., 2009; Smale et al., 2020; Sousa et al., 2020).

Limitations and scope. Our in-situ mesocosms captured short-term feeding under calm conditions; this isolates preference signals but may under-represent storm-driven breakage, seasonal tissue changes or behavioural shifts in larger shoals. The design treated the cage as the experimental unit and assumed negligible autogenic loss under calm seas, so wet-mass loss is interpreted as tissue consumed (Methods). Extending assays across seasons and wave regimes and pairing with field exclosures along upwelling/warm-edge gradients, would test the generality of the trait–preference links inferred here.

Management and monitoring implications. Because selective grazing can target foundation species and skew canopy composition, surveillance should include herbivory metrics (bite rates, selectivity indices, grazer biomass) alongside standard forest

indicators. In Portuguese warm-edge reefs, priorities include (i) long-term monitoring of *S. salpa* abundance and grazing hotspots; (ii) protection of recruitment refuges and cooler microhabitats during heat-prone months; and (iii) grazer-aware thresholds in restoration. Although our study did not quantify seasonal shifts in grazing or diet preferences, restoration timing must also consider the biological requirements of the seaweeds themselves. Future work clarifying seasonal patterns of algal availability and consumer pressure would help determine whether certain windows offer reduced grazing risk for out-planting.

5.7 Conclusions

Selective grazing by *Sarpa salpa* on habitat-forming macroalgae is non-uniform; we demonstrate a clear mechanistic link where lower tissue C:N aligns with higher intake and greater N assimilation, indicating that simple nutritional traits underpin feeding preference. In warming, tropicalising reefs, this selectivity acts as a significant factor that amplifies canopy vulnerability, accelerating the phase shift to turf-dominated states, especially at warm range margins. Managing Portuguese marine forests will benefit from grazer-aware monitoring and conservation strategy, informed by the selectivity patterns identified here, that protects recruitment windows and refuges.

5.8 Acknowledgments

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Chapter VI.

Conclusions and Remarks

6.1 General Synthesis: Synergistic Threats and Tropicalisation on the Portuguese Coast

The central objective of this PhD thesis was to characterise recent changes in the structure and functioning of habitat-forming seaweed ecosystems along the Portuguese coast and to evaluate how these systems respond to multiple stressors associated with tropicalisation. The findings presented in Chapters 2, 3, 4, and 5 provide robust evidence that tropicalisation is an active, spatially structured, and multifaceted process operating across this coastal transitional zone, which represents a sentinel system at a critical biogeographic interface between temperate and subtropical realms.

This thesis demonstrates that the degradation of temperate marine forests cannot be attributed solely to direct climatic warming. Instead, it emerges from the synergistic interaction between physical drivers (increasing temperature and altered oceanographic regimes) and biological processes, most notably herbivory by warm-affinity species. This integrative perspective highlights that understanding ecosystem responses to climate change requires explicit consideration of trophic dynamics and functional reorganization.

To address these objectives, the thesis combined large-scale observational analyses of subtidal and intertidal seaweed communities with experimental and long-term approaches focused on herbivory mechanisms and population trends of key grazers.

6.2 Community Shifts and Evidence of Tropicalisation

6.2.1 Subtidal Patterns

Chapter II provides clear evidence of ongoing tropicalisation in subtidal seaweed assemblages along the Portuguese coast. Based on a 6-year high-resolution survey (2012–2018), this process is primarily reflected by a significant increase in the Community Temperature Index (CTI), indicating a progressive shift in community composition toward species with warmer thermal affinities.

Importantly, subtidal tropicalisation is not characterised by a uniform collapse of canopy-forming forests, but rather by a directional compositional and functional reorganisation. The cold-affinity, perennial canopy-forming kelp *Laminaria hyperborea* (Order Laminariales) shows signs of regional retraction and declining dominance, particularly toward its southern distributional limits. Conversely, the fast-growing, opportunistic kelp *Saccorhiza polyschides* (Order Tilopteridales) exhibits localised increases, reflecting a shift towards more transient and unstable canopy dynamics. Simultaneously, subtidal assemblages exhibit an increasing contribution of warm-affinity taxa and opportunistic turf-forming algae, such as the invasive *Symphyocladia marchantioides*

These patterns highlight the context-dependent role of the Iberian upwelling system. In northern regions, persistent upwelling continues to provide partial thermal buffering, allowing the persistence of cold-temperate kelp forests. However, the results indicate that this buffering capacity is weakening (likely as rising sea temperatures and marine heatwaves override local cooling), leading to increasing thermal stress and functional simplification even within areas traditionally considered boreal refugia.

Conclusion 1: Subtidal marine forests along the Portuguese coast are undergoing a climate-driven compositional and functional reorganization, evidenced by rising CTI values and a gradual shift from cold-affinity canopy dominance toward warmer-affinity and opportunistic taxa. This process reflects a weakening of the buffering role of upwelling-driven refugia rather than an abrupt ecosystem collapse.

6.2.2 Intertidal Patterns

Chapter III extends the evidence of tropicalisation to intertidal habitats through a decadal comparison (2006–2021), focusing on rockpool assemblages that experience high environmental variability. In contrast to the subtidal, intertidal rockpools exhibit stronger and more rapid signals of community restructuring, consistent with their heightened exposure to temperature extremes and reduced buffering capacity during low tide.

The results demonstrate marked shifts in taxonomic and functional structure, where "deborealisation" (characterised by the collapse of cold-affinity perennial canopies, including the non-native but functionally dominant *Sargassum muticum*) is the overriding driver. Unlike the subtidal, the loss of these structural species in the intertidal was not immediately compensated for by warm-affinity habitat-formers, leading to a "canopy-to-turf" transition and a significant loss of standing biomass. These findings indicate that intertidal habitats may function as early-warning systems for climate-driven reorganization, responding to thermal tipping points more abruptly than subtidal systems.

Conclusion 2: Intertidal rockpool communities along the Portuguese coast exhibit pronounced tropicalisation signals, suggesting that highly dynamic coastal habitats act as focal points for early climate-driven restructuring rather than refugia for cold-affinity species.

6.2.3 Integrated Lessons on Community Reorganization

Comparisons between subtidal and intertidal habitats demonstrate that tropicalisation does not follow a uniform trajectory across coastal systems. Instead, ecosystem responses are strongly mediated by local biophysical context, including thermal variability, exposure, and oceanographic buffering. While the observation periods differ (capturing a 6-year "fast tropicalisation" in the subtidal and a 15-year regime shift in the intertidal) both datasets converge on a shared signal of ecosystem degradation.

Across both habitats, a common outcome is the decline of cold-affinity habitat-forming species and the increased prominence of low-structure, opportunistic algae, leading to functional simplification. The expansion of invasive turfs like *S. marchantioides* across both vertical strata suggests a process of "habitat miniaturization," where complex three-dimensional forests are replaced by flatter, less productive assemblages. These integrated findings reinforce the importance of habitat-specific environmental filters: while upwelling may delay the retreat of subtidal forests, the extreme conditions of the intertidal reveal the ultimate vulnerability of these meridional populations to a warming climate.

6.3 Herbivory as a Critical Mechanism for Ecosystem Transition

By evidencing the intensification of herbivory and its selective impact, Chapters 4 and 5 demonstrate that this is a critical biological driver that accelerates functional homogenisation, acting synergistically with climatic stress to accelerate ecosystem transitions.

6.3.1 Trends and Threat of Herbivory

Chapter IV, in particular, provided evidence that herbivory pressure from *Sarpa salpa*, a warm-affinity species, is increasing, representing a key biological threat to marine forests. The landings data revealed an upward trajectory in *Sarpa salpa* catches in the North during higher seawater temperature years, suggesting an increase in its local abundance and latitudinal occupancy shifts driven by climate change. This increased presence and its impact are further corroborated by stomach content analysis, which confirms the broad feeding preference of *S. salpa* for brown seaweeds over other algal groups. It further showed a tendency for specialisation in the consumption of large, habitat-forming kelp, a pattern modulated by canopy availability.

Conclusion 3: The increasing herbivory pressure by *S. salpa*, evidenced by both abundance trends and a general preference for brown seaweeds with a tendency toward habitat-forming (kelp) and specialisation (confirmed by stomach analysis), is a critical biological driver linking warming to the intensification of predation in seaweed communities.

6.3.2 Mechanisms Shaping Selective Impacts on Canopy Species

Chapter V complemented these findings by investigating the underlying mechanisms of selectivity using controlled experiments exclusively with brown canopy seaweed species. These experiments revealed that, even within its preferred feeding group, there is a relative preference for certain species, and that nutritional composition plays a pivotal role in dietary choice. This selective consumption has a profound ecological implication: by targeting specific canopy-forming macroalgae, the herbivore increases the rate of structural loss. In conjunction with climatic stress, this process accelerates community degradation.

Conclusion 4: *S. salpa* herbivory, through its relative preference for certain canopy species (as demonstrated in Chapter V), is a critical biological mechanism that accelerates the loss of structural biomass. This preferential consumption steers the ecosystem toward a simplified, turf-dominated state, driving functional homogenization across regions.

6.4 Ecological and Management Implications

The conclusions of this thesis emphasize that tropicalisation is an ecosystem transition process driven by the interplay of climatic and biological stressors. The loss of habitat-forming seaweeds and the subsequent functional homogenization directly threaten essential ecosystem services, including coastal productivity, biodiversity, and nursery habitats.

Management Implications:

The results of this thesis provide critical data for marine conservation. Management strategies should not only focus on reducing local stressors (e.g., pollution) but also:

- **Integrate herbivory risk** into conservation plans by monitoring and managing populations of key large herbivores.
- **Protect identified "boreal refugia"**, such as areas of persistent upwelling, ensuring these zones continue to provide environmental conditions for the persistence of cold-affinity species and limit grazing pressure from warm-water herbivores.
- **Address habitat miniaturization** by promoting restoration actions that focus on structural complexity.

Summary of the main conclusions:

1. **Active Tropicalisation:** Coastal communities along the Portuguese coast are undergoing rapid tropicalisation, with distinct spatio-temporal trajectories between subtidal and intertidal habitats.
2. **Retraction of Habitat-Formers:** Cold-affinity canopy-forming species (kelps and fucoids) are experiencing significant regional retraction, particularly at their southern distributional limits.
3. **Erosion of Boreal Refugia:** The buffering capacity of the Iberian upwelling is weakening as rising sea temperatures and marine heatwaves override local cooling, compromising traditional thermal refugia.
4. **Intensified Herbivory:** Herbivory pressure from warm-affinity fishes, notably *Sarpa salpa*, is increasing in northern latitudes, acting as a critical biological driver of forest decline.
5. **Selective Consumption and Turf Dominance:** *S. salpa* exhibits a preference for brown seaweeds; this grazing pressure, coupled with canopy availability, accelerates the loss of habitat-forming species and facilitates the transition to turf dominance.
6. **Functional Homogenisation:** The collapse of structurally complex canopies and the proliferation of opportunistic turfs are leading to a widespread homogenisation and "miniaturisation" of coastal habitats.
7. **Threat to Ecosystem Services:** The synergistic interaction of climatic and biological stressors threatens the essential ecosystem services provided by marine forests, including coastal productivity and biodiversity maintenance.
8. **Integrated Conservation Strategies:** Effective management must transcend static protection by integrating herbivory risk and focusing on the resilience of relictual cold-water populations.

6.5 Future Remarks

Future research should build upon the mechanistic foundation established by this thesis by exploring the complexity of tropicalisation impacts in dynamic coastal systems through the following avenues:

1. **Multi-Stressor Experiments:** Conduct manipulative experiments that simulate the synergy of multiple stressors, such as rising temperature, intensified herbivory, and reduced nutrient availability (predicted from the weakening of upwelling regimes), to create more accurate predictive models of marine forest responses.
2. **Genetic Vulnerability and Resilience:** Investigate the genetic variability and acclimatisation capacity of habitat-forming seaweed populations at their southern range edges. This is crucial to determining whether these retracting species possess the adaptive potential to evolve or persist under increasing environmental pressures.
3. **Regional Scale Assessment:** Expand Community Temperature Index (CTI) analysis and herbivory assessments to a broader scale across the Iberian Peninsula to map the progression of tropicalisation and identify regional vulnerability hotspots.
4. **Socio-Economic Dimensions:** Assess the implications of marine forest collapse on small-scale fisheries and other coastal ecosystem services. Translating ecological findings into socio-economic terms is essential to support evidence-based environmental policymaking and stakeholder engagement.

Overall, this thesis demonstrates that temperate marine forests along the Portuguese coast are undergoing a rapid ecological transition. By integrating community-level analyses, experimental approaches, and long-term trends, this work provides a mechanistic understanding of how climate warming and changing trophic dynamics are reshaping these ecosystems. Beyond documenting change, the thesis offers a framework for anticipating future trajectories and informs the conservation strategies that are urgently needed to safeguard the resilience of these vital coastal habitats.

Attachments.

Chapter II

Supplementary Materials

S1. Community temperature index methodology

Species data. Data for each species' occurrence were collected in Ocean Biogeographic Information System (OBIS) (<https://obis.org/>) and Global Biodiversity Information Facility (GBIF) (<https://www.gbif.org/>). A total of 424,286 georeferenced observations were retrieved from OBIS and GBIF for 94 species. From these observations, dubious records, possible misidentifications, and probable introductions were removed based on literature, as well as duplicate records within the environmental variables pixel size (5arcmin). To decrease aggregation in observations, records were randomly removed according to the Nearest Neighbour Index (NNI) and using the R package “spatialEco” (Evans & Murphy, 2022). The modeling dataset for building the models included 4259 for 94 species.

Environmental data. The environmental factors or ecogeographical variables were retrieved from Global Marine Database (Bio-ORACLE - Assis et al., 2016) using the R package ‘sdmpredictors’ (Bosch & Fernandez, 2022). A total of 14 EGVs were downloaded for the present conditions (2000 – 2014). EGVs association was tested through Pearson’s correlation test. Accordingly, ten variables (Table S1) were selected for further analyses based on the lowest correlation ($r < 0.74$). The only exception was the minimum and maximum sea surface temperatures ($r = 0.91$).

Table II-S1 Environmental variables used to assess the global area of each species' occupancy.

Name	Description	Units
Calcite (mean)	Mean concentration of calcite (CaCO ₃)	mol/m ³
Chlorophyll A (minimum)	Minimum concentration of chlorophyll A	mg/m ³
DA (maximum)	Maximum diffuse attenuation coefficient at 490 nm	m ⁻¹
pH	Water acidity	unitless
PAR (maximum)	Maximum photosynthetically available radiation	E/m ² /day
Phosphate	Reactive ortho-phosphate concentration [HPO ₄ ⁻²]	µmol/L
Nitrate	Dissolved inorganic nitrate and nitrate or nitrite concentration	µmol/L
Salinity	Dissolved salt content	PSS
SST (maximum)	Maximum sea surface temperature	°C
SST (minimum)	Minimum sea surface temperature	°C

Ecological niche models

To assess the global area of each species' occupancy, ecological Niche Models models (ENMs) were built for each species. Models were built using the Maximum Entropy approach implemented in the R package "Biomod2" (Thuiller et al., 2016), and using default parameters, with 5 replicates. They were built with 75 % of the data, and the remaining 25 % was used for testing the models. Individual models were evaluated using the Area under the curve (AUC) of the receiver-operating characteristics (ROC) plot (Fielding & Bell, 1997) the True Skill Statistic (TSS). TSS has been demonstrated as highly effective for assessing model performance (Allouche et al., 2006), and therefore, only models with TSS higher than 0.70 were kept for further analysis. To assess the global area of occupancy of each species, continuous models were converted into binary maps using the TSS threshold as it maximizes both the number of presences and absences correctly classified by the model (Vale et al., 2014).

Community Temperature Index analyses

To define the thermal “niche” of each species, the maps of the area of occupancy were used to extract the quantiles of the sea surface temperatures, for each species (Burrows et al., 2019). The median (50th percentile) of temperatures in each species-occupied area was used as the STI (Burrows et al., 2019). The Community Temperature Index (CTI) was calculated for each locality, rocky reefs, and time (all diving campaigns) as the abundance-weighted mean of all STIs (Burrows et al., 2019, 2020), as following:

$$CTI = \frac{\sum_{i=1}^N STI_i \omega_i}{\sum \omega_i}$$

where ω_i is the abundance of each species i and N is the number of species in the community.

Finally, the four processes promoting changes in CTI were assessed following the methodology of McLean et al. (2021), where (i) *tropicalization* is an increase in the abundance of warm-affinity species; (ii) *borealization* is an increase in abundance of cold-affinity species; (iii) *detropicalization* is a decrease in the abundance of warm-affinity species; and (iv) *deborealization* is a decrease in the abundance of cold-affinity species). Here, warm-affinity species are those whose STI is higher the mean of community, and the STI of cold-affinity species is lower than the mean (McLean et al., 2021).

Table II-S2 List of macroalgal taxa with corresponding biomasses measurement (g wet wt per 0.25 m², mean, SD, n = 30) by year in each region across continental Portugal. Macroalgal taxa are listed by morpho-functional groups. Climate affinity (C.A.: I, warm-water; II, cold-water; III, widely distributed).

Taxa	Viana do Castelo		Peniche		Sines		C.A.
	2012	2018	2012	2018	2012	2018	
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	
"Incrustant" group							
<i>Agissea harveyana</i>			0.119±0.394		2.372±4.246	0.026±0.142	III
<i>Codium adhaerens</i>					19.460±54.563	13.497±40.228	I
<i>Colpomenia peregrina</i>				0.043±0.184		0.097±0.534	III
<i>Cutleria adspersa</i>					0.019±0.058	2.738±5.297	I
<i>Peyssonnelia rubra</i>			1.043±3.462				III
<i>Zanardinia typus</i>	1.171±2.464	1.454±2.055	0.522±1.415	1.557±2.745	0.348±0.955	0.663±1.494	I
"Turf" group							
<i>Acrosorium ciliolatum</i>		0.096±0.437	4.761±3.791	3.331±4.129	0.062±0.234	0.95±2.607	III
<i>Acrosorium venulosum</i>					0.003±0.004		III
<i>Aglaothamnion sp.</i>		0.001±0	0.147±0.785	0.039±0.206	0.003±0.013	0.393±1.991	II
<i>Ahnfeltiopsis devoniensis</i>	0.004±0.024			0.006±0.034			III
<i>Anotrichium furcellatum</i>			0±0.002				I
<i>Asparagopsis armata</i>			27.276±40.490	6.845±18.562	1.079±2.304	74.792±97.276	III
<i>Bonnemaisonia asparagoides</i>			0±0.002	0.312±1.676	0.926±2.201	0.207±0.73	III
<i>Bornetia secundiflora</i>			0.195±0.994	0.08±0.282	0.022±0.064	0.026±0.144	I

Taxa	Viana do Castelo		Peniche		Sines		C.A.
	2012	2018	2012	2018	2012	2018	
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	
<i>Callithamnion sp.</i>					0±0.002	0.038±0.147	III
<i>Carpomitra costata</i>			0.011±0.049				III
<i>Carradoriella denudata</i>	1.686±5.014	0.014±0.037		0.039±0.093		0.091±0.332	III
<i>Ceramium ciliatum</i>					0.219±0.918		II
<i>Ceramium echionotum</i>					0.001±0.003		III
<i>Ceramium virgatum</i>			0±0.002				III
<i>Ceramium sp.</i>		0.001±0	0.001±0.003	0.001±0		0.737±2.322	III
<i>Champia parvula</i>				1.134±3.632	0.024±0.093	0.008±0.03	III
<i>Chondracanthus acicularis</i>			0.225±0.608		0.154±0.186	0.284±0.626	III
<i>Chondria dasyphylla</i>				0.323±1.67	0.24±0.409	0.008±0.031	III
<i>Chylocladia verticillata</i>						0.067±0.296	III
<i>Cladophora sp.</i>			0.001±0.003	0.191±0.34	0.265±0.362	0.558±1.38	III
<i>Cladostephus spongiosus</i>		0.001±0.004	0±0.002	0.015±0.082		4.587±11.319	III
<i>Compsothamnion thuioides</i>				0.001±0.007			III
<i>Corallina officinalis</i>	0.025±0.139	12.431±27.991		3.446±5.395	0.022±0.089	5.41±7.359	III
<i>Cryptonemia palmetta</i>		0.099±0.47		0.299±0.967			I
<i>Cryptopleura ramosa</i>	4.37±8.633	4.638±6.791		9.599±11.847	0.033±0.123	2.663±7.664	III
<i>Delesseriaceae</i>				0.002±0.01			III

Taxa	Viana do Castelo		Peniche		Sines		C.A.
	2012	2018	2012	2018	2012	2018	
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	
<i>Dictyota dichotoma</i>	9.726±26.449	11.981±15.915	2.787±3.664	2.586±3.381	3.756±4.77	7.573±21.959	III
<i>Drachiella heterocarpa</i>					0±0.002		II
<i>Drachiella spectabilis</i>			0±0.002				III
<i>Dilsea carnosa</i>	37.722±86.296						III
<i>Ellisolandia elongata</i>		1.645±5.075	0.014±0.059	3.644±9.369	6.004±10.771	0.232±1.234	III
<i>Erythroglossum laciniatum</i>	0.11±0.604		0.017±0.055		0±0.002		III
<i>Gastroclonium ovatum</i>				0.005±0.027			II
<i>Gelidium corneum</i>	0.187±0.521	31.195±71.916	0.006±0.035	14.579±55.594		0.001±0	III
<i>Gigartina pistillata</i>	0.023±0.1						I
<i>Griffithsia sp.</i>					0±0.002		I
<i>Halopithys incurva</i>		0.014±0.079	0.642±2.764	19.747±47.606		22.999±105.214	I
<i>Halopteris filicina</i>				1.522±4.861	0±0.002	0.06±0.211	III
<i>Halopteris scoparia</i>			3.136±6.162	1.928±5.997	7.388±18.292	0.989±2.503	III
<i>Halurus equisetifolius</i>		0.009±0.05	0.03±0.155	0.475±1.537	0.003±0.009	0.071±0.257	II
<i>Heterosiphonia plumosa</i>	0.07±0.383		0.94±2.085	0.068±0.276	0.033±0.127	0.934±3.55	III
<i>Hypoglossum hypoglossoides</i>	0.022±0.084	0.024±0.075	0.006±0.026	0.133±0.263	0±0.002		III
<i>Jania rubens</i>			1.253±3.697	0.153±0.702	0.146±0.332	1.904±4.125	III
<i>Lomentaria clavellosa</i>			0±0.002				III

Taxa	Viana do Castelo		Peniche		Sines		C.A.
	2012	2018	2012	2018	2012	2018	
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	
<i>Lychaete pellucida</i>			0.014±0.037		0.958±2.028	2.569±13.881	I
<i>Myriogramme minuta</i>				0.005±0.027			III
<i>Monosporus pedicellatus</i>			<0.0001±0		0±0.002		III
<i>Nitophyllum punctatum</i>			0.316±0.945	1.513±2.99	0.192±0.8	0.467±1.962	III
<i>Osmundea pinnatifida</i>				0.006±0.025	0.004±0.017	0.385±1.808	III
<i>Phycodrys rubens</i>				0.711±2.706			II
<i>Plocamium cartilagineum</i>	4.444±17.529	1.101±2.132	45.183±59.1	32.127±50.271	13.118±22.747	5.673±11.934	III
<i>Polysiphonia sp.</i>			0.049±0.246	1.192±4.352	1.445±3.596	0.027±0.146	III
<i>Porphyra umbilicallis</i>		0.01±0.047					III
<i>Pterocladiaella capillacea</i>						0.146±0.802	II
<i>Pterosiphonia complanata</i>	0.046±0.237	0.107±0.556	5.832±7.997	11.555±16.803	0.167±0.351	1.91±3.24	III
<i>Pterothamnion plumula</i>				0.072±0.356			II
<i>Pterothamnion crispum</i>				<0.0001±0	<0.0001±0	0.059±0.27	I
<i>Pterothamnion sp.</i>				<0.0001±0	<0.0001±0		III
<i>Rhodophyllis sp.</i>			0.792±2.738				III
<i>Rhodymenia ardissoni</i>						0.041±0.172	III
<i>Rhodymenia holmesii</i>	4.565±10.263	3.029±7.515	0.614±1.781		0.002±0.011		III
<i>Schizymenia dubyi</i>		0.682±3.32		0.073±0.26			II

Taxa	Viana do Castelo		Peniche		Sines		C.A.
	2012	2018	2012	2018	2012	2018	
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	
<i>Scinaia furcellata</i>	0.003±0.015	0.013±0.073					III
<i>Sphaerococcus coronopifolius</i>				3.346±10.123	18.894±40.404	49.645±115.806	III
<i>Stenogramma interruptum</i>		0.243±0.652					III
<i>Symphiocladia marchantioides</i>				42.801±57.524			III
<i>Symphyocradiella parasitica</i>		0.001±0.006	0±0.002	0.115±0.317			I
<i>Taonia atomaria</i>		11.749±30.071		0.87±2.663	0.087±0.380	9.846±29.281	III
<i>Thuretella schousboei</i>						0.477±2.294	III
<i>Ulva lactuca</i>						3.099±5.161	III
<i>Ulva rigida</i>		0.049±0.109	0.005±0.020	0.588±2.835			III
<i>Ulva sp.</i>			0.01±0.048		0.009±0.037		III
<i>Vertebrata fucoides</i>				0.004±0.022			III
<i>Xiphosiphonia ardreana</i>	0.001±0.007	0.021±0.058	0.396±0.835	1.01±2.597	0.002±0.004	0.025±0.124	I
<i>Xiphosiphonia pennata</i>		0.004±0.023	0±0.002	0.481±1.774		0.005±0.029	III
<i>Womersleyella setacea</i>				0.024±0.133		0.426±1.139	III
"Subcanopy" group							
<i>Calliblepharis ciliata</i>		0.799±3.584	0.006±0.035				II
<i>Calliblepharis jubata</i>						0.009±0.048	II
<i>Chondracanthus teedei</i>	2.747±11.492	0.680±2.840		0.227±1.195	0.033±0.181	0.333±1.223	III

	Viana do Castelo		Peniche		Sines		C.A.
	2012	2018	2012	2018	2012	2018	
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	
<i>Chondria coerulescens</i>	0.056±0.299						III
<i>Chondrus crispus</i>	6.175±21.794	0.019±0.069		0.007±0.04	0.004±0.020		II
<i>Codium tomentosum</i>		0.003±0.016		8.058±18.134	0.521±1.688	7.795±15.052	III
<i>Desmarestia ligulata</i>		0.473±2.593					II
<i>Dictyopteris polypodioides</i>	0.448±2.186	0.453±1.238			0.009±0.041	0.016±0.086	III
<i>Gynmogongrus crenulatus</i>	0.420±2.302	0.092±0.282	0.006±0.035		0.003±0.016	0.003±0.018	I
<i>Liagora distenta</i>						0.584±3.001	I
<i>Liagora viscida</i>					0.002±0.013	4.963±17.301	I
<i>Phyllophora crispa</i>	2.569±6.678	0.532±1.400	2.099±6.781	4.039±9.403	0.058±0.186	0.106±0.382	II
<i>Rhodophyllis divaricata</i>					0.004±0.024		I
<i>Rhodymenia pseudopalmata</i>	0.543±2.072	24.225±51.215	0.072±0.378	0.141±0.548			III
<i>Spatoglossum solieri</i>					0.064±0.241		III
"Canopy" group							
<i>Gongolaria baccata</i>			1.007±3.055				II
<i>Gongolaria nodicaulis</i>		1.042±5.168		5.459±11.9	0.123±0.588	0.018±0.099	I
<i>Laminaria hyperborea</i>	175.850±198.644	14.738±58.998					II
<i>Laminaria ochroleuca</i>				7.582±29.136			II
<i>Phyllariopsis brevipes</i>		5.209±6.799	0.045±0.149		0.004±0.015	13.493±32.891	I

Taxa	Viana do Castelo		Peniche		Sines		C.A.
	2012	2018	2012	2018	2012	2018	
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	
<i>Saccorhiza polyschides</i>	21.612±56.062	164.673±187.443	0.026±0.142	0.743±1.828			II
<i>Sargassum muticum</i>		0.344±1.885		0.875±4.792		0.35±1.404	I

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Attachments.

Chapter III

Supplementary Materials

S1. STI estimation

Species Temperature Indices (STI) were calculated using a model-based approach (Bowler et al., 2017). For each taxa, species distribution models were built (SDMs) using MAXENT with environmental predictors (salinity, pH, nitrate, photosynthetically active radiation, and maximum/minimum sea surface temperature); Models were built using the Maximum Entropy approach implemented in the R package “Biomod2” (Thuiller et al., 2016), and using default parameters, with 5 replicates. They were built with 75 % of the data, and the remaining 25 % was used for testing the models. Individual models were evaluated using the Area under the curve (AUC) of the receiver-operating characteristics (ROC) plot (Fielding & Bell, 1997) the True Skill Statistic (TSS). TSS has been demonstrated as highly effective for assessing model performance (Allouche et al., 2006), and therefore, only models with TSS higher than 0.70 were kept for further analysis. To assess the global area of occupancy of each species, continuous models were converted into binary maps using the TSS threshold as it maximizes both the number of presences and absences correctly classified by the model (Vale et al., 2014).

Once the ensemble model were fitted, habitat suitability across the study area using environmental similarity (Mahalanobis distance) was calculated. Later mean sea surface temperature (SST) values were extracted at locations with different suitability levels; and finally the STI was defined as the mean SST at the 90th percentile of habitat suitability predictions. This model-based STI represents the best thermal conditions of suitable habitats, accounting for multiple environmental factors and spatial biases in occurrence data

Table III-S1 List of macroalgal taxa with corresponding biomass measurements (g dry wt per 0.09 m² quadrat; 30 × 30 cm, mean ± SD, *n* = 108) by year and region across intertidal rockpools along the continental Portuguese coast.

Taxa	North		Centre	
	2006	2021	2006	2021
	mean±SD	mean±SD	mean±SD	mean±SD
<i>Acrosorium venulosum</i>	0.03 ± 0.17	0.01 ± 0.08	0.03 ± 0.17	0.08 ± 0.41
<i>Aglaothamnion</i> spp.	0 ± 0	0 ± 0	0.01 ± 0.02	0.04 ± 0.13
<i>Ahnfeltia plicata</i>	0.28 ± 1.33	0.4 ± 2.82	0 ± 0	0 ± 0
<i>Ahnfeltiopsis devoniensis</i>	0.01 ± 0.05	0.06 ± 0.26	0 ± 0.01	0 ± 0.01
<i>Antithamnionella floccosa</i>	0 ± 0	0 ± 0	0 ± 0	0.02 ± 0.11
<i>Apoglossum ruscifolium</i>	0.01 ± 0.12	0 ± 0	0.02 ± 0.03	0 ± 0
<i>Asparagopsis armata</i>	0 ± 0	0.04 ± 0.36	1.52 ± 2.92	1.51 ± 2.67
<i>Bifurcaria bifurcata</i>	3.2 ± 8.61	1.25 ± 3.84	9.87 ± 12.71	8.41 ± 11.46
<i>Bornetia secundiflora</i>	0 ± 0.01	0 ± 0	0 ± 0.01	0 ± 0
<i>Calliblepharis ciliata</i>	0 ± 0	0.01 ± 0.07	0 ± 0	0 ± 0.01
<i>Calliblepharis jubata</i>	0.13 ± 0.55	0.1 ± 0.41	0 ± 0.01	0 ± 0
<i>Callithamnion tetricum</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Callithamnion tetragonum</i>	0 ± 0.02	0.01 ± 0.08	0.01 ± 0.02	0 ± 0
<i>Carradoriella denudata</i>	0.02 ± 0.02	0.03 ± 0.14	0.01 ± 0.02	0 ± 0
<i>Carradoriella elongata</i>	0 ± 0.01	0 ± 0	0 ± 0	0 ± 0
<i>Caulacanthus ustulatus</i>	0.01 ± 0.07	0.01 ± 0.11	0 ± 0	0 ± 0.01
<i>Ceramium</i> spp.	0.46 ± 0.96	0.02 ± 0.12	0.15 ± 0.51	0.22 ± 0.55
<i>Ceramium botryocarpum</i>	0.03 ± 0.02	0 ± 0	0.03 ± 0.02	0 ± 0
<i>Ceramium ciliatum</i>	0 ± 0.01	0 ± 0	0.02 ± 0.02	0.3 ± 0.66
<i>Ceramium echionotum</i>	0.01 ± 0.02	0.37 ± 1.06	0.02 ± 0.03	0.03 ± 0.13
<i>Ceramium nodulosum</i>	0.02 ± 0.11	0 ± 0	0 ± 0	0 ± 0
<i>Ceramium pallidum</i>	0.02 ± 0.02	0 ± 0	0.01 ± 0.02	0 ± 0
<i>Ceramium secundatum</i>	0 ± 0.01	0.63 ± 1.92	0 ± 0.01	0.05 ± 0.21
<i>Chaetomorpha linum</i>	0.02 ± 0.02	0 ± 0	0.02 ± 0.03	0 ± 0.01
<i>Champia parvula</i>	0 ± 0.01	0 ± 0	0.03 ± 0.18	0.02 ± 0.17
<i>Chondracanthus acicularis</i>	0.24 ± 1.82	0.47 ± 1.71	0.01 ± 0.05	0 ± 0
<i>Chondracanthus teedei</i>	0.96 ± 2.9	0.43 ± 1.24	0.16 ± 0.3	0.05 ± 0.15
<i>Chondria dasyphylla</i>	0.03 ± 0.09	0.03 ± 0.13	0.01 ± 0.02	0 ± 0
<i>Chondrus crispus</i>	2.63 ± 6.09	0.58 ± 2.72	0 ± 0	0 ± 0
<i>Cladophora</i> spp.	0.07 ± 0.27	0.01 ± 0.05	0.53 ± 0.96	0.26 ± 1.97
<i>Cladostephus spongiosus</i>	0 ± 0.02	0 ± 0.01	0.09 ± 0.19	0.12 ± 0.85
<i>Codium tomentosum</i>	0.14 ± 1.23	0.38 ± 1.73	1.68 ± 3.69	0.31 ± 1.26
<i>Colpomenia peregrina</i>	0 ± 0	0 ± 0	0 ± 0	0.07 ± 0.15
<i>Crouania attenuata</i>	0 ± 0	0 ± 0	0 ± 0.01	0 ± 0
<i>Cryptopleura ramosa</i>	0.03 ± 0.13	0.33 ± 1.19	0.16 ± 0.34	0.13 ± 0.66

Taxa	North		Centre	
	2006	2021	2006	2021
	mean±SD	mean±SD	mean±SD	mean±SD
<i>Deltalsia parasitica</i>	0 ± 0	0 ± 0.01	0 ± 0	0.01 ± 0.06
<i>Desmarestia aculeata</i>	0 ± 0	0.04 ± 0.32	0 ± 0	0 ± 0
<i>Dictyopteris polypodioides</i>	0.26 ± 1.77	0.33 ± 1.64	0.01 ± 0.04	0 ± 0.02
<i>Dictyota dichotoma</i>	0.01 ± 0.03	0.03 ± 0.13	0.01 ± 0.03	0.14 ± 0.58
<i>Ectocarpales</i>	0 ± 0	0 ± 0	0 ± 0	0.02 ± 0.11
<i>Ericaria selaginoides</i>	0 ± 0	0 ± 0	0.34 ± 0.79	3.31 ± 7.36
<i>Erythroglossum laciniatum</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Fucus serratus</i>	0.03 ± 0.17	0 ± 0	0 ± 0	0 ± 0
<i>Gastroclonium ovatum</i>	0.01 ± 0.05	0 ± 0.01	0 ± 0.02	0 ± 0.02
<i>Gelidium corneum</i>	0.24 ± 0.54	0.19 ± 1.32	0 ± 0.01	0.01 ± 0.02
<i>Gelidium pulchellum</i>	0.01 ± 0.04	0 ± 0.03	0 ± 0	0 ± 0.05
<i>Gigartina pistillata</i>	0.07 ± 0.26	0.02 ± 0.11	0.01 ± 0.09	0 ± 0
<i>Gongolaria baccata</i>	0.23 ± 1.91	0.29 ± 1.53	3.65 ± 7.39	2.78 ± 7.74
<i>Gracilaria gracilis</i>	0 ± 0	0.01 ± 0.09	0 ± 0	0 ± 0
<i>Gracilaria verrucosa</i>	0 ± 0	0.01 ± 0.05	0 ± 0	0 ± 0
<i>Grateloupia filicina</i>	0.02 ± 0.07	0 ± 0	0 ± 0	0 ± 0
<i>Grateloupia turuturu</i>	0.91 ± 2.23	4.53 ± 16.43	0 ± 0	0 ± 0
<i>Gymnogongrus crenulatus</i>	0.09 ± 0.44	0.08 ± 0.27	0 ± 0	0 ± 0
<i>Halopteris filicina</i>	0.03 ± 0.27	0 ± 0.02	1.97 ± 3.8	2.62 ± 5.6
<i>Halurus equisetifolius</i>	0 ± 0.01	0.01 ± 0.06	0.02 ± 0.06	0 ± 0.01
<i>Heterosiphonia plumosa</i>	0.01 ± 0.02	0.01 ± 0.03	0.03 ± 0.13	0 ± 0
<i>Hypoglossum hypoglossoides</i>	0 ± 0	0.01 ± 0.05	0 ± 0	0.01 ± 0.02
<i>Laminaria ochroleuca</i>	0.07 ± 0.54	0.07 ± 0.41	0 ± 0	0 ± 0
<i>Leathesia difformis</i>	0 ± 0	0 ± 0	0 ± 0.01	0 ± 0
<i>Lomentaria articulata</i>	0.01 ± 0.02	0 ± 0.02	0 ± 0.01	0 ± 0
<i>Mastocarpus stellatus</i>	0.2 ± 1.24	0 ± 0	0 ± 0	0 ± 0
<i>Metacallophyllis laciniata</i>	0 ± 0.03	0 ± 0	0 ± 0	0 ± 0
<i>Monosporus pedicellatus</i>	0 ± 0.01	0 ± 0	0 ± 0.01	0 ± 0
<i>Nitophyllum punctatum</i>	0 ± 0	0.01 ± 0.06	0.04 ± 0.09	0.01 ± 0.03
<i>Ophidocladus simpliciusculus</i>	0.02 ± 0.18	0 ± 0	0.02 ± 0.08	0 ± 0
<i>Osmundea obtusa</i>	0 ± 0.01	0 ± 0	0.07 ± 0.23	0 ± 0
<i>Osmundea osmunda</i>	0.06 ± 0.16	0 ± 0.03	0.02 ± 0.04	0 ± 0
<i>Osmundea pinnatifida</i>	0.01 ± 0.02	0.07 ± 0.39	0.04 ± 0.07	0.02 ± 0.08
<i>Osmundea truncata</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0.01
<i>Padina pavonica</i>	0 ± 0	0 ± 0	0 ± 0	0.06 ± 0.37
<i>Phyllariopsis brevipes</i>	0 ± 0	0.01 ± 0.07	0 ± 0	0 ± 0
<i>Phyllophora crispa</i>	0 ± 0	0.15 ± 1.44	0 ± 0.04	0 ± 0
<i>Plocamium cartilagineum</i>	0.01 ± 0.03	0.11 ± 0.69	0.31 ± 0.91	0.11 ± 0.54
<i>Polysiphonia devoniensis</i>	0 ± 0	0 ± 0	0.01 ± 0.02	0 ± 0
<i>Polysiphonia opaca</i>	0.01 ± 0.02	0 ± 0	0.01 ± 0.02	0 ± 0
<i>Polysiphonia spp.</i>	0.07 ± 0.27	0 ± 0	0 ± 0	0 ± 0.01

Taxa	North		Centre	
	2006	2021	2006	2021
	mean±SD	mean±SD	mean±SD	mean±SD
<i>Pterocladia capillacea</i>	0.12 ± 1.12	0.31 ± 2.05	0 ± 0.01	0.01 ± 0.02
<i>Pterosiphonia complanata</i>	0.06 ± 0.19	0.59 ± 3.32	0.78 ± 2.41	0.6 ± 1.62
<i>Pterothamnion plumula</i>	0 ± 0	0 ± 0	0 ± 0.01	0 ± 0
<i>Rhizoclonium tortuosum</i>	0 ± 0	0 ± 0	0 ± 0	0.07 ± 0.35
<i>Rhodothamniella floridula</i>	0.05 ± 0.48	0 ± 0	0.03 ± 0.11	0 ± 0
<i>Rhodymenia holmesii</i>	0 ± 0	0.03 ± 0.27	0 ± 0	0 ± 0.01
<i>Rhodymenia pseudopalmata</i>	0.04 ± 0.27	0 ± 0.02	0 ± 0.01	0 ± 0
<i>Saccharina latissima</i>	0.01 ± 0.08	0 ± 0.03	0 ± 0	0 ± 0
<i>Saccorhiza polyschides</i>	0.01 ± 0.06	4.08 ± 11.82	0.01 ± 0.02	0.54 ± 3.35
<i>Sargassum muticum</i>	27.61 ± 55.74	0.31 ± 1.51	0.05 ± 0.29	0.11 ± 0.66
<i>Schizymenia dubyi</i>	0 ± 0.01	0.13 ± 1.37	0 ± 0	0 ± 0
<i>Scinaia furcellata</i>	0 ± 0.02	0.01 ± 0.07	0 ± 0.01	0.01 ± 0.07
<i>Symphyocladia marchantioides</i>	0 ± 0	0 ± 0	0 ± 0	2.88 ± 4.97
<i>Sphacelaria tribuloides</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0.03
<i>Taonia atomaria</i>	0 ± 0	0 ± 0	0 ± 0.04	0.04 ± 0.28
<i>Ulva clathrata</i>	0 ± 0	0.01 ± 0.08	0 ± 0	0.36 ± 1.17
<i>Ulva compressa</i>	0 ± 0	0.02 ± 0.24	0 ± 0	0.2 ± 1.93
<i>Ulva crinita</i>	0.16 ± 0.61	0 ± 0	0.01 ± 0.02	0 ± 0
<i>Ulva</i> spp.	1.89 ± 2.08	4.59 ± 5.25	2.64 ± 3.18	2.07 ± 7.31
<i>Ulvaria obscura</i>	0.01 ± 0.06	0 ± 0.03	0.02 ± 0.18	0.99 ± 1.84
<i>Vertebrata byssoides</i>	0 ± 0	0 ± 0	0 ± 0	1.48 ± 3.26
<i>Vertebrata fruticulosa</i>	0.01 ± 0.02	0 ± 0	0.42 ± 1.27	0.37 ± 1.51
<i>Vertebrata fucoides</i>	0.02 ± 0.07	0 ± 0	0 ± 0	0.01 ± 0.07
<i>Vertebrata thuyoides</i>	0 ± 0	0 ± 0	0 ± 0	0.02 ± 0.12
<i>Xiphosiphonia ardreana</i>	0.02 ± 0.02	0 ± 0.01	0.04 ± 0.06	0.05 ± 0.17
<i>Xiphosiphonia pennata</i>	0 ± 0.01	0.02 ± 0.21	0 ± 0.02	0.02 ± 0.16

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Attachments.

Chapter IV

Supplementary materials

S1. Supplementary methods

Calculation of Community-Weighted Means (CWM)

To evaluate the **functional composition** of the macroalgal community, we calculated **Community-Weighted Means (CWM)** for selected functional traits. This method integrates **species-specific trait values** with their **relative contributions to total community biomass**, resulting in a weighted average for each trait (de Bello et al., 2021).

The CWM for a given trait T was calculated as:

$$CWM_T = \sum_{i=1}^{\{n\}} p_i \cdot T_i$$

Where:

- p_i is the proportion of biomass of species i in the community (expressed as the relative abundance),
- T_i is the trait value of species i ,
- n is the total number of species in the community.

Given that traits in this study were categorical, the resulting CWM values represent the relative contribution of each trait category to the community biomass and are expressed as percentages.

CWM values were calculated using the `functcomp()` function from the dbFD R package (Laliberté & Legendre, 2010; Laliberté et al., 2014).

Sea Surface Temperature (SST) Data Processing

Sea surface temperature data were collected from the Gulf of Biscay dataset as part of the Coupled Model Intercomparison Project Phase 6 (CMIP6) (Kristiansen et al., 2024). The dataset provides daily potential temperature values at multiple depths, from which the mean, maximum, and minimum temperatures at a depth of –5 meters were extracted. Temperature data were filtered by date and geographic location, focusing specifically on the coastal marine areas adjacent to the fisheries ports studied along mainland Portugal's coast. For each port, temperature values were obtained from the nearest oceanic grid cells (horizontal resolution of $1/12^\circ \times 1/12^\circ$, approximately 8–9 km) representing the sea area immediately offshore, ensuring that the measurements reflect local marine thermal conditions relevant to the habitat of *Sarpa salpa*.

All data processing and extraction were conducted in R (v. 4.3.1) using the following packages:

- **oce** (Kelley & Richards, 2024) – for oceanographic data handling
- **ncdf4** (Pierce, 2024) – for reading and manipulating NetCDF files
- **raster** (Hijmans, 2023) – for spatial raster data processing
- **rgdal** (Bivand et al., 2023) and **sp** (Pebesma & Bivand, 2005; Bivand et al., 2013) – for spatial data transformation and coordinate operations
- **reshape2** (Wickham, 2007) – for data reshaping and reformatting
- **lubridate** (Grolemund & Wickham, 2011) – for handling date-time structures

Time Series Analysis of *S. salpa* Landings

Monthly CPUE series (kg landed per boat) were analysed using autoregressive integrated moving average (ARIMA) models to account for temporal autocorrelation. In the Centre region, where landings were highly heterogeneous, the series was log-transformed prior to analysis to stabilize variance.

To evaluate the influence of sea surface temperature (SST) on landings, we included SST as an external regressor in the ARIMA models, resulting in ARIMAX models. ARIMAX (Autoregressive Integrated Moving Average with eXogenous variables) extends the standard ARIMA framework by incorporating one or more external explanatory variables to account for additional sources of variation beyond the temporal autocorrelation structure. Separate ARIMA/ARIMAX models were fitted for each region to account for differences in temporal patterns. Model identification and selection were guided by analysing the moving average and autocorrelation structure with the autocorrelation (ACF) and partial autocorrelation (PACF) functions, respectively, and both with and without seasonal lags (12 months) for the seasonal component). The time series was made stationary when needed by testing several steps of differentiation and applying the KPSS tests. Competing models were ranked using the Akaike Information Criterion (AIC), and residual adequacy was assessed with the Box–Ljung test.

All analyses were conducted in R (v. 4.3.1) using the following packages:

- **forecast** (Hyndman et al., 2025) for ARIMA/ARIMAX modelling, moving average and autocorrelation components assessed with *acf* and *pacf* functions, detection of non-stationarity with *ndiffs* function. Model diagnostics with *Box.test* function;
- **stats** (R Core Team, 2025) for log-transformations, residual tests, and basic time series functions.
- **ggplot2** (Wickham, 2016) – for data visualization

All R scripts and code snippets used for data processing are available upon request.

Table IV-S1 List of the functional traits considered in the analyses, with their respective descriptions and categories.

Categories	Ecological relevance	Measurement	Category/Description
Pigment	Photosynthetic capacity productivity and blue carbon potential	Qualitative	Red (e.g., <i>Anhfeltiopsis devoniensis</i> , <i>Ophidocladus simpliciusculus</i>) Brown (e.g., <i>Dictyota dichotoma</i> , <i>Laminaria spp.</i>) Green (e.g., <i>Ulva spp.</i> , <i>Codium tomentosum</i>)
Vertical use	Three-level classification of vertical space use adapted from Arenas et al. (2006, "The invasibility of marine algal assemblages: role of functional diversity and identity"). Because the vertical position of algal taxa can vary among communities, species were assigned to one of the three categories based on their most commonly observed position in natural rocky shore assemblages across the surveyed areas.	Qualitative	Canopy 60-80 cm length (e.g., <i>Laminaria spp.</i> , <i>Sacchoriza polyschides</i> , <i>Cystoseira spp.</i>); Subcanopy - ~20 cm length (e.g. <i>Dictyota spp.</i> , <i>Chondrus crispus</i>); Turf – ~5 cm length (e.g., <i>Ceramium spp.</i> , <i>Vertebrata spp.</i>)
Texture	Texture defined by Littler and Littler (1980, "The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model")	Qualitative	Rubbery (e.g., <i>Laminaria spp.</i> , <i>Sacchoriza polyschides</i> , <i>Cystoseira s. l.</i>) Fleshy - Wiry (e.g., <i>Dilsea carnosa</i> , <i>Palmaria palmata</i>) Soft (e.g., <i>Ulva spp.</i> , <i>Dictyota dichotoma</i>)
Functional form (described as the dominant form)	Morpho-functional groups defined by the functional-form adapted model of Littler and Littler (1980, "The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model")	Qualitative	Thick Blade (e.g., <i>Laminaria spp.</i> , <i>Sacchoriza polyschides</i>) Thick Branched (e.g., <i>Cystoseira s. l.</i> , <i>Sargassum spp.</i>) Sheet (e.g. <i>Ulva spp.</i> , <i>Dictyota dichotoma</i>) Filamentous (e.g., <i>Rhodotamelia floricola</i> , <i>Ceramium spp.</i>) Branched (e.g., <i>Osmundea spp.</i> , <i>Sphaerococcus coronopifolius</i> , <i>Gellidium spp.</i>)

Table IV-S2 Mean ($\pm$ SD) biomass (g) of all macroalgal species identified in subtidal surveys across the three regions (North, Centre, and South). **p** indicates presence with biomass < 0.1 g.

Subtidal species	North Mean $\pm$ SD	Centre Mean $\pm$ SD	South Mean $\pm$ SD
<i>Acrosorium ciliolatum</i>	0.3 $\pm$ 1.2	3.4 $\pm$ 5.0	0.5 $\pm$ 1.2
<i>Aglaothamnion</i> spp.			
<i>Ahnfeltiopsis devoniensis</i>		0.1 $\pm$ 0.2	p
<i>Asparagopsis armata</i>		4.7 $\pm$ 13.9	70.1 $\pm$ 51.1
<i>Bonnemaisonia asparagoides</i>		p	0.1 $\pm$ 0.3
<i>Bornetia secundiflora</i>			0.1 $\pm$ 0.2
<i>Calliblepharis ciliata</i>	2.8 $\pm$ 8.9	0.2 $\pm$ 0.9	
<i>Calliblepharis jubata</i>	p		
<i>Ceramium ciliatum</i>			0.2 $\pm$ 0.5
<i>Ceramium secundatum</i>		p	
Ceraminales		0.4 $\pm$ 1.0	0.2 $\pm$ 0.6
<i>Champia parvula</i>	p	p	p
<i>Chondracanthus acicularis</i>	p	0.1 $\pm$ 0.4	0.3 $\pm$ 0.7
<i>Chondracanthus teedei</i>	7.4 $\pm$ 16.0	0.7 $\pm$ 1.8	0.5 $\pm$ 0.9
<i>Chondria</i> spp.	0.5 $\pm$ 1.4		0.3 $\pm$ 1.0
<i>Chondrus crispus</i>	4.9 $\pm$ 24.5	0.2 $\pm$ 0.9	
<i>Cladophora</i> spp.	p	p	0.1 $\pm$ 0.2
<i>Cladostephus spongiosus</i>			0.5 $\pm$ 1.0
<i>Codium adhaerens</i>			5.2 $\pm$ 13.2
<i>Codium tomentosum</i>			34.6 $\pm$ 50.1
<i>Codium vermilara</i>			4.8 $\pm$ 16.7
<i>Colpomenia peregrina</i>			p
<i>Cryptonemia crenulata</i>	2.2 $\pm$ 5.1		
<i>Cryptopleura ramosa</i>	6.4 $\pm$ 10.2	3.2 $\pm$ 4.8	3.7 $\pm$ 16.7
<i>Cutleria adspersa</i>			p
<i>Deleteria sanguinea</i>	2.4 $\pm$ 10.6		
<i>Deltalsia parasitica</i>	p		
<i>Desmarestia ligulata</i>	0.3 $\pm$ 1.5	0.2 $\pm$ 0.7	p
<i>Dictyopteris menbranacea</i>	0.1 $\pm$ 0.2	0.4 $\pm$ 0.9	0.8 $\pm$ 4.2
<i>Dictyota dichotoma</i>	6.6 $\pm$ 9.7	6.8 $\pm$ 10.8	2.1 $\pm$ 4.8
<i>Dictyota cyanoloma</i>			2.9 $\pm$ 13.2
<i>Dilsea carnosa</i>	33.1 $\pm$ 80.0		
<i>Drachiella spectabilis</i>	p		
<i>Ericaria selaginoides</i>		1.7 $\pm$ 9.1	0.1 $\pm$ 0.7
<i>Erythroglossum laciniatum</i>			p
<i>Gastroclonium ovatum</i>		p	p
<i>Gellidium corneum</i>	20.4 $\pm$ 65.3	p	
<i>Gymnogongrus crenulatus</i>	2.0 $\pm$ 4.6	0.1 $\pm$ 0.2	
<i>Kallymenia reniformis</i>		p	
<i>Halopithys incurva</i>			0.1 $\pm$ 0.3
<i>Halopteris scoparia</i>		0.6 $\pm$ 1.5	10.3 $\pm$ 13.8
<i>Halurus equisetifolius</i>		p	0.1 $\pm$ 0.5
<i>Heterosiphonia plumosa</i>		2.7 $\pm$ 3.9	0.4 $\pm$ 0.8
<i>Hypoglossum delesseriaceae</i>		p	
<i>Laminaria hyperborea</i>	3.6 $\pm$ 13.6		
<i>Laminaria ochroleuca</i>		199.0 $\pm$ 258.5	
<i>Lomentaria articulata</i>	0.6 $\pm$ 2.3		
<i>Meredithia microphylla</i>		0.6 $\pm$ 1.3	
<i>Metacallophyllis laciniata</i>	0.3 $\pm$ 1.7	3.0 $\pm$ 4.4	0.5 $\pm$ 1.2

Subtidal species	North Mean $\pm$ SD	Centre Mean $\pm$ SD	South Mean $\pm$ SD
<i>Monosporus pedicellatus</i>		<i>p</i>	<i>p</i>
<i>Nitophyllum punctatum</i>		<i>p</i>	0.1 $\pm$ 0.3
<i>Osmundea pinnatifida</i>			0.5 $\pm$ 0.9
<i>Palmaria palmata</i>	2.6 $\pm$ 5.0		
<i>Peyssonnelia coriacea</i>			0.4 $\pm$ 1.9
<i>Peyssonnelia dubyi</i>			0.1 $\pm$ 0.3
<i>Peyssonnelia harveyana</i>			<i>p</i>
<i>Phyllariopsis brevipes</i>	3.5 $\pm$ 6.5	1.2 $\pm$ 2.3	4.3 $\pm$ 8.5
<i>Phyllophora crista</i>	2.8 $\pm$ 5.3	1.5 $\pm$ 2.5	1.0 $\pm$ 2.4
<i>Phyllophora sicula</i>		0.3 $\pm$ 1.6	
<i>Plocamium cartilaginum</i>	2.9 $\pm$ 6.0	7.2 $\pm$ 15.3	3.7 $\pm$ 4.2
<i>Polysiphonia denudata</i>	<i>p</i>	0.2 $\pm$ 0.4	
<i>Polysiphonia elongata</i>	0.5 $\pm$ 2.9		
<i>Pterocladia capillacea</i>	1.0 $\pm$ 3.3		<i>p</i>
<i>Rhodomenia holmesii</i>	2.1 $\pm$ 4.2	0.1 $\pm$ 0.4	
<i>Rhodomenia pseudopalmata</i>	0.1 $\pm$ 0.4	0.2 $\pm$ 1.3	
<i>Rytiphlaea complanata</i>	<i>p</i>	12.5 $\pm$ 24.6	2.8 $\pm$ 3.1
<i>Saccorhiza polyschides</i>	64.4 $\pm$ 90.8	97.0 $\pm$ 149.5	
<i>Sargassum muticum</i>			1.1 $\pm$ 4.7
<i>Sargassum vulgare</i>			0.5 $\pm$ 1.5
<i>Spatoglossum solieri</i>		0.2 $\pm$ 0.9	0.5 $\pm$ 1.6
<i>Sphaerococcus coronopifolius</i>		1.9 $\pm$ 7.8	49.7 $\pm$ 48.3
<i>Sphondylothamnion multifidum</i>		<i>p</i>	
<i>Stenogramma interruptum</i>	27.1 $\pm$ 59.0	<i>p</i>	
<i>Symphiodia marchantioides</i>		1.5 $\pm$ 3.3	6.4 $\pm$ 15.1
<i>Taonia atomaria</i>	0.5 $\pm$ 1.5	0.9 $\pm$ 3.2	1.2 $\pm$ 3.3
<i>Treptacantha usneoides</i>			22.1 $\pm$ 36.6
<i>Ulva spp.</i>	0.3 $\pm$ 1.5	<i>p</i>	1.1 $\pm$ 2.5
<i>Vertebrata spp.</i>	2.4 $\pm$ 10.7	<i>p</i>	<i>p</i>
<i>Xiphosiphonia pennata</i>	<i>p</i>	<i>p</i>	0.1 $\pm$ 0.4
<i>Zanardinia prototypus</i>	<i>p</i>	<i>p</i>	<i>p</i>

Table IV-S3 Relative importance of prey items in the diet of *S. salpa* according to the I_o = frequency occurrence (%) and I_G = gravimetical (%) *epiphytes

Prey	North		Centre		South		Total	
	$I_o(\%)$	$I_G \pm SD(\%)$	$I_o(\%)$	$I_G \pm SD(\%)$	$I_o(\%)$	$I_G \pm SD(\%)$	$I_o(\%)$	$I_G \pm SD(\%)$
<i>Acrosorium ciliolatum</i> *			4.5	0.01 ± 0			1.5	0.01 ± 0
<i>Asparagopsis armata</i>					43.5	3.8 ± 4.7	14.9	3.8 ± 4.7
Ceraminales*			45.5	0.3 ± 0.8	4.3	1 ± 0	16.4	0.4 ± 0.8
<i>Champia parvula</i> *			9.1	0.5 ± 0.5			3	0.5 ± 0.5
<i>Chondracanthus acicularis</i>			36.4	1.8 ± 4.1			11.9	1.8 ± 4.1
<i>Chondria</i> spp.			9.1	5.3 ± 2			3	5.3 ± 2
<i>Cladophora</i> spp.			9.1	4.6 ± 3.2			3	4.6 ± 3
<i>Cladostephus spongiosus</i>			9.1	0.8 ± 0.5			3	0.8 ± 0.5
<i>Codium</i> spp.	13.6	0.4 ± 0.3	54.5	5.1 ± 4.9			22.4	4.1 ± 4.8
Corallinales			27.3	2.2 ± 4.6			9	2.2 ± 4.6
<i>Cryptonemia crenulata</i>	13.6	0.9 ± 1					4.5	0.9 ± 1
<i>Cryptopleura ramosa</i> *	63.6	3.7 ± 5.8	4.5	0.02 ± 0			22.4	3.5 ± 6.1
<i>Cystoseira</i> s.l.			18.2	15.8 ± 3.9	65.2	53.1 ± 36.9	28.4	45.2 ± 36.1
Delesseriaceae*			50	2.7 ± 3.1			16.4	2.7 ± 3.1
<i>Desmarestia ligulata</i>			22.7	4.7 ± 3.8	8.7	2.8 ± 1.1	10.4	4.1 ± 3.3
<i>Dictyopteris</i> spp.	72.7	10.1 ± 10.4					23.9	10.1 ± 10.4
Dictyotales	9.1	0.5 ± 0.2	95.5	26.5 ± 26.3	87	71.7 ± 33.8	64.2	46.6 ± 37.3
Ectocarpaceae*			90.9	22.1 ± 23	4.3	4.2 ± 0	31.3	21.2 ± 22.8
<i>Gellidium</i> spp.	36.4	2.4 ± 2.6	68.2	19.2 ± 23.7			34.3	13.3 ± 20.8
Gigartinales	13.6	4 ± 2	18.2	8.7 ± 3.2			10.4	6.7 ± 3.6
<i>Halopteris</i> spp.			4.5	0.01 ± 0	39.1	2.1 ± 2.2	14.9	1.9 ± 2.2
<i>Hypoglossum hypoglossoides</i>	4.5	0.01 ± 0	36.4	0.1 ± 0.1			13.4	0.1 ± 0.1
kelp	100	85.29 ± 14	27.3	26.6 ± 15.9			41.8	71.1 ± 27.4
<i>Lomentaria articulata</i>	9.1	0.01 ± 0.002	45.5	4.9 ± 8			17.9	4.1 ± 7.6
<i>Metacallophyllis laciniata</i>	4.5	13.4 ± 0					1.5	13.4 ± 0
<i>Osmundea</i> spp.			27.3	1.5 ± 1.6			9	1.5 ± 1.6
<i>Phyllariopsis brevipes</i>					0	3 ± 0	1.4	3 ± 7.9
<i>Plocamium cartilagineum</i>	9.1	0.05 ± 0.04	54.5	1.7 ± 2.6			20.9	1.4 ± 2.5
<i>Pterosiphonia</i> spp.*	18.2	4.5 ± 4.5	81.8	3.7 ± 5.4			32.8	3.9 ± 5.2
<i>Rhodomenia holmesii</i>	36.4	3 ± 3.4	36.4	5.8 ± 3.6			23.9	4.4 ± 3.8
<i>Sargassum muticum</i>			13.6	3.7 ± 2.8			4.5	3.7 ± 3
<i>Scinaia furcellata</i>	13.6	0.5 ± 0					1.5	2.01 ± 0
<i>Taonia atomaria</i>			27.3	4.6 ± 5.6	8.7	9.1 ± 0.8	11.9	5.7 ± 5.2
<i>Ulva</i> spp.	18.2	1.9 ± 0.5	31.8	14 ± 26.7			13.4	11.9 ± 23.9
<i>Vertebrata</i> spp.*	13.6	4.9 ± 9	31.8	0.4 ± 0.7			13.4	2.5 ± 5.8
Total epiphytes	72.1	3.9 ± 5.4	100	27.3 ± 7.8	7.8	0.5 ± 0.7	60	31.2 ± 29.8

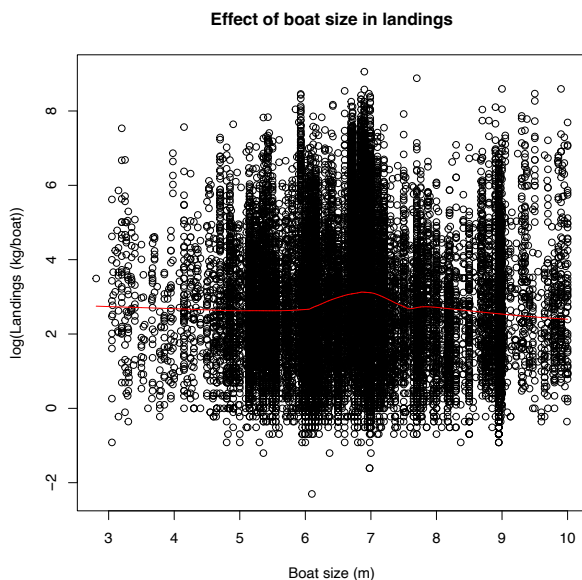


Figure IV-S1 Effect of boat size on landings. Scatterplot of log-transformed landings (kg/boat) as a function of boat size (m). The red line represents a smooth fit from a GAM model. No significant effect of boat size was detected, and thus this variable was discarded from further analyses.

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Attachments.

Chapter V

Supplementary Materials

Table V-S1 Raw macroalgal consumption data for each individual replicate cage (*Sarpa salpa*) across the experimental trials. Consumption was measured as 24-h wet mass loss and standardized to grazer biomass ($\text{g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$). Data are shown for the sequential no-choice phase (where a single algal species was offered per day) and the simultaneous choice phase (where all four species were offered together). Each value represents the consumption rate for one cage ($n = 1$ cage per measurement). Note: A dash (–) indicates that the algal species was not offered during that specific trial day. Cage identifiers (a, b, c, d) correspond to individual experimental mesocosms.

Experiment stage	Cage	Day	Absolute consumption ($\text{g} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$)			
			<i>L. ochroleuca</i>	<i>F. spiralis</i>	<i>S. muticum</i>	<i>C. humilis</i>
No-choice	a	1	74.14	-	-	-
		2	-	29.38	-	-
		3	-	-	23.12	-
		4	-	-	-	20.62
	b	1	-	5.17	-	-
		2	-	-	5.39	-
		3	-	-	-	3.65
		4	0	-	-	-
	c	1	-	-	0	-
		2	-	-	-	0
		3	0	-	-	-
		4	-	0	-	-
	d	1	-	-	-	0.83
		2	0	-	-	-
		3	-	15.64	-	-
		4	-	-	4.86	-
Choice	a	1	73.37	23.96	19.34	3.22
		2	32.42	2.64	0.61	0
	b	1	0	0	2.16	0
		2	5.16	7.53	6.92	2.18
	c	1	2.43	34.94	8.37	0
		2	4.20	8.85	3.29	1.05
	d	1	5.65	16.05	15.01	2.08
		2	2.42	11.04	9.29	1.42

Table V-S2 order of seaweeds given at no-choice

No-choice 24h trial		
Cage	Seaweed species	Date
a	<i>Sargassum muticum</i>	07/12/22
b	<i>Fucus spiralis</i>	
c	<i>Cystoseira humilis</i>	
d	<i>Laminaria ochroleuca</i>	
a	<i>Laminaria ochroleuca</i>	10/12/22
b	<i>Sargassum muticum</i>	
c	<i>Fucus spiralis</i>	
d	<i>Cystoseira humilis</i>	
a	<i>Cystoseira humilis</i>	13/12/22
b	<i>Laminaria ochroleuca</i>	
c	<i>Sargassum muticum</i>	
d	<i>Fucus spiralis</i>	
a	<i>Fucus spiralis</i>	16/12/22
b	<i>Cystoseira humilis</i>	
c	<i>Laminaria ochroleuca</i>	
d	<i>Sargassum muticum</i>	

Table V-S3 Mean ($\pm$ SD) consumption ($\text{g kg}^{-1} 24 \text{ h}^{-1}$) of each algal species in the choice and no-choice trials.

Seaweed species	No-choice Mean $\pm$ SD	Choice Mean $\pm$ SD
<i>Sargassum muticum</i>	8.34 $\pm$ 8.79	8.16 $\pm$ 6.04
<i>Fucus spiralis</i>	12.55 $\pm$ 11.23	13.13 $\pm$ 10.83
<i>Cystoseira humilis</i>	10.07 $\pm$ 8.39	6.96 $\pm$ 14.69
<i>Laminaria ochroleuca</i>	18.53 $\pm$ 32.1	15.71 $\pm$ 23.85

Figures:

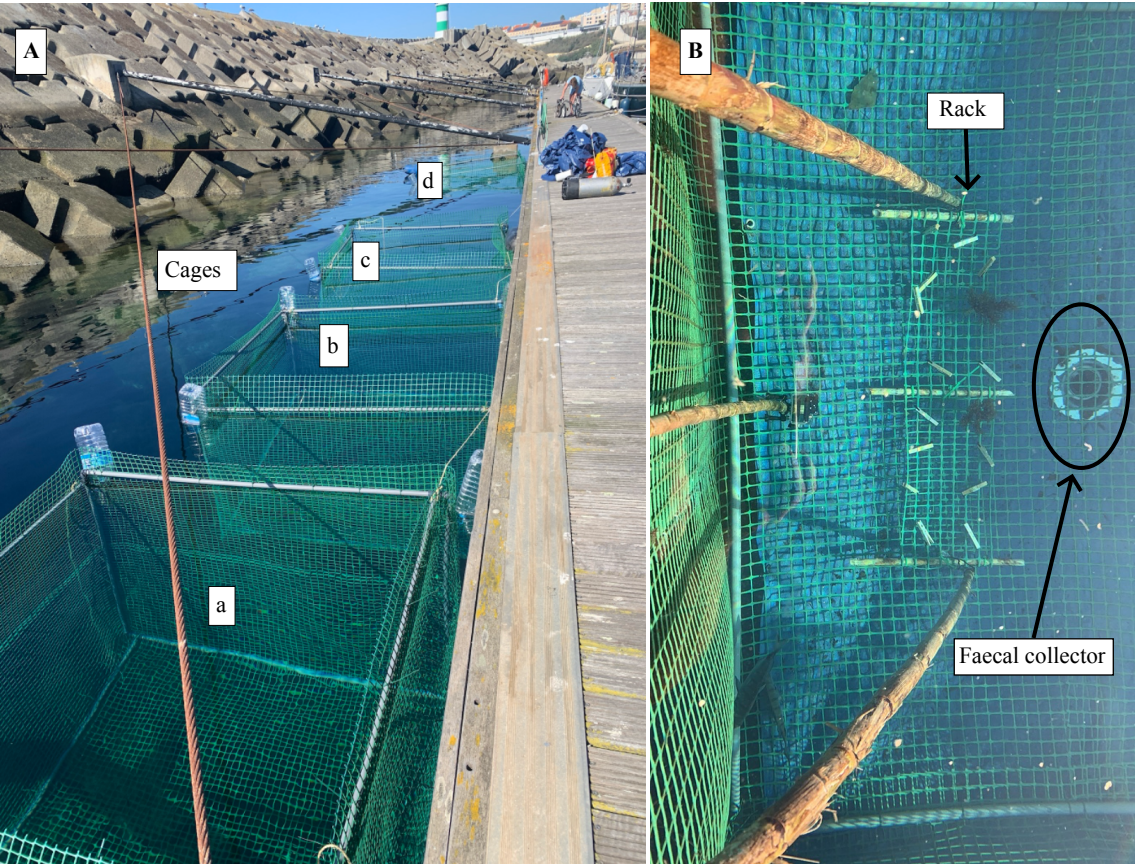


Figure V-S1 Experimental mesocosm setup at the Sines Recreational Marina. (A) Overview of the four submerged cages (a, b, c, d) moored to the sheltered side of the pier. (B) Interior view of a cage showing the polyethylene rack used to hold algal thalli. The mesh floor and the central faecal collection bottle are visible; a section of tarpaulin was placed beneath the mesh to aid in funnelling waste towards the collector.

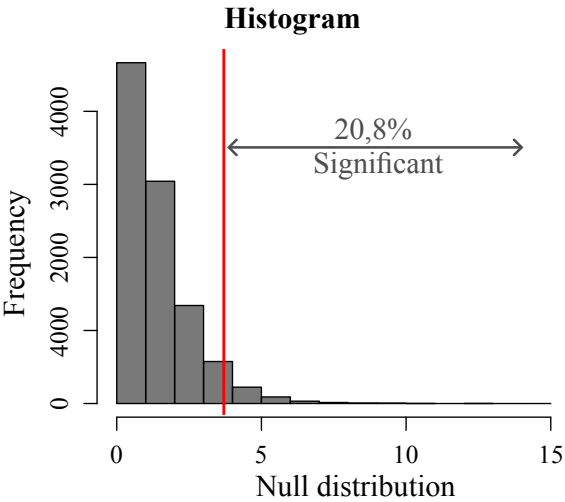


Figure V-S2 Results of the randomization test for the effect of algal species and choice on consumption by *Sarpa salpa*. The histogram shows the distribution of p-values from 1000 simulated datasets under the null hypothesis of no preference. The observed proportion of significant tests ($p < 0.05$) was 20.8%. The red vertical line denotes the conventional alpha significance level of 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

Appendix Chapter V: R Script for ANOVA Randomization

INSTRUCTIONS TO EXECUTE THE CODE FOR THE OBTAINING OF NULL AND REAL F DISTRIBUTIONS FOR THE ANOVA

Main file:

- File "ANOVA_deAzevedo" contains the code necessary to perform the analysis.

Comments: in this file, there are several chunk of codes involving randomization processes lead to the obtention of a null distribution of F and a real distribution of F. This code has been blocked with "#", and the corresponding F distributions obtained by us are provided as csv files (see below). Then, it is possible to execute the analysis with the distributions as we obtained them. This is the main purpose of this supplementary material, as well as checking the correctness of the code.

In order to execute the whole analysis "de novo" it would be necessary to remove all the "#" blocking the code. In this case, the analysis will generate very similar, but not exactly the same, distributions that might change the ultimate results in a meaningless way. This test is not important to be performed.

Software requirement: R version 4.4.x or higher.

Additional files:

- File "Null distribution of F.csv" contains the data from our data randomization leading to the obtention of the null distribution that we employed. **(Available upon request)**

- File "dados.csv" contains the real data from the experiment, and it is loaded in the script in order to perform the ANOVA analysis.

Comments: it is not necessary to load unless the analysis is to be performed "de novo", which seems unnecessary, as detailed above. **(Available upon request)**

- File "Real distribution of F.csv" contains the data from the 1000 ANOVAs performed in the interaction term by randomizations of the real data. **(Available upon request)**

SCRIPT

```
###Generate null distributions based on our experimental design and
perform 1000 ANOVA to test the significance of the interaction
```

```
##Generate null distribution without preference in the choice
```

```
#Number of samples per factor combination (two-way)
#n<-12
```

```
#Create list to store the simulated proportions of each of the 4
prey (a,b,c,d)
#consumed when there is no choice (1) and when there is choice (2)
#a1<-list(mode="vector",length=10000)
#b1<-list(mode="vector",length=10000)
#c1<-list(mode="vector",length=10000)
#d1<-list(mode="vector",length=10000)
#a2<-list(mode="vector",length=10000)
#b2<-list(mode="vector",length=10000)
#c2<-list(mode="vector",length=10000)
#d2<-list(mode="vector",length=10000)
#t1<-list(mode="vector",length=10000)
#t2<-list(mode="vector",length=10000)
#p.a1<-list(mode="vector",length=10000)
#p.b1<-list(mode="vector",length=10000)
#p.c1<-list(mode="vector",length=10000)
#p.d1<-list(mode="vector",length=10000)
#p.a2<-list(mode="vector",length=10000)
#p.b2<-list(mode="vector",length=10000)
#p.c2<-list(mode="vector",length=10000)
#p.d2<-list(mode="vector",length=10000)
```

```
#Simulate those values assuming that there is no preference in the
choice
```

```
#for(i in 1:10000){
#  a1[[i]]<-rbinom(n,25,0.5)
#  b1[[i]]<-rbinom(n,25,0.5)
#  c1[[i]]<-rbinom(n,25,0.5)
#  d1[[i]]<-rbinom(n,25,0.5)
#  a2[[i]]<-rbinom(n,25,0.5)
#  b2[[i]]<-rbinom(n,25,0.5)
#  c2[[i]]<-rbinom(n,25,0.5)
#  d2[[i]]<-rbinom(n,25,0.5)
#  t1[[i]]<-a1[[i]]+b1[[i]]+c1[[i]]+d1[[i]]
#  t2[[i]]<-a2[[i]]+b2[[i]]+c2[[i]]+d2[[i]]
#  p.a1[[i]]<-a1[[i]]/t1[[i]]
#  p.b1[[i]]<-b1[[i]]/t1[[i]]
#  p.c1[[i]]<-c1[[i]]/t1[[i]]
#  p.d1[[i]]<-d1[[i]]/t1[[i]]
#  p.a2[[i]]<-a2[[i]]/t2[[i]]
#  p.b2[[i]]<-b2[[i]]/t2[[i]]
#  p.c2[[i]]<-c2[[i]]/t2[[i]]
#  p.d2[[i]]<-d2[[i]]/t2[[i]]
#}
```

```
#Create vector to store the results of the F value for the
interaction
#null.dist<-rep(NA,10000)
```

```

#Create data frame with the variables needed for the linear model,
leaving room for
#the simulated values of consumption
#data.1<-data.frame(consum=rep(NA,times=n*2*4),
#                    species=rep(c("a","b","c","d"),each=n*2),
#                    choice=rep(c("no_choice","choice"),each=n,times=4))

#for (i in 1:10000) {
#  data.1$consum<-
#  c(p.a1[[i]],p.a2[[i]],p.b1[[i]],p.b2[[i]],p.c1[[i]],p.c2[[i]],
#    p.d1[[i]],p.d2[[i]])
#  fit<-lm(consum~species*choice,data=data.1);
#  null.dist[i]<-anova(fit)$F[3]
#}

#Plot the distribution of null F values
#hist(null.dist)

#Export these values, and import them, so there is no need to
repeat the previous loops
#write.csv(null.dist,"Null distribution of F.csv")

#Import the null distribution data
null.dat<-read.csv("Null distribution of F.csv")[,-1]
head(null.dat)

#Find the F value from the null distribution that determines p <
0.05
output1<-sort(null.dat)[10000*0.95]
length(output1[null.dat>output1])/10000 #this is the alpha = 0.05

#Plot the distribution marking the value determining alpha
hist(null.dat,col="grey")
abline(v=output1,col="red",lwd=2)

##ANOVA analysis
#Load and check data importation
#dat<-read.csv2("dados.csv",stringsAsFactors=T)
#head(dat)
#str(dat)

#Divide in 'no choice' and 'choice'
#dat_NCH<-dat[dat$Stage=="No.Choice",]
#head(dat_NCH)
#dat_CH<-dat[dat$Stage=="Choice",]
#head(dat_CH)

#Reduce the replicate from each urchin to 1 by averaging
#singleUrchin_CH.Cons<-
aggregate(dat_CH$Preference,by=list(dat_CH$Cage,dat_CH$Sp),FUN=mean
)
#dat_CH.3<-
data.frame(Cage=singleUrchin_CH.Cons$Group.1,Sp=singleUrchin_CH.Con
s$Group.2,choice=rep("choice",16),consumption=singleUrchin_CH.Cons$
x)

```

```
#Rearrange data to fit the data with choice with those with non-
choice in the same data set
#calculate the non-choice data
#p.abcd<-list(mode="vector",length=1000)
#for (i in 1:1000){
#  a<-sample(dat_NCH[dat_NCH$Sp=="S.
muticum","Consumption"],size=4,replace=T)
#  b<-sample(dat_NCH[dat_NCH$Sp=="F.
spiralis","Consumption"],size=4,replace=T)
#  c<-sample(dat_NCH[dat_NCH$Sp=="C.
humilllis","Consumption"],size=4,replace=T)
#  d<-sample(dat_NCH[dat_NCH$Sp=="L.
ochroleuca","Consumption"],size=4,replace=T)
#  p.a<-a/(a+b+c+d)
#  p.b<-b/(a+b+c+d)
#  p.c<-c/(a+b+c+d)
#  p.d<-d/(a+b+c+d)
#  p.abcd[[i]]<-c(p.a,p.b,p.c,p.d)
#}

#Create the final data frames of choice and no choice to join. But
leaving room for the randomized #p.abcd data in the "no choice"
#NC<-data.frame(Preference=rep(NA,16),Sp=rep(c("S. muticum","F.
spiralis","C. humilis","L.
ochroleuca"),each=4),Stage=rep("No.choice",times=16))
#Rearrange the choice data to fit with the previous data frame
#C<-
data.frame(Preference=dat_CH$Preference,Sp=dat_CH$Sp,Stage=dat_CH$S
tage)
#newdata<-rbind(C,NC)

#Loop the linear model to the real data
#real.dist<-rep(NA,1000)
#for (i in 1:1000){
#newdata$Preference<-p.abcd[[i]]
#fit2<-lm(Preference~Sp*Stage,data=newdata);
#real.dist[i]<-anova(fit2)$F[3]
#}
#Plot the distribution of real F values
#hist(real.dist)
#Export these values, and import them, so there is no need to
repeat the previous loops
#write.csv(real.dist,"Real distribution of F.csv")
real.dat<-read.csv("Real distribution of F.csv")[,-1]
head(real.dat)
#How many of the real F calculated are significant with the F value
determinin alpha = 0.05, calculated for the null distribution?
length(which(real.dat>=3.668805)) #204
#204/1000 = 0.204 #(20.4% of real F values are higher than the one
defining the alpha = 0.05). So, 20.4 % of significant results (take
into account that here we are analyzing the F of the interaction
only)
#Check it graphically
hist(real.dat,col="grey",breaks=100,main="",xlab="F values")
abline(v=output1,col="red",lwd=2)
text(x=100,y=500,"20.4%")
arrows(x0=100,y0=480,x1=5,y1=480,lwd=2,length=0.1)
arrows(x0=100,y0=480,x1=180,y1=480,lwd=2,length=0.1)
```