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Ecological Impact of the Invasive Algal Species *Rugulopteryx okamurae* in the Atlantic, South-west Portugal

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Abstract

Invasive alien species (IAS) are major drivers of ecosystem change, negatively impacting native communities and contributing to global biodiversity loss. The brown macroalga *Rugulopteryx okamurae*, native to the northwest Pacific, was introduced to the Mediterranean in 2002 and has become highly invasive since 2015, spreading from the Strait of Gibraltar to the northeastern Atlantic. Its rapid proliferation and competitive exclusion of other macroalgae has transformed shallow-water benthic habitats, posing significant challenges for benthic organisms. This study investigates the ecological impacts of *R. okamurae* on the benthic community in Sagres, Portugal, where the species was first documented in 2021. Using pre-invasion data from the MARSW project, collected in 2018, we conducted a comparative analysis to assess changes in benthic community composition and biodiversity following the alga's establishment. Species abundances for demersal fish, cryptic fish, macroinvertebrates and algae were recorded through scientific diving, conducting an underwater visual census (UVC) combined with the quadrat technique. Biodiversity indices were calculated to complement PERMANOVA results, providing a comprehensive analysis of ecosystem changes. Results revealed significant alterations in species composition and declining levels of biodiversity, prompted by the loss of functional macroalgae diversity. The change in biodiversity among demersal fish was driven by the dominance of *C. julis* and *C. exoletus*, whereas cryptic fish and macroinvertebrates were more strongly affected by declines in species richness. *R. okamurae* demonstrated invasive behaviour that exceeded that of other introduced species, underscoring the necessity for long-term monitoring and targeted management strategies to mitigate the alga’s detrimental impacts on marine ecosystems.

Keywords *Rugulopteryx okamurae*, Invasive Alien Species, Ecological Impact, Benthic Community Composition, PNSACV, Sagres

Resumo

As espécies exóticas invasoras (EEI) são um importante motor de mudança dos ecossistemas, com um impacto significativo nos ecossistemas afectados e contribuindo para a perda global de biodiversidade. A sua capacidade de se estabelecerem e prosperarem com sucesso em novos ambientes conduz frequentemente a alterações dramáticas nas comunidades locais, uma vez que ultrapassam as espécies nativas e perturbam os processos essenciais dos ecossistemas. Os ecossistemas marinhos são particularmente vulneráveis às EEI devido à sua natureza interligada, que facilita a propagação rápida e muitas vezes discreta dos invasores. As actividades antropogénicas, como o comércio mundial e a aquicultura, são vias fundamentais para a introdução de EEI marinhas, incluindo as macroalgas, que representam mais de 40% das invasões marinhas na Europa. A sua introdução pode conduzir à perda de biodiversidade, à homogeneização dos habitats e a perturbações significativas da estrutura e função dos ecossistemas.

A macroalga castanha *Rugulopteryx okamurae*, originária do noroeste do Pacífico, é uma das espécies invasoras mais preocupantes. Inicialmente introduzida no Mediterrâneo em 2002, não mostrou qualquer comportamento invasivo durante mais de uma década. No entanto, desde 2016, quando a alga foi detectada pela primeira vez em grandes quantidades em ambos os lados do Estreito de Gibraltar, espalhou-se extensivamente pelo nordeste do Oceano Atlântico, transformando as comunidades bentónicas de águas pouco profundas e causando impactos significativos nos organismos bentónicos. O sucesso da invasão da alga é atribuído principalmente à sua capacidade de propagação vegetativa, à sua reprodução assexuada e às suas defesas químicas contra outras espécies. A proliferação de *R. okamurae* tem o potencial de alterar drasticamente as comunidades bentónicas locais, levando a uma perda generalizada de biodiversidade e a alterações na estrutura do ecossistema.

O presente estudo tem como objetivo avaliar o impacto ecológico de *R. okamurae* na comunidade bentónica da região de Sagres do Parque Natural do Sudoeste Alentejano e Costa Vicentina (PNSACV), incidindo na zona de Proteção Parcial I do Martinhal e na área de Proteção Complementar adjacente. Esta área, caracterizada pela convergência das águas do Mediterrâneo e do Atlântico e por afloramentos localizados, suporta uma comunidade marinha rica e diversificada. No entanto, a abundância de fundos rochosos costeiros altamente complexos e os picos ocasionais de nutrientes também parecem favorecer o estabelecimento de *R. okamurae*. Os dados pré-invasão do projeto MARSW, recolhidos em 2018, que

forneceram informações sobre as comunidades marinhas no PNSACV, foram utilizados como linha de base para avaliar as alterações após a invasão. Para avaliar as contribuições de diferentes componentes da comunidade bentónica, examinámos separadamente peixes crípticos, peixes demersais, macroinvertebrados e grupos funcionais de algas em quatro locais com dois níveis de proteção diferentes. A amostragem seguiu uma abordagem sistemática, com a realização de censos visuais por mergulho (CVM) ao longo de um transecto para examinar espécies móveis como os peixes, combinado com foto-quadrados para avaliar macroinvertebrados bentónicos e algas. Estes métodos foram implementados de forma a assegurar a coerência metodológica com o projeto MARSW. Os mergulhadores registaram a abundância das espécies ao longo do transecto e no interior dos quadrantes e tiraram fotografias para estimar a percentagem de cobertura dos grupos de algas, incluindo *R. okamurae*. Os dados recolhidos foram processados e analisados separadamente pelos níveis individuais da comunidade, utilizando a Análise de Variância Multivariada Permutacional (PERMANOVA). Para garantir uma análise abrangente das alterações do ecossistema, foram calculadas métricas de biodiversidade, incluindo a riqueza de espécies, o índice de diversidade de Shannon-Wiener e o índice de uniformidade de Pielou, a partir das abundâncias observadas.

Os nossos resultados revelaram que a *R. okamurae* se tornou a espécie dominante até 2024, cobrindo uma média de 92,4% do fundo do mar estudado. A presença da alga invasora causou uma mudança significativa na composição da comunidade em todos os grupos biológicos avaliados, associada a perdas substanciais na biodiversidade global. As alterações ecológicas observadas assemelham-se fortemente às caraterísticas de uma mudança de regime, tal como definida por Scheffer et al. (2001), marcada pelo desaparecimento de vários grupos funcionais de algas e por um declínio significativo dos restantes. Esta mudança resultou numa maior homogeneização do habitat no local de estudo. A dominância de *R. okamurae* parece conduzir à exclusão competitiva através da ocupação espacial e de mecanismos de defesa altamente eficientes das algas (por exemplo, metabolitos secundários inibidores do crescimento). Uma vez que os habitats bentónicos são cruciais para o suporte da vida marinha, fornecendo serviços essenciais como abrigo e alimento, espera-se que a redução da heterogeneidade do habitat reduza a riqueza de espécies e a diversidade funcional, alterando assim os processos do ecossistema. Embora a arquitetura estrutural de *R. okamurae* possa proporcionar habitats alternativos para certas espécies, é evidente que estes benefícios não compensam os impactos negativos mais vastos.

Em resultado da invasão, duas espécies de peixes demersais, *Coris julis* e *Centrolabrus exoletus*, registaram um aumento das suas densidades, potencialmente devido ao aumento da estrutura do habitat e da diversidade das assembleias epifaunais proporcionadas por *R. okamurae*. O aumento destas duas espécies explica as alterações observadas na equidade das espécies e realça a complexidade das respostas dos ecossistemas, uma vez que alguns organismos podem beneficiar temporariamente do habitat alterado. Em contrapartida, *Diplodus vulgaris* registou um declínio acentuado da densidade, provavelmente devido ao aumento da competição por recursos e à densa copa formada por *R. okamurae*, que parece diminuir as possibilidades de abrigo para esta espécie. Os efeitos da invasão em espécies de valor comercial, como *D. vulgaris*, podem estar largamente subestimados, sendo provável que os seus impactos se estendam para além das áreas protegidas, onde se realiza a maior parte das actividades de pesca. Para os peixes crípticos, a invasão coloca grandes desafios à realização de avaliações precisas das populações. As espécies crípticas são mais difíceis de detetar sob a densa cobertura de *R. okamurae*, levando a uma potencial sobrestimação do impacto da alga. No entanto, a densa estrutura de algas pode oferecer proteção contra predadores e zonas de desova adicionais para espécies como *Parablennius pilicornis*, o que poderia potencialmente aumentar as taxas de sobrevivência e reprodução, embora sejam necessários mais estudos para confirmar estas hipóteses. O poliqueta *Polycirrus cf. aurantiacus* registou um declínio acentuado após a invasão, provavelmente devido à perturbação do habitat causada pelo crescimento de *R. okamurae*. No entanto, as perturbações do fundo do mar durante a amostragem podem também ter contribuído para este declínio.

A invasão de *R. okamurae* teve um impacto profundo e multifacetado na comunidade bentónica da região de Sagres. Embora o tamanho da amostra fosse limitado, as diferenças observadas foram altamente significativas e a consistência dos valores de p em várias permutações reforça a robustez dos nossos resultados. No entanto, a repetição deste estudo durante o inverno, quando a cobertura de algas é menor, forneceria uma visão mais clara das respostas específicas das espécies e reduziria potenciais enviesamentos de amostragem.

Este estudo é o primeiro a avaliar os impactos de *R. okamurae* nas comunidades bentónicas em Sagres, contribuindo com informações valiosas para a compreensão mais ampla desta alga invasora e a evolução da sua invasão. A redução da integridade do fundo do mar e da heterogeneidade dos habitats sublinha a importância de manter os ecossistemas bentónicos para apoiar a biodiversidade marinha e as funções dos ecossistemas. A perda de biodiversidade pode aumentar a vulnerabilidade de um ecossistema a novas perturbações, salientando a necessidade de uma monitorização contínua e de estratégias de gestão eficazes para atenuar os impactos de longo alcance de *R. okamurae*. Embora as áreas marinhas protegidas (AMP) proporcionem uma proteção essencial para a biodiversidade marinha, parecem ser insuficientes para enfrentar

os desafios colocados por espécies invasoras agressivas, como a *R. okamurae*. Ao documentar as consequências ecológicas e socioeconómicas significativas desta invasão, o estudo sublinha a necessidade urgente de estratégias abrangentes para preservar a biodiversidade marinha e as funções dos ecossistemas face às crescentes pressões antropogénicas. A monitorização contínua é crucial para compreender a dinâmica da invasão e desenvolver estratégias de mitigação eficazes. No entanto, a implementação de abordagens de gestão específicas, como a remoção física, os tratamentos químicos ou o controlo biológico, deve ser cuidadosamente avaliada para minimizar consequências ecológicas indesejadas.

Palavras-chave *Rugulopteryx okamurae*, Espécies Exóticas Invasoras, Impacto Ecológico, Composição da Comunidade Bentónica, PNSACV, Sagres

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List of Abbreviations, Acronyms and Symbols

Avg.	Average
BDBC	Banco de Datos de Biodiversidad de Canarias, ES
CBD	Convention on Biological Diversity
cf.	Latin *confer* or *conferatur*, meaning ‘compare’
CI	Confidence Interval
COP15	United Nations Biodiversity Conference
Diss	Dissimilarity
GBIF	Global Biodiversity Information Facility
GIDS	Global Invasive Species Database
GPS	Global Positioning System
GRIIS	Global Register of Introduced and Invasive Species
IAS	Invasive Alien Species
ICNF	Instituto da Conservação da Natureza e das Florestas
IPBES	Intergovernmental Platform on Biodiversity and Ecosystem Services
IUCN	International Union for Conservation of Nature
IUCN SSC	IUCN Species Survival Commission
MARSW	Name of the project carried out in the PNSACV from 2017 to 2021
MDS	non-metric multidimensional scaling
MITECO	Ministerio para la Transición Ecológica y el Reto Demográfico, ES
MPA	Marine Protected Area
MSFD	European Union Marine Strategy Framework Directive
n	Sample size
PC	Complementary Protection (PNSACV)
PERMANOVA	Permutational Multivariate Analysis of Variance
PMSACV	*Parque Marinho do Sudoeste Alentejano e Costa Vicentina*
PNSACV	*Parque Natural do Sudoeste Alentejano e Costa Vicentina*
PP	Partial Protection (PNSACV)
PPI	Partial Protection I (PNSACV)
PRIMER	Software Tool for Multivariate Analysis in Biodiversity and Community Ecology
PT	Total Protection (PNSACV)

PVC	Polyvinyl Chloride
R	Software Environment for Statistical Computing and Graphics
SD	Standard Deviation
SDG	Sustainable Development Goal
SE	Standard Error
SIMPER	Similarity Percentage Analysis
SMB	Surface Marker Buoy
SNIG	Sistema Nacional de Informação Geográfica
UN	United Nations
UVC	Underwater Visual Census

Chapter 1

General Introduction

1. Invasive Alien Species

The Convention on Biological Diversity (CBD) defines Invasive Alien Species (IAS) as species occurring outside their natural range – either through introduction or spread – that negatively impact local biological diversity and can be seen as direct drivers of biodiversity loss (CBD, 2010). The globalization of trade and travel has facilitated the movement of species beyond the barriers of their natural ranges, allowing their introduction into new regions through six major pathways: deliberate release, escape from captivity, commodity contamination, stow-away on a transport vector, anthropogenic corridor and unaided invasion from other invaded regions (Keller *et al.*, 2011; Hulme, 2015; IPBES, 2023). However, only a percentage of introduced non-native species develop into invasive species in their new environments, as species must overcome barriers at each stage of the invasion process (Blackburn *et al.*, 2011). After introduction, a species can become established by forming a viable, self-sustaining population, but is only considered invasive if it spreads and negatively impacts biodiversity or local ecosystems (IPBES, 2023).

Invasive alien species have contributed to 60% of global extinctions, and have led to increased biotic homogenization, impacting both the structure and functioning of ecosystems (IPBES, 2023). They are considered one of the most significant drivers of ecosystem change, often related to other direct drivers, such as habitat change, overexploitation, pollution and climate change (Millennium Ecosystem Assessment, 2005). IAS currently pose a threat to one in ten species listed on the IUCN Red List of threatened species (IUCN, 2022).

Apart from the vast negative impacts on local ecosystems and ecosystem services invasive alien species are also associated with significant socio-economic costs and implications for human health (e.g. through the spread of infectious pathogens) (Vilà *et al.*, 2021; Denóbile *et al.*, 2023). Between 1960 and 2020 the cost of invasive alien species was estimated to be approximately US$140.20 billion (or €116.61 billion), with Portugal alone facing around US$7.89 billion for damage, loss and management costs (prevention, control and education) (Haubrock *et al.*, 2021). The global cost of invasive alien species from 1970 to 2017 is estimated to be at least $1.288 trillion in losses and expenses (Diagne *et al.*, 2021).

There are several reasons why invasive species often become extremely successful in their new habitats and establish themselves as very competitive and highly proliferating organisms. Their success can be attributed to the ability to tolerate a broad range of environmental conditions and to reproduce very efficiently without the need for sexual reproduction, which is often paired with predator defence mechanisms (García-Gómez, 2015).

To mitigate the harmful impacts of invasive alien species on ecosystems, public health and economies it is crucial to implement effective management policies that focus on the broader implications of IAS, rather than solely on species-based control (García-Díaz *et al.*, 2021). Nevertheless, prevention remains the most cost-effective and environmentally favourable method for managing invasive alien species and should always be the primary focus. If prevention is unsuccessful, early detection and rapid response are essential, while long-term control measures should be considered only as a last resort (CBD, 2002).

1.1. Marine Invasions

Invasions in the marine realm are vastly different from terrestrial invasions, due to some general aspects of these two systems. Compared to terrestrial systems oceans are open systems with global patterns of circulation, pelagic larvae and large-scale migrations, which puts them amongst the most heavily invaded systems on earth and complicates the detection, investigation and management of invasive species (Carr *et al.*, 2003; Geburzi and McCarthy, 2018). It is practically impossible to completely prevent the introduction of future marine invasive species, as efforts to manage or eradicate them are linked to a high cost and usually prove to be ineffective (García-Gómez, 2015; Epstein and Smale, 2017).

The ocean environment, particularly in terms of temperature and currents, shows far less variability than terrestrial environments, differing by two to three orders of magnitude (Steele *et al.*, 2019). This might be another reason why invasive species seem to be much more successful in the marine realm.

Invasive species in the ocean raise significantly less public awareness than those in terrestrial environments, primarily because underwater changes are not as easily detected and often have diffuse impacts (Moorhouse and Macdonald, 2015).

1.2. Algal Invasions

Macroalgae invasions are considered one of the major threats to marine biodiversity and the proper functioning of coastal ecosystems, accounting for more than 40% of invasive alien species in the European Union (Schaffelke *et al.*, 2006; Williams and Smith, 2007; Roca *et al.*, 2022). Macroalgae are frequently introduced alongside aquaculture species and represent one of the taxonomic groups with the highest number of species released through the aquarium trade (Geburzi and McCarthy, 2018).

The increasing number of invasive macroalgae is responsible for significant ecological and economic consequences, including biodiversity loss, shifts in benthic community composition, and negative effects on coastal ecosystem services (Davidson *et al.*, 2015; Faria *et al.*, 2022). In addition to these threats, benthic macroalgae can positively impact ecosystems by acting as a habitat-forming species and providing secondary substrate for organisms like epiphytes and small invertebrates (Crooks, 2002; Jones and Thornber, 2010). The impact of each marine invasive macroalga must be evaluated individually and in the context of the specific ecosystem (Thomsen *et al.*, 2011).

2. *Rugulopteryx okamurae*

2.1. Biological Background

The algal species *Rugulopteryx okamurae* (E.Y. Dawson) Hwang, W.J. Lee & H.S. Kim, 2009, is a species of brown alga belonging to the family Dictyotaceae (Guiry and Guiry, 2024b). It is distinguished by a dichotomously branched thallus, with a medulla that is one cell thick in the center and two to three cells thick around the borders (Verlaque *et al.*, 2009) (Figure 1).

R. okamurae spreads primarily through the process of vegetative propagation and asexual reproduction, by producing vegetative propagules, tetrasporangia, and monosporangia (Altamirano Jeschke *et al.*, 2016). In its natural habit the maximum growth and development of these structures are reached when seawater temperatures are above 15°C, which may suggest that *R. okamurae* is a subtropical species that can successfully adapt to more temperate conditions (Hwang *et al.*, 2009). Gametophytes have never been observed in the natural habitat of this species, suggesting that it does not engage in sexual reproduction. (Verlaque *et al.*, 2009).

R. okamurae possesses feeding inhibitory compounds to lessen the degree of predation (Tokuda *et al.,* 1994, as cited in Verlaque *et al.*, 2009). It has also been proven to be highly toxic, due to elevated levels of sesquiterpenes, which are not present in other algae (Barcellos *et al.*, 2023). All species within the genus *Rugulopteryx* are limited to the temperate regions of the Northwest Pacific Ocean, namely Japan, Korea, China, Taiwan and the Philippines (Hwang *et al.*, 2009; Verlaque *et al.*, 2009; GBIF Secretariat, 2023). In these regions the alga grows in the shallow subtidal zone above 15 meters depth and is present year-round, although it is more prominent during the summer as its thallus reverts to a perennial stoloniferous basal system in winter (Hwang *et al.*, 2009; García-Gómez *et al.*, 2020).

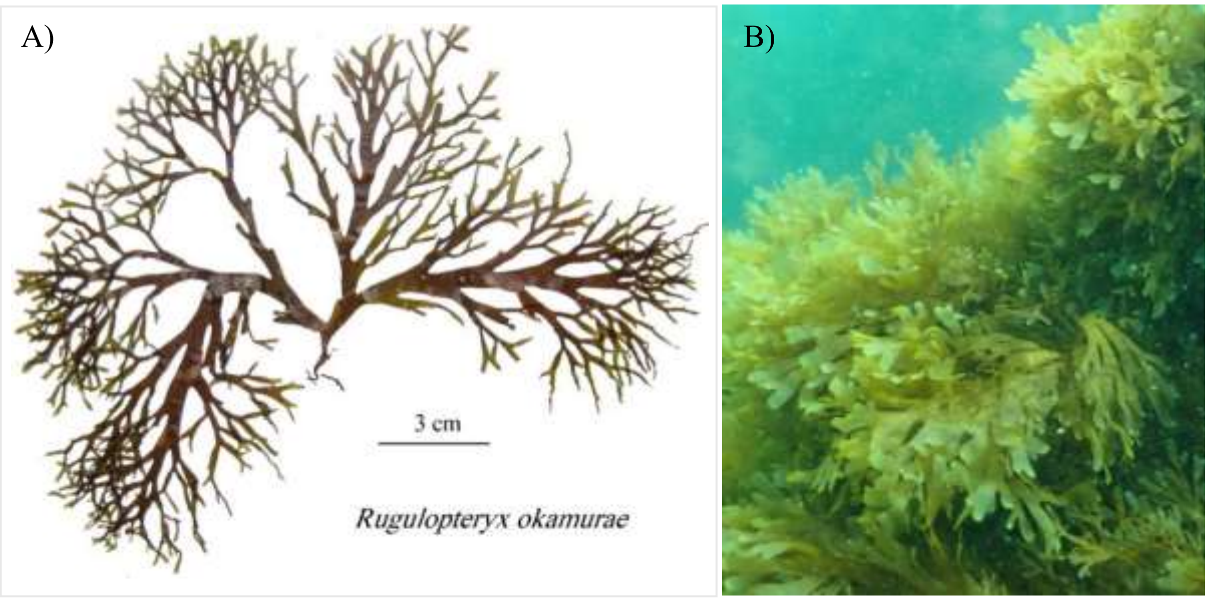

Figure 1: Rugulopteryx okamurae. A) Image from Roca et al. (2022) B) Underwater photo of the alga in Sagres, Southwest Portugal (Photo by Lea Boesiger).

2.2. Invasion

R. okamurae was first recorded in European waters in 2002, in the coastal Lagoon of Thau in France, in the Mediterranean Sea (Verlaque et al., 2009). With Japan and Korea serving as important donor regions, the Thau Lagoon is well-known as a hotspot for the introduction of marine species into Europe (Verlaque, 2001). It is believed that the introduction of *R. okamurae* to the Thau Lagoon occurred through the transport of Japanese oysters, *Magallana gigas* (Thunberg, 1793) (formerly *Crassostrea gigas* (Thunberg 1793)) (Bayne *et al.*, 2017; Guiry and Guiry, 2024a), which were brought to Europe for mariculture purposes (Verlaque *et al.*, 2009). As with other species that were introduced in the Thau Lagoon, the introduction of *R. okamurae* probably resulted from inadequacies in decontamination protocols and/or quarantine procedures of the imports (Verlaque, 2001). The assumption of species introduction from Japan is supported by the visual similarities between the European specimen and the Japanese populations of *R. okamurae*, both presenting with an undulate thallus, compared the flat thalli of Korean populations (Hwang *et al.*, 2009; Verlaque *et al.*, 2009).

In 2015 and 2016, large amounts of *R. okamurae* started washing up along both shores of the Strait of Gibraltar, marking its shift from an introduced to an invasive species (Altamirano Jeschke *et al.*, 2016, 2017; Ocaña *et al.*, 2016; Bernal-Ibáñez *et al.*, 2022). Due to its close resemblance to native species within the genus *Dictyota,* it is possible that the alga occurred in the area way before this event (Altamirano Jeschke *et al.*, 2017; Roca *et al.*, 2022). This phenomenon is referred to as a cryptic invasion, in which the alien species goes undetected during the early stages of its invasion (Altamirano Jeschke *et al.*, 2016, 2017, 2019). Since then, it has spread as far as the Azores Archipelago (Faria *et al.*, 2022), Madeira (Bernal-Ibáñez *et al.*, 2022) and the Canary Islands, which denotes the species' new southern range boundary in the Northeast Atlantic (Bernal-Ibáñez *et al.*, 2022; BDBC, 2023).

Although *R. okamurae* is a highly invasive species it is currently not represented in the two invasive species databases managed by the IUCN SSC Invasive Species Specialist Group: The global invasive species database (GIDS) and the Global Register of Introduced and Invasive Species (GRIIS).

2.3. Success

The rapid and extensive spread, dispersal, and production observed in this species can be attributed to its vegetative propagation and asexual reproduction (Altamirano Jeschke *et al.*, 2016). Suspended fragments can maintain high photosynthetic activity and are able to reattach to hard surfaces, allowing the species to spread quickly after colonizing a new area (Figueroa *et al.*, 2020; Bernal-Ibáñez *et al.*, 2022). Coupled with its strong competitive ability and spatial establishment potential, these traits make *R. okamurae* highly successful as an invasive species (Altamirano Jeschke *et al.*, 2019; García-Gómez *et al.*, 2021a, 2021b).

Additionally, the alga's potential to assimilate and store dissolved inorganic nitrogen, may enhance its presence in regions influenced by occasional spikes in nutrient levels, such as those caused by local upwelling events and seasonal coastal eutrophication (Bernal-Ibáñez *et al.*, 2022; Mercado *et al.*, 2022).

The higher winter annual temperatures experienced in Strait of Gibraltar compared to the native habitat of *R. okamurae* (Jung *et al.*, 2020; Mercado *et al.*, 2022) have not been proven to influence the success of the species. However, its persistence seems to be positively affected by the climatic conditions of the Mediterranean and the southern coasts of the North Atlantic (Verlaque *et al.*, 2009).

2.4. Ecological and Socio-Economic Impacts

Since its initial invasion, *R. okamurae* has spread along hundreds of kilometres of coastline, dominating shallow-water benthic communities and leading to substantial alterations in their structure and composition (García-Gómez *et al.*, 2020; Sempere-Valverde *et al.*, 2021; Bernal-Ibáñez *et al.*, 2022; Faria *et al.*, 2022).

The alga has not only colonized new areas but also appears to have significantly extended its vertical distribution. Compared to the bathymetrical range of the invasive species in the Pacific, which does not go beyond 15m, the species spreads up to 40m deep in the invaded areas (García-Gómez *et al.*, 2020). Other studies even suggest that the species can grow to depths of over 50 meters (Altamirano Jeschke *et al.*, 2019; Faria *et al.*, 2022). The highest densities occur between 5 and 30 meters of depth, with coverage exceeding 90% between 10 and 20 meters of depth, significantly affecting native sessile macrobiota (García-Gómez *et al.*, 2020).

The displacement of local macroalgal species is an especially problematic result of the dominant occupation of *R. okamurae* and its competitive capacity*,* leading to a homogenization of the seafloor (Altamirano Jeschke *et al.*, 2019; García-Gómez *et al.*, 2021b). A study by Navarro-Barranco *et al.* (2019) suggests that the algas' habitat-forming capacities similar to native relatives might differentially impact certain components of the ecosystem (e.g. epifauna), which emphasises the need for separate assessment of the individual components of impacted ecosystems.

So far, no species have been found to use the alga as a food source in the invaded ecosystems, further allowing it to spread (Faria *et al.*, 2022).

The accumulation of biomass resulting from the invasive behavior of *R. okamurae* poses significant social and economic challenges, as it accumulates on the coasts and negatively impacts the fishing sector by entangling in nets, reducing catch capacity, and damaging fishing gear (Mogollón *et al.*, 2024; García-Gómez *et al.*, 2021b). Coastal wrack deposits impact the recreational use of beaches and are costly to remove and dispose of (Altamirano Jeschke *et al.*, 2019). Along the Andalusian coast, the cost of managing algal deposits and their impact on the fishing sector was estimated to be 1.2 million euros over a nine-month period in 2019, although this is likely to be an underestimate (MITECO, 2022).

2.5. Mitigation Efforts

A recent review by Barcellos et al. (2023) highlights that there are only a few studies on the potential uses of *R. okamurae*, with the majority focusing on converting the alga into biogas, compost, fertilizer or bioplastics. Additionally, its potential anti-inflammatory and antimicrobial properties, along with its ability to inhibit α-glucosidase, could offer valuable opportunities for the biomedical, pharmaceutical, and food industries (Cuevas *et al.*, 2021; Barcellos *et al.*, 2023).

So far, no efforts have been made to actively remove *R. okamurae* underwater, and existing studies have primarily relied on samples from beach wrack (Cuevas *et al.*, 2021; de la Lama-Calvente *et al.*, 2021; Santana *et al.*, 2022).

3. Rationale for Studying Invasive Species

To prevent further invasions in the marine realm, it is crucial to understand the processes behind past and present invasions. Providing a baseline taxonomic study of biodiversity is essential for guiding further research and monitoring efforts, to effectively tackle the issue of invasive alien species (CBD, 2002). The development of effective management strategies for *R. okamurae* would make a valuable contribution to reaching several international agreements focusing on invasive species. These include the Sustainable Development Goal (SDG) 15.8 and the Kunming-Montreal Global Biodiversity Framework Target 6 established during COP15. Both of these agreements aim to establish strategies by 2030 to prevent the introduction of invasive species and mitigate their negative impacts (UN, 2015; CBD, 2022), which is crucial for maintaining ecosystem health and preserving biodiversity globally.

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Chapter 2

Ecological Impact of the Invasive Algal Species *Rugulopteryx okamurae* in the Atlantic, South-west Portugal

Abstract

Invasive alien species (IAS) are major drivers of ecosystem change, negatively impacting native communities and contributing to global biodiversity loss. The brown macroalga *Rugulopteryx okamurae*, native to the northwest Pacific, was introduced to the Mediterranean in 2002 and has become highly invasive since 2015, spreading from the Strait of Gibraltar to the northeastern Atlantic. Its rapid proliferation and competitive exclusion of other macroalgae has transformed shallow-water benthic habitats, posing significant challenges for benthic organisms. This study investigates the ecological impacts of *R. okamurae* on the benthic community in Sagres, Portugal, where the species was first documented in 2021. Using pre-invasion data from the MARSW project, collected in 2018, we conducted a comparative analysis to assess changes in benthic community composition and biodiversity following the alga's establishment. Species abundances for demersal fish, cryptic fish, macroinvertebrates and algae were recorded through scientific diving, conducting an underwater visual census (UVC) combined with the quadrat technique. Biodiversity indices were calculated to complement PERMANOVA results, providing a comprehensive analysis of ecosystem changes. Results revealed significant alterations in species composition and declining levels of biodiversity, prompted by the loss of functional macroalgae diversity. The change in biodiversity among demersal fish was driven by the dominance of *C. julis* and *C. exoletus*, whereas cryptic fish and macroinvertebrates were more strongly affected by declines in species richness. *R. okamurae* demonstrated invasive behaviour that exceeded that of other introduced species, underscoring the necessity for long-term monitoring and targeted management strategies to mitigate the alga’s detrimental impacts on marine ecosystems.

Keywords *Rugulopteryx okamurae*, Invasive Alien Species, Ecological Impact, Benthic Community Composition, PNSACV, Sagres

1. Introduction

Invasive alien species (IAS) are one of the most important drives of ecosystem change, negatively impacting local biological diversity and directly causing biodiversity loss (Millennium Ecosystem Assessment, 2005; CBD, 2010). IAS have contributed to 60% of global extinctions and currently endanger one in ten species on the IUCN Red List of threatened species, driving biotic homogenization and altering ecosystem structure and function (IUCN, 2022; IPBES, 2023).

Most invasive species introductions are a result of anthropogenic activities and the globalization of trade and travel and cause severe negative impacts on local ecosystems, ecosystem services, and human health (Keller *et al.*, 2011; Hulme, 2015; Vilà *et al.*, 2021; Denóbile *et al.*, 2023). The estimated global financial burden of invasive alien species from 1970 to 2017 reached at least US$1.288 trillion in damage, loss, and management costs, with US$7.89 billion attributed to Portugal alone (Diagne *et al.*, 2021; Haubrock *et al.*, 2021).

Invasive species often thrive in new habitats due to their tolerance of diverse environmental conditions, high reproductive efficiency (often without sexual reproduction), and effective predator defences (García-Gómez, 2015). Ocean systems are some of the most severely impacted ecosystems on the planet due to their open character characterized by global circulation patterns, pelagic larvae, and large-scale migrations, complicating the detection and management of invasive species (Carr *et al.*, 2003; Geburzi and McCarthy, 2018). The low variability in temperature and currents compared to terrestrial systems and the reduced public awareness further allow these species to thrive (Moorhouse and Macdonald, 2015; Steele *et al.*, 2019). Macroalgae invasions, mainly introduced through aquaculture and the aquarium trade, constitute over 40% of marine invasive alien species in the European Union, significantly threatening marine biodiversity and coastal ecosystems (Schaffelke *et al.*, 2006; Williams and Smith, 2007; Geburzi and McCarthy, 2018; Roca *et al.*, 2022). The ongoing increase in invasive macroalgae results in changes in the benthic community composition and consequently leads to biodiversity loss and habitat homogenization (Davidson *et al.*, 2015; Faria *et al.*, 2022). According to Gaertner *et al.* (2014), species capable of fundamentally reorganizing and altering ecosystems can drive regime shifts and consequently should be regarded as “most high-impact” species with priority for management action. Regime shifts are defined as noticeable transitions between two relatively stable states, resulting in abrupt and catastrophic changes in the structure and functioning of ecosystems (Scheffer *et al.*, 2001; Rietkerk *et al.*, 2004).

One notable example of a highly invasive macroalgae is the brown alga *Rugulopteryx okamurae* (E.Y. Dawson) Hwang, W.J. Lee & H.S. Kim (2009), which belongs to the family Dictyotaceae and is native to the Northwest Pacific (Hwang *et al.*, 2009; Verlaque *et al.*, 2009; GBIF Secretariat, 2023; Guiry and Guiry, 2024a, 2024b). It was first discovered in European waters in 2002, in the Thau Lagoon in France, a known hotspot for marine species introductions (Verlaque, 2001; Verlaque *et al.*, 2009). In 2016 the species began displaying invasive behaviour and accumulating in large amounts on both shores of the Strait of Gibraltar (Altamirano Jeschke *et al.*, 2016, 2017; Ocaña *et al.*, 2016; Bernal-Ibáñez *et al.*, 2022). Since then, it has rapidly spread along vast stretches of coastline, dominating shallow-water benthic communities and significantly altering their structure and composition (García-Gómez *et al.*, 2020; Sempere-Valverde *et al.*, 2021; Bernal-Ibáñez *et al.*, 2022; Faria *et al.*, 2022). In the introduced areas *R. okamurae* colonizes rocky surface areas up to over 50 meters in depth, substantially exceeding its normal vertical range (Altamirano Jeschke *et al.*, 2019; Faria *et al.*, 2022; García-Gómez *et al.*, 2021a). The greatest algal densities are found between depths of 5 and 30 meters, and often exceed 90% from 10 to 20 meters, significantly impacting native sessile macrobiota (García-Gómez *et al.*, 2020). *R. okamurae* is highly successful, largely due to its vegetative propagation and asexual reproduction, allowing it to quickly colonize new areas (Altamirano Jeschke *et al.*, 2016; Figueroa *et al.*, 2020; Bernal-Ibáñez *et al.*, 2022) The species has not been found to have any natural predators, likely due to its high toxicity and the presence of feeding-inhibitory compounds, which further facilitate its spread (Tokuda *et al.* 1994, as cited in Verlaque *et al.*, 2009; Barcellos *et al.*, 2023). As a result, the algas' highly competitive establishment leads to the displacement of local macroalgae species and the homogenization of the seafloor (Altamirano Jeschke *et al.*, 2019; García-Gómez *et al.*, 2021b). The accumulation of biomass from the invasive spread of *R. okamurae* also presents considerable social and economic difficulties, as it builds up along coastlines and disrupts the fishing industry by entangling in nets, lowering catch potential, and causing damage to fishing equipment (Mogollón *et al.*, 2024; García-Gómez *et al.*, 2021b).

The presence of *R. okamurae* in Sagres, Southwest Portugal, was reported for the first time in 2021 by Liulea *et al.* (2023). The area is part of the "Parque Natural do Sudoeste Alentejano e Costa Vicentina" (PNSACV) marine area, which is designated as a Marine Protected Area (MPA) (Presidência do Conselho de Ministros, 2011). It is influenced by the convergence of the Mediterranean and the Atlantic Ocean, along with coastal upwelling, which enhances primary productivity and supports high levels of biodiversity (Goela *et al.*, 2016; ICNF, 2024). *R. okamurae* has the ability to assimilate and store dissolved inorganic nitrogen allowing it to thrive in regions influenced by peaks in nutrient levels, such as those resulting from local upwelling (Bernal-Ibáñez *et al.*, 2022; Mercado *et al.*, 2022). The prominent presence of coastal rocky bottoms in the area further supports a diverse assemblage of marine flora and fauna by offering a variety of habitats for numerous species (Coolen *et al.*, 2015; ICNF, 2024). However, these rocky substrates also facilitate the spread of *R. okamurae*, allowing it to significantly impact these ecosystems and their biodiversity (García-Gómez *et al.*, 2021a).

The MARSW project, conducted from 2017 to 2021, provided valuable insights into the marine communities and habitats of the PNSACV prior to the invasion of *R. okamurae.* This knowledge is crucial for understanding the challenges posed by the invasive alga in this diverse marine ecosystem. Building upon the findings of the MARSW project, the present study assesses the specific impacts of *R. okamurae* on local marine benthic communities, including cryptic and demersal fish, macroinvertebrates, and algae, focusing on how its dominance affects biodiversity and community composition. The comprehensive monitoring guide developed by the MARSW project facilitates the evaluation of such ecosystem changes over time (MARSW, 2021).

To prevent further invasions in marine environments, it is essential to comprehend the mechanisms driving past and present invasions. Establishing a baseline taxonomic assessment of biodiversity is essential for guiding further research and monitoring initiatives, to effectively address the challenges posed by invasive alien species and develop effective and sustainable invasive species management strategies (CBD, 2002; Larson *et al.*, 2011).

Consequently, this study broadens our understanding of the ecological consequences of the invasion of *R. okamurae*, marking the first evaluation of its impacts on the local benthic community in Sagres. By understanding the ecological repercussions of this invasion, the study establishes an essential foundation for advancing knowledge that will ultimately guide future management strategies for this species.

The main objective of this study is to understand how the benthic community has changed or potentially adapted in response to the significant structural shift in the ecosystem caused by the invasion of *R. okamurae*. Establishing this baseline will provide a foundation for future research focused on the invasion and potential mitigation strategies for the invasive species. Given the trends observed in other regions in recent years (García-Gómez *et al.*, 2021a), we hypothesize that the invasion of *R. okamurae* in Sagres has led to substantial ecosystem degradation, with the invasive algae dominating the seafloor and consequently displacing or covering native species. Such shifts in community structure can profoundly impact local biodiversity and ecosystem functioning, requiring targeted conservation efforts (Simberloff *et al.*, 2013).

2. Materials and Methods

2.1. Study Area

The '*Parque Natural do Sudoeste Alentejano e Costa Vicentina'* (PNSACV), established in 1988, spans the southwestern coastline of Portugal from S. Torpes, south of Sines, to Burgau (Ministério do Planeamento e da Administração do Território, 1988). It covers an area of 89,568.77 hectares, with about one third designated as a marine reserve. This two-kilometre-wide coastal strip features a wide range of habitats and species (ICNF, 2024), prompting the establishment of the '*Parque Marinho do Sudoeste Alentejano e Costa Vicentina'* (PMSACV) in 2011, as part of the revised Management Plan for the Natural Park. The '*Plano de Ordenamento'* incorporates areas of complementary, partial, and total protection (Figure 2A), in which fishing is either partially or completely prohibited, and other human activities are subject to regulation (Presidência do Conselho de Ministros, 2011; Castro *et al.*, 2021). The PMSACV is the largest Marine Protected Area (MPA) along the Portuguese coastline (Gonçalves *et al.*, 2021).

The present study was conducted in the Martinhal Islands Partial Protection I (PPI) zone and the adjacent Complementary Protection (PC) area (Figure 2B). The seafloor around the islands and near the cliffs predominantly consists of rocky substrates, characterized by blocks of various sizes and rocky platforms, which extend to depths of about 20 to 25 meters (Gonçalves *et al.*, 2021). These complex coastal rocky bottoms increase the structuring of the seafloor, providing habitat for various species (Monteiro *et al.*, 2012; Coolen *et al.*, 2015), including *R. okamurae* (García-Gómez *et al.*, 2021a). Additionally, the area is characterized by localized upwelling, which enhances primary productivity and supports highly productive fisheries (Hutchings *et al.*, 1995; Goela *et al.*, 2016). However, these occasional spikes in nutrient levels may further contribute to the proliferation of *R. okamurae,* making this a crucial area for studying the impacts of the invasion.

Consequently, this specific region was selected due to the strong presence of *R. okamurae* and the availability of pre-invasion data from the MARSW project sampled in 2018, which allowed for a comparative analysis of the ecosystem state before and after the invasion.

Four sampling locations, ranging in depth from 7 to 20 meters, were chosen for this study. Two locations are situated in the PPI zone (Martinhal Islands 37.01525, -8.91737; Ponta dos Caminhos 37.02265, -8.90763) and two in the adjacent PC zone (Atalaia 37.00814, -8.92507;

Barranco 37.03276, -8.89832) (Figure 2B). The distinction between protection levels (PPI and PC) adds a layer of detail, providing a clearer understanding of how protection levels may influence the benthic ecosystem and shape the outcomes of the invasion of *R. okamurae.*

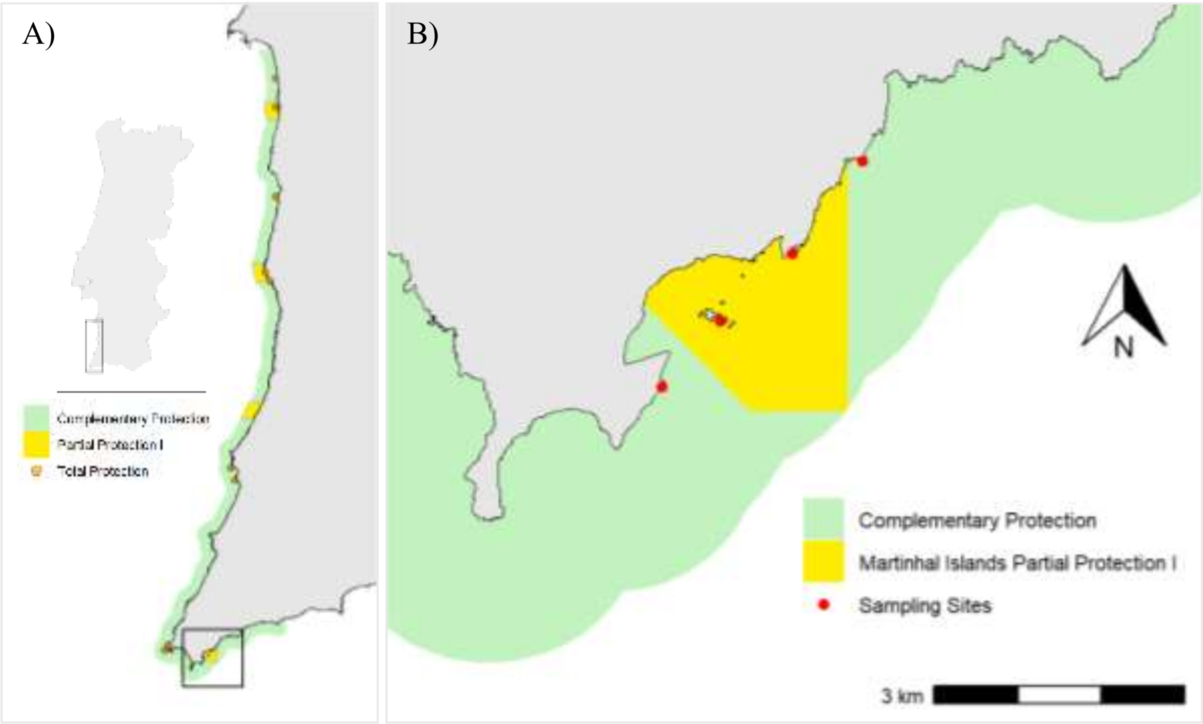

Figure 2: A) Total extent of the PMSACV along the southwestern coast of Portugal, including Complementary Protection zones (PC; green), Partial Protection I zones (PPI; yellow) and Total Protection zones (PT; orange) B) Closeup of the Martinhal Islands PPI zone and the surrounding PC zone. Two sampling sites were located within the PPI zone ("Martinhal Islands" and "Ponta dos Caminhos") and two in the adjacent PC zone ("Atalaia" and "Barranco"). Figure adapted from Belackova et al. (2023) and Castro et al. (2021), with coordinates from ICNF (2024b). Map created using the R software environment (version 4.4.1) (R Core Team, 2024), with landmass data sourced from SNIG (2024).

2.2. Sampling Methodology

To compare the community composition before and after the invasion of *R. okamurae*, the applied methodology was identical to the one used in the MARSW project (Castro *et al.*, 2021). The following paragraphs are based on the methodology described in this source.

Current data were collected through scientific diving during the summer season, under calm conditions with an average visibility of five meters and negligible currents, which allowed for clear observation of the seafloor. The sampling consisted of two dives per site and was carried out by a single pair of divers (including the author), following a systematic approach. The dive sites were located using their exact GPS location (provided by the MARSW data) and marked with a surface marker buoy (SMB), attached to the seafloor on the first descent.

Four groups—demersal fish, cryptic fish, macroinvertebrates, and algae—were surveyed in each location. To monitor changes in the community composition and structure within those groups, two conventional methods were employed:

1. **Underwater visual census (UVC)**: The UVC method is commonly used for evaluating shallow water species and habitats up to a depth of 20 meters. This approach is straightforward to implement and provides an efficient collection of data on species abundance, size, and diversity, which is generally used for fisheries conservation and management (Lowry *et al.*, 2012; Castro *et al.*, 2021; López-González *et al.*, 2022). In this study, this method was applied to survey demersal and cryptic fish during the first dive.
2. **Quadrat Technique**: The quadrat technique is a widely used method for collecting quantitative data on benthic species and habitats. This non-invasive method is only suitable for sessile organisms or species with low motility and is less effective at identifying rare or cryptic species (Lessios, 1996; Johnson and Newman, 2011; Castro *et al.*, 2021). Additionally, (fixed) quadrats can be utilized to monitor reactions of sessile bioindicator species to ecological changes, such as invasive species (García-Gómez, 2015). Consequently, the quadrat technique was used to survey macroinvertebrates and algae during the second dive.

The primary objective of the sampling process was to assess the benthic community composition and structure in the area. To achieve this, the different species and their relative abundances were documented, to gain insight into the benthic biodiversity at the time of sampling.

2.2.1. Data Collection

During the first dive, the team of divers extended a fiberglass measuring tape to cover a distance of 90 meters along a predetermined heading, ensuring consistency with the MARSW project data (Castro *et al.*, 2021). Demersal fish and cephalopods (cuttlefish and squids) were surveyed along the transect in three sections of 20 by 4 meters each (80 m^2); from 10 to 30 m, from 40 to 60 m and from 70 to 90 m (Figure 3). The presence of juveniles within these transects was also noted. However, species and abundances of juveniles were not incorporated in the analysis.

Figure 3: Demersal fish census conducted along a 90 m transect, with three replicates of 20 x 4 meters (80 m²) each (light grey), sampled in the direction from 0 to 90 m. Figure created in Drawboard PDF.

During the same dive, cryptic fish, commercially valuable crustaceans (such as crabs, spider crabs, and lobsters), and octopuses were sampled in the opposite direction along the 90-meter transect. Cryptic species were surveyed in three sections of 10 by 1 meter each (10 m^2); from 80 to 70 m, from 60 to 50 m, and from 40 to 30 m (Figure 4). As for the demersal fish census, the presence of juveniles was noted, but species or their abundances were not considered.

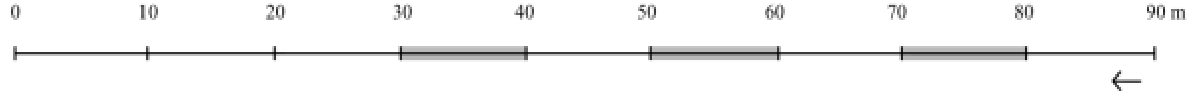

Figure 4: Cryptic fish census conducted along a 90 m transect, with three replicates of 10 x 1 meters (10 m²) each (light grey), sampled in the direction from 90 to 0 m. Figure created in Drawboard PDF.

As part of the second dive, macroinvertebrates were surveyed at five pre-established points, namely 10, 15, 20, 25, 30 metres along the previously established transect. Using a quadrat of 0.5 by 0.5 meters (0.25 m^2) made of lightweight PVC material, a total area of 1 m^2 was sampled at each sampling point (Figure 5).

The dense canopy formed by the invasive species was removed prior to sampling, to enhance visibility and ensure accurate observations and only individuals above 5 cm in length were recorded. To confirm the observations made in situ, each square was photographed following the collection of records. Additionally, organisms that were difficult to identify in situ (e.g., Porifera and Bryozoa) were photographed for later identification with the assistance of experts.

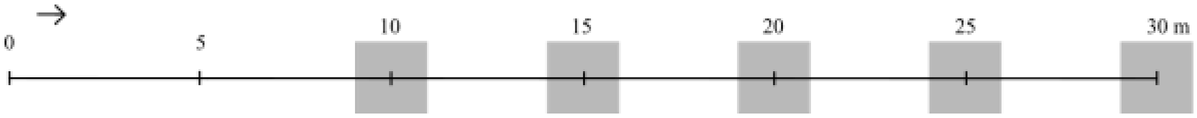

Figure 5: Macroinvertebrate census conducted along a 30 m transect, with five replicates of 1 m^2 each (light grey). Figure created in Drawboard PDF.

The algae census was carried out simultaneously to the macroinvertebrate census, by the other diver. At each of the five sampling points along the transect, a 0.25 m^2 sampling quadrat (identical to the one used for the macroinvertebrate census) was systematically placed 0.5 meters away from the transect, using the quadrats of the macroinvertebrate census as a guide (Figure 6). Each quadrat was photographed before and after the removal of *R. okamurae,* to assess the layer of canopy forming species as well as any incrusting algae or smaller species beneath. Percentage coverage for all species, including *R. okamurae,* was later estimated from these photographs.

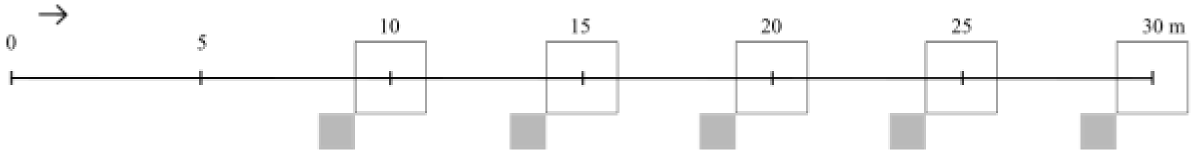

Figure 6: Algae census conducted along a 30 m transect, with five replicates of 0.25 m^2 each, systematically placed 0.5 m away from the transect (light grey). White squares show the adjacent macroinvertebrate census used as a measure for the placement of the quadrat. Figure created in Drawboard PDF.

2.3. Data Processing and Analysis

The data obtained from sampling the different groups – demersal fish, cryptic fish, macroinvertebrates and algae – was processed and analysed separately to evaluate the potential impact of the invasive algae on the individual components of the ecosystem. Photographs of in-situ unidentified species in both algae and macroinvertebrate samples were cross-referenced with experts to ensure accurate classification to the smallest possible taxonomic level.

Since *R. okamurae* was absent in the previous data and the sampled squares showed minimal variability, with almost complete coverage by the invasive species (Figure 7A), we categorized the samples based on its presence. Because the overwhelming dominance of *R. okamurae* in the samples left little room for the presence of other species, further analysis of this set of pictures was not conducted. The second set of pictures taken to estimate the coverage of algal species below the layer of invasives (Figure 7B), were analysed using the Coral Point Count software from the US's National Coral Reef Institute (Kohler and Gill, 2006). To ensure comprehensive species detection in the images, a total of one hundred randomly distributed sampling points was selected for each analysis. This number of points was shown to enhance the reliability of the data, while maintaining a manageable effort per sample. Since it was not possible to fully remove *R. okamurae* prior to taking the photographs (see Figure 7B), areas with remnants of the invasive alga were classified as non-defined, as were blurry spots.

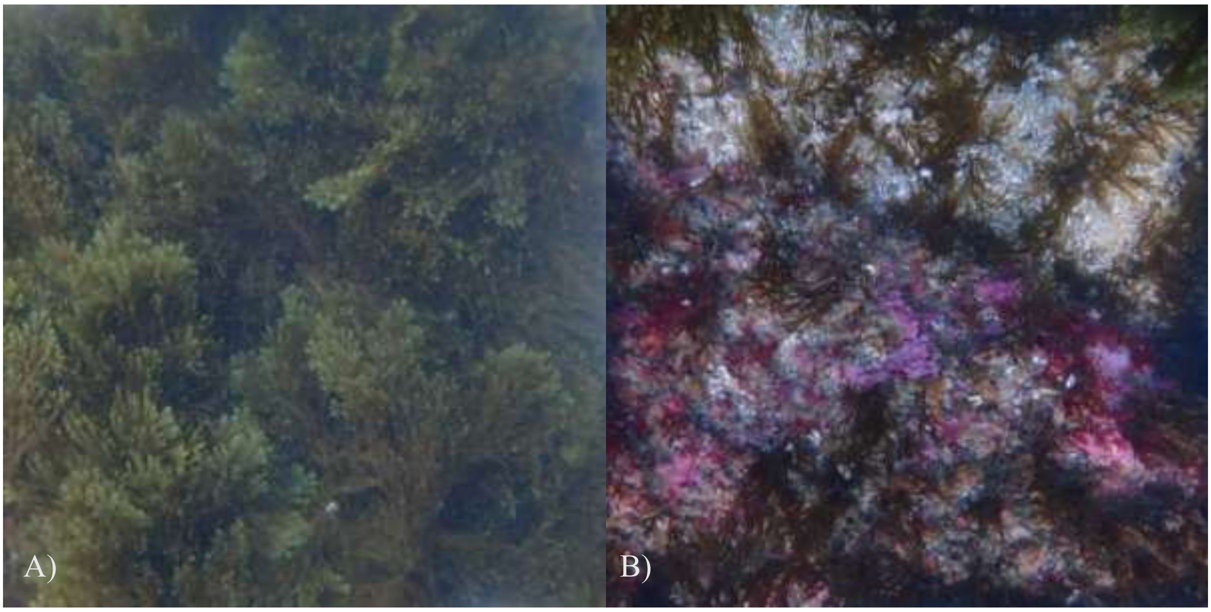

Figure 7: Example of the algal census. A) Photograph of the sampling square showing the top layer of algae, dominated by R. okamurae; B) Photograph of the algae beneath the invasive species, processed in Coral Point Count. Photographs by Lea Boesiger.

The data obtained from the visual census and quadrat methods were entered into separate excel sheets and then combined with the data from the MARSW project. To ensure the accuracy of the direct comparison of the two years, only the MARSW dives from the locations that were repeated in 2024 were included in the analysis.

To quantify the impact to the invasive algae on the biodiversity with the groups assessed, species richness, Shannon-Wiener Diversity Index and Pielou's Evenness Index were calculated (mean ± 95% confidence interval), as done in the MARSW project (Castro *et al.*, 2021), and compared between the two sampling years.

Species richness is calculated as the number of species per sampling unit (transect/ quadrat). Considering both species richness and evenness, the Shannon-Wiener Diversity Index (Equation 1) serves to estimate overall diversity by summing the individual proportion (p_i) of each species relative to the total number of individuals in the community (Clarke and Warwick, 2001):

$$Shannon - Wiener\ Diversity\ Index\ (H') = -\sum_{i=1}^{S} p_i \ln (p_i) \qquad (1)$$

Pielou's Evenness Index (Equation 2) quantifies the evenness of species distribution within a community, providing an estimate of how equally individuals are distributed across species. It is calculated by dividing the Shannon-Wiener Diversity Index (H') by the natural logarithm of species richness, which represents the maximum value of H' (H'max) when all species are equally abundant, indicating maximum evenness (Clarke and Warwick, 2001):

$$Pielou's\ Evenness\ Index\ (J') = H' / \ln (S) = H' / H'max \qquad (2)$$

The statistical methods used in this thesis were consistent with the approaches used in the MARSW project (Castro *et al.*, 2021). Following the established statistical framework, the multivariate data analysis was performed using PRIMER version 6.1.11 (Clarke and Gorley, 2008) with PERMANOVA+ version 1.0.1 (Anderson *et al.*, 2008). The species abundance matrixes needed as a basis for the PRIMER software were created using the R software environment (version 4.4.1) (R Core Team, 2024). The matrixes for each group, as outlined above, were imported into PRIMER along with the two factors ‘Protection Level’ (‘PPI’ or ‘PC’) and “Presence Invasive Species’ (‘Not Present’ or ‘Present’). The levels of the latter were consistent with the two sampling years (2018 = ‘Not Present’; 2024 = ‘Present’). The individual abundances were square root transformed to minimize the influence of the most dominant species. Following the approach used in the MARSW project, the Bray-Curtis similarity index was used to calculate a resemblance matrix (Clarke and Warwick, 2001). Based on this, a Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2005) was performed for each of the four biological groups, to determine whether the two factors significantly influenced the observed differences between the samples ($p < 0.05$). To further explore the data, an ordination was performed using non-metric multidimensional scaling (MDS). Additionally, a similarity percentage analysis (SIMPER) was conducted for each group to identify which species contributed most significantly to the differences detected in the PERMANOVA, thereby highlighting the species most impacted by the invasive species.

Changes in the mean biodiversity metrics – species richness, Shannon-Wiener Biodiversity Index, and Pielou’s Evenness Index – before and after the invasion of *R. okamurae* were statistically analysed using R (version 4.4.1) (R Core Team, 2024). P-values were calculated using either a paired t-test (for normally distributed data, as assessed by the Shapiro-Wilk test) or a paired Wilcoxon test (for non-normally distributed data).

3. Results

3.1. Community-Wide Trends

The data analysis revealed that *R. okamurae*, which was entirely absent in 2018, has since become the dominant species in the re-sampled sites, leading to a substantial shift in community structure. The mean percentage coverage of the alga across the sampled locations was 92.4%, with coverages ranging from 80.2% to 98% among different locations. This marks an increase of 92.4% from 2018 to 2024, highlighting the alga's potential to spread rapidly across suitable habitats. For each of the biological groups investigated – demersal fish, cryptic fish, macroinvertebrates and algae – the PERMANOVA revealed a significant difference in the species composition between the two years (Table 1; p = 0.001), driven by the presence of the invasive alga. The widespread coverage of *R. okamurae* is a key factor influencing the abundance and diversity of taxa across the surveyed locations.

Due to the small sample size in the different groups (n = 12 for demersal fish and cryptic fish; n= 20 for macroinvertebrates and functional algae groups), p-values were calculated using 999, 4999, and 9999 permutations, to improve the accuracy and reliability of the results. However, the p-values obtained from 999 permutations were consistent with those from 4999 and 9999 permutations, indicating that increasing the number of permutations did not substantially affect the results. For simplicity, the results from 999 permutations are presented here (Table 1). The results for 4999 and 9999 permutations are provided in Appendix I for reference (Tables 11 and 12).

Table 1: Results of PERMANOVA analysis (999 permutations) showing p-values for the effects of ***Presence of Invasive Species****,* ***Protection Level****, and their interaction (****Presence x Protection****) on community composition across different biological groups. Significant p-values (p < 0.05) are highlighted with an asterisk (*). PERMANOVA results for algae functional groups were supplemented with analysis using adonis2 (cursive). The interaction term includes two values, representing the two sampling years. Layout based on Belackova* et al. *(2023).*

Biological Group	Presence Invasive Species	Protection Level	Presence x Protection
Demersal Fish	0.001*	0.205	0.734
Cryptic Fish	0.001*	0.021*	0.062
Macroinvertebrates	0.001*	0.046*	0.175
Algae Functional Groups	0.001* / _0.001*_	0.473 / _0.811_	_0.972 / 0.197_

The MDS plots for the factor “Presence Invasive Species” (Figure 8) effectively illustrate these findings, revealing distinct grouping patterns among the samples. These plots provide a visual representation of the underlying data structure, complementing the statistical results from PERMANOVA. While the significant p-values indicate clear differences between 2018 and 2024, the MDS plots reveal patterns of similarity and dissimilarity, making these results more interpretable. Additionally, MDS plots can highlight trends that may not be fully captured by statistical analysis, offering a clearer, more intuitive understanding of the data.

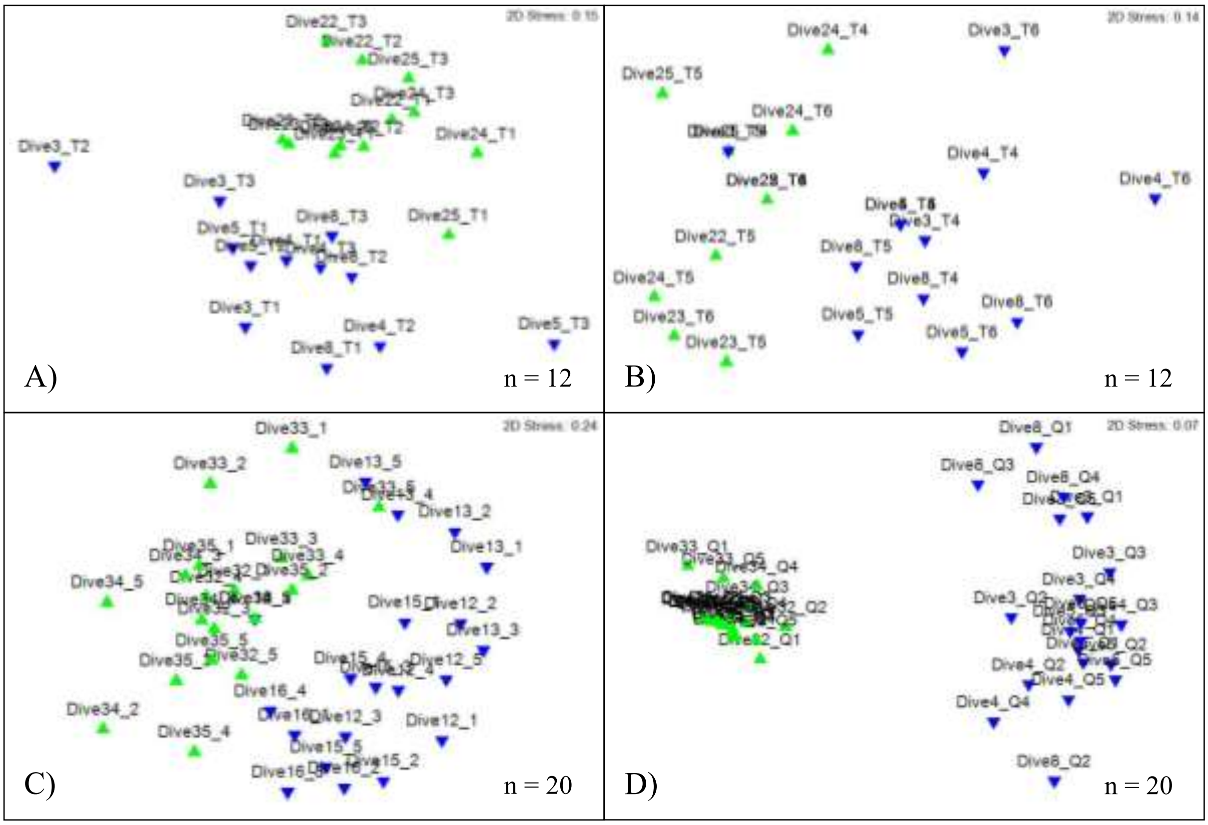

Figure 8: MDS plots for the analysed groups, visualizing the differences in the community structure between the corresponding samples with the factor “Presence Invasive Species” for A) demersal fish (n=12), B) cryptic fish (n=12), C) macroinvertebrates (n=20) and D) algae functional groups (n=20). The green triangles represent presences of the algae/ full coverage, while the blue triangles represent absence of the invasive species. The data was square root transformed, and the resemblance matrix was calculated using the Bray-Curtis similarity. MDS plots generated in PRIMER version 6.1.11 (Clarke and Gorley, 2008).

The analysis of species richness, the Shannon-Wiener Diversity Index, and Pielou’s Evenness Index revealed substantial shifts in community composition following the invasion of *R. okamurae.* Significant changes were observed in the mean Shannon-Wiener Diversity Index, and mean Pielou’s Evenness Index for demersal fish (p = 0.031 and 0.007, respectively; Table 2). In contrast, for cryptic fish, only the mean species richness showed a significant change (p = *0.036*), with no significant differences in the diversity indices between 2018 and 2024 (p =

0.075 and p = *0.130)*. For macroinvertebrates and functional algae groups, both the mean species richness and the mean Shannon-Wiener Diversity Index showed significant changes (*p* < 0.001; *macroinvertebrate p-values were approximated*; Table 2). In calculating these mean indices, most p-values were approximated (with exact = FALSE; denoted in cursive) due to the presence of zero values and ties in the data. However, according to Bellera *et al.* (2010), this should not affect the accuracy of the results.

Table 2: *P-values obtained from statistical comparisons of biodiversity metrics (Species Richness, Shannon-Wiener Diversity Index, and Pielou's Evenness Index) between 2018 and 2024 for the different biological groups assessed. Significant p-values (<0.05) are indicated by an asterisk (*), reflecting differences between the two years. Cursive p-values were approximated due to the presence of zero values and ties in the data.*

Biological Group	Species Richness (S)	Shannon-Wiener Diversity Index (H')	Pielou's Evenness Index (J')
Demersal Fish	*0.152*	0.031*	0.007*
Cryptic Fish	*0.036**	*0.075*	*0.130*
Macroinvertebrates	*<0.001**	*<0.001**	*0.074*
Algae Functional Groups	<0.001*	<0.001*	*0.421*

3.2. Demersal Fish

While the presence of the invasive species significantly impacted the community composition of demersal fish (n = 12 samples), individual species showed differential responses, with some positively reacting to the structural changes in the ecosystem. Contrary to our hypothesis, the SIMPER analysis revealed that the two species primarily responsible for the average dissimilarity of 72.65% between the years, exhibited a substantial rise in population density. *Coris julis*, which contributed 26.91% to the observed differences, increased dramatically from a density of 53.13 individuals per 1000m^2 in 2018 to 491.67 individuals per 1000m^2 in 2024, representing an amplification of nearly ten-fold (Table 3). Similarly, *Centrolabrus exoletus,* contributing 10.67% to the dissimilarity, surged from 2.08 individuals per 1000m2 in 2018 to 109.38 individuals per 1000m2 in 2024, marking an even larger increase of over fifty-fold. In contrast, *Diplodus vulgaris,* which contributed 10.66% to the total dissimilarity, showed a decline in density from 82.29 individuals per 1000m^2 in 2018 to 16.67 individuals per 1000m^2 in 2024, consistent with our hypothesis. Overall, the average similarity between the individual samples increased from 39.19% to 51.41% between 2018 and 2024.

Table 3: SIMPER results displaying changes in densities of demersal fish species (individuals per 1000m^2) and their contributions to the dissimilarity between 2018 and 2024. The table presents average densities (Avg. Density) with standard errors (SE) for each species in both years, as well as the dissimilarity-to-standard deviation ratio (Diss/SD) and each species' percentage contribution (%) to the overall dissimilarity.

	2018	2024		
Species	Avg. Density (SE)	Avg. Density (SE)	Diss/SD	Contribution %
Coris julis	53.13 (13.59)	491.67 (69.77)	1.85	26.91
Centrolabrus exoletus	2.08 (1.34)	109.38 (62.93)	1.03	10.67
Diplodus vulgaris	82.29 (18.31)	16.67 (6.64)	1.48	10.66

These above-mentioned trends were reflected in broader diversity measures. Although the mean species richness showed a slight increase from 4 to 5 species per sample between the two years (Table 4), this change was not statistically significant (p = *0.152*; Table 2). In contrast, both the mean Shannon-Wiener Diversity Index and Pielou's Evenness Index were significantly lower in 2024 compared to 2018 (p = 0.031 and p = 0.007, respectively; Table 2), with mean values decreasing from 1.209 to 0.913 and from 0.823 to 0.531, respectively (Table 4), marking a strong shift in community composition and species evenness.

Table 4: Species Richness (S), Shannon-Wiener Diversity Index (H'), and Pielou's Evenness Index (J') for the demersal fish community in the years 2018 and 2024. Mean and 95% confidence intervals were calculated using bootstrapping. An asterisk (*) denotes significant differences between 2018 and 2024 (p < 0.05). Note: Values for Species Richness (S) are rounded down to the nearest whole number for clarity in the main text.

	2018	2024
Biodiversity Metric	Mean (95% CI)	Mean (95% CI)
Species Richness (S)	4.859 (1, 7)	5.416 (3, 7)
Shannon-Wiener Diversity Index (H’)	1.209 (0.963, 1.400) *	0.913 (0.687, 1.126) *
Pielou’s Evenness Index (J’)	0.823 (0.791, 0.858) *	0.531 (0.419, 0.629) *

Apart from the considerable impact of the presence of the invasive alga on the community composition for demersal fish, the protection level did not contribute to the observed differences. The p-values for both the main effect (p = 0.205; MDS plot Figure 9, Appendix I) as well as for the interaction (p = 0.734) of the two factors, were not statistically significant (Table 1).

3.3. Cryptic Fish

Within the cryptic fish community (n = 12 samples), the average dissimilarity between the two years was 67.05%, reflecting the overall changes in community composition observed over this period. Of this total dissimilarity, *Parablennius pilicornis* accounted for 19.34%, demonstrating its significant contribution to the observed differences between the two years (Table 5). The average density of *P. pilicornis* declined from 108.33 individuals per 1000m^2 in 2018 to complete absence in 2024 (Table 5). Other species included in the SIMPER analysis failed to show consistent contributions to the strong distinction between the two years (dissimilarity-to-standard-deviation ratio (Diss/SD) < 1). Overall, the average similarity between all samples within cryptic fish increased slightly from 46.50% in 2018 to 53.24% in 2024.

Table 5: SIMPER results showing changes in the density of Parablennius pilicornis (individuals per 1000m^2) and its contribution to the dissimilarity between 2018 and 2024. The table presents average densities (Avg. Density) with standard errors (SE) for the species in both years, as well as the dissimilarity-to-standard-deviation ratio (Diss/SD) and percentage contribution (%) to the overall dissimilarity.

	2018	2024		
Species	Avg. Density (SE)	Avg. Density (SE)	Diss/SD	Contribution %
Parablennius pilicornis	108.33 (21.92)	0.00 (0.00)	1.29	19.34

While there was a significant decline in mean species richness within the cryptic fish community, with the average number of species per sample decreasing from 3 to 1 (Table 6; p = *0.036*; Table 2), the changes in mean Shannon-Wiener Diversity Index and mean Pielou’s Evenness Index did not yield significant p-values (p = *0.075* and p = *0.130*; Table 2). However, the observed differences for both indexes – the mean Shannon-Wiener Diversity Index decreasing from 0.922 to 0.384, and the mean Pielou’s Evenness Index dropping from 0.929 to 0.545 – suggest a potential negative trend.

Table 6: Species Richness (S), Shannon-Wiener Diversity Index (H'), and Pielou's Evenness Index (J') for the cryptic fish community in the years 2018 and 2024. Mean and 95% confidence intervals were calculated using bootstrapping. Two asterisks (**) indicate significant differences between 2018 and 2024 (p < 0.05), based on an approximated p-value. Note: Values for Species Richness (S) are rounded down to the nearest whole number for clarity in the main text.

	2018	2024
Biodiversity Metric	Mean (95% CI)	Mean (95% CI)
Species Richness (S)	3.397 (1, 8) **	1.335 (0, 3) **
Shannon-Wiener Diversity Index (H’)	0.922 (0.586, 1.236)	0.384 (0.140, 0.660)
Pielou’s Evenness Index (J’)	0.929 (0.836, 0.988)	0.545 (0.222, 0.859)

In addition to the significant changes observed in the cryptic fish community due to the presence of the invasive alga, the factor "Protection Level" significantly affected the community structure (p-value = 0.021; Table 1; MDS plot Figure 9, Appendix I). However, a significant interaction between the two factors, "Protection Level" and "Presence Invasive Species" could not be inferred, as the interaction term was not statistically significant (p = 0.062; Table 1).

3.4. Macroinvertebrates

The invasion of *R. okamurae* significantly altered the macroinvertebrate community composition (n = 20 samples), resulting in an average dissimilarity of 77.87% between the years. The total dissimilarity was attributable to changes in the average densities of multiple species with very small individual contributions (below 8%). The only species exceeding this threshold, contributing 8.12% to the observed differences was *Polycirrus* cf. *aurantiacus* (Table 7). This species experienced a significant decline in density, dropping from 1.05 individuals per square meter in 2018 to just 0.10 individuals per square meter in 2024. Indicating a shift towards greater similarity in community structure after the invasion of *R. okamurae*, the average similarity within the macroinvertebrate community composition increased from 28.78% to 34.80% from 2018 to 2024.

Table 7: SIMPER results showing changes in the density of Polycirrus cf. aurantiacus (individuals per m^2) and its contribution to the dissimilarity between 2018 and 2024. The table presents average densities (Avg. Density) with standard errors (SE) for the species in both years, as well as the dissimilarity-to-standard-deviation ratio (Diss/SD) and percentage contribution (%) to the overall dissimilarity.

	2018	2024		
Species	Avg. Density (SE)	Avg. Density (SE)	Diss/SD	Contribution %
Polycirrus cf. aurantiacus	1.05 (0.28)	0.10 (0.10)	1.04	8.12

The invasive algas' dominant coverage of the seafloor led to drastic changes in macroinvertebrate diversity, with both mean species richness and the mean Shannon-Wiener Diversity showing substantial declines from 2018 to 2024 (p < *0.001*; Table 2). Specifically, species richness dropped from an average of 5 to 2 species per sample, while the Shannon-Wiener Diversity Index diminished from 1.579 to 0.801 respectively (Table 8). However, the mean Pielou's Evenness Index did not show any significant change between 2018 and 2024 (p = *0.074*; Table 2), suggesting that the species distribution remained relatively stable over time, with species being rather evenly distributed (0.888 to 0.821; Table 8). This pattern further supports the results from the SIMPER analysis, which indicate that the total dissimilarity between the years was primarily driven by the low individual contributions of multiple species, with the highest contribution being just 8.12% (Table 7).

Table 8: Species Richness (S), Shannon-Wiener Diversity Index (H'), and Pielou's Evenness Index (J') for the macroinvertebrate community in the years 2018 and 2024. Mean and 95% confidence intervals were calculated using bootstrapping. Two asterisks (**) indicate significant differences between 2018 and 2024 (p < 0.05), based on approximated p-values. Note: Values for Species Richness (S) are rounded down to the nearest whole number for clarity in the main text.

	2018	2024
Biodiversity Metric	Mean (95% CI)	Mean (95% CI)
Species Richness (S)	5.942 (0, 10) **	2.854 (0, 7) **
Shannon-Wiener Diversity Index (H')	1.579 (1.343, 1.786) **	0.801 (0.524, 1.073) **
Pielou's Evenness Index (J')	0.888 (0.781, 0.963)	0.821 (0.648, 0.949)

Similarly to the cryptic fish community, the factor 'Protection Level' significantly influenced the macroinvertebrate community composition (Table 1; p = 0.046; MDS plot, Figure 9, Appendix I). However, this effect did not seem to be altered by the influence of the invasive species presence, as the interaction term was non-significant (Table 1; p-value = 0.175).

3.5. Algae Functional Groups

The composition of algae functional groups (n = 20 samples) was significantly affected by the invasion of *R. okamurae*, which fully dominates the upper algal layer, competitively occupying the seafloor. This led to a substantial increase in density for corticated foliose or foliose algae from 0.037 m^2 per m^2 of substrate in 2018 to 0.939 m^2 per m^2 in 2024, contributing 43.68% to the dissimilarity observed between the two years (Table 9). Consequently, the presence of the invasive algae resulted in a decrease in the density of other algal types classified as corticated foliose or foliose, from 0.037 m^2 per m^2 in 2018 to 0.015 m^2 per m^2 in 2024. Similarly, corticated macrophytes, which accounted for 24.86% of the total dissimilarity, decreased from 0.275 m^2 per m^2 in 2018 to 0.006 m^2 per m^2 in 2024. Algae groups growing beneath the dominant invasive layer, including crustose (14.28% contribution) and prostrate (8.29% contribution) algae, were also strongly impacted, leading to density changes from 0.518 m^2 per m^2 to 0.234 m^2 per m^2 for crustose algae, and from 0.051 m^2 per m^2 to 0.002 m^2 per m^2 for prostrate algae. The additional loss of functional groups such as articulated calcareous algae, filamentous algae, and leathery macrophytes resulted in a notable increase in average similarity within the functional groups, from 72.81% to 91.46%, suggesting that the sampled areas became nearly identical in terms of their functional group composition.

Table 9: SIMPER results displaying changes in densities (proportion of area covered in m^2 per m^2) of functional algae groups (Corticated foliose or foliose, Corticated macrophytes, Crustose, and Prostrate) and their contributions to the dissimilarity between 2018 and 2024. The table shows average densities (Avg. Density) with standard errors (SE) for each functional group in both years, as well as the dissimilarity-to-standard-deviation ratio (Diss/SD) and each group's percentage contribution (%) to the overall dissimilarity.

	2018	2024		
Species	Avg. Density (SE)	Avg. Density (SE)	Diss/SD	Contribution %
Corticated foliose or foliose	0.037 (0.010)	0.939 (0.028)	4.85	43.68
Corticated macrophytes	0.275 (0.030)	0.006 (0.003)	2.70	24.86
Crustose	0.518 (0.046)	0.234 (0.018)	1.82	14.28
Prostrate	0.051 (0.018)	0.002 (0.002)	1.06	8.29

Since species richness, the Shannon-Wiener Diversity Index, and Pielou's Evenness Index are generally used to assess diversity at the species level, each functional group of algae was treated as if it were a distinct species. This approach enabled a meaningful comparison of biodiversity metrics across years, capturing shifts in the composition of functional groups of algae. The comparison revealed that the dominance exhibited by *R. okamurae* led to significant reductions in both the mean species richness and the mean Shannon-Wiener Diversity Index ($p < 0.001$; Table 2). More precisely, species richness decreased from an average of 4 functional groups per sample in 2018 to just 2 in 2024, while the Shannon-Wiener Diversity Index dropped from 0.924 to 0.515 in the same period (Table 10). In contrast, the mean Pielou's Evenness Index showed a slight increase from 0.624 in 2018 to 0.662 in 2024, though this change was not statistically significant (p = *0.421*; Table 2).

Table 10: Species Richness (S), Shannon-Wiener Diversity Index (H'), and Pielou's Evenness Index (J') for the functional groups of algae in the years 2018 and 2024. Mean and 95% confidence intervals were calculated using bootstrapping. An asterisk (*) denotes significant differences between 2018 and 2024 (p < 0.05). Note: Values for Species Richness (S) are rounded down to the nearest whole number for clarity in the main text.

	2018	2024
Biodiversity Metric	Mean (95% CI)	Mean (95% CI)
Species Richness (S)	4.459 (3, 6) *	2.347 (2, 4) *
Shannon-Wiener Diversity Index (H')	0.924 (0.810, 1.045) *	0.515 (0.484, 0.546) *
Pielou's Evenness Index (J')	0.624 (0.563, 0.689)	0.662 (0.593, 0.726)

Apart from the strong effect of the presence of the invasive species, the factor "Protection Level" did not show a significant effect on the functional algae group composition. However, an interaction between the factors "Presence Invasive Species" and "Protection Level" could not be identified, as PRIMER was not able to provide a p-value for the interaction term, due to a negative pseudo-F value likely related to the small sample size. To address this issue, the PERMANOVA for this group was recalculated using the adonis2 function from the vegan package in R (Oksanen *et al.*, 2024). The function returns a single p-value for the entire model rather than individual p-values for each main effect and the interaction term, prioritizing the significance of the overall model over the individual contributions. To estimate the interaction between the invasive species' presence and the protection level, the effect of the protection level was tested separately for each year (representative of the presence of the invasive algae), allowing for a clearer interpretation of the effects of the two variables on each other. The notable difference in p-values (from 0.965 in 2018 before the invasion to 0.167 in 2024 after the invasion), suggests that the impact of the protection level on the functional algae group composition may not be consistent across years. However, since both p-values are above 0.05, it is impossible to conclude that there is a significant interaction between the presence of the invasive species and the protection level. Additionally, the effects of the protection level and the presence of the invasive algae were tested separately to elucidate any potential differences between the analysis with PRIMER and adonis2.

To ensure comparability with PRIMER, the data were square root transformed, and the distance matrix was calculated using the Bray-Curtis dissimilarity index (Clarke and Warwick, 2001). Although adonis2 operates on dissimilarity matrices instead of similarity matrices, as used in PRIMER, the p-values generated by both approaches can be interpreted in the same way. While the values within the resemblance matrix are inverted (i.e., a high dissimilarity value corresponds to low similarity), they fundamentally represent the same relationships among samples. Additionally, sequential testing was applied to all analyses performed with adonis2, and the number of permutations was matched to align the analytical approach with PRIMER's method for conducting PERMANOVA.

Due to slight differences in the implementation of the statistical tests and permutation procedures, the p-values generated by PRIMER and adonis2 may vary in magnitude (Anderson *et al.*, 2008; Oksanen *et al.*, 2024) ($p = 0.473$ in PRIMER vs. $p = 0.811$ in adonis2 for the factor “Protection Level”; see Table 1)

4. Discussion

4.1. Impacts on the Local Ecosystem

The invasion of *R. okamurae* in Sagres has profoundly altered the ecosystem structure, resulting in a fundamental shift in community composition and an overall loss in native biodiversity. The alga's rapid proliferation and near-complete coverage of the seafloor since its first appearance in the area in 2021 (Liulea *et al.*, 2023), highlights its capacity to dominate suitable habitats within a relatively short timeframe. Although our study was strongly limited by sample size, the heterogeneity in the data was proven to be extremely high, leading to the detection of highly significant differences. The consistency in p-values across different numbers of permutations improves the reliability of our results, suggesting that an increase in sample size would probably not lead to drastic changes in the results.

The catastrophic change in the structure of the studied ecosystem, marked by the loss of several functional groups of algae and a significant decrease in the remaining ones, is characteristic of a regime shift (Scheffer et al., 2001; Rietkerk et al., 2004). The clear separation between the samples from the two years, as illustrated by MDSs plots, confirms this assumption. *R. okamurae* is perceived to play a critical role as an ecosystem engineer, inducing physical changes in ecosystem structure and drastically altering habitat availability (Jones *et al.*, 1994). Its ability to induce regime shifts classifies it as a "high-impact" species, which, according to Gaertner *et al.* (2014), warrants prioritization for management action. Coastal ecosystems are especially vulnerable to state changes, because of their exposure to anthropogenic pressures and climate change (Diaz *et al.*, 2004; Thrush *et al.*, 2021b).

The loss of certain functional groups and the sharp decline in others are likely driven by competitive exclusion, influenced by spatial constraints and the defensive mechanisms of the invasive alga (e.g. growth-inhibiting secondary metabolites; Bernal-Ibáñez *et al.*, 2022). Before the invasion, the seafloor was characterized by an extensive coverage of crustose algae, unmatched by any other functional group, which explains the non-significant change in mean Pielou's Evenness Index between the two years. Connell (2003) showed that the extent of incrusting algae was not limited by canopy formation, which suggests that other factors are responsible for the decrease in average density for this group.

However, the shading resulting from the dense cover could potentially affect other taxa, such as small corticated macrophytes, corticated foliose, and foliose algae (Reed and Foster, 1984), and light-dependent benthic invertebrates.

The alternative stable state of the ecosystem is characterized by a pattern of increased average similarity across the whole benthic community, marking a trend toward habitat homogenization (Muthukrishnan and Larkin, 2020). A reduction in habitat heterogeneity can have cascading effects on other levels of the community, resulting in a loss of functional diversity and species richness (Tews *et al.*, 2004; Thrush *et al.*, 2006), which explains the observed pattern for demersal fish, cryptic fish, and macroinvertebrates. Our SIMPER results did not display significant changes in other habitat-structuring organisms, such as gorgonians and Porifera, indicating that the invasion is directly responsible for the observed changes.

Benthic habitats are a vital compartment for marine life, providing shelter, nursery areas, feeding, and breeding grounds for coastal fish and benthic invertebrates (Kritzer *et al.*, 2016; MSFD, 2017; Henseler *et al.*, 2019). They support higher trophic levels through the process of benthic-pelagic coupling, which is crucial for maintaining ecosystem functions such as nutrient cycling and energy transfer in food webs (Griffiths *et al.*, 2017). The heterogeneity of rocky habitats in temperate regions is largely defined by the composition of macroalgae, which guides the composition of associated species and mediates trophic interactions (Sebens, 1991; Norton *et al.*, 1996; Ware *et al.*, 2019). This highlights the significance of habitats characterized by different algae morphotypes (Hinz *et al.*, 2019), as macroalgae are an essential component of healthy rocky shore ecosystems (Norton *et al.*, 1996). Therefore, *R. okamurae's* dominance presumably disrupts the structure and functioning of food webs by influencing species interactions.

Although *R. okamurae* drastically decreases habitat heterogeneity, it might provide an alternative habitat for certain species due to its structural architecture (Navarro-Barranco *et al.*, 2019; Ware *et al.*, 2019). Sebens (1991) emphasizes, that canopy-forming algae establish stratified structures that create microhabitats for mobile invertebrates and establish a suitable environment for the growth of low-light adapted algae. In the Strait of Gibraltar, Navarro-Barranco *et al.* (2019) discovered that compared to its sympatric native species *Dictyota dichotoma, R. okamurae* hosted a more diverse assemblage of epifauna, almost comparable to more complex macroalga in the area. This shows that both habitat heterogeneity and habitat structure are crucial for maintaining community diversity and species coexistence (Sebens, 1991), although it is unclear to what extent the increase in habitat structure might balance the observed negative impacts (Bernal-Ibáñez *et al.*, 2022).

Overall, maintaining seafloor integrity is highly important to preserve marine biodiversity (MSFD, 2017), as most benthic habitats of the northeast Atlantic provide biodiversity maintenance services (Galparsoro *et al.*, 2014). According to Thrush *et al.* (2021), seafloor biodiversity is a vital component of ecosystem functioning, defining how marine ecosystems drive and react to change. Consequently, the loss of biodiversity can lead to increased susceptibility to disturbances (Biggs *et al.*, 2020), although functional diversity seems to be the main factor in maintaining ecosystem processes and health (Bolam *et al.*, 2002; Ware *et al.*, 2019).

The significant increases in densities for *Coris julis* and *Centrolabrus exoletus*, suggest that these two species may benefit from the establishment of the invasive alga. Since both species have a carnivorous diet consisting of benthic invertebrates (Sayer *et al.*, 1996; Pinnegar and Polunin, 2000), *R. okamurae* can be excluded as an alternative food source, on top of its high toxicity levels and the presence of feeding inhibitory compounds (Tokuda *et al.* 1994, as cited in Verlaque *et al.*, 2009; Barcellos *et al.*, 2023).

Cheminée *et al.* (2017) found, that *Coris julis* was more commonly associated with lower coverage of corticated macrophytes (e.g. *Cystoseira* spp.) and showed higher densities of juveniles in algae coverages characterized by Dictyotales and Sphacelariales. This might explain the increased abundance in the presence of *R. okamurae* due to its similar morphology (Cheminée *et al.*, 2017), likely providing more shelter and food for juveniles. Some of the observed juveniles were visually identified as *C. julis*, though further investigation is required to draw accurate conclusions. In a study by Cheminée *et al.* (2016), *C. julis* was shown to settle in *Caulerpa taxifolia* meadows in higher densities than other species, although larval recruitment significantly decreased with the reduction in habitat complexity. Observed individuals showed antioxidant adaptation mechanisms in the liver to prevent oxidative damage caused by the toxic levels of Caulerpenyne present in *Caulerpa* spp (Sureda *et al.*, 2006). This ability may provide *C. julis* with a competitive advantage in coping with high toxicity levels compared to other demersal fish.

The demersal fish species *Centrolabrus exoletus* is a carnivorous grazer of epifauna and has been shown to occupy a similar habitat as *C. julis,* characterized by rocky algal assemblages (Darwall *et al.*, 1992; Sayer *et al.*, 1996). A small portion of its diet consists of parasitic items, as this species has been observed to facultatively clean other fish species, including *C. julis* (Galeote and Otero, 1998). This potential food source combined with the species' grazing behavior might explain the increased density of this species in our study, as *R. okamurae* seemed to host a rather diverse assemblage of epifauna (Navarro-Barranco *et al.*, 2019).

Diplodus vulgaris' sharp decrease in density might be partly explained by the added resource competition with *C. julis* and *C. exoletus* combined with the shift from more complex algae like *Cystoseira* spp. to less structured Dictyotales, such as *R. okamurae,* that generally have lower prey densities (Hinz *et al.*, 2019). Furthermore, the dense algal cover might provide an unsuitable habitat for *D. vulgaris,* as this species does not hide inside but rather behind algae, using them as a "parkour of obstacles" to evade human predators (Hinz *et al.*, 2019). This is particularly concerning since *D. vulgaris* is subject to intense fishing pressure and protected areas are considered crucial for ensuring the species' protection during the spawning season (Alós *et al.*, 2012). Additionally, the effects of the invasion likely extend beyond the PMSACV, leading to an underestimation of its impact on commercially valuable species, such as *D. vulgaris,* that are caught outside the protected area. The decrease in *D. vulgaris* could potentially have cascading effects on the benthic community, as this species has been found to prey on the sea urchin *Paracentrotus lividus* (Gil Fernández *et al.*, 2016).

The significant decreases in the mean Shannon-Wiener Diversity and Pielou's Evenness Index stress the dominant role exerted by *C. julis* and *C.exoletus,* accompanied by an overall decrease in biodiversity. Our results highlight that species-specific responses to environmental changes do not necessarily reflect the overall trends observed in the community. However, the true reasons for changes in fish densities are hard to observe given the dense alga coverage, which only allows for speculation. Therefore, it would be beneficial to repeat the sampling in winter, when the alga is less dense (García-Gómez *et al.*, 2020). Demersal fish tend to be territorial (Lejeune, 1987; Sayer *et al.*, 1996), suggesting that the individuals sampled in summer will most likely be encountered in the same locations in winter. However, the different abiotic conditions in winter might also impact other species and influence their dynamics, by introducing an additional variable.

Compared to demersal fish, cryptic fish are much harder to detect due to their successful camouflage on the seafloor (Encel and Ward, 2021), making it nearly impossible to observe them beneath the dense canopy of *R. okamurae*. As a results, the presence of empty samples can artificially increase the dissimilarity between the years, leading to a potential overestimation of the perceived impact. Consequently, the coverage of *R. okamurae* is likely the main reason for the observed differences and the significant reduction in mean species richness. The non-significant patterns observed for the mean Shannon-Wiener Diversity and mean Pielou's Evenness Index could be caused by the considerable number of empty samples, providing insufficient variability for the pair-wise test applied.

We hypothesize that the increased habitat structure could potentially even have a positive impact on the survival and reproduction of cryptic fish, similar to *C. julis* and *C. exoletus.* The dense canopy formed by *R. okamurae* could provide additional protection from predators, since *P. pilicornis,* like other blennies, uses small holes for shelter and as spawning grounds (Nieder, 1997). Since *P. pilicornis* has a highly diverse omnivorous diet that is characterized by a high degree of seasonal variability (Nieder, 1997), this species should adjust rather flexibly to changing food sources.

To correctly estimate the impact of *R. okamurae* on the cryptic fish community, it would be beneficial to repeat the present study during winter, when the alga is less dense (García-Gómez *et al.*, 2020).

The terebellid polychaete *Polycirrus cf. aurantiacus* sharply declined in density after the invasion of *R. okamurae.* The growth of the invasive alga potentially impacts the habitat of *P. cf. aurantiacus,* as this species is generally found under stones and in rock crevices. However, the observed impact on *P. cf. aurantiacus* might be slightly overestimated due to the removal of *R. okamurae* prior to sampling. *P. aurantiacus* shows a great number of retractable tentacles, that are highly sensitive to touch (Dales, 1955). Therefore, the disturbance of the seafloor immediately before recording the abundance of this species might be responsible for the observed reduction in density in 2024. Consequently, it would be beneficial to repeat the study in winter, to reduce the potential impact of the sampling method on the surveyed organisms, while considering the influence of this added factor on other species.

The low individual contributions of multiple species to the total dissimilarity between the years implies that the invasion impacts multiple species equally, rather than triggering species-specific responses. This pattern might be caused by the dense formation of algal canopy, which can decrease water circulation, potentially restricting food supply for sessile invertebrates living on the substrate (Sebens, 1991). Many grazers primarily consume smaller, less complex algae, that are generally found beneath the canopy (Steneck and Dethier, 1994). Additionally, the establishment of benthic invertebrates may be impeded by a 'whiplash' effect of the invasive alga, caused by surge action (Dayton, 1975; Sebens, 1991). However, it is also important to notice, that some quadrats sampled in 2024 did not contain any individuals, adding to the dissimilarity between the years without offering any additional information.

The significant declines in mean species richness and mean Shannon-Wiener Diversity Index indicate a substantial loss in biodiversity, driven by the presence of fewer species, rather than species evenness, which remained relatively stable. This aligns with the assumption that the total dissimilarity between the years was driven by multiple species rather than a few dominant ones.

The invasion of *R. okamurae* might have several biological implications that were not assessed in the scope of this study. García-Gómez *et al.* (2018) reported, that besides displacing autochthonous food sources and important nursery areas for the sea urchin *Sphaerechinus granularis,* the presence of the alga apparently led to a reduction in gonad size. In another study Casal-Porras *et al.* (2021) found, that the high concentration of the secondary metabolite Dilkamural in the invasive alga not only displayed feeding-inhibitory characteristics but also induced harmful or even lethal effects on the grazing sea urchin *Paracentrotus lividus.*

Importantly, accurate species identification in situ requires extensive experience, especially for hard-to-identify taxa such as Porifera and Bryozoans. This task becomes even more challenging in difficult conditions with limited visibility, such as those present in Sagres. As a result, some species were identified solely from in-situ photographs, and even with expert assistance, could not be identified at the species level. Broad expertise in the occurring taxa, paired with high image resolution would mitigate some of these issues.

4.2. Comparison with Previous Research

This is the first study assessing the ecological impact of *R.* okamurae on the benthic subtidal communities in mainland Portugal. Our findings contribute to a growing body of research, supporting the results of previous studies by García-Gómez *et al.* (2020, 2021a) and Faria *et al.* (2022), who found similar trends in coastal areas of the El Estrecho Natural Park (Biosphere Reserve) in the Strait of Gibraltar and in the Azores Archipelago, respectively. In both studies, *R. okamurae* has been shown to dominantly occupy the available habitat, severely modifying the structure of resident communities and causing decreases in species richness, species abundance, and natural variability. Recent studies in the Mediterranean and the North African coast of the Strait of Gibraltar present paralleling results (Ruitton *et al.*, 2021; Sempere-Valverde *et al.*, 2021).

Consistent with our findings, the impact of *R. okamurae* on native macroalgae seems to be the most devastating (García-Gómez *et al.*, 2018). Recent studies report substantial reductions in the abundance of native macroalgae, prompted by competition for space and resources (Bernal-Ibáñez *et al.*, 2022; Faria *et al.*, 2022; García-Gómez *et al.*, 2021b). In fact, Faria *et al.* (2022) found massive declines in several functional groups of algae, including articulated coralline algae, corticated algae, and corticated foliose algae, following the invasion. The same trend was observed in the Strait of Gibraltar, characterized by alterations of the coralligenous community structure (Sempere-Valverde *et al.*, 2021). These results confirm that the loss of habitat is by far the greatest threat to macroalgae (Norton *et al.*, 1996).

The recent introduction of *R. okamurae* in Madeira, documented by Bernal-Ibáñez *et al.* (2022), could give important insights into the spatial variations of the species' establishment by underlining factors that limit its distribution. The authors predict a medium-high risk of invasion in the area, although it is uncertain whether the oligotrophic conditions of the island will support equally explosive quantities of biomass as demonstrated in other invaded regions. Our findings align with the observation that invasive macroalgae predominantly alter competitive relationships, characterized by space monopolization and changed community composition (Davidson *et al.*, 2015). The assumption that invasive species, along with global change, will eventually result in biotic homogenization (Schaffelke *et al.*, 2006) has been confirmed in this study. Consequently, the homogenization of the seafloor is not only caused by physical destruction and selective removal of habitat-forming species but also by the introduction of competitive habitat-forming species (Thrush *et al.*, 2006).

Inside the PMSACV, Gil Fernández *et al.* (2016) found that algal assemblages in the Martinhal Islands PPI zone and the adjacent PC zone significantly differed from each other. However, this pattern was not observed for the invasive species *Asparagopsis armata.* Comparably, MPAs in the Azores Archipelago hosted more diverse shallow-water macroalgal communities than non-protected areas but did not seem to influence the distribution of *A. armata* (Cacabelos *et al.*, 2020). These results align with our findings for *R. okamurae*, suggesting that MPAs might be insufficient conservation measures to counteract invasive species, which could lead to an increased degree of habitat homogenization and emphasizes the need for more targeted management strategies, such as physical removal, chemical control or bio-control (Schaffelke *et al.*, 2006).

Most studies on *R. okamurae* focus on its adverse effects on the recipient communities, and it is undetermined whether certain dynamics might balance these negative impacts (Bernal-Ibáñez *et al.*, 2022). Navarro-Barranco *et al.* (2019) pointed out that *R. okamurae* could still provide ample resources for predators, such as many demersal fish if it supports a relatively diverse and abundant invertebrate community (Taylor, 1998; Jiménez-Prada *et al.*, 2015). In a review by Davidson *et al.* (2015) only 9 out of 158 published case studies for marine macroalgae invasions showed an increased diversity of epibionts or benthic macrofauna, highlighting that this scenario is rather rare and might not be sufficient to balance the negative impacts of marine macroalgae invasions.

Consequently, further research is necessary to fully understand the range of the invasions ecological effects and its persistence over time (Faria *et al.*, 2022), as the individual components of the community might be affected differently (Navarro-Barranco *et al.*, 2019). It is crucial to consider that studies on invasive macroalgae are often short-term, focusing solely on the expansion phase of the invasion, which does not account for possible variations in invasive pressure in later stages (Fernández, 2020). As species invasions are an irreversible process (Boudouresque *et al.*, 2005), it is important to consider their evolution on a global scale to identify possible patterns of the invasion process (Boudouresque and Verlaque, 2012). Importantly, past invasions can offer useful insights into possible trajectories of current and future invasions.

4.3. Invasion Kinetics

When focusing on the histories of other invasive algae it becomes apparent that the trajectories of invasion are rather variable for distinct species. However, according to Boudouresque and Verlaque (2012), two models are generally used to describe the evolution of biological invasions: The ‘natural fluctuations model’ and the ‘boom and bust model’. Invasions classified under the former exhibit a ‘persistence phase’, after the species has successfully occupied all available habitats within its entire accessible geographical range, where species abundances naturally fluctuate (Boudouresque *et al.*, 2005; Boudouresque and Verlaque, 2012). The boom and bust model is a well-known concept in invasion biology, describing how invaders experience a strong population growth (“boom” phase, expansion phase) with a subsequent decline in population size (“bust” phase), suggesting that their impacts on ecosystem processes and native species are reversible to some extent (Lockwood *et al.*, 2013; Strayer *et al.*, 2017). According to Boudouresque and Verlaque (2012), natural declines in population size for invasive species are rather rare and can generally be explained by species interactions (e.g. predator-prey), low genetic diversity, climatic variation, or the misinterpretations of natural variation in abundance during the persistence phase.

Studying the evolution of some of the worst macroalgae invasions in Europe and worldwide (Lowe *et al.*, 2000; Streftaris and Zenetos, 2006) can provide important insights into the development of invasions across spatial and temporal scales, and might be useful for predicting the trajectory of the recent invasion of *R. okamurae*:

- The red macroalga Asparagopsis armata, native to Southern and Western Australia, as well as New Zealand (Harvey, 1855; Horridge, 1951), was first introduced to the Atlantic and Mediterranean in the 1920s, and has since spread to areas from the British Isles to Senegal and to other areas of the world (Cacabelos *et al.*, 2020; Pinteus *et al.*, 2021; Silva *et al.*, 2021). In the north of Spain, the area occupied by *A. armata* has substantially expanded in the last few years and its abundance largely increased in the southwestern part of the Bay of Biscay between 2014 and 2021 (Ramos *et al.*, 2020; Carreira-Flores *et al.*, 2023). However, research on the temporal variation in these abundances across the invaded regions remains limited (Carreira-Flores *et al.*, 2023).
- The red alga *Grateloupia turuturu,* originating from Japan, has extended its distribution to the northeastern and northwestern Atlantic coasts, the Mediterranean, as well as to Australia and New Zealand (Araújo *et al.*, 2011), after its earliest observation in British marine water in 1969 (Farnham and Irvine, 1973, as cited in Petrocelli *et al.*, 2020). There is a lack of research on the evolution of *G. turuturu's* invasion, but a recent study has shown an extension in its distribution along the southwestern coast of the Bay of Biscay between 2014 and 2021 (Carreira-Flores *et al.*, 2023).
- The green macroalga *Caulerpa taxifolia,* originally from the Caribbean Sea, was introduced to the Mediterranean in the 1980s (Meinesz and Hesse, 1991), where it became extremely invasive until the 2000s when its abundance naturally declined to a very low level ('bust') (Montefalcone *et al.*, 2015). Various *Caulerpa* species have shown similar patterns of rapid proliferation followed by complete disappearance or decline (Jaubert *et al.*, 2003).

  Caulerpa prolifera, a congener of *C. taxifolia* and native to the Mediterranean, is generally not considered invasive; however, Parreira *et al.* (2021) found that the recent infestation of unvegetated bottoms in the Ria Formosa coastal lagoon in Portugal has triggered notable ecological changes, similar to other invasive species. The discovery of *C. prolifera* in California in 2021 raises serious concerns, given that the highly invasive *C. taxifolia* was successfully eradicated from this region in 2006 (McHaskell, 2024).
- The invasive green macroalgae *Codium fragile,* native to Japan (Silva, 1955), was first discovered in Europe before 1900, subsequently spreading along the shores of the north-

east and north-west Atlantic, the Mediterranean, the north-east Pacific and Australasia (Trowbridge, 2001; Provan *et al.*, 2005). Chapman (1998) found that despite the persistence and continued dispersal, the species experiences notable variations in abundance and community dominance across these regions, occurring in rather separated, small populations in Europe while exhibiting much more substantial impacts in other regions.

  Trowbridge *et al.* (2013) have shown that *C. fragile* abundances in the British Isles and Ireland have substantially declined after phases of strong proliferation between the 1940s and 1970s. These findings are contrary to the persistence of the alga on the Atlantic coasts of Canada and the United States (Trowbridge *et al.*, 2013).
- Native to the northwest Pacific, the invasive kelp *Undaria pinnatifida* was first discovered in the Mediterranean in the 1970s (Perez *et al.*, 1981) and has since progressively extended its distribution worldwide (Epstein and Smale, 2017). In New Zealand, *U. pinnatifida* experienced a population collapse in 2018, a decade after the onset of its expansion phase, marking the first 'invasion front collapse' of this alga (Lorkin, 2019). Yet, in 2021, *U. pinnatifida* was first observed along the southwestern coast of the Bay of Biscay (Carreira-Flores *et al.*, 2023), indicating continued dispersal.
- The canopy-forming brown macroalga *Sargassum muticum*, native to Asia, was introduced to North America during the 1940s and to Europe in the early 1970s (Farnham *et al.*, 1973; Critchley *et al.*, 1990; Pinteus *et al.*, 2021). Fernández (2020) identified that in northern Spain, at the site where the invasion was first detected, *S. muticum* experienced a 'boom' phase from 1990 to 2002, followed by a 'bust' phase between 2002 and 2010, which resulted in a complete population collapse. Contrary to these findings, the population on the southwestern coast of the Bay of Biscay showed a general increase in abundance (Carreira-Flores *et al.*, 2023).

Regarding *R. okamurae,* only the blooms of pelagic Sargassum spp., specifically *Sargassum fluitans* and *Sargassum natans* (Franks *et al.*, 2011), in the western Atlantic appear to resemble its invasive behavior (García-Gómez *et al.*, 2020). The authors relate the blooms of Sargassum spp. to the presence of the "Great Atlantic Sargassum Belt", representing the greatest macroalgal bloom globally (Wang *et al.*, 2019; García-Gómez *et al.*, 2020). Although both *S. fluitans* and *S. natans* are native to the North Atlantic, unlike *R. okamurae*, their ecological impacts are equally devastating (van Tussenbroek *et al.*, 2017). García-Gómez *et al.* (2020) hypothesize that the uncontrolled biomass proliferation of *R. okamurae* might be caused by supplementary nutrient inputs, similar to the bloom of *Sargassum spp*.

4.4. Future Research

The histories of these macroalgae invasions show that marine macroalga invasions are very dynamic, characterized by frequent fluctuations in abundance and distribution (Fernández, 2020). They often deviate from model predictions, emphasizing the need for continued monitoring (Carreira-Flores *et al.*, 2023). Additionally, the outcome of species introductions is highly unpredictable, as it is largely influenced by the environmental conditions of the receiving habitat (Petrocelli *et al.*, 2020).

Many invasive species possess similar characteristics responsible for their invasive success, namely rapid proliferation and efficient dispersion, great photosynthetic activity, high reproductive capacity, rapid uptake of nutrients, high competitive capacity, etc. (Pinteus *et al.*, 2021). However, results from Faria *et al.* (2022) emphasize that the severe invasive nature of *R. okamurae* is unmatched by other, better-known invasive macroalgae. García-Gómez *et al.* (2020) observed, that when *R. okamurae* interacts with other invasive species in the Strait of Gibraltar, such as *A. armata, Asparagopsis taxiformis*, and *Caulerpa cylindracea,* it dominantly displaces all three. Only in 6% of cases surveyed, *R. okamurae* was observed as a basibiont (i.e., host to a macroalgal epibiont) (García-Gómez *et al.*, 2021b). In Madeira, *R. okamurae* was more commonly observed as an epiphyte of *A. taxiformis* (Bernal-Ibáñez *et al.*, 2022). Moreover, *R. okamurae* accumulations on beaches in the Algarve have also been observed far more frequently than those of other invasive species, such as *A. armata* and *Ulva spp.,* which were only recorded in spring and summer instead of year-round (Herrero *et al.*, 2023).

The increased susceptibility of the ecosystem due to the changes in biodiversity could potentially facilitate further species introductions in a process referred to as 'invasional meltdown' (Simberloff and Von Holle, 1999), although it is plausible that *R. okamurae* would rapidly outcompete the new invaders considering its interactions with other invasive species.

It is crucial to consider that studies on invasive macroalgae are often short-term and focus on the expansion phase of the invasion (Fernández, 2020). For most of them, not enough time has passed since the introduction event to accurately evaluate the prevailing invasions dynamics (Boudouresque and Verlaque, 2012). Despite this, it is almost certain that *R. okamurae* has not completed the expansion phase of its invasion. Although it has successfully invaded the rocky central and western beaches of the Algarve (Herrero *et al.*, 2023), the alga has not stopped spreading along the Portuguese West coast. It was recently recorded for the first time in the Professor Luiz Saldanha Marine Park in Sesimbra (personal communication). According to distribution models, the coastlines of the Mediterranean Sea, North Africa, and the Iberian

Peninsula seem suitable for the continued expansion of *R. okamurae* (MITECO, 2022; Herrero *et al.*, 2023), although this might not necessarily represent the eventual outcome (Carreira-Flores *et al.*, 2023). This reinforces the need for continued monitoring of the invasion to examine how its impact might change over time, and to assess what will happened if all available habitat is occupied. If the invasion enters the “persistence phase”, which is highly likely, its impacts are not expected to diminish over time and with increased distance (Boudouresque *et al.*, 2005). Therefore, it is crucial to integrate research on the potential long-term impacts of *R. okamurae* on benthic ecosystems and the evolution of its abundance and distribution. Future studies should aim to build upon these findings by incorporating longer-term monitoring and expanding the geographical scope to include adjacent areas, providing a more comprehensive understanding of invasion dynamics. Continued monitoring and targeted conservation efforts, particularly for species directly impacted by the invasion, will be essential for understanding and mitigating the long-term ecological consequences of *R. okamurae*. However, Thrush *et al.* (2006) underscores that habitat management offers greater potential for the conservation of the seafloor compared to species management. Potential restoration strategies for impacted species, and particularly for functional groups of algae, could help to improve the ecosystem's resistance to change, by mitigating the negative effects caused by the structural change. Additionally, it could be beneficial to evaluate the effectiveness of various management strategies for controlling the alga, including the exploration of new removal techniques, while considering their logistical and economic aspects. It remains uncertain whether it would be beneficial to invest efforts into the eradication of the species, as Ojaveer *et al.* (2014) highlight that successful eradication attempts of marine invasive species are a rather rare event. Also, it is still unknown whether an ecosystem can fully recover after the successful eradication of an invasive species (Boudouresque *et al.*, 2005). Moreover, the removal and control of the invasive species through physical intervention require a vast amount of effort and time and are therefore associated with a significant financial burden. However, the potential uses of *R. okamurae* in biomedical, pharmaceutical, and food industries (Barcellos *et al.*, 2023), could provide a compelling financial incentive to pursue such efforts.

In several instances, *R. okamurae* has been observed to detach from the substrate and accumulate in rock crevices (personal observation). In addition to complicating the assessment of the benthic community in these locations, the loose algae may have additional impacts on the ecosystem that were beyond the scope of this study. Studies have shown that detached fragments are able to reattach to hard surfaces (Figueroa *et al.*, 2020; Bernal-Ibáñez *et al.*, 2022) or even establish themselves as epibionts of other species (e.g. gorgonians) (Sempere-

Valverde *et al.*, 2021; García-Gómez *et al.*, 2021b), further promoting the alga's dispersal. Alga fragments that remain free-floating can results in the decomposition of large quantities of biomass near the coast and in deeper water, which has been shown to further degrade local ecosystems resulting in the increased mortality of fish and other organisms (MITECO, 2020; Bernal-Ibáñez *et al.*, 2022). The affected areas are commonly referred to as "dead zones" (Thrush *et al.*, 2006). In conclusion, it is unsurprising that the detached alga strongly impacts the fishing sector in our study area, with local fishermen voicing concerns about fewer catches, fewer fishing grounds and their nets being covered in algae (personal communication). Future research could focus on these changes in the fishing sector, expanding the monitoring efforts and impact studies to areas where commercial fishing takes place, and potentially identifying patterns of benthic-pelagic coupling.

In a recent study, García-Gómez *et al.* (2021c, 2021b) showed, that *R. okamurae* is also capable of colonizing a broad range of artificial substrates, including plastic. Since floating plastic debris is a well-known and effective vector for marine species introductions and secondary spread (Rech *et al.*, 2016), this observation is highly concerning, making it a critical focus for future research.

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Appendix I – Figures and Tables

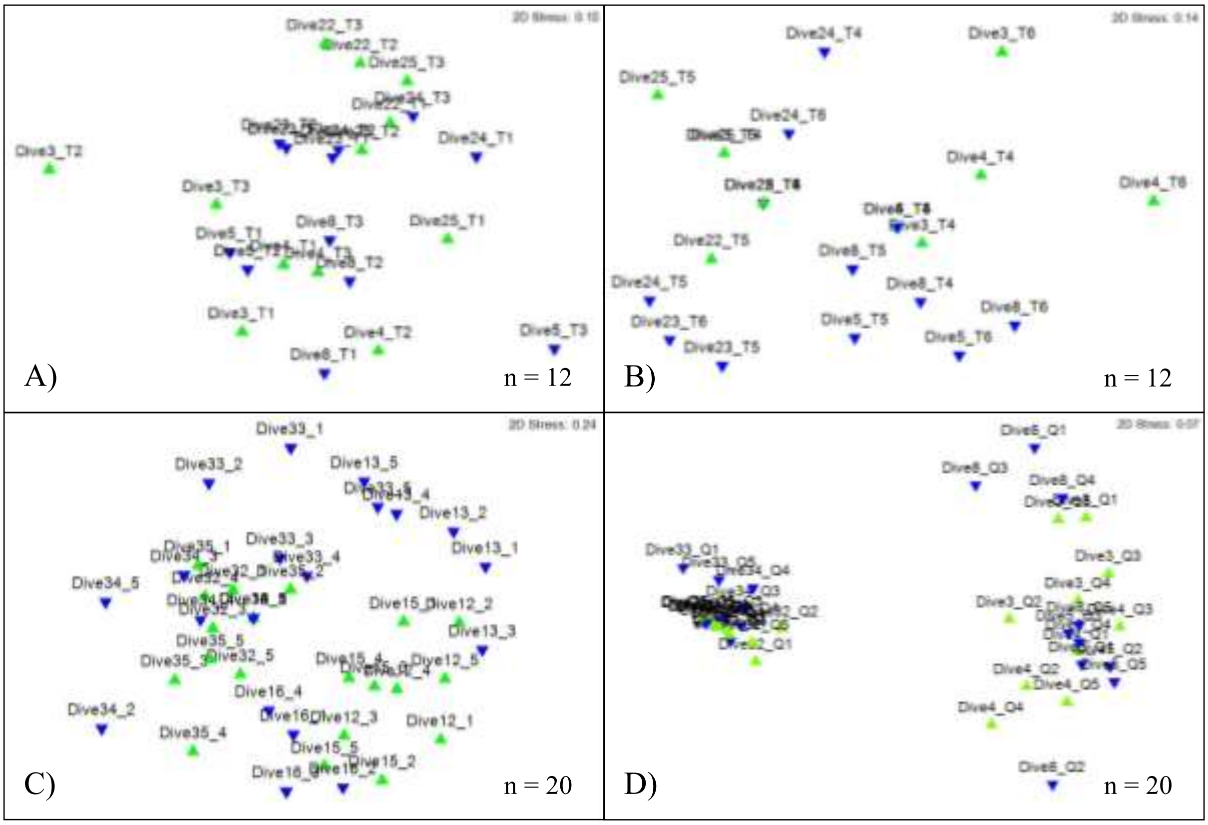


Figure 9: MDS plots for the different groups, visualizing the differences in the community structure with the factor protection level for A) demersal fish, B) cryptic fish, C) macroinvertebrates and D) algae functional groups. The green triangles represent Partial Protection I (PPI), while the blue triangles represent Complementary Protection (PC). The data was square root transformed, and the resemblance matrix was calculated using the Bray-Curtis similarity. MDS plots generated in PRIMER version 6.1.11 (Clarke and Gorley, 2008).

*Table 11: Results of PERMANOVA analysis (4999 permutations) showing p-values for the effects of **Presence of Invasive Species**, **Protection Level**, and their interaction (**Presence x Protection**) on community composition across different biological groups. Significant p-values ($p < 0.05$) are highlighed with an asterisk (*). Layout based on Belackova* et al. *(2023).*

Biological Group	Presence Invasive Species	Protection Level	Presence x Protection
Demersal Fish	0.0002*	0.1834	0.7386
Cryptic Fish	0.0002*	0.0238*	0.069
Macroinvertebrates	0.0002*	0.0478*	0.1898
Algae Functional Groups	0.0002* / *0.0002**	0.486 / *0.8084*	*0.9658 / 0.1732*

Table 12: Results of PERMANOVA analysis (9999 permutations) showing p-values for the effects of ***Presence of Invasive Species****,* ***Protection Level****, and their interaction (****Presence x Protection****) on community composition across different biological groups. Significant p-values ($p < 0.05$) are highlighed with an asterisk (*). Layout based on Belackova* et al. *(2023).*

Biological Group	Presence Invasive Species	Protection Level	Presence x Protection
Demersal Fish	0.0001*	0.1712	0.7189
Cryptic Fish	0.0001*	0.0258*	0.0615
Macroinvertebrates	0.0001*	0.0464*	0.1881
Algae Functional Groups	0.0001* / *0.0001**	0.4767 / *0.8131*	*0.9652 / 0.1865*

Appendix II – Complete SIMPER Results

Table 13: Complete SIMPER results for demersal fish, showing the changes in average abundance (Av. Abund) between the two sampling years 2018 and 2024, average dissimilarity (Av. Diss), the dissimilarity-to-standard deviation ratio (Diss/SD), the percentage contribution of each species to the total dissimilarity (Contrib %), and their cumulative percentage contribution (Cum. %).

	2018	2024				
Species	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib %	Cum. %
Coris julis	1.71	6.03	19.55	1.85	26.91	26.91
Centrolabrus exoletus	0.17	2.00	7.75	1.03	10.67	37.58
Diplodus vulgaris	2.24	0.73	7.75	1.48	10.66	48.24
Boops boops	1.43	0.64	7.14	0.78	9.83	58.07
Diplodus sargus	1.26	0.35	5.06	1.11	6.96	65.04
Labrus mixtus	0.00	0.71	3.14	1.14	4.32	69.35
Ctenolabrus rupestris	0.49	0.69	3.08	1.09	4.24	73.59
Serranus cabrilla	0.65	0.50	3.07	1.16	4.23	77.82
Symphodus melops	0.08	0.59	2.99	0.78	4.12	81.94
Diplodus puntazzo	0.56	0.08	2.76	0.63	3.80	85.74
Labrus bergylta	0.33	0.43	2.42	0.89	3.33	89.06
Symphodus sp.	0.00	0.35	1.73	0.54	2.38	91.44

Table 14: Complete SIMPER results for cryptic fish, showing the changes in average abundance (Av. Abund) between the two sampling years 2018 and 2024, *average dissimilarity (Av. Diss), the dissimilarity-to-standard deviation ratio (Diss/SD), the percentage contribution of each species to the total dissimilarity (Contrib %), and their cumulative percentage contribution (Cum. %).*

	2018	2024				
Species	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib %	Cum. %
Parablennius pilicornis	0.89	0.00	12.97	1.29	19.34	19.34
Tripterygion delaisi	0.65	0.00	9.05	0.96	13.49	32.83
Gobius xanthocephalus	0.43	0.00	5.22	0.61	7.78	40.61
Labrus mixtus	0.00	0.40	5.09	0.66	7.59	48.19
Parablennius rouxi	0.37	0.00	4.29	0.60	6.40	54.59
Gobius paganellus	0.08	0.17	3.40	0.41	5.07	59.66
Scorpaena porcus	0.25	0.08	2.99	0.55	4.46	64.12
Scomber colias	0.42	0.00	2.66	0.30	3.97	68.09
Parablennius gattorugine	0.17	0.08	2.58	0.50	3.85	71.94
Symphodus sp.	0.17	0.08	2.36	0.53	3.52	75.47
Serranus cabrilla	0.08	0.08	2.09	0.41	3.12	78.59
Oblada melanura	0.32	0.00	2.06	0.30	3.08	81.67
Ctenolabrus rupestris	0.00	0.17	1.95	0.43	2.92	84.58
Gobius cruentatus	0.17	0.00	1.61	0.44	2.40	86.98
Boops boops	0.19	0.00	1.60	0.30	2.39	89.37
Coris julis	0.00	0.12	1.53	0.29	2.28	91.65

Table 15: Complete SIMPER results for macroinvertebrates, showing the changes in average abundance (Av. Abund) between the two sampling years 2018 and 2024*, average dissimilarity (Av. Diss), the dissimilarity-to-standard deviation ratio (Diss/SD), the percentage contribution of each species to the total dissimilarity (Contrib %), and their cumulative percentage contribution (Cum. %).*

	2018	2024				
Species	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib %	Cum. %
Polycirrus cf. aurantiacus	0.76	0.07	6.32	1.04	8.12	8.12
Bryozoa sp.	0.00	0.66	5.19	0.84	6.67	14.79
Leuconia johnstoni	0.53	0.00	4.19	0.66	5.38	20.17
Sarcotragus foetidus	0.29	0.17	3.48	0.58	4.47	24.64
Holothuria (Panningothuria) forskali	0.38	0.14	3.28	0.68	4.22	28.85
Scopalina lophyropoda	0.33	0.15	3.21	0.65	4.12	32.97
Holothuria (Holothuria) mammata	0.34	0.10	2.98	0.65	3.83	36.80
Paracentrotus lividus	0.40	0.00	2.89	0.61	3.72	40.52
Ophioderma longicauda	0.10	0.27	2.84	0.51	3.64	44.16
Anemonia sulcata	0.27	0.00	2.64	0.54	3.40	47.56
Holothuria (Roweothuria) arguinensis	0.25	0.00	2.57	0.53	3.30	50.86
Sphaerechinus granularis	0.19	0.15	2.41	0.56	3.09	53.95
Aplidium punctum	0.34	0.00	2.40	0.46	3.08	57.04
Phorbas fictitius	0.10	0.29	2.30	0.50	2.96	59.99
Serpula vermicularis	0.30	0.00	2.27	0.60	2.91	62.91
Leptogorgia sarmentosa	0.20	0.16	2.11	0.57	2.72	65.62
Ophiocomina nigra	0.28	0.00	2.01	0.45	2.59	68.21
Eunicella verrucosa	0.15	0.15	1.95	0.54	2.50	70.71
Porifera n. id.	0.00	0.26	1.59	0.46	2.05	72.75
Polychaeta n. id.	0.00	0.23	1.54	0.40	1.98	74.73
Crambe crambe	0.15	0.07	1.39	0.46	1.78	76.51
Eunicella gazella	0.15	0.05	1.37	0.46	1.76	78.27
Octopus vulgaris	0.15	0.00	1.37	0.38	1.75	80.03
Marthasterias glacialis	0.17	0.00	1.30	0.41	1.67	81.70

Table 15 (continued): Complete SIMPER results for macroinvertebrates.

	2018	2024				
Species	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib %	Cum. %
Filograna implexa	0.17	0.00	1.24	0.40	1.59	83.29
Cliona viridis	0.12	0.00	0.98	0.32	1.26	84.55
Aiptasia diaphana	0.15	0.00	0.95	0.31	1.22	85.77
Sarcotragus fasciculatus	0.12	0.00	0.73	0.32	0.94	86.71
Protula intestinum	0.10	0.00	0.73	0.32	0.94	87.65
Calliactis parasitica	0.00	0.11	0.70	0.22	0.90	88.56
Lanice conchilega	0.00	0.11	0.70	0.22	0.89	89.45
Diplosoma cf. listerianum	0.00	0.07	0.65	0.21	0.83	90.28

Appendix III – Dive Plan

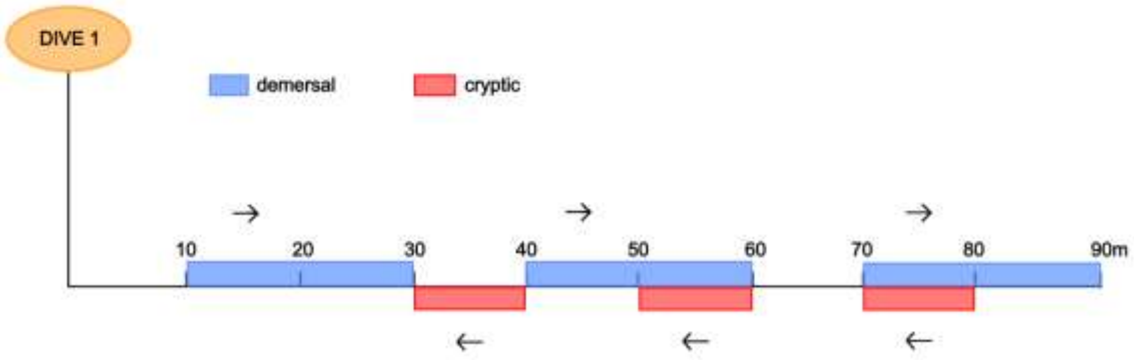
Table of Contents

1. Dive sites

Table 16: Geographic coordinates, protection levels, depth characteristics, and transect headings of sampled locations in Sagres, Southwest Portugal. Protection levels are designated as PPI (Partial Protection I) and PC (Complementary Protection).

Location	Protection Level	Latitude	Longitude	Heading	Average Depth	Maximum Depth
PontadosCaminhos	PPI	37.02265	-8.90763	240	9.7	12.6
Atalaia	PC	37.00814	-8.92507	270	16.4	20
Martinhal Ilhas	PPI	37.01525	-8.91737	100	11.5	14
BarrancoW	PC	37.03276	-8.89832	270	11.5	14.3

A. Dive 1: Fish census

Study variables	Fish abundance / diversity
Factors	Location Protection Level Year/ Presence invasive species Average Depth Substrate Temperature
Sampling design	Systematic band transects for fish census Demersal 20x4m: From 10 to 30m, 40 to 60m and 70 to 90m (3 pseudo-replicates) Cryptic 10x1m: From 80 to 70m, 60 to 50m and 40 to 30m (3 pseudo-replicates)
Illustration of the dive  	

GUE EDGE

G oal	Fish census for demersal and cryptic fish along a transect, assessment of abundance and diversity
U nified Team	Two divers
E qipment	Special equipment see list below
E xposure	Depth: depending on site, 10 -20m, Time: ~ 40 min
D ecompression	Stay withing no deco limits, 9m/min until half the depth, steps of 3m/min until surface
G as	Depending on depth of dive site 10m: Turning pressure 120 bar, Minimum gas 40 bar 20m: Turning pressure 130 bar, Minimum gas 60 bar Rule of thirds: Turning pressure 140 bar (do if possible) (stay conservative!) **
E nvironment	Awareness of rocks, Boat entry

** Turning pressure:

full tank – ((full tank – reserve) / 2)

10m: 200 – ((200bar – 40bar) / 2) = 120 bar

20m: 200 – ((200bar – 60bar) / 2) = 130 bar

OR rule of thirds: turning pressure 200 – (200/3) = ~140 (more conservative)

Equipment

- Datum
- Compass
- Transect (two fibreglass measuring tapes of 50 m each)
- Wet notes with pencils for one diver
- 1 kg to fix transect

Tasks

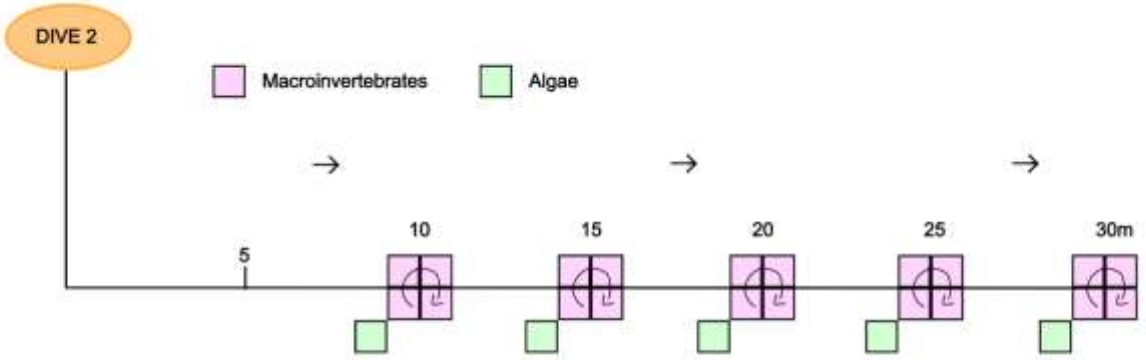
1. Set Datum (with correct GPS coordinates of dive site), connect transect to datum
2. Identify heading (defined in MARSW project)
3. Swim in correct heading slowly unrolling the spool/measuring tape
4. Visually note demersal fish along transect in triplicates of 4m width (from 10 to 30m, 40 to 60m and 70 to 90m)
5. At 90m (total length of the transect) turn around and start rolling back the transect
6. Note cryptic species in triplicates of 1m width from 80 to 70m, 60 to 50m and 40 to 30m
7. At 30 m, secure the transect (for Dive 2)
8. Return to surface

Direction	X	→	→	X	→
D1	T2	T4	T6		T8
D2	T1	T3	T5	T7	T8

B. Dive 2: Macroinvertebrates and Algal census

Study variables	Macroinvertebrate abundance/ Diversity Algae abundance/ Diversity
Factors	Location Protection Level Year/ Presence invasive species Average Depth Substrate Temperature
Sampling design	Photo quadrats for Macroinvertebrate and Algae census Sampling points at 10, 15, 20, 25, 30m along a 30m transect resulting in 5 pseudo-replicates each per dive site (Macroinvertebrates – 1m^2, Algae – 0.25m^2)

Illustration of the Dive



GUE EDGE

G oal	Macroinvertebrate and Algae census using photo quadrats and collecting algal samples along a transect, assessment of abundance and diversity
U nified Team	Two divers – Daniel and Lea
E qipment	Special equipment see list below, some equipment already set during Dive 1 (Datum, Transect)
E xposure	Depth: depending on site, 10 -20m, Time: ~1 hour
D ecompression	Stay withing no deco limits, 9m/min until half the depth, steps of 3m/min until surface
G as	Depending on depth of dive site No turning pressure, use all available, Minimum gas depending on depth of dive site: 10m – 40 bar, 20m – 60 bar (stay conservative!)
E nvironment	Awareness of rocks, boat entry

Equipment

- Wet notes with pencils for each diver
- Photo quadrat for each diver
- Two cameras (Olympus TG5 and TG6) with housing
- Sampling cups for algae samples

Tasks

Using the same transect as Dive 1

1. Starting at the datum, swim along the transect setting the photo quadrat with one corner touching the first sampling point at 10m
2. Remove the algae within the square (Macroinvertebrates)
3. Take a picture of the square holding one finger up (to identify picture later), count and identify all macroinvertebrates > 5 cm (write down in wet notes)
4. Repeat steps 2. and 3. for the other three squares around the sampling point to create an area of $1m^2$
5. Set the photo-quadrat for the algal census outside of the first Macroinvertebrate square, touching one corner
6. Take a picture of the square (holding one finger up to identify picture later) and collect the different algal species inside sampling tubes (except *Rugulopteryx okamurae*)
7. Remove algae, repeat photo (for incrusting algae)
8. Assist in algal removal inside macroinvertebrates squares
9. Move along transect to next sampling point and repeat the same steps (sampling points at 10, 15, 20, 25, 30m), holding up the corresponding number of fingers in the picture (2-5 for replicate 2-5)

Direction	→ X	X	X	X	→ X
D1	T1	T2	T3	T4	T9
D2	T5	T6	T7	T8	T9