Jéssica Rodrigues Louro

Assessing differences on the epifaunal assemblages of an invasive and native macroalgae in São Miguel Island Marine Protected Areas

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2023

Jéssica Rodrigues Louro

Assessing differences on the epifaunal assemblages of an invasive and native algae in São Miguel Island Marine Protected Areas

Mestrado em Biologia Marinha

Supervisor:
Joana Maria dos Reis Franco Cruz

Co-supervisor:
Andrea Zita Costa Botelho

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2023

Declaração de autoria de trabalho

Assessing differences on the epifaunal assemblages of an invasive and native algae in São Miguel Island Marine Protected Areas

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

I hereby declare to be the author of this work, which is original and unpublished. Authors and works consulted are properly cited in the text and included in the reference list.

(Jéssica Rodrigues Louro)

(Jéssica Rodrigues Louro)

Abstract

Marine macroalgae play an important role acting as ecosystem engineers in intertidal coastal waters. Their connection with epifaunal assemblages is crucial, since food resources are provided, as well as protection from physical stress, and shelter from predators. The structure and composition of epifaunal communities can be distinctively influenced by different macroalgae species.

Over the years, the introduction of non-native species has increased, and impacts resulting from biological invasions have been responsible for significant changes to ecosystem structure and functioning. Being a remote oceanic island, the Azores can be highly influenced by the introduction of new species, affecting the specific ecological existent niche. In this context, marine protected areas allow to preserve biodiversity and maintain ecosystems. Due to this reason, it is imperative to maintain a constant monitoring, whether assessing the evolution of the already identified invasive species or preventing and control the introduction of new ones, that can seriously damage the native populations and even affect socioeconomic factors and human health.

The present study aimed to test the following hypotheses: 1) The associated epifaunal assemblages inhabiting algae in rockpools inside and outside marine protected areas are different, and 2) There are differences in abundance, richness and diversity in those communities between indigenous and non-indigenous macroalgal species.

To test the hypotheses, *Rugulopteryx okamurae* (non-indigenous) and *Halopteris scoparia* (native) were collected in rockpools inside and outside MPAs, and macroinvertebrates inhabiting macroalgae were posteriori counted and separated in taxonomic groups.

The results showed that no significant differences were found for abundance, richness and diversity of macroinvertebrates assemblages regarding in and out MPAs, as well as between native and invasive algae. The present study results suggest that MPAs might not being effective and might have insufficient management conservation efforts and *R. okamurae* might have a similar functional role as *H. scoparia* not affecting the epifaunal structure inhabiting rock pools.

Keywords:

Marine conservation, Ecological impact, Macroinvertebrate communities, *Halopteris scoparia*, *Rugulopteryx okamurae*

Resumo

Espécies não-indígenas (ENI), também conhecidas como espécies exóticas ou não-nativas, são organismos introduzidos de forma intencional ou acidental em zonas fora da sua distribuição geográfica natural, com dispersão geralmente mediada por fatores antropogénicos. Certas características, tais como a tolerância ambiental, variabilidade genética, alta capacidade reprodutiva e preferências alimentares, permitem que estas espécies colonizem e se adaptem com sucessos em novas áreas, tornando-se invasoras. Por sua vez, estas propagam-se rapidamente, provocando mudanças consideráveis no novo ecossistema, incluindo alterações de habitat, substituição de espécies nativas através de predação e/ou competição, transmissão de doenças e consequentes efeitos na saúde humana e impactos ecológicos e sócio-económicos. Com o aumento drástico destas espécies em novas áreas, bem como a capacidade de estabelecerem populações auto-sustentáveis, surge a contínua preocupação e necessidade de investigação dos possíveis impactos nos ecossistemas marinhos.

As invasões biológicas são, geralmente, caracterizadas por quatro fases: a introdução da espécie, o seu estabelecimento, a sua distribuição ou propagação e, por fim, o equilíbrio da espécie na comunidade, e consequentes impactes. Desta forma, a suscetibilidade das invasões depende da prevalência de ENI, bem como das características do habitat, sendo que ecossistemas mais desequilibrados ou que apresentam menor riqueza de espécies serão mais propensos ou vulneráveis a alterações. No entanto, a presença de ENI não significa necessariamente que ocorram efeitos negativos nas comunidades indígenas e impactes na estrutura e funcionalidade do ecossistema.

Recentemente, um aumento drástico da presença de ENI tem sido verificado, especialmente em regiões tropicais e temperadas, tais como na Macaronésia, incluindo os arquipélagos da Madeira e Açores, ilhas Canárias e Cabo Verde. A introdução destas espécies nestas áreas deve-se essencialmente às águas de lastro e à incrustação no casco das embarcações marítimas, associadas principalmente à navegação comercial. Adicionalmente, alterações climáticas e o aumento da temperatura da superfície do oceano têm estado na origem da expansão e distribuição das espécies para novas zonas.

O arquipélago dos Açores é composto por nove ilhas vucânicas, vários ilhéus e numerosos montes submarinos. Localiza-se entre as latitudes 36˚ 45’ N e 39˚ 43’ N; e as longitudes 24˚ 45’ W e 31˚ 17’ W, na dorsal mesoatlântica, o que lhe permite ser um ponto de encontro para uma enorme

variedade de fauna e flora marinha de diferentes origens. Sendo uma área remota, é característico de um nicho ecológico distinto com diversidade genética limitada, comunidades marinhas isoladas e limitada disponibilidade de habitat, tornando-o altamente vulnerável a espécies invasoras. Adicionalmente, devido à sua localização central entre a América e a Europa e o Atlântico Oriental e Ocidental, facilita a introdução de espécies invasoras através das rotas comerciais internacionais. São Miguel, a maior ilha deste arquipélago, serve como principal ponto de entrada para o comércio, abastecendo as outras ilhas e contribuindo significativamente para a economia dos Açores. A crescente globalização da navegação comercial originou um aumento proporcional da propagação de espécies não indígenas.

Assim, a criação das Áreas Marinhas Protegidas (AMP) visa a conservação da biodiversidade marinha e recuperação do ecossistema afetado tanto pelas atividades antropogénicas, bem como contribuir para a utilização sustentável dos recursos marinhos. Desta forma, o planeamento, gestão e monitorização eficazes são essenciais para definir os objetivos da AMP e fazer cumprir a legislação, abordando aspetos ecológicos e sócio-económicos.

A utilização das macroalgas marinhas no presente estudo, surge da sua importância nos ecossistemas marinhos, servindo como habitat e oferecendo proteção a uma grande biodiversidade marinha. Recentemente, no arquipélago dos Açores, cerca de 444 espécies de macroalgas foram identificadas, sendo 31 não-índigenas, e 11 classificadas como invasoras. A composição e estrutura das comunidades de macroinvertebrados é influenciada por diferentes macroalgas. Sendo assim, é importante estudar os efeitos da introdução de espécies não indígenas de macroalgas nas comunidades epifaunais, tais como a recente *Rugulopteryx okamurae*, uma vez que ecossistemas intertidais costeiros poderão ser desestabilizados e alterados em termos das comunidades associadas.

A macroalga *R. okamurae* tem vindo a aumentar rapidamente a sua área de distribuição, dominando macroalgas nativas na Ilha de São Miguel, situação que pode afetar negativamente a macrofauna endémica e vulnerável existente e, consequentemente todo o ecossistema onde estão inseridas. Assim, compreender e comparar os efeitos das macroalgas nativas e não nativas na abundância, riqueza e diversidade de comunidades bentónicas em áreas específicas, dentro e fora de AMP, pode ser uma forma de avaliar os diferentes impactos, e desta forma, avaliar as medidas de conservação e de gestão destas zonas.

O presente estudo analisou as comunidades epifaunais associadas a duas espécies de algas

castanhas, pertencentes à classe Phaeophyceae, a nativa *Halopteris scoparia* e a não nativa *Rugulopteryx okamurae*, em amostras recolhidas aleatoriamente em poças de marés localizadas em zonas intertidais de costa rochosa, tanto dentro como fora de AMP. Os objetivos centraram-se em responder às seguintes questões: 1) Existem diferenças na estrutura das comunidades epifaunais dentro e fora das AMP? e 2) Existem diferenças significativas na estrutura dos macroinvertebrados associados entre a macroalga nativa e a não-nativa?

Os resultados obtidos tanto para a análise comparativa da estrutura das comunidades epifaunais dentro e fora das AMP (1), tal como a abundância (N), riqueza (S) e diversidade (H') dos macroinvertebrados associados às algas em estudo (2), revelou que não existem diferenças significativas. Isto indica que as AMP poderão não ser eficazes, e revela a ausência de planos de gestão ou a implementação e monitorização ineficientes, pelo que é essencial o reforço de supervisão nestas áreas, especialmente tratando-se de áreas remotas, como os Açores. Em relação às comunidades epifaunais associadas às algas, *R. okamurae* apresentou semelhante composição e estrutura que a *H. scoparia*, o que poderá demonstrar a sua capacidade de desempenhar papéis ecológicos e funcionais idênticos. A arquitetura e complexidade da alga poderão influenciar significativamente a comunidade dos invertebrados, tal como fatores associados à variação sazonal.

Estudos anteriores também compararam modificações nas comunidades epifaunais associadas a algas nativas e algas não-nativas, ou mesmo invasoras, no entanto, ainda é um desafio prever as consequências de invasões biológicas num dado local. Pesquisas futuras devem priorizar a investigação de variações sazonais em diversos locais e incluir uma gama mais ampla de espécies de algas nativas. Esta abordagem será essencial para alcançar uma compreensão abrangente das alterações existentes causadas por espécies invasoras.

Palavras-Chave:

Conservação Marinha, Impacto ecológico, Comunidades de macroinvertebrados, *Halopteris scoparia, Rugulopteryx okamurae*

Acknowledgments

This was an adventure that took longer than expected, but I am very happy and proud to have finally completed another stage in my life.

So, I want to express my gratitude to my supervisors. Zita, for the opportunity and support to work and develop a project in the Azores, together with CIBIO and the University of Azores and Joana for her willingness to accompany me as a supervisor on this journey and help me with anything I needed. To Clara Cordeiro, thank you for being available to help me with the statistics of the thesis.

To Afonso Prestes, my most sincere and enormous thanks from the bottom of my heart. I can't thank you enough for all your help. Whenever I was struggling you always found time in your schedule to help me out.

Also, I couldn't fail to thank my friends and colleagues, who put up with me a lot during this difficult phase, and always gave me strength, encouragement and support when I needed the most.

Finally, a huge thank you to my family, who have always believed in me and supported me unconditionally in this decision to start a master's degree. And to you, Vítor, for giving me the strength to work on my thesis a little bit every day even when I didn't feel like it.

Insist, persist, resist and never give up.

Table of Contents

List of abbreviations, acronyms and symbols

Al – Algae
AP – Água de Pau
CE – Cerco
DW – Dry Weight
ENI – Espécies Não-Indígenas
ET - ETAR
ETAR – Estação de Tratamento de Resíduos
FE – Ferraria
inMPA – Inside Marine Protected Areas
LA – Lagoa
Lo – Location
MB – Milicias Beach
MO – Mosteiros
MPAs – Marine Protected Areas
NE – Northeast
NIS – Non-Indigenous Species
outMPA – Outside Marine Protected Areas
SC – Santa Clara
SE – Standard Error
Si – Site

Chapter 1: State of Art

1.1 Introduction of Non-Indigenous Species

Non-indigenous species (NIS), alien, foreign, or non-native species are defined as species introduced intentionally or accidentally outside their natural habitat geographic range, as well as outside their natural dispersal potential, by human-mediated vectors (Pysek *et al.*, 2009). Additionally, these organisms can be considered as established or not, whether there are self-sustaining populations, at least at a specific location in one of the areas considered, or just a small number of live specimens found on a single location, respectively (Chainho *et al*., 2015). Globally, the occurrence of NIS is becoming more frequent, which may have potential ecological, and socio-economic risks (Aamri *et al*., 2018). According to Richardson & Ricciardi (2013), the number of articles published regarding biological invasions have been increasing proportionally along the years (1998-2012), which shows the growing concern related to the consequences on biological diversity, ecosystem functioning, and socio-economic values caused by NIS.

A biological invasion is characterized by having four different main stages: arrival, establishment, dispersal or spreading and resultant impacts (Travizi *et al*., 2019). The NIS can successfully colonize new areas, become established and consequently invasive due to particular characteristics, such as environmental tolerance, high genetic variability, early sexual maturity, high reproductive capacity and a distinct diet (Travizi *et al*., 2019). Those invasive organisms usually exhibit massive spreading rates causing major changes to the new environment, involving the alteration of habitats, replacement of native species associated with predation and competition, as well as the transmission of diseases and the later effects on human health and economy (Costa *et al*., 2021). Furthermore, the susceptibility of invasions is determined by their prevalence, which will also depend on the habitat characteristics (Essink *et al.*, 2002). Therefore, the poor the ecosystem (more disturbed or lower in species richness), the more susceptible to invasions (Chainho *et al*., 2015). However, being one of the drivers of global biodiversity changes, it does not necessarily mean that it will cause harmful and long-term effects to the ecosystem integrity and functioning of indigenous communities (Travizi *et al*., 2019). Besides negative effects, such as decreasing biodiversity and trigger ecosystem shifts, these establishments can have some advantages as well, profiting fisheries, strengthening of genetic complexity and the replacement of lost ecological functions (Kleitou *et al.*, 2021). For an accurate estimation of the potential damage and impacts on indigenous populations, adequate data regarding the environmental pressures, native communities' structure, composition of species and functional diversity in the invaded areas is required.

1.2 Impacts on ecosystems

Ecosystems consist of communities of organisms which involve physical, chemical and geological environmental interactions in a unit of space (Frederick Grassle, 2013). The marine ecosystems are considered as main components of ecological function on the marine environments (Frederick Grassle, 2013), which not only they represent the major part of the Earth's total biological system, but also their study has been increasing to better understand their trophic dynamics, a key role of the marine ecosystem functioning (Du Pontavice, 2019). The relationship between ecosystem functioning and structure allows to combine all the developed biotic and abiotic elements to produce ecosystem services. Consequently, an imbalance in an ecosystem generally distresses the whole structure, resulting in cascading consequences, impacts on food webs, trophic interactions, habitats and biodiversity (species, genetic diversity and community complexity) as well as in the fisheries sector (Hodgson *et al.*, 1998, Curtin *et al.*, 2010; Gaspar *et al*., 2012). Regarding marine ecosystems, it has been demonstrated that anthropogenic disturbances are the additional contributing factor for some seaweeds invasions (Mercado *et al*., 2022). Thus, major threats include anthropogenic and environmental impacts, as a result of intense extraction of resources, pollution, eutrophication, climate change and introduction of invasive species (Castilla, 2001). In fact, the combination of these impacts, such as climate change together with eutrophication or acidification can be determinant and play an important role determining the success of non-native species colonization (Mercado *et al*., 2022).

Maritime transportation and associated port activities have been acknowledged as growing sources of the threats mentioned above (Travizi *et al*., 2019). Shipping is nowadays the most contributing vector for the introduction of marine NIS, which for centuries allowed for the unintentional transportation of several species across huge biogeographical barriers (Chainho *et al*., 2015; Costa *et al*., 2021; Kleitou *et al*., 2021). Globally, 55.5-69.2% of the currently established coastal and estuarine NIS were due to hull fouling, a sub-vector of shipping (Parretti *et al*., 2020). Chainho *et* *al*. (2015) assessed that most non-native species found in Portuguese coastal areas, coastal lagoons, estuaries and islands (Azores and Madeira) are due to shipping and might have been introduced by ballast water discharges and fouling, with percentages of 28% and 57%, respectively. Besides it, aquaculture and fisheries human actions, aquarium trade, recreational boating, as well as the construction of canals or other mobile structures also creates major pathways for the arrival of NIS to non-expected locations, allowing them to attach to vessels and marine structures and then be transported (Chainho *et al*., 2015; Costa *et al*., 2021; Kleitou *et al*., 2021). In fact, with the increase of tourism along with the search for recreational diving activities, according to Parretti *et al*. (2020), aquatic environments with recreational boating or yachting activities displayed a higher abundance of alien species. Furthermore, within islands, due to biofouling, recreational boats constitute a higher risk vector in the dispersion of marine NIS (Parretti *et al*., 2020).

1.3 Study Area – The archipelago of Azores

The increasing of NIS has been reported in the past few years, mostly, in regions affected by a latitudinal gradient of temperatures from south tropical areas to more temperate environments, characteristics of the oceanic Macaronesia area, which includes the following volcanic archipelagos in the NE Atlantic Ocean: Madeira, Canary Islands, Cape Verde and Azores (Bernal-Ibáñez *et al.*, 2022). These records were mostly in coastlines of these remote islands and considered to be a result of ballast waters and hull fouling of commercial shipping (Bernal-Ibáñez *et al.*, 2022). Apart from that, these islands have been exposed to a process known as tropicalisation, as a result of climate change and warming ocean temperatures, which may benefit the arrival, survival and reproduction of many NIS (Bernal-Ibáñez *et al*., 2022). This process is characterized by the expansion of range of tropical or subtropical species to areas that have, usually, more temperate or cooler climates (Afonso *et al*., 2013).

The Azores archipelago is an autonomous region located on the mid-Atlantic ridge between the latitudes 36˚ 45’ N and 39˚ 43’ N and the longitudes 24˚ 45’ W and 31˚ 17’ W, about 1500 km off mainland Portugal and composed of nine oceanic islands and several islets (Morton *et al*., 1998). Due to this significant distance from a continental source across the open ocean, the geographic location of the Azores archipelago hinders natural dispersal and species colonization and benefits from a sustainable practice of fisheries (Afonso *et al*., 2020). Additionally, since it lacks estuaries and coastal lagoons and have a short continental shelf, it is considered as an island ecosystem type with an exclusive biological diversity of high ecological and evolutionary interest. It holds the most isolated coastal marine communities in the North Atlantic and have one of the main MPA networks in the Atlantic, covering coastal, oceanic and deep-sea habitats (Chainho *et al.*, 2015; Afonso *et al.*, 2018). Consequently, this particular ecological niche represents features that allow for oceanic species to be extremely vulnerable to invasive species, such as small populations with limited genetic diversity, restricted habitat availability and lack of competitors and predators (Costa *et al.*, 2021). Moreover, being in the middle of America and Europe and linking Eastern and Western Atlantic margins, permits for migratory crossings, providing a constant connectivity with international trades, which facilitates the routes of entrance of invasive species (Costa *et al.*, 2021) (Figure 1.1).

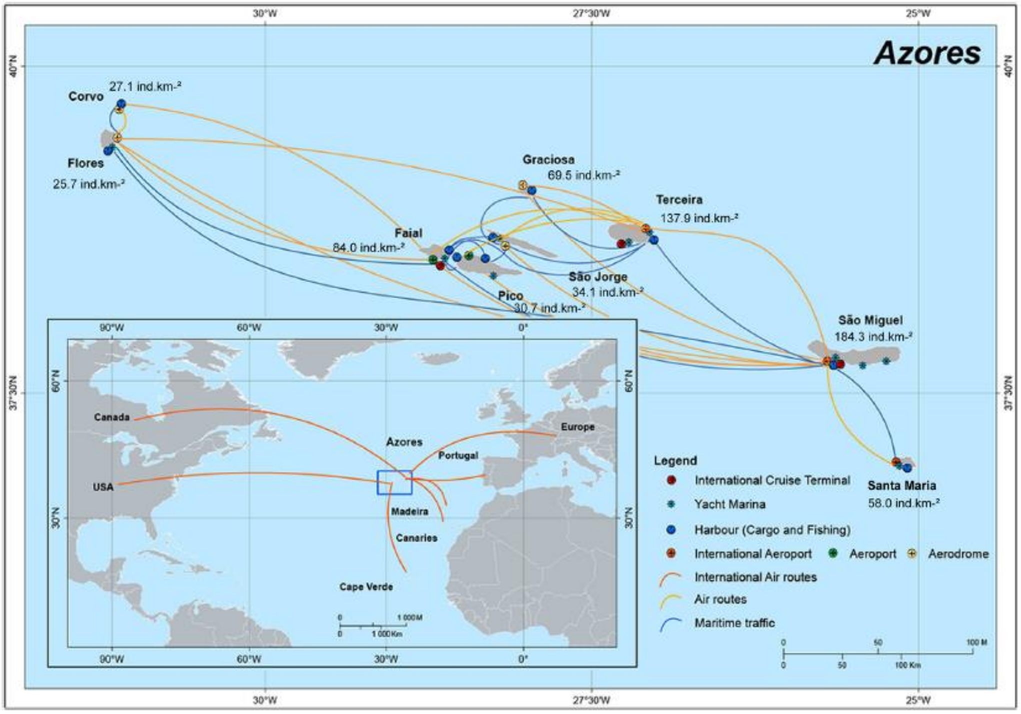

Figure 1.1 - Location of Azores Archipelago on North Atlantic Ocean with the principal routes of entry of NIS. (Costa *et al*. 2021)

As mentioned before, the fact that NIS have ecological traits that offer high plasticity and adaptation capabilities, along with the specific environmental conditions of this remote oceanic island, such as the moderate climate, which has no significant oscillations during the year, allows for an easier adaptation to the fluctuating conditions by several species. Chainho *et al.*, (2015) studied the NIS in Portuguese Coastal Areas, Coastal Lagoons, Estuaries and Islands and concluded that most of them are originary from Indo-Pacific regions. However, shipping routes connecting to the Indo-Pacific region are occasional, meaning that the species derive from secondary introductions. Additionally, island ecosystems revealed a greater vulnerability to invasions, with a higher number of NIS found (85 different species recorded in both Azores and Madeira archipelagos).

Recent data gathered from the NIS Monitoring Program in the region of Azores (PIMA - Implementation of the Marine Strategy framework Directive – monitoring program for marine invasive species of the Azores), describes the assessment of NIS as well as the inventory/report along the years (Marine Strategy Framework Directive (DQEM); Governo dos Açores (2020)). In 2020, 92 NIS have already been identified in which 72 were considered established, 19 species indetermined and only 1 was considered not established, being the new records mainly from Santa Maria Island. Given the high degree of adaptability of invasive species and since introduction pathways and vectors might be due to both natural and human-mediated factors, identifying them is imperative to minimize potential impacts in the indigenous populations, and so prioritize management effort by monitoring its development and distribution (Cacabelos *et al*., 2019). As referred above, the main introductory vectors are mainly shipping and human activities, therefore the number of invasive species in a given area could be correlated with the increase of marine traffic and tourism associated within that same area (Kötter-Lange *et al.*, 2023). Taking that into consideration, many scientific researches and publications have been focusing, along the years, on studying these susceptible ecosystems to preserve the native biota (Aamri *et al.*, 2018; Costa *et al.*, 2021; Castro *et al.*, 2022; Faria *et al.*, 2022). This involves the creation of numerous strategy instruments and guidelines through the application of preventive measures, specifically through the management of those potential pathways and vectors. The aim is the protection of major ecosystems and species across regions and the implementation and integration of Marine Protected Areas (Cardigos *et al*., 2006; Calado *et al*., 2011; Abecasis *et al*., 2015).

1.3.1 São Miguel Island

São Miguel is the largest island in the archipelago of Azores (Figure 1.2) with an area of 759,41 km^2, 65 km long and 16 km wide, and thus the most inhabited (56% of the total population of Azores in 2017 (Azevedo, 2017). Given these reasons, is considered as the main entrance point of trading, supplying the remaining islands of the archipelago (Costa *et al*., 2021) and representing the major contribution to Azores economy due to economic indicators such as importation and exportation of agriculture and fisheries' products, as well as the enhancing levels of tourism (Azevedo, 2017; Costa *et al*., 2021). It is expected that the increase of globalization in commercial shipping (i.e. includes marine trade and maritime traffic) will be proportional to the rapid spread of NIS (Castro *et al*., 2022). The increase of biological invasions may be a consequence of the main introductory vector in this oceanic island, the classic Atlantic stop-over for transatlantic yachting (Chainho *et al*., 2015). It is important to highlight that the construction of artificial structures, which might be correlated to anthropogenic pressures intended for developing maritime conditions, also contributes to the introduction of NIS (Castro *et al*., 2022).

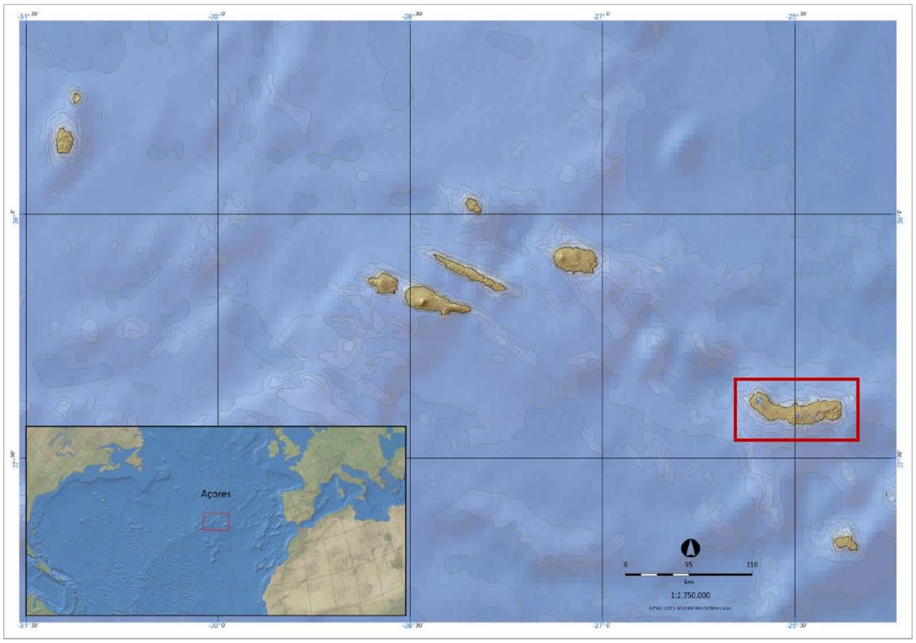

Figure 1.2 - Archipelago of Azores with focus on São Miguel Island (Botelho, 2013)

1.4 Marine Protected Areas

Marine Protected Areas (MPAs) are geographically delimited areas which have the purpose of protect and conservate marine biodiversity, including endangered species and allow for the recovering of species generally affected by human actions (Afonso *et al*., 2018). The promotion of MPAs depends not only on the demand to preserve the species and ecosystems, but also on the contribution to a sustainable use of the marine resources according to its marine ecosystems and ecological changes (Commonwealth Department of Environment and Heritage, 2003). Therefore, monitorization and assessment by management organisations are needed to define goals for each MPA. Moreover, specific legislations are established and regulated in order to accomplish its aims, not only related with ecological aspects but also socioeconomic. (Edgar *et al*., 2007). In case of appropriate management, MPAs are a crucial tool that will allow for the overturning of the degradation of marine life along with the recovery of the ecosystem complexity and integrity (Kleitou *et al*., 2021). Besides, the important keystone species and habitats will be protected, and the ecosystem will be improved, developing resistance to stresses such as NIS and climate changes (Kleitou *et al*., 2021). As mentioned before, island ecosystems, such as Azores, are particularly critical due to their vulnerability to the anthropogenic pressures and invasive species. In addition, they have some limitations on institutional capacity to cope with environmental threats, along with a high dependency on marine resources. So, the use of MPAs is often prioritized to achieve fisheries management and conservation benefits (Abecasis *et al*., 2013).

According to the Azores Government, each island of the archipelago maintains what is called Island Natural Park (Parque Natural de Ilha), which integrates the protected areas of Azores for the conservation of biodiversity and nature. Coastal Marine Protected Areas in São Miguel are designated as “Protected Area of Management Resources” (Área Protegida de Gestão de Recursos) (Decreto Legislativo Regional n.º 15/2012/A, de 2 de abril de 8 de Julho da Região Autónoma dos Açores – Assembleia Legislativa, 2012). As observed in Figure 1.3, in total, São Miguel counts with 5 MPAs with the following designations: Caloura, Costa Este (East Coast); Ponta do Cintrão; Porto das Capelas and Ponta da Ferraria.

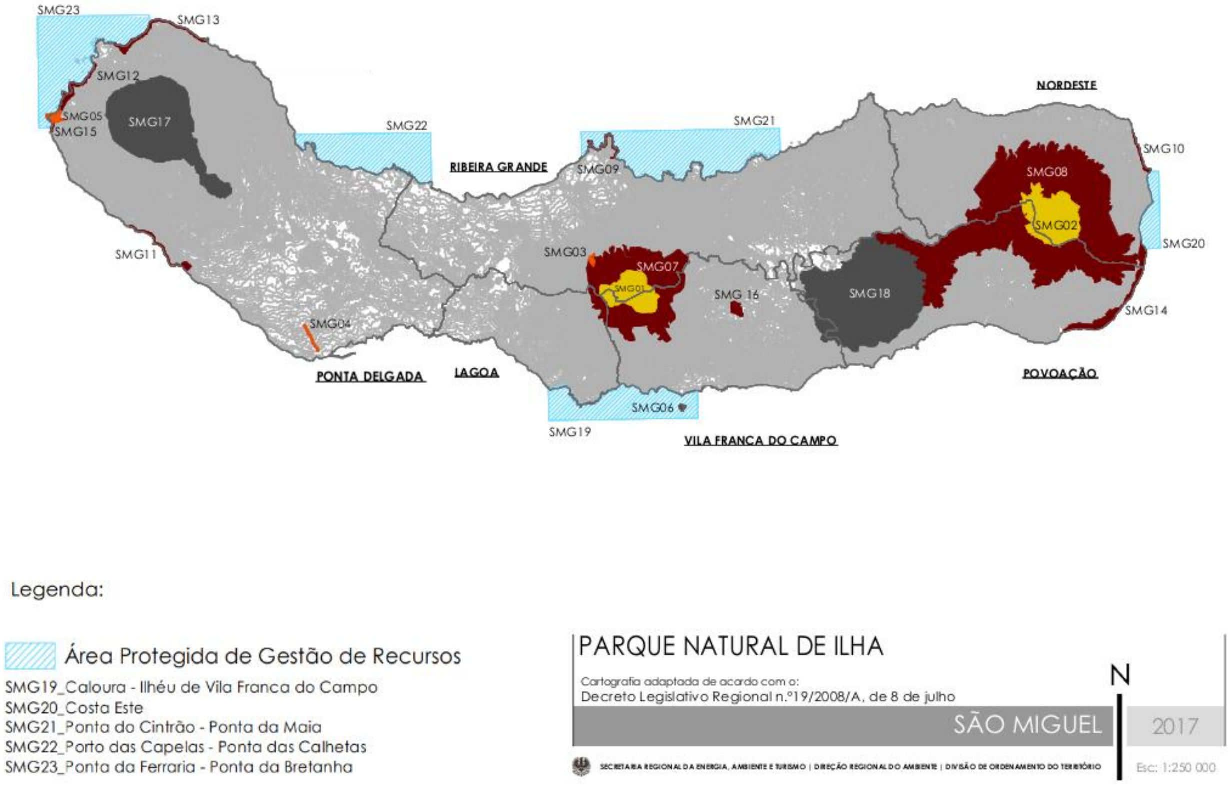

Figure 1.3 - Map of São Miguel indicating the areas of the Protected Area of Management Resources (Adapted from ot.azores.gov.pt)

Parretti *et al*. (2020) evaluated the risk of marine NIS dispersal in Azores and Madeira archipelagos that exhibit a high international intensity of shipping, as well as an increase in the marine tourism activities, such as recreational diving activities (e.g. scuba diving). In this study, São Miguel MPAs were analysed and compared with diving sites outside MPAs to understand the impact of aquatic activities on the introduction of NIS and whether the conservation value of MPAs could be compromised. The study showed that diving boats recurrently visiting the protected areas can cause a higher risk of introduction of NIS when comparing to non MPAs, highlighting the importance of a management focused on reducing the risk caused by regular vessel visits.

1.5 Marine macroalgae

Marine macroalgae are represented by a diverse group of seaweeds which have important functions in the marine ecosystems: act as ecosystem engineers increasing the substrate heterogeneity, add spatial complexity to the substratum, modulate the resources available and can provide shelter to mobile and sessile invertebrates (Navarro-Barranco *et al*., 2019; Cacabelos *et al*., 2020). This relationship between the epifaunal assemblages and associated macroalgae is crucial, particularly in coastal areas, where it act as habitat, refuge from physical stress, protection from predators and food resource (Gestoso *et al*., 2012).

For the archipelago of Azores, according to the Monitoring Program (PIMA - Implementation of the Marine Strategy framework Directive – monitoring program for marine invasive species of the Azores), in January 2020, 444 species of marine macroalgae had been identified, in which 31 were considered non-indigenous, including 80.6% Rhodophyta (red algae), 12.9% Chlorophyta (green algae) and 6.5% Ochrophyta (brown algae). In this group of NIS, 11 species were considered as invasive, such as *Acrothamnion preissii, Asparagopsis armata, Asparagopsis taxiformis, Bonnemaisonia hamifera, Caulerpa prolifera, Caulerpa webbiana, Codium fragile subsp. fragile, Grateloupia turuturu, Halimeda incrassata, Lophocladia trichoclados* and *Symphyocladia marchantioides*. However, this document is not up to date, meaning that the number of non-indigenous seaweed existent nowadays and classified as invasive is more likely higher than the year of 2020. This situation can already be observed with the non-indigenous *Rugulopteryx okamurae,* which is used in this study and is referred in subchapter 1.5.2.

The structure and composition of epifaunal assemblages can be influenced by different macroalgae and thus depend on them (Gestoso *et al*., 2010). Gestoso *et al*. (2010), for example, studied the variability of epifaunal assemblages associated with native (*Bifurcaria bifurcata)* and invasive (*Sargassum muticum*) seaweed, and concluded that significant differences between the epifaunal assemblages were found, being predation the most important factor affecting individual epifaunal species. Moreover, epifaunal composition differed between macroalgae in terms of abundance of macroinvertebrate, while the diversity (number of species) was not affected. The results concluded that the epifauna inhabiting the native algae successfully colonized the invasive algae *S. muticum*. Another particularity is the potential to start both trophic and habitat cascades, which significantly increase coastal biodiversity. In this way, the associated species will not only show complex trophic interactions but as well as acting as an additional habitat-forming species (Navarro-Barranco *et al*., 2019). Therefore, disturbances in these ecosystems, triggered by anthropogenic impacts (e.g. biological invasions), as mentioned above, may cause changes in the macroalgal communities, thus altering the whole structure and functioning. Navarro-Barranco *et al*. (2019)

compared the response of the epifauna assemblages to two high morphological similar habitat-forming species, namely, *Rugulopterix okamurae* (NIS) and *Dictiota dichotoma* (native). The conclusions are quite interesting as, even though these algaes are similar in their morphology, both of them hosted different mobile epifaunal assemblages. Hence, according to the conclusions, morphological similarity does not mean equivalent functionality. Therefore, negative impacts cannot be inferred or suggested in this case.

These scenarios of exploring the ecological impacts of marine invasions on marine benthic ecosystems have been even more studied nowadays, in order to predict the positive or negative impacts of the non-native marine macroalgae species in the ecosystem.

Identical to the examples of studies carried out, two highly different morphological species of brown seaweed will be used in this work, the native *Halopteris scoparia* and the NIS *Rugulopterix okamurae* with focus on analysing the impact in São Miguel Island, inside and outside MPAs.

1.5.1 *Halopteris scoparia* (Sphacelariales, Phaeophyceae) (Linnaeus) Sauvageau, 1904

Halopteris scoparia (Linnaeus) Sauvageau, 1904 (Phaeophyta, Sphacelariales) is a perennial brown algae species widely distributed along the Mediterranean and Atlantic coasts and predominantly found from intertidal pools to the infralittoral, mainly on rocks and sandy bottoms (Sánchez-Moyano *et al*., 2002). In terms of complexity and structure, *H. scoparia* is an erect, flexible coarsely / corticated branched algae, where the main branches have dense ramifications, having an appearance of overlapping conical brooms (Angulo, 1980; Sánchez-Moyano *et al.*, 2000). Such characteristics enclose very small spaces, interstices, and allows for the existence of microhabitats, as well as a good trap of sediments, which harbor a significant number of macroinvertebrates (Angulo, 1980; Sánchez-Moyano *et al*., 2000). Moreover, the algae individuals can grow up to 15 cm in length and are typically attached to rock substrate and areas with high luminosity (Campos *et al*., 2019). In the Azores, it is a native algal species present throughout the whole year and mainly found in shallow-water rocky bottom groups inhabiting rockpools along the island (Figure 1.4) (Patarra *et al*., 2017; Faria *et al.*, 2021).

Figure 1.4 - *Halopteris scoparia* attached to the rock substrate. (Campos *et al*., 2019)

1.5.2 *Rugulopteryx okamurae* (Dictyotales, Phaeophyceae) (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim, 2009

Rugulopteryx okamurae (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim, 2009 is a brown algae species that belongs to the Dictyotaceae family and it is originated from the temperate Northwestern Pacific Ocean (Asia) specifically, Philippines, China, Japan and Korea, inhabiting the sublittoral rocky substrata, extending over a large area and colonizing habitats from 0 to 35 meters depth (Roca *et al*., 2022). In 2002, for the first time, this seaweed was found outside its native distribution areas, precisely in European waters, Mediterranean Sea, in the coastal lagoon of Thau (Occitania, France, Mediterranean), establishing self-sustaining populations (Aamri *et al*., 2018). Afterwards, in 2015, its distribution expanded to the northern Bay of Ceuta (southern sector of the Strait of Gibraltar) (Ruitton *et al*., 2021). Rapidly dispersed along the shore being reported for the first time in the Northwest Mediterranean Sea, Marseille, France, in 2018, followed by a 9,5 km coastline population extension in 2020, that grew over most indigenous algal species (Ruitton *et al*., 2021). Regarding its morphology it is characterized by having a yellowish-brown colour which tends to become dark brown, as well as an erect, dichotomously branched thallus which can reach up to 14 cm in diameter with the fronds developing into a dense flabellum up to 20 cm in height culminating in obtuse apices with smooth margins (Hwang *et al*., 2009; Aamri *et al*., 2018; Faria *et al*., 2021).

The highly efficient mechanism of vegetative multiplication in this species has been studied, suggesting the staggering ability of *R. okamurae* to replicate by cloning, with a single individual being capable enough to generate more than thousands of specimens as well as the main cause for the high potential to be an invasive species (Jeschke *et al*., 2019; Faria *et al.*, 2021). Moreover, it is able to adapt to conditions absent in the native ecosystems, such as higher levels of salinity and temperature, displaying an optimal growth (observed for the Strait of Gibraltar) and remaining active during the whole seasonal cycle (Mercado *et al*., 2022). It also survives in darkness conditions for weeks, allowing for an easy dispersion through discharged ballast waters by vessels (Faria *et al*., 2021).

In the Atlantic Ocean, it was first sighted in the Northeast Atlantic Islands (NE Atlantic), in the Azores archipelago, on the south coast of São Miguel Island. It is assumed that in early 2019, it was already part of the island's ecosystem since it may have been initially mistaken as *Dictyota* sp. due to their structural similarity (Faria *et al*., 2021). This situation was therefore confirmed after the investigation of a video recorded in April 2019, near the main harbor of São Miguel Island, suggesting that ballast waters, or fouling may have been the introductory vectors of this macroalgae (Faria *et al*., 2021).

Equally important to the exponential colonization of intertidal and subtidal zones, which is a threat to the ecosystems, affecting not only native algae, but also fish communities and other organisms, is the accumulation of fragments of this macroalgae, for example on the beaches and shores, which tend to become massive amounts of waste biomass. Floating fragments disperse in the ocean and accumulate in the coastal areas as a consequence of the continuously detachments due to hydrodynamic effect (e.g. wave action and currents) and may even deposit in the seafloor affecting fishery activities (e.g blocking fishing net; entanglement in the fishing gear) (García-Gomez *et al.*, 2021; Barcellos *et al*., 2023). These accumulations have a disagreeable smell and cause significant socioeconomical issues, impacting tourism and concerning local population (Figure 1.5) (Faria *et al*., 2021; Ruitton *et al*., 2021; Barcellos *et al.*, 2023).

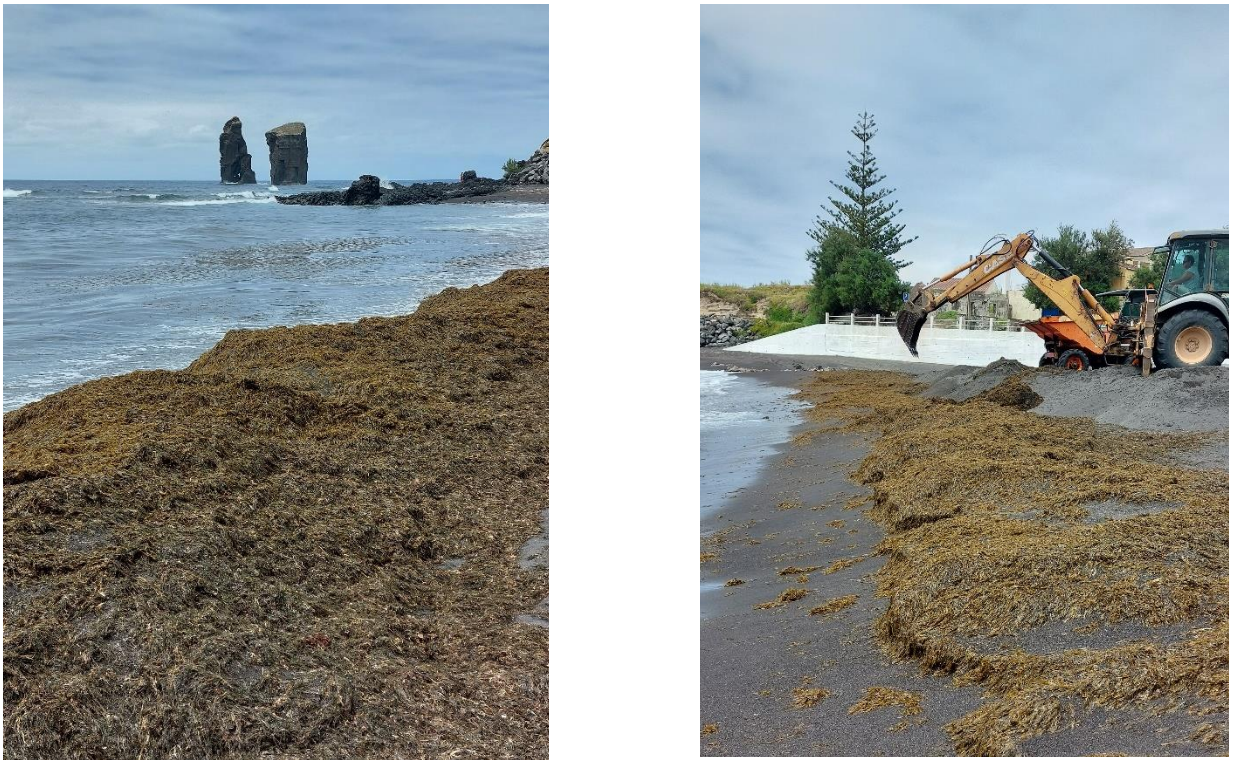

Figure 1.5 - Accumulation of Rugulopteryx okamurae (on the left side) on the beach and machinery trying to remove the seaweed (on the right side) at Mosteiros Beach. (Louro, 2023)

In the Strait of Gibraltar, *R. okamurae* has expanded to an extent that has caused significant ecological impacts in the ecosystem, such as the removal or substantial displacement of native species, impacting severely the native biota (García-Gómez *et al*., 2020). This seaweed had the capacity to cover 90% of the area between 10 and 20 meters of depth in the North Bay of Ceuta, as well as the wide distribution along the Atlantic and Mediterranean coast between 2018-2019 (García-Gómez *et al*., 2020). As referred above, the high proliferation ability and successful colonization allowed for this species to rapidly expand, establish, and become the most dominant, affecting the size and number of individuals within indigenous algae populations (Figure 1.6).

Figure 1.6 – Comparison between algae individuals: *Halopteris scoparia* (on the left side) and *Rugulopteryx okamurae* (on the right side). Individuals taken from the rockpools at Ferraria. (Louro, 2023)

Therefore, groups of native seaweed like the ones observed in Figure 1.4 are not commonly seen currently.

In the South coast of São Miguel Island, it also covered over than 90% between 10-20 meters depth, from 2019 to 2021, as observed in Figure 1.7, (Faria *et al*., 2021) and extended the distribution to the North coast of the island between 2022 and 2023 (author personal observation). Furthermore, in 2022, Bernal-Ibáñez *et al*., (2022) publicized *R. okamurae* recent discovery in Madeira Archipelago, demonstrating a new southern dispersal limit of this species in the NE Atlantic European waters.

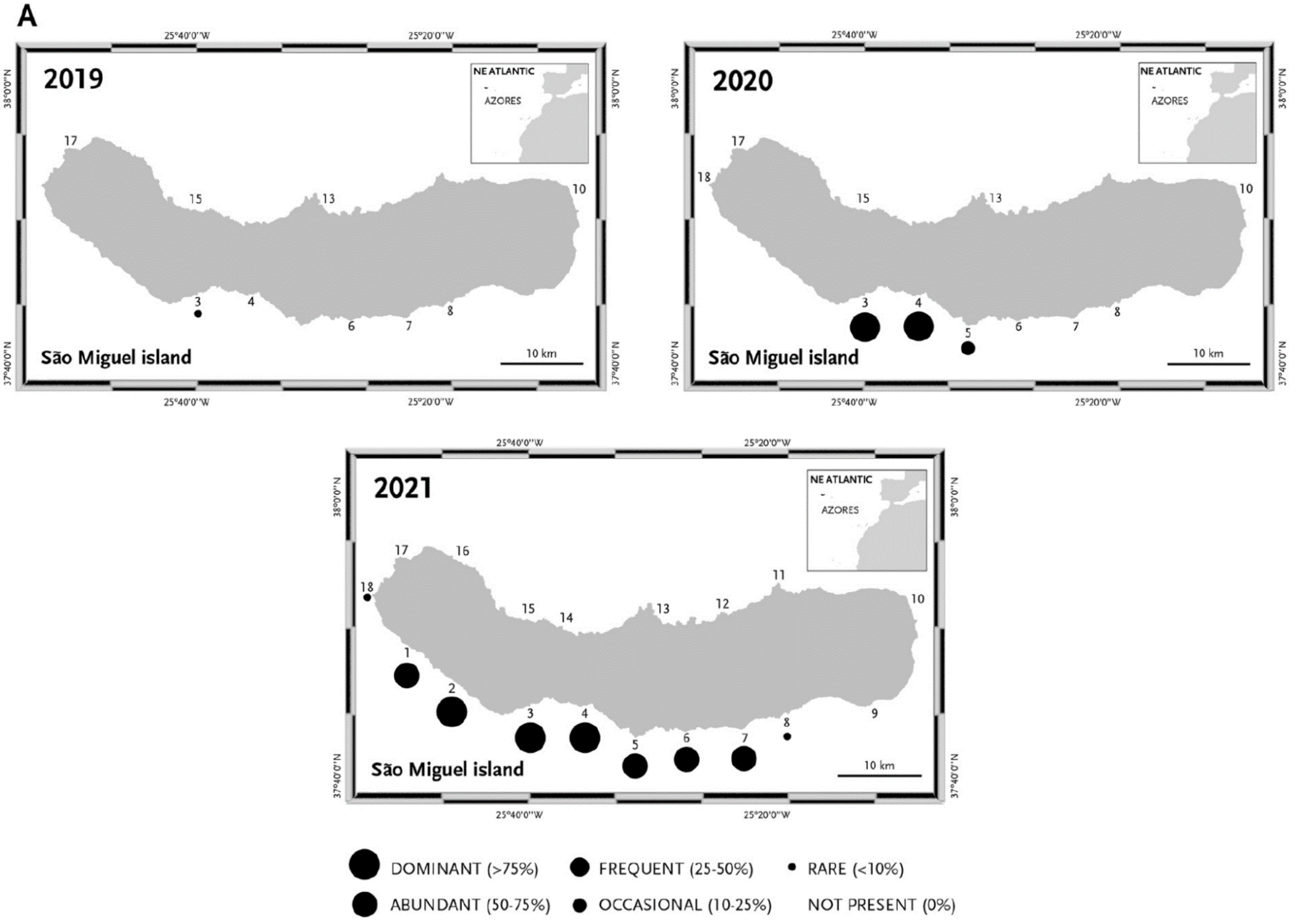

Figure 1.7 - Map of São Miguel Island (A) with locations (numbers) sampled for the presence of *R. okamurae from* 2019 to 2021. Black circles indicate the evolution of the occurrence and abundance of the species. (Faria *et al*., 2021)

1.6 Objectives

The archipelago of Azores, constituted by the most isolated oceanic islands in the North Atlantic, gathers a specific marine biodiversity with endemic species, particular conditions, and unique functional characteristics, becoming an oasis for many marine species. With the increasing of biological invasions worldwide, more studies have been assessing the potential impact of the introduction of non-indigenous algal species into non-native areas, evaluating the possible consequences that may occur to the associated assemblages, ecosystem structure, as well as outcomes in a socioeconomical level. In order to protect and maintain the ecosystem integrity, function and resilience, assessment and management of the affected and impacted marine ecosystems and respective biodiversity associated is required, such as planning of mitigation and preventive measures, as well as the establishment of Marine Protected Areas.

R. okamurae has been increasing rapidly its percentage cover and its distribution range, aggressively replacing the native macroalgae of São Miguel Island, situation that may negatively affect the endemic and vulnerable macrofauna existent and consequently the whole ecosystem where they are inserted on, with consequent cascading effects.

Thus, understanding and comparing the effects of native and NIS macroalgae on the diversity and variability of epifaunal assemblages in specific areas (MPA and nMPA), can be a way of evaluating the different impacts and hence, be aware of the importance in establishing conservation measures.

This study analysed the epifaunal assemblages associated with native, *Halopteris scoparia*, and non-indigenous algal species, *Rugulopteryx okamurae*, on random collected samples in rockpools located inside and outside MPA. Objectives were focused on the following questions: (1) Are there differences in the structure of epifaunal assemblages inside and outside MPAs? (2) Are there significant differences in the structure: abundance, richness and diversity of the individuals between the native and non-indigenous macroalgae?

1.7 References

Aamri, F. E. L., Mohamed, I., and Mohamed, N. T. (2018). Occurrence of the Invasive Brown Seaweed Rugulopteryx Okamurae (E . Y . Dawson) I . K . Hwang , W . J . Lee & H . S . Kim (Dictyotales , Phaeophyta) in Morocco (Mediterranean Sea). *Mediterranean fisheries and aquaculture research* 1:92–96.

Abecasis, R. C., Afonso, P., Colaço, A., Longnecker, N., Clifton, J., Schmidt, L., Santos, S. R. (2015). Marine Conservation in the Azores: Evaluating Marine Protected Area Development in a Remote Island Context. *Frontiers in Marine Science* 2:1–16. doi: 10.3389/fmars.2015.00104.

Abecasis, R. C., Longnecker, N., Schmidt, L., and Clifton, J. (2013). Marine Conservation in Remote Small Island Settings: Factors Influencing Marine Protected Area Establishment in the Azores. *Marine Policy* 40(1):1–9. doi: 10.1016/j.marpol.2012.12.032.

Afonso, P., Porteiro, F., Fontes, J., Tempera, F., Morato, T., Cardigos, F., and Santos, R. S. (2013). New and Rare Coastal Fishes in the Azores Islands: Occasional Events or Tropicalization Process? *Journal of Fish Biology* 83(2):272–94. doi: 10.1111/jfb.12162.

Afonso, P., Fontes, J., Giacomello, E., Magalhães, M. C., Martins, H. R., Morato, T., Neves, V., Prieto, R., Santos, R. S., Silva, M. A., and Vandeperre, F. (2020). The Azores: A Mid-Atlantic Hotspot for Marine Megafauna Research and Conservation. *Frontiers in Marine Science* 6:1–8. doi: 10.3389/fmars.2019.00826.

Afonso, P., Schmiing, M., Fontes, J., Tempera, F., Morato, T., and Santos, R. S. (2018). Effects of Marine Protected Areas on Coastal Fishes across the Azores Archipelago, Mid-North Atlantic. *Journal of Sea Research* 138:34–47. doi: 10.1016/j.seares.2018.04.003.

Angulo, R., 1980. Sistematica de las algas marinas de la costa vasca. *Sociedad Cultural INSUB, San Sebastian.* Publicación 1.

Azevedo, F. (2017). Research for REGI Committee:The Economic, Social and Territorial Situation of the Azores (Portugal).

Barcellos, L., Pham, C. K., Menezes, G., Bettencourt, R., Rocha, N., Carvalho, M., and Felgueiras, H. P. (2023). A Concise Review on the Potential Applications of Rugulopteryx Okamurae Macroalgae. *Marine Drugs* 21(1):1–15. doi: 10.3390/md21010040.

Bernal-Ibáñez, A., Chebaane, S., Sempere-Valverde, J., Faria, J., Ramalhosa, P., Kaufmann, M., Florido, M., Albert, A., Canning-Clode, J., Gestoso, I., and Cacabelos, E. (2022). A Worrying Arrival: The First Record of Brown Macroalgae Rugulopteryx Okamurae in Madeira Island and Its Invasive Risk. *BioInvasions Records* 11(4):912–24. doi: 10.3391/bir.2022.11.4.10.

Botelho, A. Z. (2013). Planeamento espacial marinho em Áreas Marinhas Protegidas: O caso de estudo da área protegida de gestão de recursos Caloura - Ilhéu de Vila Franca do Campo. Tese de Doutoramento. Universidade dos Açores.

Cacabelos, E., Faria, J., Martins, G., Mir, C., Parente, M. I., Gabriel, D., Sánchez, R., Altamirano, M., Costa, A. C., Prud'Homme Van Reine, W., and Neto, A. (2019). First Record of Caulerpa Prolifera in the Azores (NE Atlantic). *Botanica Marina* 62(2):155–60. doi: 10.1515/bot-2018-0075.

Cacabelos, E., Martins, G. M., Faria, J., Prestes, A. C. L., Costa, T., Moreu, I., and Neto, A. (2020). Limited Effects of Marine Protected Areas on the Distribution of Invasive Species, despite Positive Effects on Diversity in Shallow-Water Marine Communities. *Biological Invasions* 22(3):1169–79. doi: 10.1007/s10530-019-02171-x.

Calado, H., Ng, K., Lopes, C., and Paramio, L. (2011). Introducing a Legal Management Instrument for Offshore Marine Protected Areas in the Azores-The Azores Marine Park. *Environmental Science and Policy* 14(8):1175–87. doi: 10.1016/j.envsci.2011.09.001.

Campos, A. M., Matos, J., Afonso, C., Gomes, R., Bandarra, N. M., and Cardoso, C. (2019). Azorean Macroalgae (Petalonia Binghamiae, Halopteris Scoparia and Osmundea Pinnatifida) Bioprospection: A Study of Fatty Acid Profiles and Bioactivity. *International Journal of Food Science and Technology* 54(3):880–90. doi: 10.1111/ijfs.14010.

Cardigos, F., Tempera, F., Ávila, S., Gonçalves, J., Colaço, A., and Santos, R. S. (2006). Non-Indigenous Marine Species of the Azores. *Helgoland Marine Research* 60(2):160–69. doi: 10.1007/s10152-006-0034-7.

Castilla, J. C. (2001). Marine Ecosystems, Human Impacts On. *Encyclopedia of Biodiversity: Second Edition* 4:27–36. doi: 10.1016/B978-0-12-384719-5.00425-1.

Castro, N., Gestoso, I., Marques, C. S., Ramalhosa, P., Monteiro, J. G., Costa, J. L., and Canning-Clode, J. (2022). Anthropogenic Pressure Leads to More Introductions: Marine Traffic and Artificial Structures in Offshore Islands Increases Non-Indigenous Species Introduction. *Marine Pollution Bulletin* 181. doi: 10.1016/j.marpolbul.2022.113898.

Chainho, P., Fernandes, A, Amorim, A., Ávila, S. P., Canning-Clode, J., Castro, J. J., Costa, A. C., Costa, J. L., Cruz, T., Gollasch, S., Grazziotin-Soares, C., Melo, R., Micael, J., Parente, M. I., Semedo, J., Silva, T., Sobral, D., Sousa, M., Torres, P., Veloso, V., and Costa, M. J. (2015). Non-Indigenous Species in Portuguese Coastal Areas, Coastal Lagoons, Estuaries and Islands. *Estuarine, Coastal and Shelf Science* 167:199–211. doi: 10.1016/j.ecss.2015.06.019.

Commonwealth Department of Environment and Heritage, (2003). The benefits of Marine Protected Areas. *Commonwealth of Australia*. Australian Government.

Costa, A. C., Balibrea, A., Raposeiro, P. M., Santos, S., Souto, M., and Gonçalves, V. (2021). Non-Indigenous and Invasive Freshwater Species on the Atlantic Islands of the Azores Archipelago. *Frontiers in Ecology and Evolution* 9:1–19. doi: 10.3389/fevo.2021.631214.

Curtin, R., Prellezo, R. (2010). Understanding marine ecosystem based management: A literature review. *Marine Policy,* 34: 821-830.

Decreto Legislativo Regional n.º 15/2012/A, de 2 de abril de 8 de Julho da Região Autónoma dos Açores – Assembleia Legislativa, 2012

Du Pontavice, H. (2019). Changing Biomass Flows in Marine Ecosystems: From the Past to the Future. *Predicting Future Oceans: Sustainability of Ocean and Human Systems Amidst Global Environmental Change* 121–28. doi: 10.1016/B978-0-12-817945-1.00012-5.

EC [European Commission] Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Off. J. Eur. Union L164, 19–40.

Edgar, G. J., Russ, G. R., Babcock, R. (2007). Marine protected areas. Marine Ecology. 533-555.

Essink, K. & Dekker, R. (2002). General patterns in invasion ecology tested in the Dutch Wadden Sea: the case of a brackish-marine polychaetous worm. *Biological invasions*, 4: 359–368

Faria, J., Prestes, A. C. L., Moreu, I., Cacabelos, E., and Martins, G. M. (2022). Dramatic Changes in the Structure of Shallow-Water Marine Benthic Communities Following the Invasion by *Rugulopteryx Okamurae* (Dictyotales , Ochrophyta) in Azores (NE Atlantic). 175. doi: 10.1016/j.marpolbul.2022.113358.

Faria, J., Prestes, A. C. L., Moreu, I., Martins, G., Neto, A. I. & Cacabelos, E. (2021). Arrival and proliferation of the invasive seaweed *Rugulopteryx okamurae* in NE Atlantic islands. Botanica Marina. 65. 10.1515/bot-2021-0060.

Frederick Grassle, J. 2013. Marine Ecosystems. Pp. 45–55 in *Encyclopedia of Biodiversity*. Elsevier.

García-Gómez, J. C., Florido, M., Olaya-Ponzone, L., Díaz de Rada, J. R., Donázar-Aramendía, I., Chacón, M., Quintero, J. J, Magariño, S., and Megina, C. (2021). Monitoring Extreme Impacts of *Rugulopteryx Okamurae* (Dictyotales, Ochrophyta) in El Estrecho Natural Park (Biosphere Reserve). Showing Radical Changes in the Underwater Seascape. *Frontiers in Ecology and Evolution* 9. doi: 10.3389/fevo.2021.639161.

García-Gómez, J. C., Sempere-Valverde, J., González, A. R., Martínez-Chacón, M., Olaya-Ponzone, L., Sánchez-Moyano, E., Ostalé-Valriberas, E., and Megina, C. (2020). From Exotic to Invasive in Record Time: The Extreme Impact of *Rugulopteryx Okamurae* (Dictyotales, Ochrophyta) in the Strait of Gibraltar. *Science of the Total Environment* 704:0–2. doi: 10.1016/j.scitotenv.2019.135408.

Gaspar, M., Carvalho, S., Cúrdia, J., Santos, M . N, and Vasconcelos, P. (2012). *Restoring Coastal Ecosystems from Fisheries and Aquaculture Impacts*. Vol. 10. Elsevier Inc.

Gestoso, I., Olabarria, C., and Troncoso, J. S. (2010). Variability of Epifaunal Assemblages Associated with Native and Invasive Macroalgae. *Marine and Freshwater Research* 61(6):724–31. doi: 10.1071/MF09251.

Gestoso, I., Olabarria, C., and Troncoso, J. S. (2012). Effects of Macroalgael Identity on Epifaunal Assemblages: Native Species versus the Invasive Species *Sargassum Muticum*. *Helgoland Marine Research* 66(2):159–66. doi: 10.1007/s10152-011-0257-0.

Governo dos Açores (2020). Directiva Quadro Estratégia Marinha (DQEM) - Relatório do 2º ciclo (Parte D), pp 94-144)

Hodgson, J. G., Thompson, K., Wilson, P. J., Bogaard, A. (1998). Does biodiversity determine ecosystem function? The ecotron experiment reconsidered. *Functional Ecology*, Vol. 12, No.5, pp. 843-848.

Hwang, I., Lee, W., Kim, H. and De Clerck, O. (2009). Taxonomic Reappraisal of Dilophus okamurae (Dictyotales, Phaeophyta) from the Western Pacific Ocean. *Phycologia*, 48, 1-12. http://dx.doi.org/10.2216/07-68.1

Jeschke, M. A., De la Rosa, J., Muñoz, A. R., & Ros, M. (2019). Workshop de especies invasoras marinas: construyendo una propuesta para Andalucía Conclusiones de la mesa redonda. 55, 41-43.

Kleitou, P., Crocetta, F., Giakoumi, S., Giovos, I., Hall-Spencer, J. M., Kalogirou, S., Kletou D., Moutopoulos, D. K., and Rees, S. (2021). Fishery Reforms for the Management of Non-Indigenous Species. *Journal of Environmental Management* 280. doi: 10.1016/j.jenvman.2020.111690.

Kötter-Lange, K., Lienhoop, N., and Buschbaum, C. (2023). Effects and Perception of Marine Introduced Species by Stakeholders in the Wadden Sea—an Exploratory Approach. *Marine Biodiversity* 53(5):1–16. doi: 10.1007/s12526-023-01358-9.

Mercado, J. M., Gómez-Jakobsen, F., Korbee, N., Aviles, A., Bonomi-Barufi, J., Muñoz, M., Reul, A., and Figueroa, F.L. (2022). Analyzing Environmental Factors That Favor the Growth of the Invasive Brown Macroalgae *Rugulopteryx Okamurae* (Ochrophyta): The Probable Role of the Nutrient Excess. *Marine Pollution Bulletin* 174. doi: 10.1016/j.marpolbul.2021.113315.

Morton, B. S, Britton, J. C., Martins, A. M. F. (1998). Coastal ecology of the Azores. *Sociedade Afonso Chaves*, Ponta Delgada, Azores

Navarro-Barranco, C., Muñoz-Gómez, B., Saiz, D., Ros, M., Guerra-García, J. M., Altamirano, M., Ostalé-Valriberas, E.,and Moreira, J., (2019). Can Invasive Habitat-Forming Species Play the Same Role as Native Ones? The Case of the Exotic Marine Macroalgae *Rugulopteryx Okamurae* in the Strait of Gibraltar. *Biological Invasions* 21(11):3319–34. doi:

10.1007/s10530-019-02049-y.

Parretti, P., Canning-Clode, J., Ferrario, J., Marchini, A., Botelho, A. Z., Ramalhosa, P., and Costa, A. C. (2020). Free Rides to Diving Sites: The Risk of Marine Non-Indigenous Species Dispersal. *Ocean and Coastal Management* 190. doi: 10.1016/j.ocecoaman.2020.105158.

Patarra, R. F., Carreiro, A. S., Lloveras, A. A., Abreu, M. H., Buschmann, A. H., Neto, A. I. (2017). Effects of light, temperature and stocking density on *Halopteris scoparia* growth. *J Appl Phycol* 29, 405–411. https://doi.org/10.1007/

Pysek, P., Hulme, P.E., Nentwig, W. (2009). Glossary of the Main Technical Terms Used in the Handbook, Handbook of Alien Species in Europe. Springer, pp. 375–379.

Richardson, D. M., and Ricciardi, A. (2013). Misleading Criticisms of Invasion Science: A Field Guide. *Diversity and Distributions* 19(12):1461–67. doi: 10.1111/ddi.12150.

Roca, M., Dunbar, M. B., Román, A., Caballero, I., Zoffoli, M. L., Gernez, P., and Navarro, G. (2022). Monitoring the Marine Invasive Alien Species *Rugulopteryx Okamurae* Using Unmanned Aerial Vehicles and Satellites. *Frontiers in Marine Science* 9:1–15. doi: 10.3389/fmars.2022.1004012.

Ruitton, S., Blanfuné, A., Boudouresque, C. F., Guillemain, D., Michotey, V., Roblet, S., Thibaut, D., Thibaut, T., and Verlaque, M. (2021). Rapid Spread of the Invasive Brown Algae *Rugulopteryx*.” *Water* 13(2306).

Sánchez-Moyano, J. E., Estacio F. J., García-Adiego, E. M., and García-Gómez J. C. (2000). “The Molluscan Epifauna of the Algae *Halopteris Scoparia* in Southern Spain as a Bioindicator of Coastal Environmental Conditions. *Journal of Molluscan Studies* 66(4):431–48. doi: 10.1093/mollus/66.4.431.

Sánchez-Moyano, J. E., García-Adiego, E. M., Estacio, F., and García-Gómez, J. C. (2002). Effect of Environmental Factors on the Spatial Variation of the Epifaunal Polychaetes of the Algae *Halopteris Scoparia* in Algeciras Bay (Strait of Gibraltar). *Hydrobiologia* 470:133–48. doi: 10.1023/A:1015680106097.

Travizi, A., Balković, I., Bacci, T., Bertasi, B., Cuicchi, C., Flander-Putrle, V., Grati, F., Grossi, L., Jaklin, A., Lipej, L., Mavrič, B., Mikac, B., Marusso, V., Montagnini, L., Nerlović, V., Penna, M., Salvalaggio, V., Santelli, A., Scirocco, T., Spagnolo, A., Trabucco, B., and Vani, D., (2019). Macrozoobenthos in the Adriatic Sea Ports: Soft-Bottom Communities with an Overview of Non-Indigenous Species. *Marine Pollution Bulletin* 147:159–70. doi: 10.1016/j.marpolbul.2019.01.016.

Chapter 2 : Manuscript

Assessing differences on the epifaunal assemblages of an invasive and native algae in São Miguel Island in Marine Protected Areas

Louro J.[1], Cordeiro C.[1], Cruz J.[2], Botelho A. Z.[3]

[1] Universidade do Algarve, Faculdade de Ciências e Tecnologias (FCT) – Campus de Gambelas, 8005-139 Faro, Portugal

[2] CCMAR, Centro de Ciências do Mar (Building 7), Universidade do Algarve – Campus de Gambelas, 8005-139 Faro, Portugal

[3] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Lab Associado, Universidade dos Açores – Faculdade de Ciências e Tecnologias, 9501-801 Ponta Delgada, Portugal

Author e-mail: jessicarlouro@hotmail.com

Keywords:
Marine conservation, Ecological impact, Macroinvertebrate communities, *Halopteris scoparia*, *Rugulopteryx okamurae*

2.1 Abstract

Marine macroalgae play an important role acting as ecosystem engineers in intertidal coastal waters. Their connection with epifaunal assemblages is crucial, since food resources are provided, as well as protection from physical stress, and shelter from predators. The structure and composition of epifaunal communities can be distinctively influenced by different macroalgae species.

Over the years, the introduction of non-native species has increased, and impacts resulting from biological invasions have been responsible for significant changes to ecosystem structure and functioning. Being a remote oceanic island, the Azores can be highly influenced by the introduction of new species, affecting the specific ecological existent niche. In this context, marine protected areas allow to preserve biodiversity and maintain ecosystems. Due to this reason, it is imperative to maintain a constant monitoring, whether assessing the evolution of the already identified invasive species or preventing and control the introduction of new ones, that can seriously damage the native populations and even affect socioeconomic factors and human health. The present study aimed to test the following hypotheses: 1) The associated epifaunal assemblages inhabiting algae in rockpools inside and outside marine protected areas are different, and 2) There are differences in abundance, richness and diversity in those communities between indigenous and non-indigenous macroalgal species.

To test the hypotheses, *Rugulopteryx okamurae* (non-indigenous) and *Halopteris scoparia* (native) were collected in rockpools inside and outside MPAs, and macroinvertebrates inhabiting macroalgae were posteriori counted and separated in taxonomic groups.

The results showed that no significant differences were found for abundance, richness and diversity of macroinvertebrates assemblages regarding in and out MPAs, as well as between native and invasive algae. The present study results suggest that MPAs might not being effective and might have insufficient management conservation efforts and *R. okamurae* might have a similar functional role as *H. scoparia* not affecting the epifaunal structure inhabiting rock pools.

2.2. Introduction

Worldwide, biological invasions by Non-Indigenous Species (NIS) have been affecting numerous marine habitats leading to ecological impacts, such as habitat damage and loss of biodiversity, which is associated with the weakening of ecosystem functionality, composition and structure and the spread of NIS (Gestoso *et al.*, 2010, 2012). Given the limited ecological niche in Azores and being a hotspot for a large biodiversity of species, it is crucial to maintain, protect and ensure the conservation of marine ecosystems (Afonso *et al*., 2020, Costa *et al*., 2021). Therefore, delimited marine areas are managed to ensure their protection as well as reduce the degradation and impacts on the habitats by having an effective monitoring and management approaches to mitigate the impact of invasive species, allow for the maintenance and functioning of the ecosystem (Cacabelos *et al*., 2020).

Marine macroalgae are an essential part of the ecosystem, acting as ecosystem engineers, since a spatial complexity to the substratum is added, the resources available adjusted, and a shelter to a substantial number of sessile and mobile invertebrates is provided (Navarro-Barranco *et al*., 2019; Cacabelos *et al*., 2020). In intertidal coastal areas, the relationship between epifaunal assemblages and its associated seaweed is crucial, as they give protection from predators, refuge from physical stress and availability of food resources (Gestoso *et al*., 2012). Hence, an introduction of a non-indigenous species in a new environment, may alter the associated species, impact the ecosystem, affect the native marine fauna, and consequently create local concerns with influences in a socioeconomical level (Faria *et al*., 2021; Barcellos *et al*., 2023). *Halopteris scoparia* is a perennial brown alga, abundant along the Mediterranean and Atlantic shores and found mainly from intertidal pools to the infralittoral, on rocks and sandy bottoms (Sánchez-Moyano *et al*., 2002). Being a coarsely branched algae with dense ramifications, is one of the few that can host a greater quantity of macroinvertebrates (Sánchez-Moyano *et al*., 2000, Prestes, 2021). On the other hand, the brown algae *Rugulopteryx okamurae* has its origin in the East Asia (China, Japan and Korea), inhabiting the sublittoral rocky substrata (Mercado *et al.*, 2022). Over the years this alga has shown a rapid distribution, being recorded in European Waters for the first time in 2002 and dispersing intensively to the Mediterranean Sea (Aamri *et al*., 2018; Barcellos *et al*., 2023).

The archipelago of Azores is composed of nine volcanic islands, several islets, and numerous seamounts. Its geographic position between latitudes 36˚ 45’ N and 39˚ 43’ N and the longitudes

24˚ 45’ W and 31˚ 17’ W, on the mid-Atlantic ridge in the north-central Atlantic Ocean, allows it to be a “meeting point” to a variety of marine fauna and flora of different origins (Morton *et al*., 1998). However, it also encompasses constant exchanges between the archipelago and other countries, such as Portugal on the European continent and Boston on the North American continent, as well as between islands (Morton *et al.*, 2018; Costa *et al.*, 2021). Consequently, the risk of being exposed to potential vectors that allow for the introduction of NIS (e.g. marine traffic/shipping) is higher, in addition to the favorable temperate climate that the island itself provides. Evidence of increased impacts on ecosystems by biological invasions due to commercial shipping and recreational boats are mentioned by Castro *et al*. (2022).

In 2019, *R. okamurae* was reported in the Atlantic Ocean at Azores, successfully spreading, and covering approximately 90% of the South coast of São Miguel, between 2019-2021, causing ecological impacts on the ecosystems (Faria *et al*., 2021, 2022; García-Gómez *et al*., 2021). Additionally, it has also been registered in Madeira islands, as well as in the southern Portugal, Algarve, in 2021, with large accumulations at the beaches and an estimated spread rate of 87 km per year (Liulea *et al*., 2023). Studies have been analyzing the reproduction cycle of *R. okamurae* which revealed the incredible ability of producing numerous individuals through the cloning process, even with dispersed fragments of the algae (Jeschke *et al.*, 2019; Faria *et al*., 2021). In addition to this, the seaweed can adapt to extreme conditions, such as living in the darkness for a certain period and tolerate high levels of salinity and temperature, reasons that explain the successful colonization and establishment in non-native extents (Faria *et al*., 2021; Mercado *et al*., 2022)

As macroalgae support habitat for numerous organisms, modifications in the ecosystem by non-indigenous species can significantly alter the native assemblages and existent epibiota (Gestoso *et al*., 2010). Factors associated with complexity and structure of the alga, as well as seasonal variation, might originate different responses from macroinvertebrates communities (Bertocci *et al*., 2012; Veiga *et al.*, 2014; Faria *et al*., 2022). Intertidal macroalgae in affected regions had shown a decrease in abundance and richness of macroalgae assemblages with the invasion of *R. okamurae* (Faria *et al*., 2022). However, the presence of NIS may not result, exclusively, in negative ecological impacts, but can also play similar native species ecological roles, reflected in terms of abundance, richness and diversity of the associated species assemblages. For example, a negative response was not observed in some seaweeds, which suggests the coexistence and development combined with the invasive algae, reduced species abundance and richness of intertidal macroalgae in impacted regions (Liulea *et al*., 2023). Moreover, according to Lutz *et al*. (2019) epibionts do not exactly have a preference on their habitat host, being generally non-specific, and thus, able to associate with different macroalgae species. Therefore, in this situation, the occurrence of a NIS in a certain area will influence less of this type of epibionts since hosts can be selected according to the highest protection and habitat benefit.

So, this study will test the following hypotheses: 1) There will be differences in the structure of epifaunal assemblages (abundance, taxon richness and diversity) associated with native and NIS macroalgae inside and outside an MPA. 2) The abundance, taxon richness and diversity of epifaunal assemblages (macrofauna) associated with NIS will differ from the native ones.

2.3. Methods

2.3.1. Study and sampling area

The study was carried out between June and July 2022 at eight different rocky shores located between the Western end, Mosteiros, and the South of São Miguel, Cerco, along 51 km. The selected rocky shores sites covered four different locations inside MPA (Água de Pau; Cerco; Ferraria and Mosteiros) and four different locations outside MPA (Lagoa; ETAR Ponta Delgada, Milicias Beach and Santa Clara) (Figure 2.1) – Table with coordinates of each location in Annex A (Table A1 and Table A2). These locations worked as replications of site and were chosen since the access to the rock pools during low tide was feasible, and both native *H. scoparia* and non-indigenous *R. okamurae* were present in those areas.

Samples of the two brown macroalgae target species (i.e. the native *H. scoparia* and the invasive *R. okamurae*) were collected from tide pools during low tide in each site of study to quantify their macrofauna. In all eight shores, different areas were sampled, and each replicate was collected from different pools with a total of 5 replicates per macroalgae with 5 randomly chosen individuals each per replicate, giving a total of 25 individuals of each macroalgae per location (i.e. 200 individuals of each macroalgae in total).The fauna sampling method was the same for all locations, which involved the careful placement of 5 individuals in a plastic bag for each replicate and macroalgae.

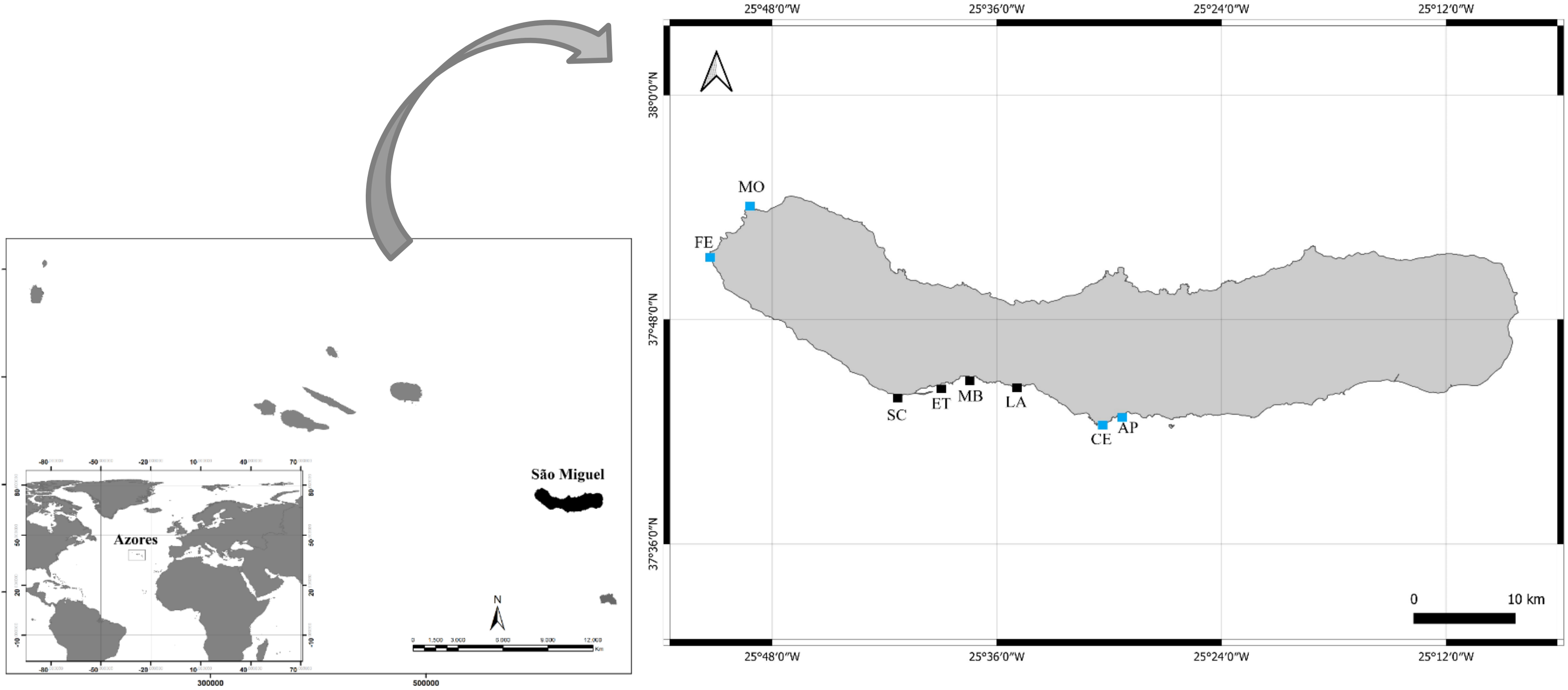

Figure 2.1 - The study area locations of the sampled shores at São Miguel. Blue squares represent the samples inside MPA (Mosteiros (MO); Ferraria (FE); Cerco (CE) and Água de Pau (AP)) and black squares the samples outside MPA (Santa Clara (SC); ETAR Ponta Delgada (ET); Milicias Beach (MB) and Lagoa (LA) (adaptation from Álvaro, 2022)

2.3.2. Laboratory Methodologies

In the laboratory, the plastic bags with the samples were filled with tap water, shaken vigorously and placed in a container for about 1 hour, allowing the macrofauna to free itself from the macroalgae samples. Furthermore, individual thallus were washed in freshwater and carefully examined to collect all visible animals with the use of forceps. The water was sieved (0.5 mm mesh size) to retain the remaining macrofauna. All macrobenthic organisms were stored in 70% ethanol until their identification under a stereomicroscope. The taxa were identified in different taxonomic levels, such as Phylum, Class, Order and Family and abundance was quantified. Each macroalgae replicate were dried at 50.5 °C for at least 72h to constant biomass (dry weight), ensuring the elimination of water, and determined to the nearest milligram (Gestoso *et al.*, 2012).

2.3.3. Data Analysis

The data obtained was consolidated in a simple and summarized table (Table 1.1).

Table 2.1 - Summary of the independent and dependent variables in study.

Independent Variables		Dependent Variables
Site	Inside Marine Protected Areas (inMPA) Outside Marine Protected Areas (outMPA)	Abundance (N) - individuals g DW^{-1} of algae
Location	Mosteiros (MO) Ferraria (FE) Cerco (CE) Água de Pau (AP) Santa Clara (SC) ETAR (ET) Milicias Beach (MB) Lagoa (LA)	Taxa Richness (S) – Taxonomic groups per algae
Algae	*Halopteris scoparia* (native) *Rugulopterix okamurae* (NIS)	Diversity (Shannon-Wiener Index, H')

From a total of 80 samples (n =80), 40 were collected inside MPAs and 40 were collected outside MPAs (5 samples of each alga in each location).

Since each sample included 5 different individuals of the same alga (*Rugulopteryx okamurae* or *Halopteris scoparia*), which may vary in size and/or weight, a comparison unit per gram of DW (dry weight) algae was done, to determine the abundance of macroinvertebrates that exist per gram of seaweed. Due to this reason, the abundance data of the different macrofauna taxonomic groups was based on standardised per gram of dry algal biomass (i.e. number of individuals per gram of DW algae) according to Gestoso *et al*. (2010) and Torres *et al*. (2015).

Seventeen taxonomic groups were established according to the macroinvertebrates identified: Amphipoda, Annelida, Arachnida, Asteroidea, Bivalvia, Caprellidae, Chironomidae, Copepoda, Decapoda, Echinoidea, Gastropoda, Isopoda, Ophiuroidea, Polyplacophora, Pycnogonida, Sipuncula and Tanaidacea.

To investigate both hypotheses, data was consolidated in a worksheet and statistically analyzed using the software Primer 6, version 6.1.11 & PERMANOVA +, version 1.0.1 (Clarke & Gorley, 2006) and a 5% significant level was considered. Shannon diversity Index (*H'*), representing the macrofauna diversity, was computed using the mentioned software (Clarke & Warwick, 1994). Data was analyzed based on two similarity indexes: Bray-Curtis similarity matrix for the number of invertebrates (abundance) and Euclidean distance regarding richness and diversity. Moreover, each established taxonomic group was analyzed using Euclidean distance. Before each statistical inference, data was checked for homogeneity of variances, using PERMDISP test, a multivariate analogue to Levene's test (Anderson, 2004). When needed, data was transformed to remove heterogeneity of variances, by using square root, fourth root and log (x+1). When this was not possible, untransformed data was analyzed and results were considered robust with a significant value of 1% (Underwood, 1996).

1) To examine differences in the structure of the macroinvertebrate community (N, S and H') within Sites (inMPA or outMPA) and associated algae species (*R. okamurae* and *H. scoparia*), a Permutational multivariate analysis of variance (PERMANOVA) was performed (Anderson, 2001). The experimental design included three orthogonal factors: Site (fixed, 2 levels, orthogonal); Location (random, nested in Site, 8 levels) and Algae (fixed, 2 levels, orthogonal) – Table 2.1.

Data was analyzed based on two similarity indexes: Bray-Curtis similarity matrix for the number of invertebrates (abundance) and Euclidean distance regarding richness and diversity. Before each statistical inference, data was checked for homogeneity of variances, using PERMDISP test, a multivariate analogue to Levene's test (Anderson, 2004). When necessary, data was transformed to remove heterogeneity of variances, by using square root, fourth root and log (x+1). When this was not possible, untransformed data was analyzed and results were considered robust with a significant value of 1% (Underwood, 1996). Moreover, the Monte Carlo method was used when the number of possible permutations was low (Anderson, 2001).

To allow a visualization of the pattern of the epifaunal communities across sites and algae, non-metric multi-dimensional scaling (nMDS) with a two-dimensional plot was used. Similarity Percentage analyses (SIMPER), based on Bray-Curtis matrix, were performed to identify the taxonomic groups that contributed the most to the similarity between the two habitats.

2) To examine differences in the structure of macrofaunal assemblages (N, S and H') between native and non-indigenous algae a PERMANOVA was also performed (Anderson, 2001). The

model included two orthogonal factors: Algae (fixed, 2 levels, orthogonal) and Location (random, 8 levels).

A nMDS was performed allowing for a visualization of the pattern of the epifaunal communities between algae. Moreover, SIMPER analyses, based on Bray-Curtis matrix, were performed to identify the taxonomic groups that most contributed to the similarity between the two seaweeds.

2.4. Results

General description of epifaunal assemblages inMPA and outMPA

Inside MPA, a total of 32 291 macroinvertebrates belonging to the 17 taxonomic groups were counted, with *Rugulopteryx okamurae* having more individuals (50.9%) than *Halopteris scoparia* (49.1%), as well as hosting macroinvertebrates from all the taxa, contrarily to the native algae (Figure 2.2 and Annex B: Table B1). Although present in extremely low quantities in the invasive algae, Asteroidea, Decapoda and Polyplacophora were not found in *H. scoparia*.

On the other hand, in non MPA, a total of 38 151 individuals were identified with *H. scoparia* covering more individuals than *R. okamurae,* 61.5% and 38.5% of the total number of macroinvertabres, respectively (Figure 2.2). The 17 taxonomic groups were harboured by the invasive algae, while no decapods were present in the native one (Annex B: Table B2).

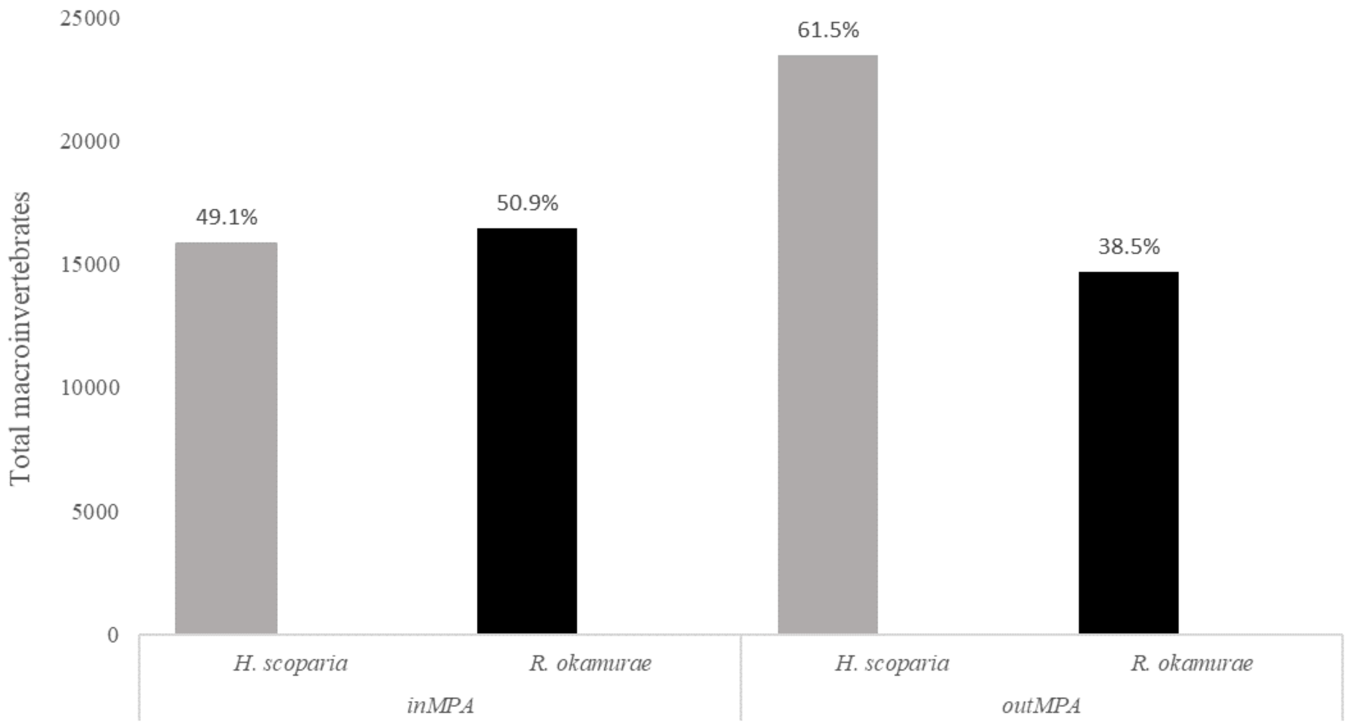

Figure 2.2 – Total number of macroinvertebrates and percentage counted in each alga, native *H. scoparia*, and non-indigenous *R. okamurae* for each site: inside Marine Protected Areas and outside Marine Protected Areas.

Either inside and outside MPA, Amphipoda, Annelida and Gastropoda were the most dominant groups in both habitats (Figure 2.3), however, gastropods were more associated with the native algae, while amphipods to the invasive one (Figure 2.4). Outside MPA for *H. scoparia*, both Isopoda and Caprellidae groups have increased their number of about 930 and 2650 individuals, respectively (Table 2 and Table 3).

Amphipoda was the most abundant group both inside and outside MPA, with 31% and 34% of the total abundance correspondingly, followed by Gastropoda and Annelida groups (Figure 2.3). The total abundance of Caprellidae differed outside the MPA, with a value of 9.5%, when compared to the 3.7% in the MPA.

When comparing both habitats, the native algae, *H. scoparia*, harboured more individuals than the invasive, where the dominant group were Gastropoda, with 33.3% of the total macroinvertebrates, followed by 26.9% of Amphipoda. This difference was greater when observing the results for amphipods living in *R. okamurae*, corresponding to 39.8% of the total macroinvertebrates, followed by Gastropoda 25.7%. In addition, Copepoda was replaced by Tanaidacea in the native macroalgae (Figure 2.4, Annex B: Table B1 and B2).

Shannon-Wiener diversity index (H’) of the macroinvertebrate fauna varied between 0.42 – 1.81 for *R. okamurae* and 0.56-1.83 for *H. scoparia* in each replicate. The average value for inMPA in *R. okamurae* was 1.38 and *H. scoparia* 1.32; whereas for outMPA in *R. okamurae* 1.41 and *H. scoparia* 1.31. This index considers the abundance as well as the richness of the sample, indicating that for higher H’ values, more equilibrated and distributed is the community of invertebrates. The results are very similar, although the NIS macroalgae obtained for both sites an average higher value.

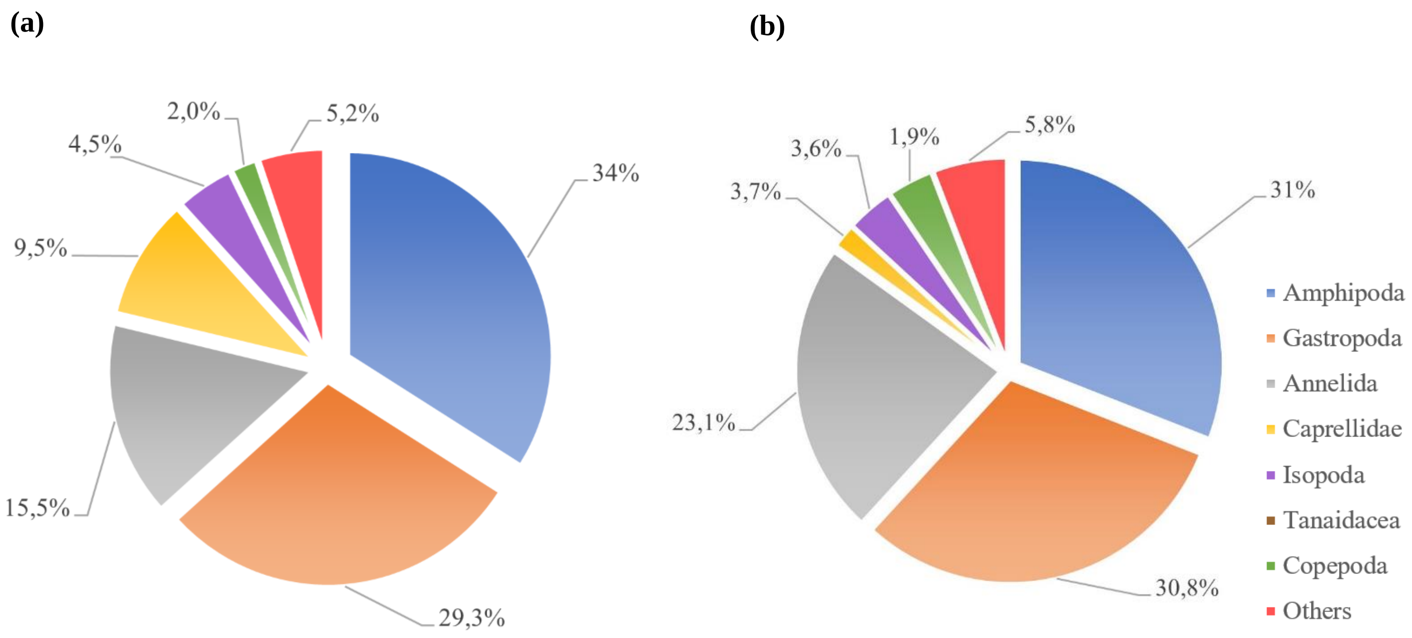

Figure 2.3 - Percentage of most abundant taxonomic groups identified in epifaunal assemblages. (a) Total percentage of main taxonomic groups in the two algae outside the Marine Protected Area. (b) Percentage of main taxonomic groups in the two algae inside the Marine Protected Area. “Others” represent the sum of percentages for lower abundant taxonomic groups (see Annex: Index C)

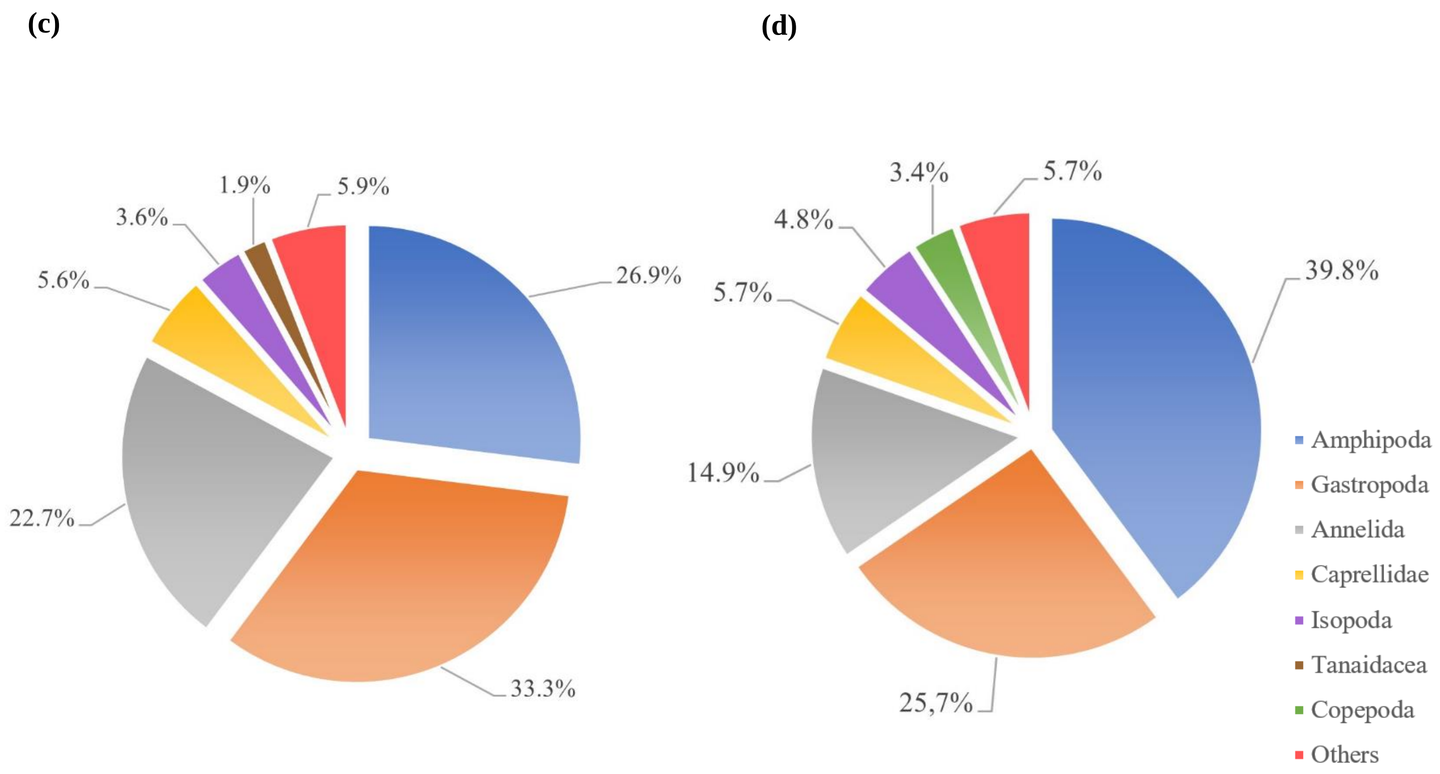

Figure 2.4 - Percentage of most abundant taxonomic groups identified in epifaunal assemblages. (c) Total percentage of main taxonomic groups in *Halopteris scoparia*. (d) Total percentage of main taxonomic groups in *Rugulopteryx okamurae*. "Others" represent the sum of percentages for lower abundant taxonomic groups (see Annex C: Table C1)

Multivariate analysis of epifaunal assemblages inMPA and outMPA

According to Table 2.2, the results suggest no significant statistical differences regarding abundance (*p-value* > 0.05), richness (*p-value* > 0.05) and diversity (*p-value* > 0.05) of associated macrofauna assemblages between site and algae. Therefore, both *R. okamurae* and *H. scoparia* supported similar variability of macroinvertebrates in the studied rock pools inside and outside MPA.

The number of invertebrates inhabiting the native and invasive species of alga (Figure 2.5), the presence of individuals from each taxonomic group established (Figure 2.6), as well as the diversity (Figure 2.7) were similar in each site.

Table 2.2 - Summary of PERMANOVA analysis for abundance (N), richness (S) and Shannon-Wiener diversity index (H') of epifaunal assemblages within sites. Significant statistical differences represented in bold (p - value < 0.05). Untransformed used data for S (p – *value* < 0.01 considered)

		N			**S**			**H'**		
Parameters	d*f*	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Si	1	2443.9	0.40776	0.796	1.25	0.044709	0.757	0.002854	0.005956	0.878
Al	1	6917.5	3.044	0.094	16.2	1.3288	0.309	0.12493	1.8624	0.208
Lo(Si)	6	5993.4	8.0323	**0.001**	27.958	10.236	**0.001**	0.4792	6.2839	**0.001**
Si x Al	1	2937.1	1.2925	0.278	0.05	0.004101	0.944	0.006793	0.10126	0.775
Lo(Si) x Al	6	2272.5	3.0455	**0.001**	12.192	4.4638	**0.002**	0.06708	0.87965	0.513
Residuals	64	746.16			2.7313			0.076258		
PERMDISP				0.124			0.002			0.378

Si – Site; Al – Algae; Lo – Location
d*f* – Degrees of freedom; MS – Mean squares

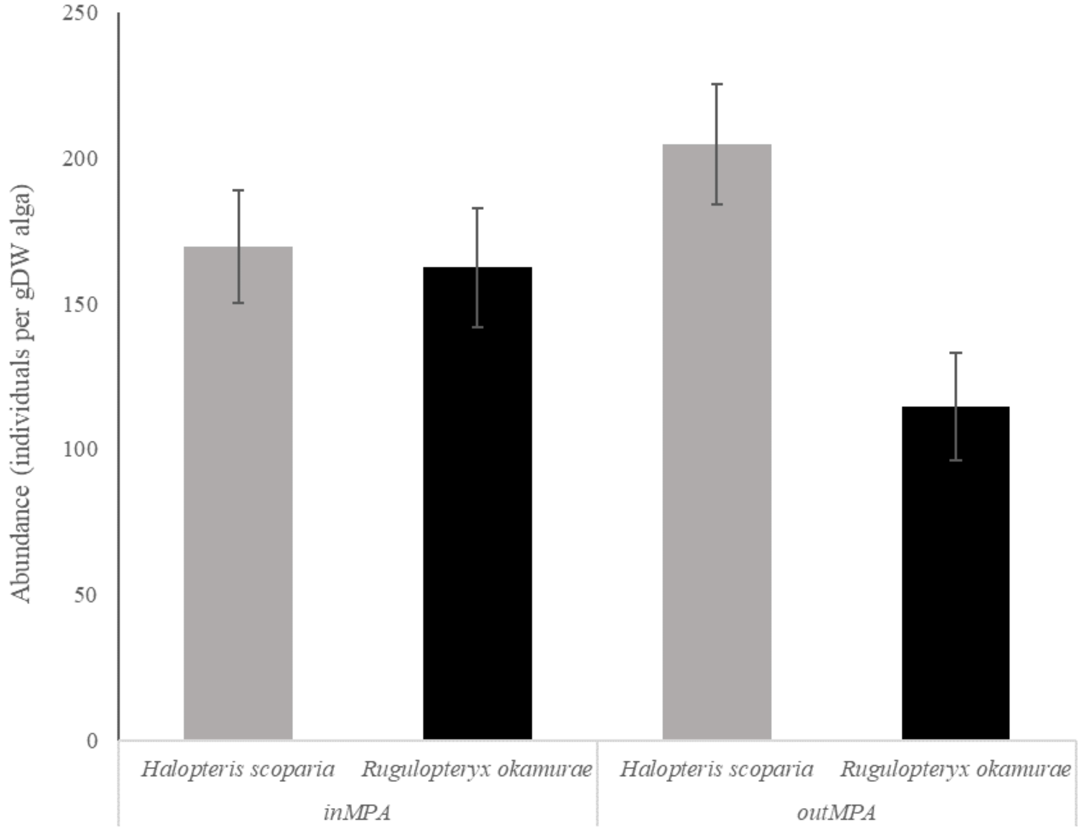

Figure 2.5 - Average abundance (N) individuals per gDW alga ± Standard Error (SE) for each alga, native *H. scoparia*, and non-indigenous *R. okamurae*, and location inside and outside Marine Protected Areas.

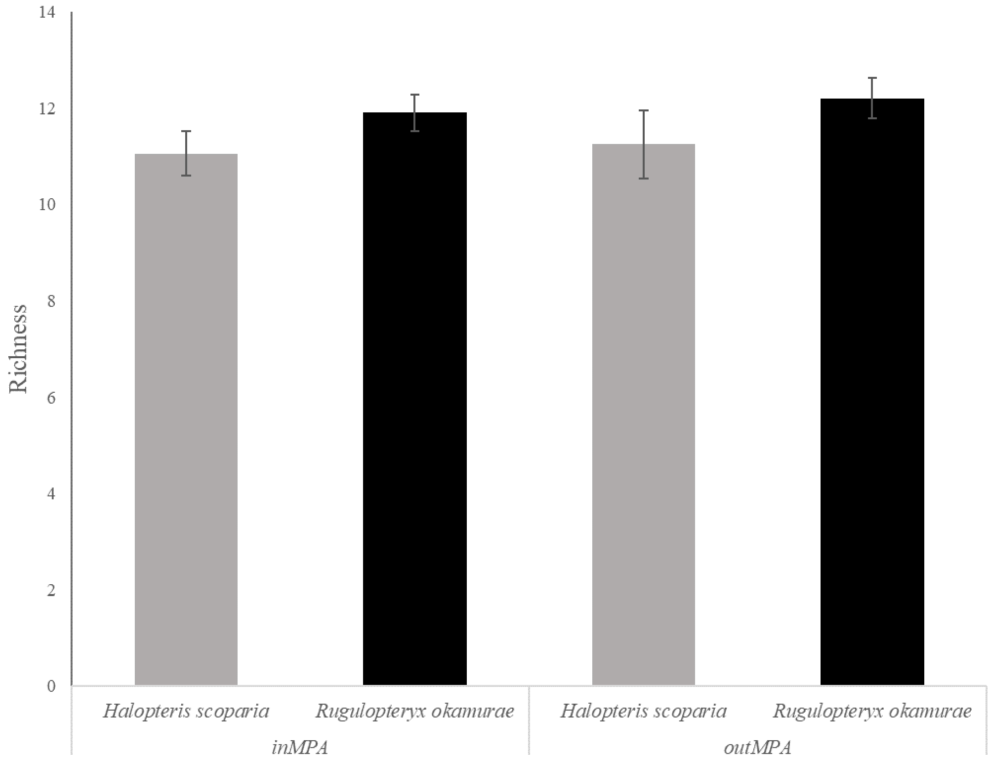

Figure 2.6 – Average Richness (S) ± Standard Error (SE) for each alga, native *H. scoparia*, and non-indigenous *R. okamurae*, inside and outside Marine Protected Areas.

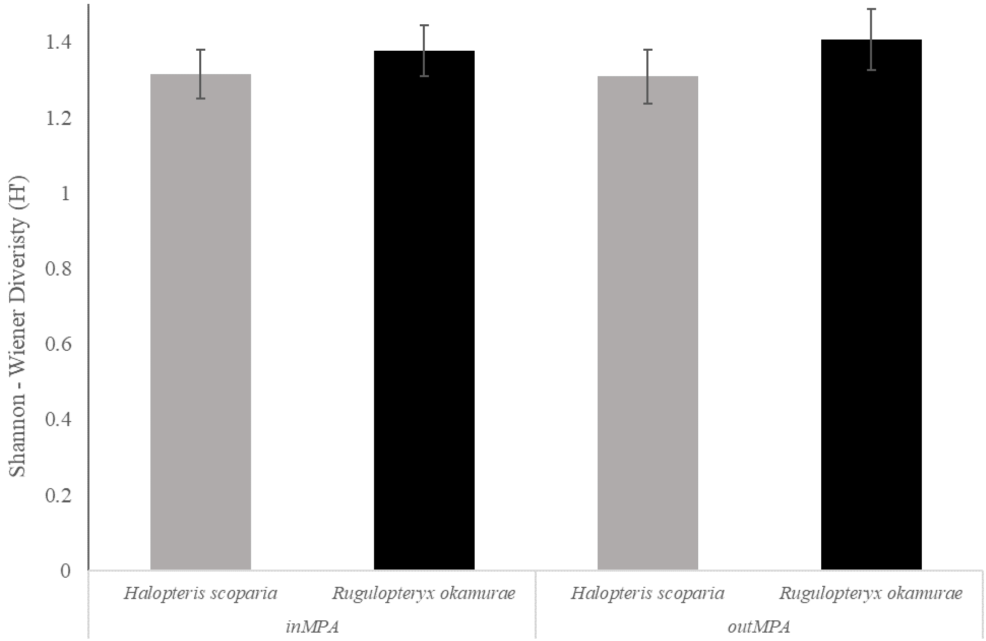

Figure 2.7 - Average Shannon – Wiener Diversity (H') values ± Standard Error (SE) per alga, native *H. scoparia*, and non-indigenous *R. okamurae*, inside and outside Marine Protected Areas.

The nMDS plot (Figure 2.8) clearly showed the similarity of the different samples across algae within sites, reinforcing the statistical analysis results that no significant differences were observed for the variability of community of invertebrates in the different algae.

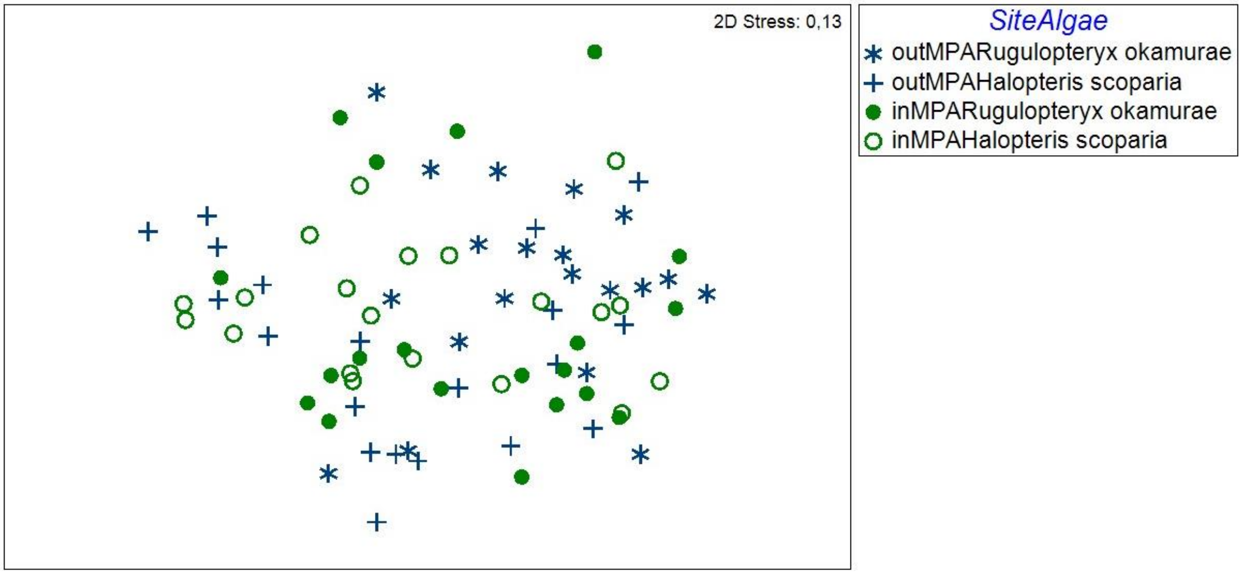

Figure 2.8 – Non-metric multidimensional scaling (nMDS) for epifaunal assemblages associated with the invasive *R. okamurae* and the native *H. scoparia* inside (inMPA) and outside Marine Protected Areas (outMPA).

SIMPER analysis results (Table 2.3 and Table 2.4) indicated that within the 17 taxonomic groups defined, six are responsible for approximately 90% of the differences between macroalgae in both sites.

Gastropoda and Amphipoda corresponded to the most contributors for 50% of the dissimilarities, with very similar contribution. Amphipoda revealed to be the most abundant group in and out MPA for *R. okamurae*, while Gastropoda was the most abundant for *H. scoparia*.

Table 2.3 - SIMPER analysis showing the percentage contribution of each taxonomic group inMPA.

inMPA

Average dissimilarity = 49,14%

	R. okamurae	***H. scoparia***				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Gastropoda	42.44	59.87	15.98	1.32	32.52	32.52
Amphipoda	62.83	39.9	13.23	1.33	26.92	59.45
Annelida	28.88	47.88	10.09	1.31	20.54	79.99
Isopoda	8.29	3.92	2.51	0.78	5.11	85.09
Copepoda	6.44	5.6	1.95	0.87	3.96	89.06
Caprellidae	4.26	2.17	1.62	0.67	3.3	92.36

Table 2.4 - SIMPER analysis showing the percentage contribution of each taxonomic group outMPA.

outMPA

Average dissimilarity = 53,15%

	R. okamurae	***H. scoparia***				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Gastropoda	28.59	64.84	16.58	1.1	31.19	31.19
Amphipoda	47.46	61.06	14.05	1.31	26.43	57.61
Annelida	12.38	36.93	8.57	1.16	16.12	73.73
Caprellidae	11.5	18.92	6.57	0.84	12.36	86.08
Isopoda	4.89	9.51	2.73	0.97	5.14	91.22

Multivariate analysis of epifaunal assemblages associated with native and invasive algae

According to Table 2.5, the results obtained indicate that no significant statistical differences were verified in the macrofaunal communities between native and the invasive algae for the number of hosted invertebrates (*p-value* > 0.05), richness (*p-value* > 0.05) and diversity (*p-value* > 0.05). Both studied algae supported similar variability of macroinvertebrates independently of the site or the typology, as observed in the nMDS 2-dimensional plot (Figure 2.9).

Table 2.5 - Summary of PERMANOVA analysis for abundance (N), richness (S) and Shannon-Wiener diversity index (H') of epifaunal assemblages between algae. Significant statistical differences represented in bold (*p* - value < 0.05). Untransformed used data for S (*p – value* < 0.01 considered)

		N			**S**			**H'**		
Parameters	d*f*	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Al	1	6917.5	2.922	0.075	16.2	1.5492	0.255	0.12493	2.1368	0.183
Lo	7	5486.3	7.3527	**0.001**	169	8.8395	**0.001**	0.41115	5.3915	**0.001**
Al x Lo	7	2367.4	3.1728	**0.001**	73.2	3.8284	**0.004**	$5.85\text{x}10^{-2}$	0.76671	0.628
Residuals	64	746.16			2.7313			$7.63\text{x}10^{-2}$		
PERMDISP				0.391			0.01			0.399

Al – Algae; Lo – Location
d*f* – Degrees of freedom; MS – Mean squares

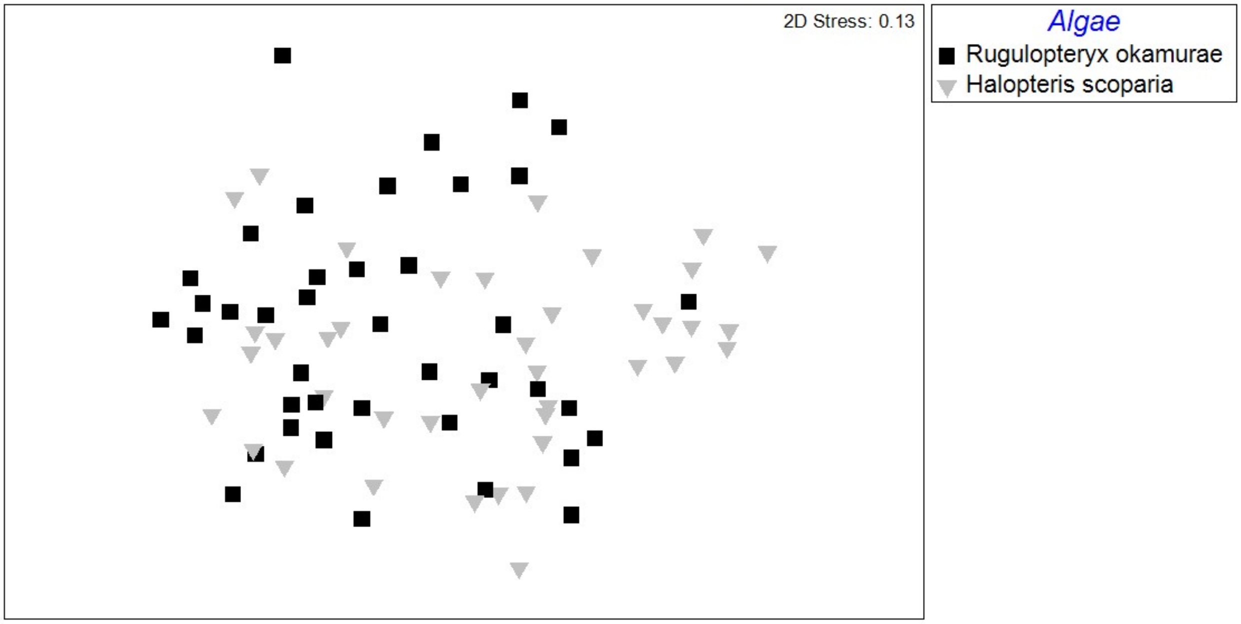

Figure 2.9 - Non-metric multidimensional scaling (nMDS) for epifaunal assemblages associated with the two macroalgae: native *H. scoparia*, and non-indigenous *R. okamurae*.

SIMPER analysis results (Table 2.6) indicated that within the 17 taxonomic groups defined, six are responsible for approximately 90% of the differences between native and invasive seaweeds. Gastropoda and Amphipoda corresponded to the most contributors for 50% of the dissimilarities, with very similar contribution. Amphipoda revealed to be the most abundant group in the non-invasive alga, *R. okamurae*, while Gastropoda was the most abundant for the native, *H. scoparia*.

Table 2.6 - SIMPER analysis showing the percentage contribution between native (*H. scoparia*) and invasive (*R. okamurae*) algae.

Average dissimilarity = 51,07%

	R. okamurae	***H. scoparia***				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Gastropoda	35.52	62.36	16.17	1.21	31.66	31.66
Amphipoda	55.14	50.48	13.25	1.32	25.95	57.6
Annelida	20.63	42.4	9.7	1.24	19	76.6
Caprellidae	7.88	10.54	4.15	0.68	8.14	84.74
Isopoda	6.59	6.71	2.51	0.89	4.92	89.66
Copepoda	4.76	4.47	1.59	0.9	3.12	92.78

Analysing the different taxonomic groups of macroinvertebrates (Table 2.7 and Figure 2.10), in the 17 groups, 4 of them obtained significant statistical differences regarding the relative abundance (individuals per gDW alga) between native and non-native algae: Annelida, Arachnida, Ophiuroidea and Tanaidacea.

Moreover, significant statistical differences were obtained for the interaction algae x location, in the following taxonomic groups: Amphipoda, Annelida, Bivalvia, Caprellidae, Chironomidae, Copepoda, Gastropoda, Isopoda, Ophiuroidea, Pycnogonida, Sipuncula and Tanaidacea.

Table 2.7 - PERMANOVA analysis with Euclidean distance (similarity index) of taxonomic groups of macroinvertebrates within algae. Significant values are in bold (*p-value* < 0.05 and *p-value* < 0.01). Untransformed used data for Decapoda (*p – value* < 0.01 considered); **Square root transformation; *** Fourth root transformation.

		Amphipoda			Annelida			Aracnida			Asteroidea		
Parameters	d*f*	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Al	1	434.74	0.15006	0.67	76.834	15.617	**0.008**	13.072	16.272	**0.01**	5.1983x10^{-}	0.81047	0.521
Lo	7	3539	3.0776	**0.011**	21.497	9.9229	**0.001**	17.308	20.403	**0.001**	2.3294x10^{-}	2.4454	**0.017**
Al x Lo	7	2897.1	2.5194	**0.03**	4.92	2.2711	**0.039**	0.80336	0.947	0.509	6.4139x10^{-}	0.67334	0.741
Residuals	64	1149.9			2.1663			0.84832			9.5255x10^{-}		
PERMDISP				0.86			0.134**			0.426			0.491

		Bivalvia			Caprellidae			Chironomidae			Copepoda		
Parameters	d*f*	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Al	1	0.11531	0.138	0.757	142.47	0.10845	0.781	4.0696	0.75662	0.432	1.6722	4.4639x10^{-2}	0.837
Lo	7	6.9887	18.464	**0.001**	2309.4	21.197	**0.001**	4.8081	3.0763	**0.008**	85.157	3.0482	**0.01**
Al x Lo	7	0.83558	2.2076	**0.047**	1313.7	12.058	**0.001**	5.3786	3.4413	**0.003**	37.461	1.3409	**0.24**
Residuals	64	0.37851			108.95			1.5629			27.936		
PERMDISP				0.282			0.303			0.226			0.615

		Decapoda			Echinoidea			Gastropoda			Isopoda		
Parameters	d*f*	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Al	1	9.2016x10^{-3}	1.8044x10^{-3}	0.02	1.7836x10^{-3}	1.8044x10^{-3}	0.892	14406	3.1163	0.127	0.29807	1.7882x10^{-3}	0.967
Lo	7	9.1859x10^{-4}	0.47012	0.878	2.1582	7.4785	**0.001**	14797	20.37	**0.001**	206.33	4.1464	**0.001**
Al x Lo	7	9.1859x10^{-4}	0.47012	0.874	9.8851x10^{-2}	0.34253	0.935	4622.9	6.3642	**0.001**	166.68	3.3496	**0.003**
Residuals	64	0.28859			0.28859			726.38			49.761		
PERMDISP				0.001			0.735			0.074			0.79

		Ophiuroidea			Polyplacophora			Pycnogonida			Sipuncula		
Parameters	d*f*	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Al	1	8.7292	9.4113	**0.027**	1.3637x10^{-4}	5.6369x10^{-3}	0.938	0.49224	2.3402	0.162	2.2573	1.0096	0.307
Lo	7	5.4208	22.858	**0.001**	8.4619x10^{-2}	4.022	**0.004**	0.35734	9.9414	**0.001**	2.0183	2.8817	**0.015**
Al x Lo	7	0.92752	3.9112	**0.002**	2.4193x10^{-2}	1.1499	0.34	0.21034	5.8517	**0.001**	2.2359	3.1923	**0.003**
Residuals	64	0.23714			2.1039x10-2			3.5945x10^{-2}			0.7004		
PERMDISP				0.222**			0.723			0.001			0.197

		Tanaidacea		
Parameters	d*f*	MS	Pseudo-F	P(perm)
Al	1	1.264	5.7755	**0.049**
Lo	7	0.76753	7.6071	**0.001**
Al x Lo	7	0.21885	2.1691	**0.047**
Residuals	64	0.1009		
PERMDISP				0.193***

Al – Algae; Lo – Location
d*f* – Degrees of freedom; MS – Mean squares

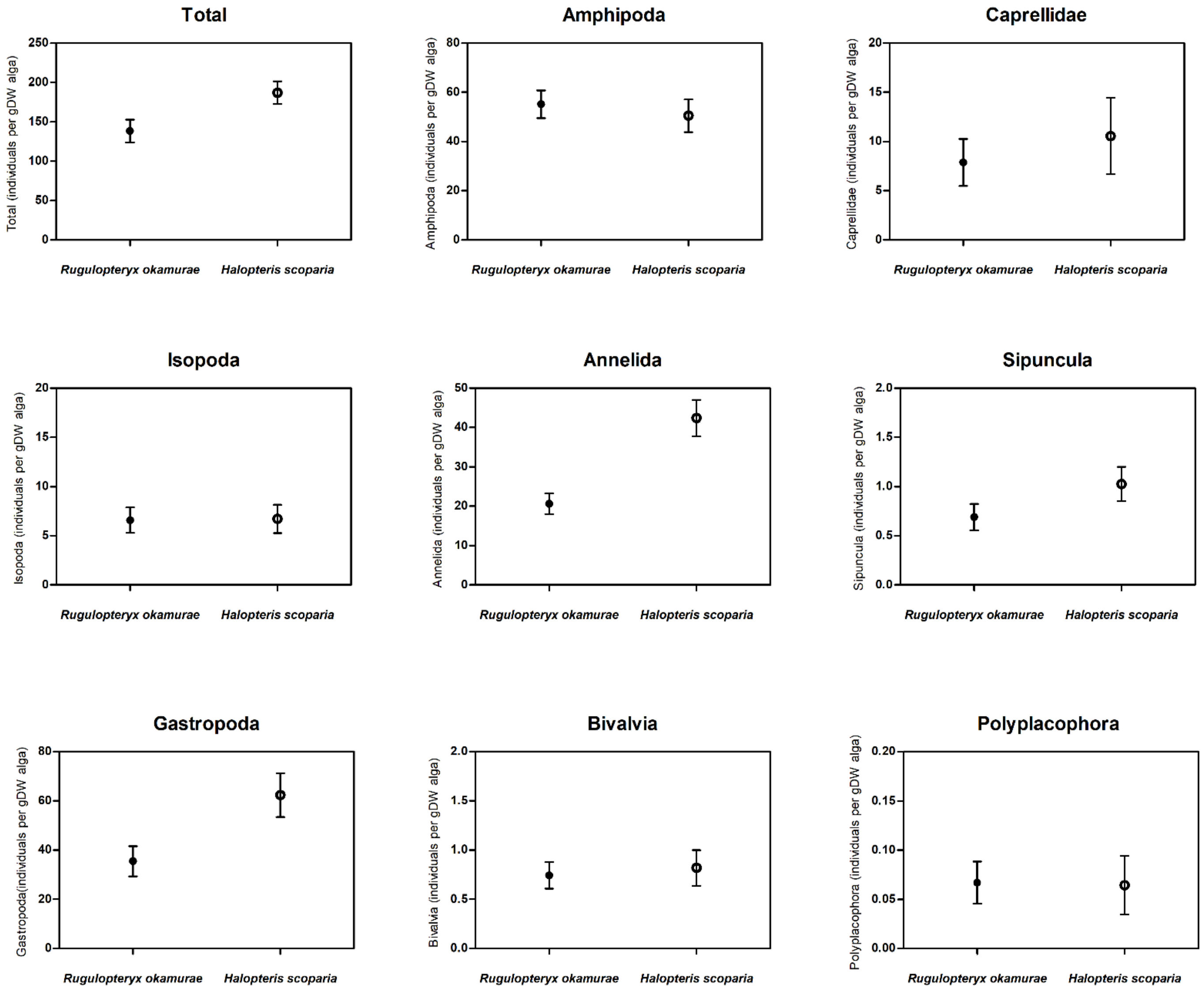

Figure 2.10 – Abundance (average number of ind. per gDW ± SE) of taxonomic groups identified in each alga species (note the different y-axis scale for plots).

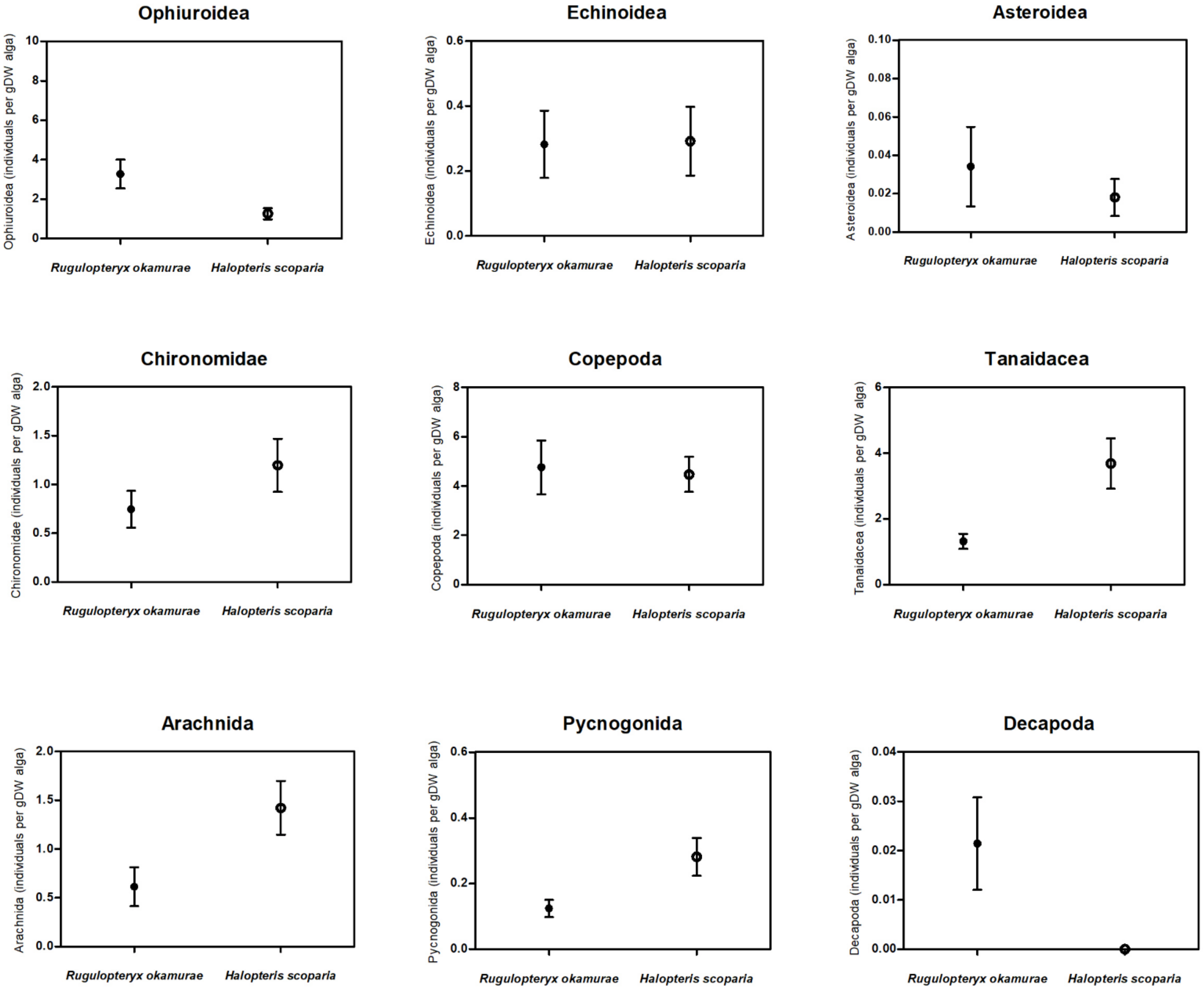

Figure 2.10 – (Cont.) Abundance (average number of ind. per gDW ± SE) of taxonomic groups identified in each alga species (note the different y-axis scale for plots).

2.5. Discussion

Regarding the results obtained in the present study and considering descriptive analysis, the total abundance of invertebrates hosted outside marine protected areas, as well as taxonomic groups were higher than inside marine protected areas. Additionally, *H. scoparia* has shown a tendency to support a higher number of invertebrates outside MPA. However, regarding statistical analysis, the structure of epifaunal assemblages (N, S and H') did not show any significant differences between sites and macroalgae in the rockpools.

Effects of MPAs tend to be positive for marine coastal habitats, regarding macroinvertebrates species, as well as macroalgal functional groups (Cacabelos *et al.*, 2020). MPAs are established to maintain ecosystem biodiversity, implying management and assessment of marine ecosystems integrity, and mitigation of the introduction of non-native species, which can impact the community structure and composition (Leenhardt *et al.*, 2015). Therefore, these areas should provide support and conservation to epifaunal communities, meaning more protection and thus, increase in abundance, diversity and richness of marine coastal habitats (Gaines *et al.*, 2010; Leenhardt *et al.*, 2015). Additionally, another theory is that specific functional characteristics, such as body size, feeding behavior, reproductive mechanisms and habitat preferences are maintained in MPAs (Leenhardt *et al.*, 2015). However, evidence provided in this study revealed similar results of epifaunal assemblages for protected and non-protected areas, in both macroalgae. This can be a non-surprising outcome, since MPAs are not effective, lack explicit management plans and have insufficient conservation efforts, and so, are less likely to have significant differences (Abecasis *et al.*, 2015). Absence of management plans or inefficient implementation, application and monitoring of MPAs leads to poor managing, being crucial, especially in remote areas, such as Azores, the need to focus and reinforce the supervision in these areas (Maestro *et al.*, 2020). On the other hand, it is still unclear the role that MPAs have in terms of controlling biological invasions, as well as the consequential impacts (Cartagena *et al.*, 2018).

Regarding epifaunal assemblages inhabiting native and invasive algal species in the studied rockpools, the non-indigenous macroalgae did not affect the macroinvertebrates inhabiting the area, since the community structure did not show any significant differences concerning abundance, richness and diversity. Hence, the non-native algae might be functionally similar to the native ones and play ecological similar roles. Prior studies have also compared modifications in the macrofaunal communities living in native versus invasive seaweeds, since macroinvertebrates can have different responses (negative, neutral, positive) difficult to predict (Navarro-Barranco *et al.*, 2018). In one hand, epifaunal communities might alter in structure with the introduction of non-indigenous macroalgae (Faria *et al*., 2021, 2022; García-Gómez *et al*., 2021; Ruitton *et al*., 2021) with the architecture being the major influence that shape the macroinvertebrates community (Gestoso *et al.,* 2012; Güner *et al*., 2019; Faria *et al*., 2022). Moreover, more complexity is associated with a higher variety of food resources and better protection from predators, being a critical factor for predicting the influence of invasive macroalgae on epifaunal communities (Veiga *et al.,* 2014). For Gestoso *et al*. (2012) the invasive macroalgae obtained a higher number of individuals, whilst the native had more diversity. Similarly, according to Lutz *et al.* (2019), habitat features, as well as spatial variation were the main responsible for the differences observed between native and non-indigenous macroalgae, with the latter hosting considerable fewer taxa, but having higher abundance. Prestes (2021), observed significant structural and compositional differences in the macroinvertebrate's community for *H. scoparia,* where the native macroalga supported less associated abundance, but similar or higher taxa number than the non-indigenous macroalgae. On the other hand, no harmful effects, or low impacts with no substantial modifications have been observed (Thomsen *et al*., 2009). Navarro-Barranco *et al.* (2018) reported less density, diversity and richness in the non-indigenous than in *H. scoparia*. Veiga *et al.* (2014) concluded that the introduction of a non-indigenous macroalgae which is less complex than the native algae, is less likely to change the composition of epifauna associated, and vice-versa.

The fact that in this study *H. scoparia* supported as much or even a higher number of invertebrates than *R. ok*amurae might indicate that the structure of the native seaweed is compensated by the greater number of ramifications when compared to the invasive one, which facilitates enhanced adhesion to the indigenous algae, providing habitat for a larger number of individuals (Faria *et al*., 2022; Güner *et al*., 2019; Vázquez-Luis *et al*., 2012). However, the effects that the introduction of non-native species have in the structure and functionality of native mobile invertebrates' assemblages remain a challenge to forecast (Veiga *et al*., 2014).

Both algae supported similar epifaunal communities, and no differences were observed, which indicates the possibility of mobile invertebrates being not host-specific (Navarro-Barranco *et al.*, 2018), and are equally inhabiting both algae, independently of their typology.

Considering that in Azores, size and cover of *R. okamurae* follows a seasonal fluctuation with larger and more abundant algae individuals from mid-winter until summer (Faria *et al.*, 2022), which includes the months of this study, it is a favourable outcome that *H. scoparia* persisted with similar or more abundance of macrofauna in the rock pools. According to Faria *et al*. (2022), *R. okamurae* covered a high percentage of São Miguel coast, replacing previous dominant species, which emphasizes the seriousness risk of extinction and total colonization by the invasive algae, leading to significant changes in the structure of shallow-water benthic communities. It is notable the domination of the invasive macroalgae in the tide pools, given the difficulty in finding *H. scoparia* among the non-native algae (author personal observation). Liulea *et al.* (2023), showed the significant impact of *R. okamurae* regarding the intertidal macroalgae community, which decreased species richness and abundance. Nevertheless, ten species out of the fifty-four surveyed were able to co-exist and did not show any negative response to the presence of *R. okamurae*.

As indicated by Bertocci *et al.* (2012), the structure of alga and benthic assemblages have seasonal fluctuations between winter and summer in rock pools that may be driven by hydrodynamic patterns, intraspecific competition, life cycles, patchy algal recruitment and colonization, heterogeneity of substratum, grazing and temperature. Moreover, variation may be associated with other factors, such as epiphytes load, morphological complexity (as referred above), nutritional content, toughness and chemical defences (Schreider *et al*., 2003; Mcdonald, 2010; Prestes, 2021). Wai *et al.* (2006) studied the variability of Mollusca and found temporal patterns associated with season and tides, as well as physical stress and temperature. Additionally, Mateo-Ramirez *et al.* (2018), observed that decapods obtained higher values of abundance when *H. scoparia* development was minimum, showing a temporal variability of Decapoda assemblages.

So, identifying and understanding the taxonomic groups existent in the two habitats as well as recognizing patterns is key for predicting changes in ecology, dispersal, and diversity due to stressors and anthropogenic impacts. Therefore, it is of high importance that future research works, include seasonal and temporal variation along with additional locations and probably consider more species of native algae, for a more comprehensive conclusion of the existent alterations, as well as prediction of ecological impacts.

2.6 Acknowledgements

The development of this study would not be possible without the support of CIBIO (Centro de Investigação em Biodiversidade e Recursos Genéticos), as well as the University of Azores in providing all the necessary conditions and material to carry out the experiment. We would like to express our sincere gratitude to Manuela Parente for the identification of macroalgal species, Ana Costa for macroinvertebrates identification and Afonso Prestes that helped in different stages of this research.

2.7 References

Aamri, F. E. L., Mohamed, I., and Mohamed, N. T. (2018). Occurrence of the Invasive Brown Seaweed Rugulopteryx Okamurae (E . Y . Dawson) I . K . Hwang , W . J . Lee & H . S . Kim (Dictyotales , Phaeophyta) in Morocco (Mediterranean Sea). *Mediterranean fisheries and aquaculture research* 1:92–96.

Abecasis, R. C., Afonso, P., Colaço, A., Longnecker, N., Clifton, J., Schmidt, L., Santos, S. R. (2015). Marine Conservation in the Azores: Evaluating Marine Protected Area Development in a Remote Island Context. *Frontiers in Marine Science* 2:1–16. doi: 10.3389/fmars.2015.00104.

Afonso, P., Fontes, J., Giacomello, E., Magalhães, M. C., Martins, H. R., Morato, T., Neves, V., Prieto, R., Santos, R. S., Silva, M. A., and Vandeperre, F. (2020). The Azores: A Mid-Atlantic Hotspot for Marine Megafauna Research and Conservation. *Frontiers in Marine Science* 6:1–8. doi: 10.3389/fmars.2019.00826.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecol 26*, 32–46.

Anderson, M. J. (2004). PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests. University of Auckland, New Zealand

Barcellos, L., Pham, C. K., Menezes, G., Bettencourt, R., Rocha, N., Carvalho, M., and Felgueiras, H. P. (2023). A Concise Review on the Potential Applications of Rugulopteryx Okamurae Macroalgae. *Marine Drugs* 21(1):1–15. doi: 10.3390/md21010040.

Bertocci, I., Araujo, R., Incera, M., Arenas, F., Pereira, R., Abreu, M., Larsen, K., Sousa, I. P. (2012). Benthic assemblages of rock pools in northern Portugal: Seasonal and between-pool variability. *Scientia Marina*. 10.3989/scimar.03669.21A.

Cacabelos, E., Martins, G. M., Faria, J., Prestes, A. C. L., Costa, T., Moreu, I., & Neto, A. I. (2020). Limited effects of marine protected areas on the distribution of invasive species, despite positive effects on diversity in shallow-water marine communities. *Biological Invasions*, *22*(3), 1169–1179. https://doi.org/10.1007/s10530-019-02171-x

Cartagena, A. B., Troncoso, J. S., Olabarria, C., Lemos, M. F. L. (2018). The effect of protection and wave-exposure of two marine protected areas have opposite trends on macroalgae invasiveness. *PeerJ Preprints 6:e26679v2* https://doi.org/10.7287/peerj.preprints.26679v2

Castro, N., Gestoso, I., Marques, C. S., Ramalhosa, P., Monteiro, J. G., Costa, J. L., and Canning-Clode, J. (2022). Anthropogenic Pressure Leads to More Introductions: Marine Traffic and Artificial Structures in Offshore Islands Increases Non-Indigenous Species Introduction. *Marine Pollution Bulletin* 181. doi: 10.1016/j.marpolbul.2022.113898.

Costa, A. C., Balibrea, A., Raposeiro, P. M., Santos, S., Souto, M., and Gonçalves, V. (2021). Non-Indigenous and Invasive Freshwater Species on the Atlantic Islands of the Azores Archipelago. *Frontiers in Ecology and Evolution* 9:1–19. doi: 10.3389/fevo.2021.631214.

Clarke, K. R., Gorley, R. N. (2006). PRIMER V6: User Manual/Tutorial. PRIMER-E, Plymouth

Clarke, K. R., Warwick, R. M. (1994). An approach to statistical analysis and interpretation. PRIMER-E Ltd., Plymouth.

Faria, J., Prestes, A. C. L., Moreu, I., Cacabelos, E., and Martins, G. M. (2022). Dramatic Changes in the Structure of Shallow-Water Marine Benthic Communities Following the Invasion by *Rugulopteryx Okamurae* (Dictyotales , Ochrophyta) in Azores (NE Atlantic). 175. doi: 10.1016/j.marpolbul.2022.113358.

Faria, J., Prestes, A. C. L., Moreu, I., Martins G., Neto, A. I. & Cacabelos, E. (2021). Arrival and proliferation of the invasive seaweed *Rugulopteryx okamurae* in NE Atlantic islands. Botanica Marina. 65. 10.1515/bot-2021-0060.

García-Gómez, J. C., Florido, M., Olaya-Ponzone, L., Díaz de Rada, J. R., Donázar-Aramendía, I., Chacón, M., Quintero, J. J, Magariño, S., and Megina, C. (2021). Monitoring Extreme Impacts of *Rugulopteryx Okamurae* (Dictyotales, Ochrophyta) in El Estrecho Natural Park (Biosphere Reserve). Showing Radical Changes in the Underwater Seascape. *Frontiers in Ecology and Evolution* 9. doi: 10.3389/fevo.2021.639161.

Gaines, S.D., Lester, S.E., Grorud-Colvert, K., Costello, C., Pollnac, R. (2010). Evolving science of marine reserves: New developments and emerging research frontiers. *PNAS 107*:18251–18255.

Gestoso, I., Olabarria, C., and Troncoso, J. S. (2010). Variability of Epifaunal Assemblages Associated with Native and Invasive Macroalgae. *Marine and Freshwater Research* 61(6):724–31. doi: 10.1071/MF09251.

Gestoso, I., Olabarria, C., and Troncoso, J. S. (2012). Effects of Macroalgael Identity on Epifaunal Assemblages: Native Species versus the Invasive Species *Sargassum Muticum*. *Helgoland Marine Research* 66(2):159–66. doi: 10.1007/s10152-011-0257-0.

Güner, A, Nalbantsoy, A., Sukatar, A., and Yavaşoğlu, N. U. K. (2019). Apoptosis-Inducing Activities of Halopteris Scoparia L. Sauvageau (Brown Algae) on Cancer Cells and Its Biosafety and Antioxidant Properties. *Cytotechnology* 71(3):687–704. doi: 10.1007/s10616-019-00314-5.

Hwang, I., Lee, W., Kim, H. and De Clerck, O. (2009.) Taxonomic Reappraisal of Dilophus okamurae (Dictyotales, Phaeophyta) from the Western Pacific Ocean. *Phycologia*, 48, 1-12. http://dx.doi.org/10.2216/07-68.1

Jeschke, M. A., De la Rosa, J., Muñoz, A. R., & Ros, M. (2019). Workshop de especies invasoras marinas: construyendo una propuesta para Andalucía Conclusiones de la mesa redonda. 55, 41-43.

Leenhardt, P., Low, N. H. N., Pascal, N., Micheli, F., Claudet, J. (2015). The role of marine protected areas in providing ecosystem services. In: *Aquatic functional biodiversity - An ecological and evolutionary perspective*. p 211–239.

Liulea, S., Serrão, E., Santos, R. (2023). Spread and impact of the invasive brown Algae *Rugulopteryx okamurae* on the Algarve Coast, Southern Portugal (NE Atlantic). 10.2139/ssrn.4446622.

Lutz, M. L., Minchinton, T., and Davis, A. R. (2019). Differences in Architecture between Native and Non-Indigenous Macroalgae Influence Associations with Epifauna. *Journal of Experimental Marine Biology and Ecology* 514–515:76–86. doi: 10.1016/j.jembe.2019.03.006.

Maestro, M., Chica-Ruiz, A. J., Pérez-Cayeiro, M. L. (2020). Analysis of marine protected area management: The Marine Park of the Azores (Portugal), *Marine Policy*, Volume 119, 104104. https://doi.org/10.1016/j.marpol.2020.104104.

Mateo-Ramírez, Á., Urra, J., Rueda, J.L., Marina, P., García Raso, J.E., (2018). Decapod assemblages associated with shallow macroalgal communities in the northwestern Alboran Sea: Microhabitat use and temporal variability, *Journal of Sea Research, Volume 135*, Pages 84-94, ISSN 1385-1101, https://doi.org/10.1016/j.seares.2018.02.009

McDonald, P. S., Bingham, B. L. (2010). Comparing macroalgal food and habitat choice in sympatric, tube-building amphipods, *Ampithoe lacertosa* and *Peramphithoe humeralis*. *Mar Biol 157*, 1513–1524. https://doi.org/10.1007/s00227-010-1425-5

Mercado, J. M., Gómez-Jakobsen, F., Korbee, N., Aviles, A., Bonomi-Barufi, J., Muñoz, M., Reul, A., and Figueroa, F.L. (2022). Analyzing Environmental Factors That Favor the Growth of the Invasive Brown Macroalgae *Rugulopteryx okamurae* (Ochrophyta): The Probable Role of the Nutrient Excess. *Marine Pollution Bulletin* 174. doi: 10.1016/j.marpolbul.2021.113315.

Morton, B. S., Britton, J. C., Martins, A. M. F. (1998). Coastal ecology of the Azores. *Sociedade Afonso Chaves*, Ponta Delgada, Azores

Morton, B. S., & Martins, A. M. F. (2018). The azores. *World Seas: An Environmental Evaluation Volume I: Europe, the Americas and West Africa*, *January 2019*, 501–530. https://doi.org/10.1016/B978-0-12-805068-2.00025-5

Navarro-Barranco, C., Florido, M., Ros, M., González-Romero, P., Guerra-García, J. M. (2018). Impoverished mobile epifaunal assemblages associated with the invasive macroalga *Asparagopsis taxiformis* in the Mediterranean Sea. *Mar Environ Res* 141:44–52.

Navarro-Barranco, C., Muñoz-Gómez, B., Saiz, D., Ros, M., Guerra-García, J. M., Altamirano, M., Ostalé-Valriberas, E.,and Moreira, J., (2019). Can Invasive Habitat-Forming Species Play the Same Role as Native Ones? The Case of the Exotic Marine Macroalgae *Rugulopteryx okamurae* in the Strait of Gibraltar. *Biological Invasions* 21(11):3319–34. doi: 10.1007/s10530-019-02049-y.

Prestes, A. C. L. (2021). Impacte da alga invasora *Asparagopsis armata* nas comunidades litorais dos Açores. Tese de doutoramento. Faculdade de Ciências e Tecnologia, Departamento de Biologia, Universidade dos Açores. xxiii+160 pp.+Anexos.

Ruitton, S., Blanfuné, A., Boudouresque C. F., Guillemain, D., Michotey, V., Roblet, S., Thibaut, D., Thibaut, T., and Verlaque, M. (2021). Rapid Spread of the Invasive Brown Algae *Rugulopteryx*." *Water* 13(2306).

Sánchez-Moyano, J. E., Estacio F. J., García-Adiego, E. M., and García-Gómez J. C. (2000). The Molluscan Epifauna of the Algae *Halopteris Scoparia* in Southern Spain as a Bioindicator of Coastal Environmental Conditions. *Journal of Molluscan Studies* 66(4):431–48. doi: 10.1093/mollus/66.4.431.

Sánchez-Moyano, J. E., García-Adiego, E. M., Estacio, F., and García-Gómez, J. C. (2002). Effect of Environmental Factors on the Spatial Variation of the Epifaunal Polychaetes of the Algae *Halopteris Scoparia* in Algeciras Bay (Strait of Gibraltar). *Hydrobiologia* 470:133–48. doi: 10.1023/A:1015680106097.

Schreider, M. J., Glasby, T. M., Underwood, A. J. (2003). Effects of height on the shore and complexity of habitat on abundances of amphipods on rocky shores in New South Wales, Australia. J Exp Mar Bio Ecol 293:57–71.

Thomsen, M. S., Wernberg, T., Tuya F., Silliman, B. R. (2009). Evidence for impacts of nonindigenous macroalgae: A meta-analysis of experimental field studies 1. *J. Phycol., 45 (4),* pp. 812-819

Torres, A. C., Veiga, P., Rubal, M., Sousa-Pinto, I. (2015). The role of annual macroalgal morphology in driving its epifaunal assemblages. *Journal of Experimental Marine Biology and Ecology*, 464: 96-106.

Underwood, A. J. (1996) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.

Vázquez, L., M., Borg, A. J., Sanchez-Jerez, P., Bayle – Sempere, J. T., (2012). Habitat colonisation by amphipods: Comparison between native and alien algae. *Journal of Experimental Marine Biology and Ecology, Volumes* 432-433. Pages 162-170. https://doi.org/10.1016/j.jembe.2012.07.016.

Veiga, P., Rubal, M., Sousa-Pinto, I., (2014). Structural complexity of macroalgae influences epifaunal assemblages associated with native and invasive species, *Marine Environmental*

Research, Volume 101, Pages 115-123, ISSN 0141-1136, https://doi.org/10.1016/j.marenvres.2014.09.007.

Wai, T. C., Williams, G. A. (2006). Monitoring spatio-temporal variation in molluscan grazing pressure in seasonal, tropical rock pools. Mar Biol 149, 1139–1147. https://doi.org/10.1007/s00227-006-0291-7

Annexes

Annex A: Coordinates of locations inside and outside Marine Protected Areas.

Table A1 - Correspondent coordinates of the sampled locations outside Marine Protected Areas.

Location	Coordinates
Santa Clara (SC)	37°43'57.8"N 25°41'01.1"W
ETAR Ponta Delgada (ET)	37°44'31.2"N 25°38'56.8"W
Lagoa (LA)	37°44'32.9"N 25°35'15.6"W
Milicias Beach (MB)	37°44'58.8"N 25°37'22.1"W

Table A2 - Correspondent coordinates of the sampled locations inside Marine Protected Areas.

Location	Coordinates
Água de Pau (AP)	37°42'57.3"N 25°29'12.3"W
Cerco (CE)	37°42'27.5"N 25°30'31.5"W
Ferraria (FE)	37°51'31.7"N 25°51'10.5"W
Mosteiros (MO)	37°54'00.8"N 25°49'07.2"W

Annex B: Summary of total number of invertebrates (N) and richness (S) in the native and invasive macroalgae inMPA and outMPA.

Table B1 - Total abundance of each macrofauna taxon collected in each algae sp. inside the MPA.

Taxa	***H. scoparia***	***R.okamurae***	**Total**
Amphipoda	4233	6634	10867
Annelida	4268	2864	7132
Arachnida	170	104	274
Asteroidea	0	3	3
Bivalvia	61	60	121
Caprellidae	200	502	702
Chironomidae	132	65	197
Copepoda	506	627	1133
Decapoda	0	2	2
Echinoidea	19	13	32
Gastropoda	5344	4145	9489
Isopoda	326	762	1088
Ophiuroidea	114	409	523
Polyplacophora	0	3	3
Pycnogonida	25	13	38
Sipuncula	92	73	165
Tanaidacea	362	160	522
N Total	15852	16439	32291
S Total	14	17	

Table B2 - Total abundance of each macrofauna taxon collected in each algae sp. outside MPA.

Taxa	***H. scoparia***	***R.okamurae***	**Total**
Amphipoda	6010	6095	12105
Annelida	3409	1708	5117
Arachnida	103	22	125
Asteroidea	6	7	13
Bivalvia	156	115	271
Caprellidae	2849	1195	4044
Chironomidae	152	89	241
Copepoda	357	328	685
Decapoda	0	3	3
Echinoidea	57	51	108
Gastropoda	8557	3885	12442
Isopoda	1250	645	1895
Ophiuroidea	108	282	390
Polyplacophora	6	14	20
Pycnogonida	47	19	66
Sipuncula	63	74	137
Tanaidacea	342	147	489
N Total	23472	14679	38151
S Total	16	17	

Annex C: List of taxa identified in *R. okamurae* and *H. scoparia*.

Table C1 – List of taxonomic groups identified in the eight sampled locations in the algae *R. okamurae* and *H. scoparia*.

	Água de Pau		Cerco		ETAR		Ferraria	
Taxonomic Groups	*H. scoparia*	*R. okamurae*	*H. scoparia*	*R. okamurae*	*H. scoparia*	*R. okamurae*	*H. scoparia*	*R. okamurae*
Amphipoda	1561	1790	1723	1450	386	940	462	1249
Annelida	1511	887	696	447	1223	357	539	276
Aracnida	31	7	33	7	15	5	15	7
Asteroidea	0	0	0	0	5	7	0	3
Bivalvia	48	31	3	4	29	29	4	5
Caprellidae	170	373	14	96	44	49	9	12
Chironomidae	67	26	28	7	115	24	4	2
Copepoda	192	101	37	75	104	52	81	164
Decapoda	0	1	0	0	0	1	0	0
Echinoidea	19	11	0	1	8	2	0	1
Gastropoda	1886	721	356	247	5666	1398	762	245
Isopoda	110	241	5	56	861	270	22	172
Ophiuroidea	14	29	0	11	41	81	11	40
Polyplacophora	0	0	0	1	0	2	0	2
Pycnogonida	19	7	2	1	15	5	1	2
Sipuncula	22	13	33	9	11	9	12	38
Tanaidacea	294	115	13	9	126	20	25	13

	Milicias Beach		Mosteiros		Lagoa		Santa Clara	
Taxonomic Groups	*H. scoparia*	*R. okamurae*	*H. scoparia*	*R. okamurae*	*H. scoparia*	*R. okamurae*	*H. scoparia*	*R. okamurae*
Amphipoda	3837	1772	487	2145	494	1854	1293	1529
Annelida	1116	484	1522	1254	256	541	814	326
Aracnida	66	7	91	83	5	6	5	0
Asteroidea	1	0	0	0	0	0	0	0
Bivalvia	124	68	6	20	1	10	2	8
Caprellidae	2778	613	7	21	2	501	25	32
Chironomidae	35	16	33	30	0	49	2	0
Copepoda	180	81	196	287	1	153	72	42
Decapoda	0	1	0	1	0	0	0	1
Echinoidea	49	40	0	0	0	9	0	0
Gastropoda	2159	1080	2340	2932	107	1111	625	296
Isopoda	329	222	189	293	27	134	33	19
Ophiuroidea	50	63	89	329	1	92	16	46
Polyplacophora	0	4	0	0	2	2	4	6
Pycnogonida	32	7	3	3	0	3	0	4
Sipuncula	12	4	25	13	30	22	10	39
Tanaidacea	87	36	30	23	13	48	116	43