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LOSS OF BIODIVERSITY IN SEA URCHIN PATCHES IN THE RIA FORMOSA, DUE TO THE INVASIVE BEHAVIOUR OF *CAULERPA*?

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Loss of Biodiversity in Sea Urchin patches in the Ria Formosa, due to the invasive behaviour of *Caulerpa*?

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Declaration of authorship of work

I declare I am the author of this work, titled “Loss of Biodiversity in Sea Urchin patches in the Ria Formosa, due to the invasive behaviour of *Caulerpa*?”, which is original and unpublished. The sources consulted have been duly cited in the text and included in the list of references.

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Resumo

Keywords: Hotspots de biodiversidade, complexidade comunitária, mergulho científico, impacto de espécies invasoras

A Ria Formosa é um sistema lagunar pouco profundo e com várias barreiras, localizado ao longo da costa sul de Portugal, entre Loulé e Vila Real de Santo António, é amplamente reconhecida como uma zona húmida de importância ecológica e de elevado valor económico sendo classificada como parque natural desde 1987. A lagoa, funciona como um berçário crucial para um conjunto diversificado de espécies marinhas, incluindo peixes, crustáceos e moluscos. Embora seja reconhecida a sua importância para a biodiversidade local e para os serviços ecossistémicos, certos habitats dentro da lagoa permanecem pouco estudados, como é o caso das manchas de biodiversidade dominadas por ouriços-do-mar. Encontradas em fundos arenosos da lagoa e próximo de zonas com ervas marinhas e macroalgas, estas manchas contêm uma grande variedade de espécies e são marcadas por uma concentração de ouriços-do-mar e, por isso, são designadas por manchas de ouriços-do-mar. No entanto, este habitat enfrenta ameaças crescentes devido à invasão de espécies não nativas, como a *Caulerpa prolifera*, uma alga invasora de rápida disseminação que representa um grande desafio para a conservação da biodiversidade e a estabilidade do ecossistema na região.

De modo a investigar o impacto da invasão de *Caulerpa prolifera* na biodiversidade da lagoa, foi realizado um intenso trabalho de campo, que incluiu várias campanhas de mergulho para recolher amostras biológicas, e posteriormente a análise das mesmas, com o intuito de avaliar a a composição e distribuição da fauna e da flora em dois tipos de habitat: manchas dominadas por ouriços-do-mar e aquelas invadidas por *Caulerpa prolifera*. Os resultados deste estudo demonstram que as manchas invadidas por *Caulerpa prolifera* suportam um conjunto de espécies distinto em comparação com as áreas nativas dominadas por ouriços-do-mar. Especificamente, durante o estudo a fauna amostrada nestas áreas incluiu quatro espécies de Annelida, que foram encontradas predominantemente nas manchas invadidas por *Caulerpa prolifera*. Relativamente às populações de artrópodes, foi observado o oposto, eram mais abundantes nas manchas de ouriços-do-mar, com uma ausência notável nas áreas invadidas por *Caulerpa prolifera*. Os artrópodes, incluindo uma variedade de crustáceos, são membros importantes do ecossistema e a sua presença reduzida nas zonas invadidas pode indicar alterações significativas nas interações tróficas.

Os cordados, incluindo peixes e ascídias, foram outro grupo predominantemente encontrado nas manchas de ouriços-do-mar. Os moluscos, representando várias ordens, distribuíram-se uniformemente ambas as manchas, dominadas por ouriços-do-mar e pelas manchas invadidas por *Caulerpa prolifera*, embora a sua distribuição não tenha seguido qualquer padrão distinto. Por fim, os equinodermes, principalmente os próprios ouriços-do-mar, também estavam em grande parte restritos às manchas de ouriços-do-mar, com apenas uma exceção registada nas áreas invadidas por *Caulerpa prolifera*, salientando a potencial exclusão destas espécies-chave dos habitats invadidos.

Para além da alteração da composição faunística, o estudo revelou outro aspeto preocupante da transformação do habitat. A análise da flora mostrou que a ameaça representada pelas espécies invasoras se estendia para além da *Caulerpa prolifera*. Nas manchas que se esperava que fossem dominadas por espécies de algas nativas, como *Ulva sp.* e *Codium sp.*, a alga invasora *Rugulopteryx okamurae* foi, em vez disso, dominante. Esta sobreposição de múltiplas espécies invasoras complica a avaliação das alterações da biodiversidade, uma vez que se torna cada vez mais difícil atribuir as alterações do ecossistema aos efeitos de um único invasor. A invasão simultânea de *Caulerpa prolifera* e *Rugulopteryx okamurae* cria um desafio complexo para os esforços de conservação, uma vez que as interações entre estas espécies invasoras e o seu impacto coletivo no ecossistema nativo continuam a ser mal compreendidas.

Os resultados deste estudo sublinham o impacto ecológico significativo que espécies invasoras como a *Caulerpa prolifera* podem ter nos habitats marinhos. Ao alterarem a composição e a abundância da flora e da fauna, as espécies invasoras não só reduzem a biodiversidade como também perturbam potencialmente o funcionamento do ecossistema e os serviços prestados pela lagoa. A presença de múltiplas espécies invasoras acrescenta uma camada adicional de complexidade a estes impactos, complicando ainda mais as estratégias de conservação e gestão destinadas a proteger a biodiversidade nativa.

Um dos maiores desafios apontados por este estudo é a falta de dados de referência para a Ria Formosa, particularmente no que respeita à distribuição e composição histórica das manchas de ouriços-do-mar antes da chegada das espécies invasoras. Esta lacuna no conhecimento torna difícil compreender plenamente as consequências a longo prazo destas invasões e a resiliência do ecossistema nativo a tais perturbações. Os resultados sublinham a necessidade urgente de uma monitorização e investigação contínuas, não só para documentar as alterações em curso, mas também para considerar a inclusão de estratégias de gestão.

Para investigações futuras, seria ideal incorporar estratégias de amostragem espacial e temporal de modo a incluir na análise alterações sazonais na dinâmica do ecossistema e os factores abióticos, como as flutuações de temperatura, a disponibilidade de nutrientes e as forças hidrodinâmicas, que podem influenciar a estrutura e a função das manchas de ouriços-do-mar. A compreensão da forma como estes factores interagem com a invasão de espécies como *Caulerpa prolifera* e *Rugulopteryx okamurae* é crucial para o desenvolvimento de estratégias abrangentes de conservação e gestão. Além disso, esta investigação aponta para a importância de compreender os efeitos ecológicos mais alargados das espécies invasoras. Ao colmatar estas lacunas de conhecimento, estudos futuros poderão contribuir para a gestão sustentável da Ria Formosa e para a preservação da sua biodiversidade única no parque natural.

Abstract

Keywords: Biodiversity hotspots, community complexity, scientific diving, impact of invasive species

Ria Formosa, a shallow, multi-barrier lagoon system located along Portugal's southern coast between Loulé and Vila Real de Santo António, is recognized as an ecologically significant wetland with substantial economic value. Protected as a natural park since 1987, the lagoon serves as a vital nursery ground for various species of fish, crustaceans, and molluscs. Despite its importance, certain habitats within the lagoon, particularly with high biodiversity, known as sea urchin patches, have remained understudied. Spread in the less vegetated bottoms, spatially restricted patches of seagrass can be found, marked by the dominance of sea urchins and are here referred to as sea urchin patches. However, this habitat is increasingly threatened by the invasion of non-native species, such as *Caulerpa prolifera*, which has become an emerging concern for biodiversity conservation and ecosystem functioning in the region.

Extensive fieldwork was conducted, including diving campaigns and sample analysis, to access the changes in fauna composition and distribution in the sea urchin patches dominated by sea urchins and those invaded by *Caulerpa prolifera*. The results revealed that *C. prolifera* invaded patches support a distinct assemblage of species compared to sea urchin-dominated areas. The sampled fauna included Annelida species, which were predominantly found in *C. prolifera* invaded patches. In contrast, arthropods were more frequently encountered in sea urchin patches, with a notable absence in *C. prolifera* invaded areas. Chordates were primarily present in sea urchin patches. Molluscs, including various orders, were distributed across all patches without a clear pattern. Echinoderms were also predominantly found in sea urchin patches, with only a one exception in *C. prolifera* invaded areas. The flora samples revealed that not only the patches dominated by *C. prolifera* are affected by an invasion. Unexpectedly, the remaining patches mainly presented an invasive alga, the recently described *Rugulopteryx okamurae*, instead of the expected native algae species *Ulva sp.* and *Codium sp*. This complicates the evaluation of biodiversity changes due to multiple overlapping invasions and highlights the lack of data for this specific habitat of the Ria Formosa lagoon. The analyses indicate the significant ecological impact of *Caulerpa prolifera* invasion that alters the species composition and abundance in marine habitats, possibly affecting the diversity and distribution of organisms. The observed alteration in flora and fauna highlights the ecological

impact of invasive species on local biodiversity and the need for further research on this habitat. Moreover, the simultaneous presence of multiple invasive species complicates efforts to manage and protect the native biodiversity of this ecosystem.

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1. Introduction

1.1. Context of the work

The Ria Formosa, situated in Faro, Portugal, is a lagoon system inhabited by a diverse wildlife. Within this dynamic environment, seagrass and macroalgae serve as pivotal elements, hosting a variety of fish and invertebrates. This intricate network creates a multi-tiered food web where higher-level predators feed on organisms from the lower trophic levels, shaping interconnected communities within what is commonly referred to as an ecosystem (Hanley & La Pierre. 2015) Seagrass meadows form a base for a well-diversified community and represent one of the most productive types of coastal vegetation, playing a crucial role in sediment stabilization and enrichment (Duarte & Chiscano. 1999; Duarte, 2000) and are frequently found on muddy and sandy substrate (Cabaço *et al.* 2010). A diverse range of different taxa and species is presented in the seagrass beds (Parreira *et al.* 2021). The seagrasses are found both in meadows and smaller accumulations within spatially restricted patches (Guimarães *et al.* 2012). According to the team's experience and knowledge of previous years, there are also spatially restricted patches found in the lagoon with accumulation of shells and macroalgae where an expressive presence of sea-urchins and other macrobenthic invertebrates such as ascidians and nudibranchs occurs (Correia. 2015; Fragkopoulou. 2016; Hoett. 2019; Reis. 2018). These macroinvertebrates have dominated the reported patches and are, throughout this report, referred to as sea urchin patches. The aim of this study is to assess the biodiversity of the sea urchin patches and to gain a better understanding of their dynamic and the influence of the Caulerpa invasion, a macroalgae that is spreading by shallow bottoms of Ria Formosa.

1.2. Contribution to Science

During a series of diving campaigns, extensive data was collected from the sea urchin patches, providing raw information on sediment, macrofauna and macroflora. By analysing the data, a comprehensive depiction of the lagoonal biodiversity of this special habitat can be presented. The exploration of the structure and ecological relationships may uncover previously unknown or poorly understood aspects, particularly within the sea urchin patches. Gaining a deeper understanding of this environment is crucial for effective management and preservation, especially with in an increasing presence of an invasive species *Caulerpa prolifera*. Additionally, the thesis is part of the MaréFormosa project, focused on Tide propagation and energy dissipation in the Ria Formosa Coastal lagoon system (Portugal), hosted at CIMA, University of Algarve, contributing to the program's overarching goals.

1.3. Organisation and structure

For an enhanced understanding of the structure, a visual representation of the organization of the work is presented (Figure 1). The visual organization is a guideline for the following report, in which a detailed explanation of each step, the tasks involved, and how the various steps and resulting data are interconnected, is provided.

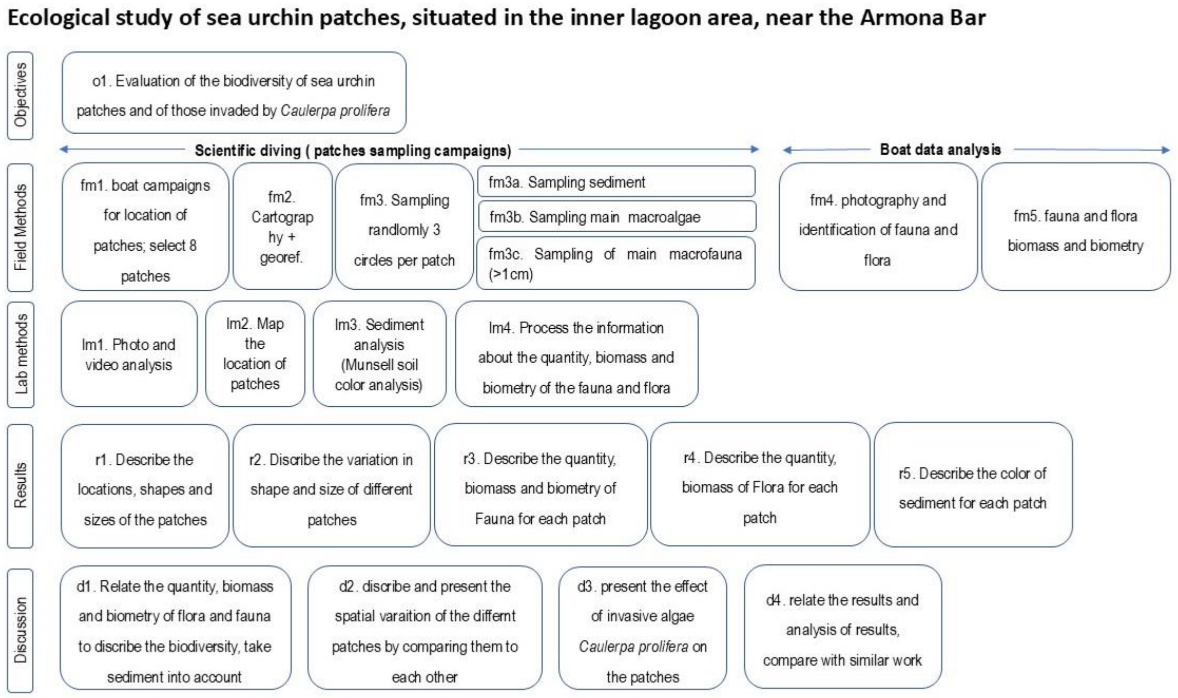

Figure 1: Schematic overview of the thesis organisation. The relation of each step and the required organization is explained throughout the report.

1.4. Objectives and scope

The primary objective of this study is to evaluate the biodiversity within the sea urchin patches located near the Armona inlet in Ria Formosa (Figure 1) and infer the effect of invasive macroalgae. To comprehensively achieve this goal, the observations were extended spatially to gather information for assessing the impacts of invasive species, such as *Caulerpa prolifera*. To accomplish this scope, several steps were taken to define the composition of the identified patches. In the field campaigns, ten selected patches near the Armona inlet were analysed in detail. These areas were monitored through dive campaigns aimed at observing and documenting the biodiversity. Key parameters, including the shape and size of the patches, were measured during each campaign, and variations in these aspects contribute to the overall description. For the flora, the biomass was quantified and for the fauna the biomass and the biometry of the larger specimens were estimated, together with a quantification of the density of all invertebrate specimens larger than one centimetre. Additionally, the sediment underneath each patch was extracted during each observation, for colour and grain size analysis. These systematic steps are crucial for an evaluation and detailed description of sea urchin patches in Ria Formosa. Beyond that, the study aims to enhance the understanding of the invasion due to *Caulerpa prolifera* associated with these patches.

1.5. Limitation of the project

The project followed a diving schedule to systematically collect all essential information required for the study during the diving campaigns. Initially the campaigns were planned to start in spring and continue into summer. A two-month delay of the campaigns postponed the project unexpectedly, due to a long wait for the licence and permission to conduct scientific dives in the natural park Ria Formosa. To accommodate the project's time constraints, these campaigns were rescheduled for May and June. This schedule is influenced by the anticipation of very low biodiversity activity during winter, when compared to the summer months.

When accessing biodiversity, only the range or number of different species for macroflora and macrofauna were taken into consideration. The research area of this work was already well defined and limited to the sea urchin patches. Therefore, considering the diversity of different habitats to access biodiversity is, for this work, negligible. Research on a genetic level to access genetic diversity, giving a deepening inside on the biodiversity, was, due to limitation in time and financial reason, not possible.

In the initial observation campaign, ten patches near the Armona inlet, suitable for further analysis, were identified. Each diving campaign involved the placement of three circular plastic rings, with a 30-centimeter diameter, randomly on each patch. Organisms larger than one centimetre were considered during the sampling process, while smaller organisms were not counted and measured individually but their number was estimated whenever found while sampling. Since the organisms were re-released after sampling, time was limited. Sediment analyses was restricted to granulometry analysis, due to practical and financial reasons.

2. State of the Art

2.1. Ria Formosa

Ria Formosa, a shallow, multi barrier lagoonal system situated along the southern coast of Portugal between Loulé, Faro, Olhão, Tavira and Vila Real de Santo António municipalities, exhibits distinctive hydrodynamical, biological and ecological features (Cabaço *et al.* 2010; Erzini *et al.* 2022; Falcão & Vale 1990; Gamito 1998; Lock & Mees 1999). The bottom shows grain sediment size, that ranges from muddy sediments in the inner areas to coarse sand in the inlet channels (Falcão & Vale. 1990). In some areas the bottom composition is altered due to bivalve farming. Sand is transported from the beaches to the clam growth banks, situated on the intertidal flats within the lagoon, for an efficient farming ground (Falcão & Vale. 1990). There is no significant freshwater input to the lagoon, and therefore the salinity remains around 36 ppt throughout the year, except during occasional short runoff periods (Ferreira *et al.* 2012). The same authors state that the water temperature fluctuates annually between 12 to 26 °C. The dissolved oxygen in the water column consistently stays close to or above saturation levels, and there is no development of an anoxic water layer (Falcão & Vale. 1990). Notably, the semidiurnal tidal show a significant variation, ranging from 0.5m during neap tides to 3.5m during spring tides (Falcão & Vale. 1990; Lock & Mees. 1999) The intense water exchange, ranging from 50-70% between tides, results in continuous water mass movements (Pacheco *et al.* 2010). During high tides, an area of 14.1 to 63.1 km2 is submerged (Lock & Mees. 1999; Pacheco *et al.* 2010). Recognized as a significant natural wetland with substantial economic value, it has held the status of a natural park since 1987 and is designated as a Ramsar and Natura 2000 site (Newton *et al.* 2003). This dynamic ecosystem, approximately 60 kilometer in length, serves as a crucial nursery ground for various fish, crustacean, and mollusks species (Falcão & Vale. 1990). However, the natural park faces human exploitation due to extensive fisheries and aquaculture, salt extraction, intensive agriculture, and coastal engineering (Gamito *et al.* 2003).

2.2. Sea grass meadows forming sea urchin patches

Seagrass meadows are among the most productive coastal vegetation types, playing a vital role in sediment stabilization and enrichment. They support a complex detritus-based food chain, serving as breeding and nursery grounds for finfish and shellfish, while providing a crucial food resource and habitat for diverse marine organisms as well (Duarte & Chiscano.

1999; Duarte. 2000). The increased biological diversity observed within seagrass patches stems from their importance as habitats for species that rely on seagrass and benefit from the abundant detritus they produce, serving as food sources (Guimarães *et al.* 2012; Duarte. 2000). In Portugal, the Ria Formosa lagoon system houses one of the most important populations of seagrass, amongst the native seagrass species in the lagoon system are *Cymodocea nodosa* and *Zostera marina* (Parreira *et al.* 2021). Additionally, *Zostera noltii* is describe as a commonly spread sea grass species in the lagoon, predominantly in the low intertidal zone (Guimarães *et al.* 2012). Spread in the less vegetated, bare bottom, spatially restricted patches can be found, surrounding and interweaving accumulation of shells which, according to the team's experience of previous years, indicate the presence of a wide range of species and have previously been introduced as sea urchin patches. Over time the sea urchin patches could experience a shift in flora, being Ulva sp. the preferred food source and hence is trapped by the sea urchins (Vital. 2022; Prato et al. 2018). Additionally, the epifauna in these patches likely includes three organisms (Correia. 2015; Cotrim et al. 2021; Fragkopoulou. 2016; Hoett. 2019; Reis. 2018; Rothe. 2017; Schmid. 2019): sea urchins, ascidians, and nudibranchs, among others.

Sea urchins are the most found metazoan in the small spatially restricted patches, for this reason these patches are labelled "sea urchin patches" (Fragkopoulou. 2016). Herbivorous sea urchins play a crucial role in the overall functioning of ecosystems, often acting as the primary determinant of the abundance and distribution of photoactive organisms, especially seagrass, in shallow-water marine environments (Boudouresque & Verlaque. 2013). At moderate population densities, sea urchins can influence plant species composition and enhance species diversity through selective feeding. Their grazing behaviour has the potential to reshape the structure and dynamics of benthic communities by eliminating certain algae species due to extensive feeding, leading to an overall shift of the ecology structure (Hereu *et al.* 2012). Conversely, a high abundance can result in the formation of "sea urchin barren grounds" (Clemente & Hernández. 2008). Their preferred habitat predominantly consists of solid rocks, boulders (Boudouresque & Verlaque. 2013). This phenomenon could be attributed to the unsuitability of the sandy bottom between *C. nodosa* shoots for locomotion or the influence of high predation pressure. Conversely, *Paracentrotus lividus* is rarely found on sandy bottoms (Boudouresque & Verlaque. 2013; Clemente & Hernández. 2008). In such environments, *P. lividus* individuals tend to cluster on isolated stones, large shells, and various debris

(Boudouresque & Verlaque. 2013; Clemente & Hernández. 2008). Notably, the absence or limited presence of *P. lividus* in meadows of *C. nodosa* seagrass is of interest, considering that this seagrass is a preferred food source (Boudouresque & Verlaque. 2013). Controversy, sea urchins were found in Ria Formosa in such habitats, forming what we have denominated the sea urchin patches (Fragkopoulou. 2016; Reis. 2018; Rothe. 2017). Sea urchin patches in Ria Formosa are reported to host following species: *Paracentrotus lividus, Sphaerichinus granularis and Psammechinus miliaris* (Ruiz. 2015).

Ascidians, or commonly known as sea squirt, include approximately 3000 described species (Shenkar & Swalla. 2011). According to these authors, despite its sessile adult form, ascidians have a broad geographical distribution, ranging from polar and temperate to tropical environments, in both shallow and deeper habitats. These filter feeders are invertebrate chordates belonging to the earliest branch of in the phylum of the chordates, a subphylum called tunicates, which the ascidians belong to (Corbo *et al.* 2001). The subphylum name "tunicates" derives from their cellulose- like polysaccharide exoskeleton or tunic (Matos & Antunes. 2021). This tunic can differentiate solitary from colonial individuals (Matos & Antunes. 2021; Holland. 2016). These author state that, solidary species possess their own tunic, whereas colonial organisms can share theirs. Colonial ones must not share their tunic, but can be attached at the base to each other, without any further contact. Several studies proof the long-term presence of ascidians in Ria Formosa, but they were not the focus of any of the studies, limiting specific information about the ascidians in this lagoonal environment (Gamito. 2008; Pack *et al.* 2015; Sprung. 1994; Vasconcelos *et al.* 2007).

Nudibranchs, often referred to as sea slugs, are a marine group of gastropods. Their name translates to 'naked gills' and indicates the exposure of their gills on the back, due to the lack of an external shell (Dean & Prinsep. 2017). The same authors state that these gastropods play an important role as carnivores in the ecosystem since they mainly feed upon sessile organisms like sponges, cnidarians, tunicates and bryozoans and utilize a chemical defence system to defeat predator, due to the lack of a protective shell. The documented diversity within the taxa of nudibranch counts almost 5000 different species (Dean & Prinsep. 2017). While they are distributed across the world's oceans at different depths, including tidal pools and coral reefs, they are particularly abundant and diverse in shallow tropical waters (Wägele & Klussmann-Kolb. 2005; Valdés. 2004). Nudibranchs play significant roles in benthic marine ecosystems, often observed grazing on corals, sponges, and macroalgae or moving across rocks and various substrates (Valdés. 2004; Behrens & Valdés. 2004). In similar research campaigns to this one in previous years, a diversity and high abundance of nudibranchs was described in the Ria Formosa and has shown that these gastropods are present in the patches (Hoett 2019).

2.3. Biodiversity and its importance

The term "biodiversity" has its roots in the Greek word "bios," signifying life, and the Latin word "diversitas," indicating variety or difference (Venturelli *et al.* 2023). According to the same author, the "National Forum on Biodiversity" has been using this term, since the early 1980s, to encompass the richness of life, showing diversity within species, among species, and the variety of habitats. A different approach of the concept of biodiversity, adopted by the Convention of Biological diversity sign in 1993 in Rio de Janeiro (https://www.cbd.int/intro), encompasses three distinct levels within the natural system (Alho. 2008; Chivian. 2002):

Diversity of Species: This refers to the variety or range of living species present in an ecosystem.

- Genetic Diversity: It involves the variety of genetic information found in all distinct populations of plants, animals, and microorganisms of the same species. This includes differences between populations. Genetic diversity forms the foundation for ongoing adaptation to changing environmental conditions.
- Ecosystem Diversity: This level relates to the variety of habitats or different structural forms within an ecosystem.

As already state in the limitation of the project, only the diversity of species was considered to access the biodiversity. Since the study focuses on one specific habitat, the sea urchin patches, the number of different habitats is, in this case, negligible for the evaluation of the biodiversity. Furthermore, genetic diversity cannot be accessed, due to a lack of time, technical and financial means. The biodiversity or more specifically the species diversity plays a pivotal role in shaping ecosystem productivity, stability, nutrient dynamics, and tolerance against invasion (Schulze & Mooney. 2012; Tilman *et al.* 2014). Numerous studies across terrestrial, aquatic, and marine ecosystems consistently demonstrate that systems with high diversity exhibit approximately double the productivity compared to monocultures (Tilman *et al.* 2014). The positive impact of increased diversity, according to the same reference, stems from factors such as interspecific complementarity, more efficient use of limiting resources, reduced herbivory and disease incidence, and nutrient-cycling feedback, which contribute to nutrient storage and supply rates overall.

2.4. Issue regarding *Caulerpa prolifera* invasion

The genus *Caulerpa* has a circum-tropical to warm water geological distribution (Zubia *et al.* 2020). Due to the extension of the natural habitat impacted by some *Caulerpa* species, the invasive species attracted much more attention (Zubia *et al.* 2020). This behaviour can be observed in the Mediterranean Sea, where *Caulerpa racemosa var. cylindracea* and *Caulerpa taxifolia* were the cause of major ecological disturbances (Meinesz *et al.* 2001). Australia and California, western USA, reported similar invasive interferences (York *et al.* 2006; Anderson. 2005). The occurring issue, caused by the invasion, is the highly competitive characteristic of some *Caulerpa* species with native seagrass and macro algae species (Pérez-Ruzafa *et al.* 2012; Piazzi *et al.* 2005), which can lead to significant changes in the sediment properties (Piazzi *et al.* 2005; Pérez-Ruzafa *et al.* 1991). The more severe impact of the invasive spreading occurs, when *Caulerpa sp*. overcomes the native seagrass species and conquers their habitat. Seagrass meadows are rated as highly valuable ecosystems, due to their great biodiversity and ecological services, which is provided by them (Fourqurean *et al.* 2012; Duffy *et al.* 2019). The loss of this diversity and services can, according to the same authors, present a serious issue. Research on the invasion in the Atlantic has been done and shows similar, negative impacts on the biodiversity, as seen in the Mediterranean Sea and other costal systems (Meinesz *et al.* 2001). The problem of invasion applies to the Ria Formosa. The green seaweed spreads unpredictably since 2015 in the lagoon, but the extent of the impact remains uncertain (Vital. 2022). This issue needs to be evaluated if it includes a loss of biodiversity and ecological services. Furthermore, the change in biodiversity towards a *Caulerpa prolifera* dominated environment can influence the composition of sediment, since anoxic conditions are created in the sediment (Duffy *et al.* 2019). Anoxic conditions can lead to an increased accumulation of sulphides and herbivory deterrent metabolites (Duffy *et al.* 2019). The same author states, that this results in a change of composition of the detritus and restricts the recirculation of the biomass to the food chain. The exact impacts and consequences of biodiversity alternations associated to the *Caulerpa prolifera* expansion in the Ria Formosa for commercial species remain largely unknown.

2.5. Commercial use of Ria Formosa

Traditional aquaculture in Portugal involves, among other practices, the extensive cultivation in specially constructed ponds or water reservoirs within Salinas (Gamito. 1998; Ferreira *et al.* 2012). Among the crucial activities, shellfish and finfish aquaculture stand out prominently

(Ferreira *et al.* 2012). According to the same author, most producers primarily focus on cultivating shellfish, in 2001, there were 1245 leases, with 1224 dedicated to bivalves. By 2010, the number had slightly decreased to 1122, indicating a consistent pattern over the years (Ferreira *et al.* 2012). The average bivalve production was around 2500 tons and average fish production was exceeding 2800 tons in 2010. (Ferreira *et al.* 2012). The productivity of this lagoon system relies on existing benthic populations, as nearly all commercially valuable fish species feed on these organisms (Gamito. 1998). To increase the production of fish of commercial value, it is a common practice to restock the earthen ponds with juveniles caught in the Ria Formosa (Gamito. 1998), presenting the importance of Ria Formosa for the fishing industry in Portugal. The addressed seagrass meadows in Ria Formosa create conditions for aquaculture farming, including natural geomorphology, climate, and minimal industrial and urban pollution (Carvalho *et al.* 2006; Falcão & Vale, 1990). As a result of the conditions around 10 km^2 of the lagoon's intertidal flats are utilized for clam cultivation, primarily of *Ruditapes decussatus* (Carvalho *et al.* 2006; Falcão & Vale, 1990). Ria Formosa is used for more than 1500 cultivation concessions with this species, contributing to an annual estimated production of up to 7000 tons (Carvalho *et al.* 2006). The establishment of bivalve aquaculture has resolved in clearing *Z. noltii* meadows, and the transport of sand from local beaches (Falcão & Vale, 1990). The economic value attributed to Ria Formosa relies on the presence of flora and the necessary nutrients. To sustain these conditions and characteristics of Ria Formosa, a well-balanced and rich biodiversity is essential, as elaborated earlier. Preserving the lagoonal environment, and consequently the biodiversity, is most important not only for the sake of natural environmental protection but also for the sustenance of fish and bivalve production.

This literature review highlights the existing gap of research regarding the sea urchin patches. Notably, a lack of information about certain species in a specific environment, such as Ria Formosa, is most evident regarding the sea urchins and its role in the ecology of the lagoon. While extensive information exists about some individual species and biodiversity within these communities, the study of the sea urchin patches showcases a very limited understanding. The current extent of invasion remains unknown, hindering an understanding of its impact on biodiversity. Information about the biodiversity in Ria Formosa is essential to enable commercial and economic utilization in the future, as a balanced ecosystem relies on a stable and balanced biodiversity within it. Furthermore, effective management and preservation of the lagoonal system depend on an accurate assessment of the biodiversity. The listed points justify the necessity of accessing biodiversity in Ria Formosa and its contribution to the scientific research world. Since examining the entire biodiversity of a complex system like Ria Formosa requires an approach, that is separated in multiple steps, this study focuses on one specific part of the lagoonal system. The goal is to contribute to the overall understanding of biodiversity in Ria Formosa, by adding knowledge about the biodiversity of the sea urchin patches.

3. Methodology

Data collection was divided into several diving campaigns. During the dives, the sampling and measurements were taken place, while a part of the analysis was conducted on a boat. This approach allows for an immediate release of the sampled organisms back into the lagoon once all the required measures were completed. Any tasks that do not involve the analysis of living organisms were carried out in the laboratory. The diving team for the fieldwork included Boat skipper Alexandra Pereira (UAlg), Supervisor Duarte Duarte (CIMA, Certification: CMAS M2 and Scientific Diving instructor), Duarte Fortunato assisting diver (Certification: Advanced Open Water Diver, Marine and Coastal System Student, a78722) and myself, Tim Hartmann (Certification: Advanced Open Water Diver, Marine and Coastal Systems student). On board Supervisor Sofia Gamito (CCMAR), Inês Ribeiro (a77696) and Alexandra Haslett (a78755) assisted with the sample processing and analysis.

3.1. Survey Site

The region designated for researching biodiversity within sea urchin patches is situated in the Ria Formosa lagoon system, specifically in the proximity to the Armona Inlet (Figure 2).

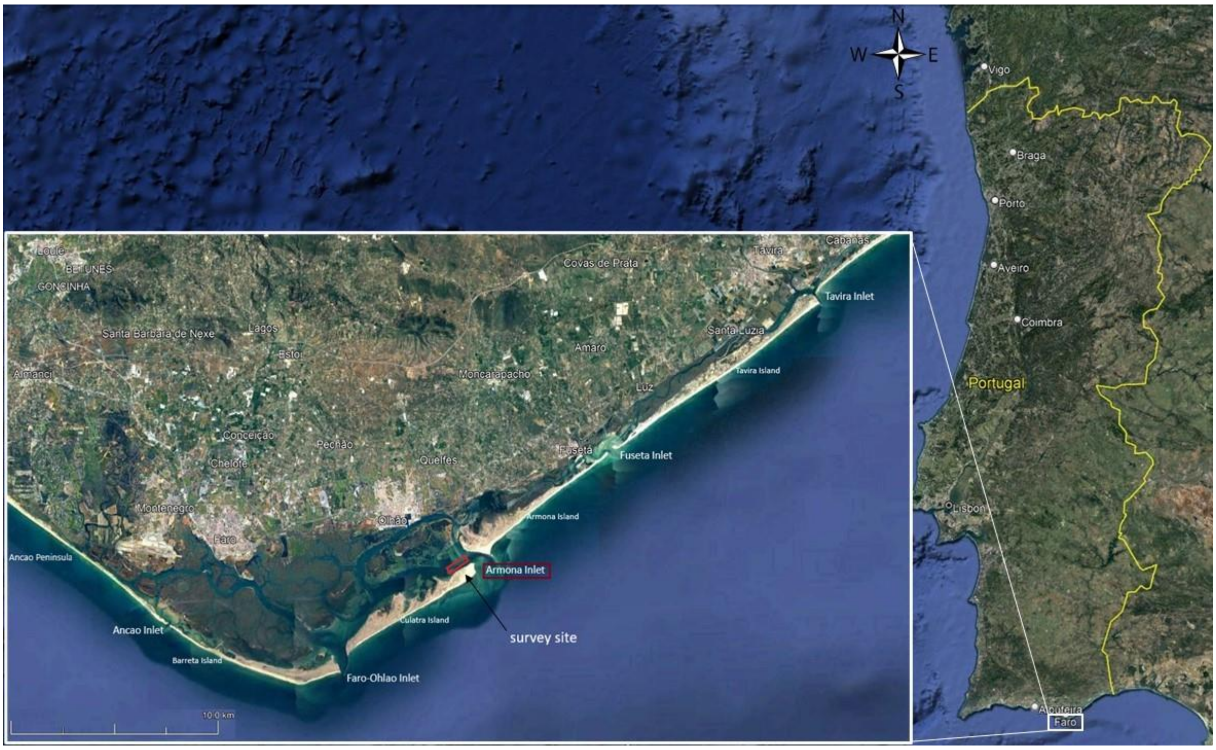

Figure 2: An overview of Portugal and Faro, located in the south, is presented. The Ria Formosa area is enhanced in the left bottom side. The different barrier islands and the inlets are labelled. In red the Armona Inlet is highlighted and nearby the red rectangle marks the study area. (Image courtesy of Google Earth Pro; Google Earth Pro Version 10.40.0.2, Portugal, 7°54'W, 37°N, 2D map, viewed 04 Dezember 2023 (http://www.google.com/earth/index.html)).

3.2. Field Methodology

Fieldwork commenced by first identifying and locating the sea urchin patches. Of these ten patches were selected: three patches affected by *Caulerpa prolifera* invasion and seven others showing a non-invaded condition. Three patches for each state are the minimum required for conducting statistical comparisons between patches within the same state, and a reliable comparison between the two conditions. The sea urchin patches were expected to show a wider range of biodiversity then the patches exhibiting a *Caulerpa prolifera* invasion. Sampling a significant higher amount of sea urchin patches allows to cover the anticipated higher biodiversity of the non-invaded patches. Throughout each campaign, the same parameters were measured to enable a reliable comparison of the gathered data. The dives were scheduled according to the weather and sea conditions, to monitor and sample each patch.

3.2.1. Locating suitable sea urchin patches

The initial step in this process was the identification of the sea urchin patches and their location, the requirement for any further fieldwork. The team met at the harbor of Olhão, to access the Armona bar by boat. This area was assumed to exhibit a high concentration of these patches, based on the team experiences of the previous years. A camera, mounted on a pole, was used from the boat, allowing for an efficient observation of the lagoon bottom and of the epibenthic organisms and a rough estimation of the patch locations. The boat navigated the inner channel near the Armona inlet, cruising along west-easterly routes parallel to the barrier island (Figure 3). During this time, the bottom was scanned with a camera setup, allowing for an effective observation of the bottom in order to identify the most suitable patches.

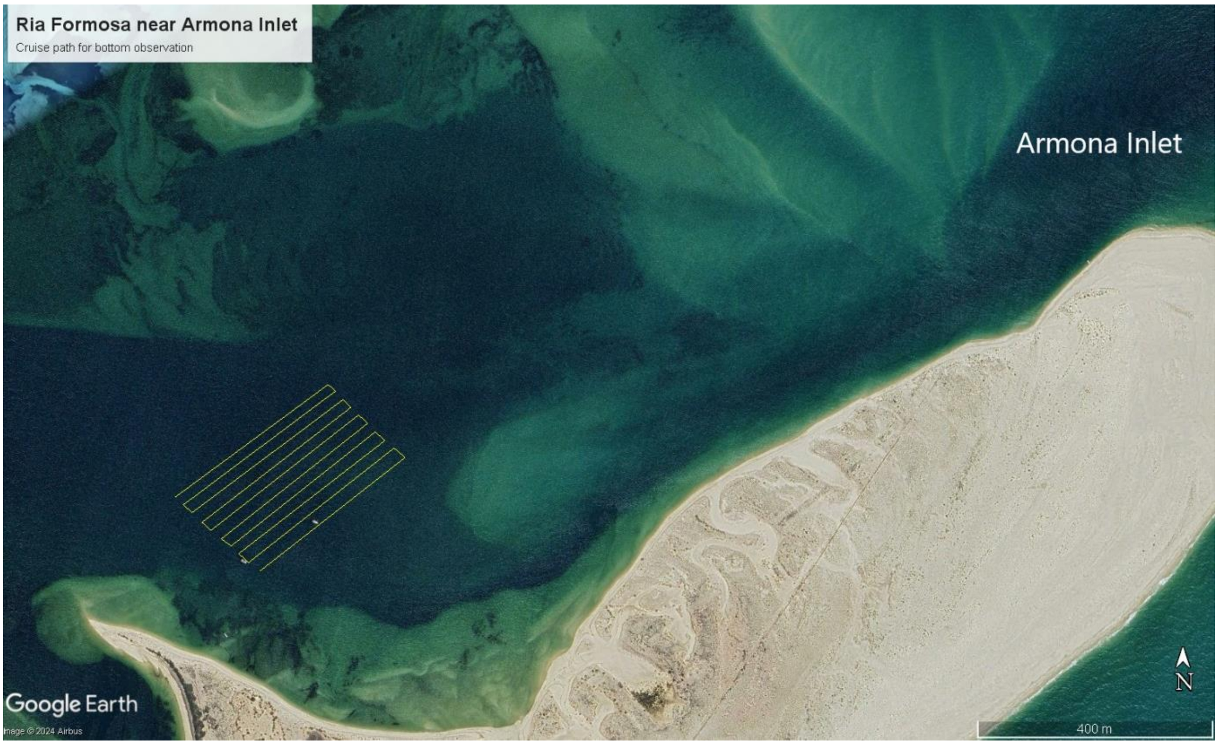

Figure 3: A Section of Ria Formosa, close to the Armona Inlet, is shown. The yellow line represents the route, that was followed by the boat during the first survey. (Image courtesy of Google Earth Pro; Google Earth Pro Version 10.40.0.2, Portugal; 37° 00'11''N, 7°48'40''W (WGS 84)/ 29S 605218m N, 4095984m E (UTM); 2D map; reviewed 10.06. 2024 (http://www.google.com/earth/index.html).

During this time, the bottom was scanned with the camera setup, allowing for effective observation to identify the most suitable patches.

3.2.2. Cartography and georeferencing of the sea urchin patches

After the patch selection, they must be georeferenced, and their sizes mapped. Georeferencing under water enabled cartographical mapping afterwards. During the dives, a metal stick was positioned at the centre of the patch, and labelled with a tag, referred to as a cookie. A surface marker buoy was then attached to the stick, serving for georeferencing at the surface using a DGPS. The precise position of each surface buoy is needed as a reference point for mapping of each patch later. Once the reference point is established, divers attached a measure tape to the reference stick and dived towards the edge of the patch, navigating with the aid of a compass. One diver measured the exact distance of the edge of the patch to the reference point at a certain angle and documented the certain angle using a compass. Proceeding in an anticlockwise direction along the edge, the diver continued measuring distances and exact angles, recording the results. By employing the angle and distance data from each measurement, the area of each patch can be subsequently calculated.

3.2.3. Sampling to access biodiversity

The other two divers were sampling the sediment, flora and fauna, and were documenting each sampling site on pictures. The following step involved assessing the biodiversity of the patches by collecting samples. Three 30-centimeter diameter plastic rings were randomly placed on each patch, and all organisms within these areas were collected. Sediment samples were taken, before the sediment is resuspended, using a modified syringe. Only the first top ten centimetres of sediment were sampled. Before removing any flora and fauna, the areas within the circles were video and photo documented. Following this step, fauna larger than one centimetre and flora was collected and transported to the boat using mash bags. On the boat, the collected flora and fauna was identified and photographed for documentation purposes. The biomass of each species was quantified as well, and biometric data was recorded. The sediment samples were transported in plastic bags for further analysis on the boat and in the laboratory.

3.2.4. On-boat analysis

To ensure an efficient workflow, samples were delivered onto the boat using a transportation box, placed on the bottom near the divers. The attached rope allowed immediate transport of the samples, while the divers remained underwater to continue their diving tasks. On the boat, before sunlight and exposure to air alters the properties of the sediment, the samples were analysed for colour using the Munsell Soil colour charts (Munsell Color, 1994). The pH value was measured with a pH probe. All sampled flora and fauna species were identified. The height, diameter and wet weight of the sea urchins was recorded, and the length of their longer and shorter spines was measured. Height, width and thickness of the remaining larger fauna was recorded. Smaller specimens were identified and counted. Additionally, the macroalgae and seagrass were scaled. All values were noted in a previous prepared table. Throughout the process, fauna, flora and sediment were photographed. The documented photos were numbered and included in the same table.

3.3. Diving organisation and equipment

3.3.1. Preparation of equipment

Preparing and verifying the diving equipment stands as one of the initial tasks on a scientific diving session. Consequently, each diver used a gear box containing all the necessary equipment required for the planned session. To set up the components associated with gas, the process begins with strapping the buoyancy compensator onto the tank by securing two buoyancy compensator straps around it. Subsequently, the tank is lifted with the buoyancy compensator using the shoulder straps to ensure the tightness and stability of the buoyancy compensator straps. Before moving on to the next step, the spare part of the straps is used to fix the needed weight. The tank valve cover and the dust cover of the first stage regulator must be removed to connect the two parts to each other. The inflator hose is then attached to the intake valve of the buoyancy compensator to utilize the compensator for buoyancy control.

The following steps involve positioning the gauge face downward and slowly opening the tank valve, followed by checking the air pressure gauge for the standard pressure of 220bar. Moving forward, the regulator and the octopus (second regulator for gas-sharing scenarios) must be tested by pressing the purge buttons and breathing through them. Prior to entering the water, divers must put on their wetsuits and the team must go through the pre-dive briefing and safety check. Each diver prepares the mask, fins, gloves, boots, and any additional, session-specific equipment (e.g. wet notes, etc.). A shot line must be lowered into the water to mark the starting and meeting point of the diving area for the divers, providing an orientation reference. After fully inflating the buoyancy compensator with gas, the diving team enters the water. Once the entire team is assembled in the water, bubble and valve checks are conducted before the descent begins. Dives require specific gear for the dives, for the tasks during the dives and first aid equipment (Table 1).

Table 1: Diving gear required for the diving campaigns, including the needed equipment for specific tasks and first aid equipment.

Diving Gear			
Standard gear	Cartography	Sampling	First Aid
Mask	Compass	Mash bags	Oxygen tank
Fins	Measure tape	plastic bags	First Aid Kid
Wetsuit	additional weights	plastic circles	
Hood	Wet notes	Video camera	
Gloves	Cookies	Transportation box	
Boots	Metal stick	grid board (for sea horse samples)	
Wing	Buoy + Spool	Syringe for sediment sample	
Regulator			
12L Stell Tank			
Knife			
Flashlight			
Depth gauge			
Manometer			
Weights			
Whistle			
Surface Marker Buoy (SMB)			

3.3.2. Training diving skills

Prior to the open water lagoonal dives in the Ria Formosa, each team member had to participate in pool training sessions. In a controlled environment each diver had to accomplish the following requirements to ensure safe and efficient scientific dives and guarantee the conversation and preservation of the natural habitat:

- Acquiring the proper horizontal trim position to maintain optimal buoyancy and prevent disturbances to the environment (plants, animals, sediment, etc.) (Figure 4).

Figure 4: Schematic presentation of the trim position for most efficient buoyancy control. The body is in a horizontal Position. Ankles and knees are bend in an 90° angle. Arm remain free to perform tasks while diving. (Figure courtesy of Integration and Application Network (ian.umces.edu/media library)).

- Gaining control of the buoyancy using the equipment to its full potential and locomotion with fins to allow weightless dives and prevent disturbances of the environment
- Acquiring effective propulsion techniques to reduce energy consumption and move efficiently.
- Training in underwater and surface emergency procedures.
- Gaining confidence in the utilization of underwater cartographic, sampling and photographing materials to apply confidentiality in

3.3.3. Safety plan

Every member of the diving team had to prepare and train diving skills during pool sessions. The team had to be well-acquainted with the pre-dive safety plan, encompassing details about the dive site location and proximity to the nearby port for emergency services. Mobile phones for emergency communication and safety equipment, including an oxygen unit and a first aid kit, must be available on the boat.

3.4. Laboratory methodology

3.4.1. Mapping and georeferencing the sea urchin patches

The data, obtained throughout the diving campaigns, and its processing involved several steps. Firstly, a map displaying the eight patches had to be generated using the computer program Microsoft Excel. To compute maps of the patches using simple trigonometric calculations is used implementing the measured edge-points from each patch. The coordinates for point A (Figure 5) were determined by putting the distance from the referenced centre in relation with the circular shape of the patch, using the following equations:

$$\frac{1}{\cos(\theta)} = \frac{r}{x} \qquad ; \qquad \frac{1}{\sin(\theta)} = \frac{r}{y}$$

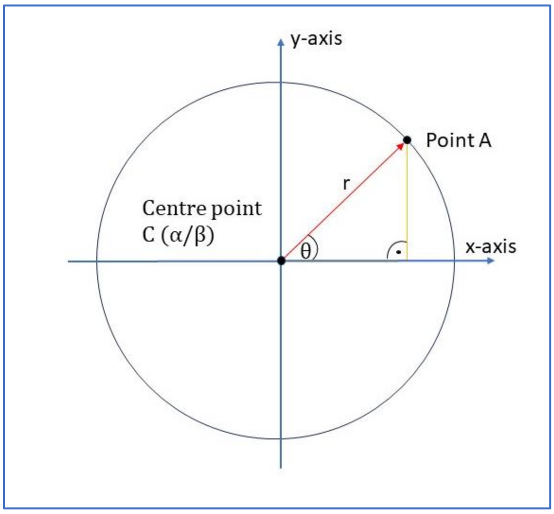

Figure 5: Scheme for visualization of the trigonometric calculations, with Point A (x/y) being a measured point at the edge of the patch and centre point C being the marked and reference centre of a patch. For simplification the patch shape is displayed as a circle.

From here, the coordinates for Point A (x,y) could be calculated using the measured coordinates (α,β) from centre point C, applying the following equation:

$$x = r * \alpha * \cos(\theta + 90) \qquad y = r * \beta * \sin(\theta + 90)$$

Finally, the coordinates, in UTM system, could be plotted in a map, revealing the shape of the patch in relation to the corresponding coordinates. Ultimately, a separate map for each patch was created. Applying the same technique, the location of the three circles within a patch can be obtained. After the calculation the outline of each patch, the coordinates were imported into Google Earth to obtain the covered area (Google Earth Pro. 2022).

3.4.2. Data analysis for biodiversity evaluation

The information collected during the onboard analysis, including data on the quantity and biomass of the sampled fauna and flora from each diving campaign, was collected into Excel tables. The diversity and the density or biomass of the main species within patches were established and possible differences tested. For an accurate description of difference in biodiversity of the patches the Shannon-Wiener Index (H'bits) (Shannon. 1948; Fedor & Zvaríková. 2019) and the Simpson Index (D-1) (Simpson 1949; Somerfield *et al.* 2008; Fedor & Zvaríková. 2019) were estimated. A non-metric multidimensional scaling (MDS) was preformed using the software PRIMER (PRIMER-e Empowering Research. 2024) to extract possible patterns and similarities among patches. The analysis of biodiversity in different habitats was based on the number of different taxa presented in the patches. Potential differences in the distribution of different measurements in the data set were calculated using the Welch two sampled T-test and the Pearson's Chi-squared test in the statistical computing software R (R Core Team. 2024). Using this methodology, the similarities or differences in the composition of the patches can be presented and discussed, and possible disturbances on the biodiversity inferred.

3.4.3. Sediment analysis

In the Laboratory, sediment samples were analysed for grain size using a polydisperse analysis. Particles differing in size or density were dispersed in a viscous fluid and were measured in a Laser Granulometer, more specifically the Malvern Instrument Mastersizer Mirco Version 2.18. This instrument enabled the determination of the grain size distribution within each sample (Basson et al., 2009). For further analysis and graphical representation, the Folk and Ward Method (Folk & Ward, 1957) was applied using an Excel extension developed by Blott (2010).

4. Results

During the diving campaigns ten patches, seven of which were sea urchin patches, were located, georeferenced, measured using underwater cartography and sampled. The remaining three patches were affected by the previous mentioned *Caulerpa prolifera* invasion, that the lagoonal environment is experiencing. All patches showed a well-defined flora layer, that was growing, regardless of the flora type, on muddy substrate. Noticeable, across all patches of both states, was the absence of a shell layer. To study the patches and collected samples, each was assigned a short identifier. The first sea urchin patch in the project was labelled S1, the second S2, and so on. The patches exhibiting mainly the invasive macroalgae *Caulerpa prolifera* are for simplicity referred to as *Caulerpa*-invaded patches and labelled accordingly with C1, C2 and C3. Within each patch, three circles were sampled: the first circle was designated A, the second B, and the third C.

4.1. Location of the sea urchin patches

All patches were in close proximity to each other near the Armona Inlet in Ria Formosa. The exact locations of the centre point of each sea urchin patches are displayed in Figure 6. The coordinates for each centre point of a patch were obtained by using a DGPS (Annex Table A 1).

Figure 6: The Section of Ria Formosa close to the Armona Inlet is presented. The ten pins indicate the location of the centre of each patch. The short term corresponds to a specific patch. The first sea urchin patch in the project was labelled S1, the second S2, and so on. The patches exhibiting mainly the invasive macroalgae Caulerpa *prolifera were labelled accordingly with C1, C2 and C3. (Image courtesy of Google Earth Pro; Google Earth Pro Version 10.40.0.2, Portugal; 37° 00’11’’N, 7°48’40’’W (WGS 84)/ 29S 605218m N, 4095984m E (UTM); 2D map; reviewed 12.07. 2024 (http://www.google.com/earth/index.html).*

4.2. Mapping and georeferencing the sea urchin and *Caulerpa*-invaded patches

The sea urchin patches were georeferenced as indicated above. Using these measurements along with data from the underwater cartography (Annex Table A 2, table A 3), the outlines of sea urchin patches 1 to 7, were reconstructed and placed in a geographical context (Figure 7).

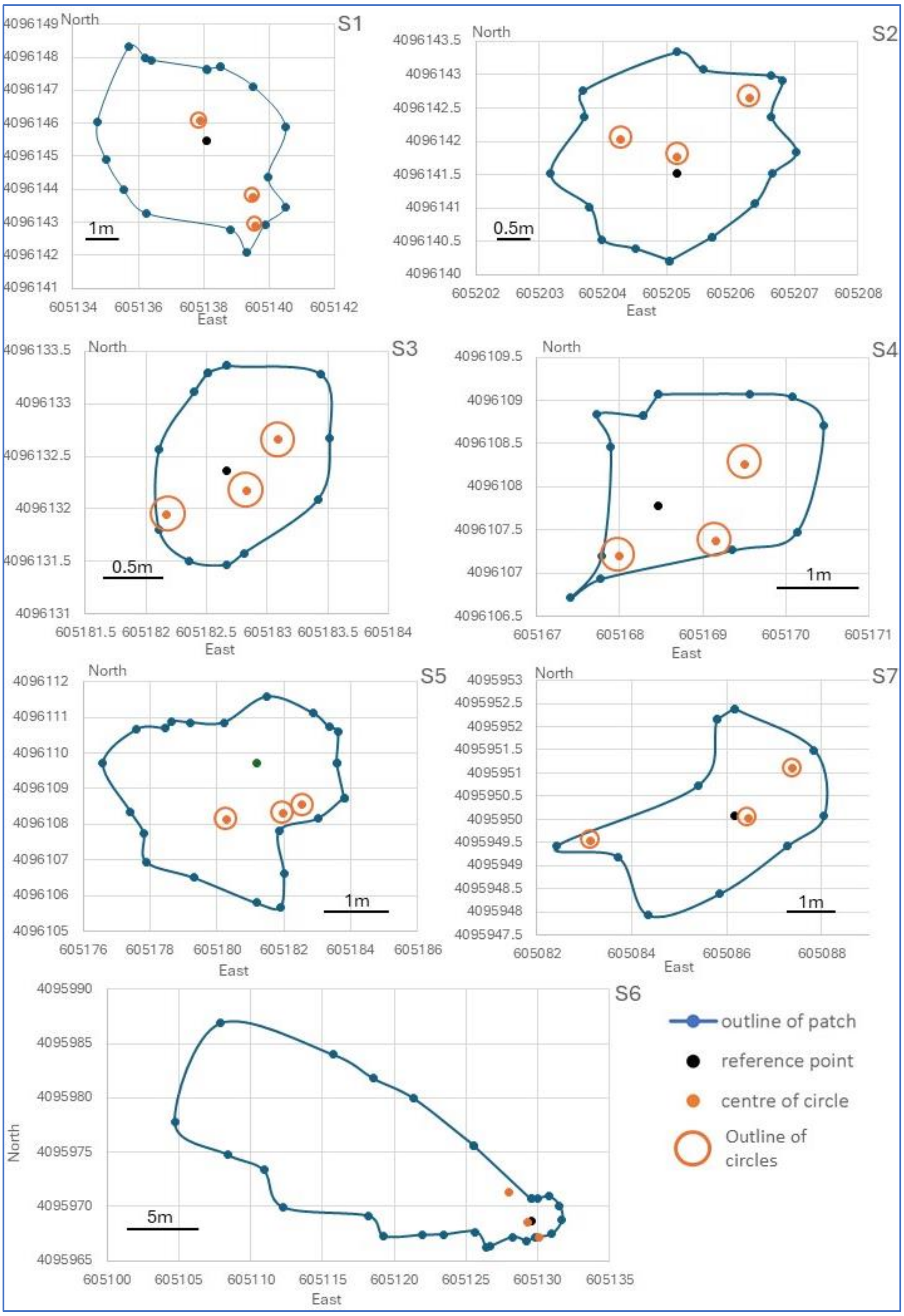

Figure 7: Shape and location of the sea urchin patches (S1 to S7). The labelled reference for each patch is indicated at the top right. The y-axis represents the east coordinate and the x-axis the north coordinate in UTM system.

The last three patches, dominated by the invasive algae *C. prolifera*, were labelled accordingly as C1, C2, and C3. Their shapes were reconstructed in the same manner (Figure 8), utilizing the underwater measurements and georeferenced points (Annex Table A 4).

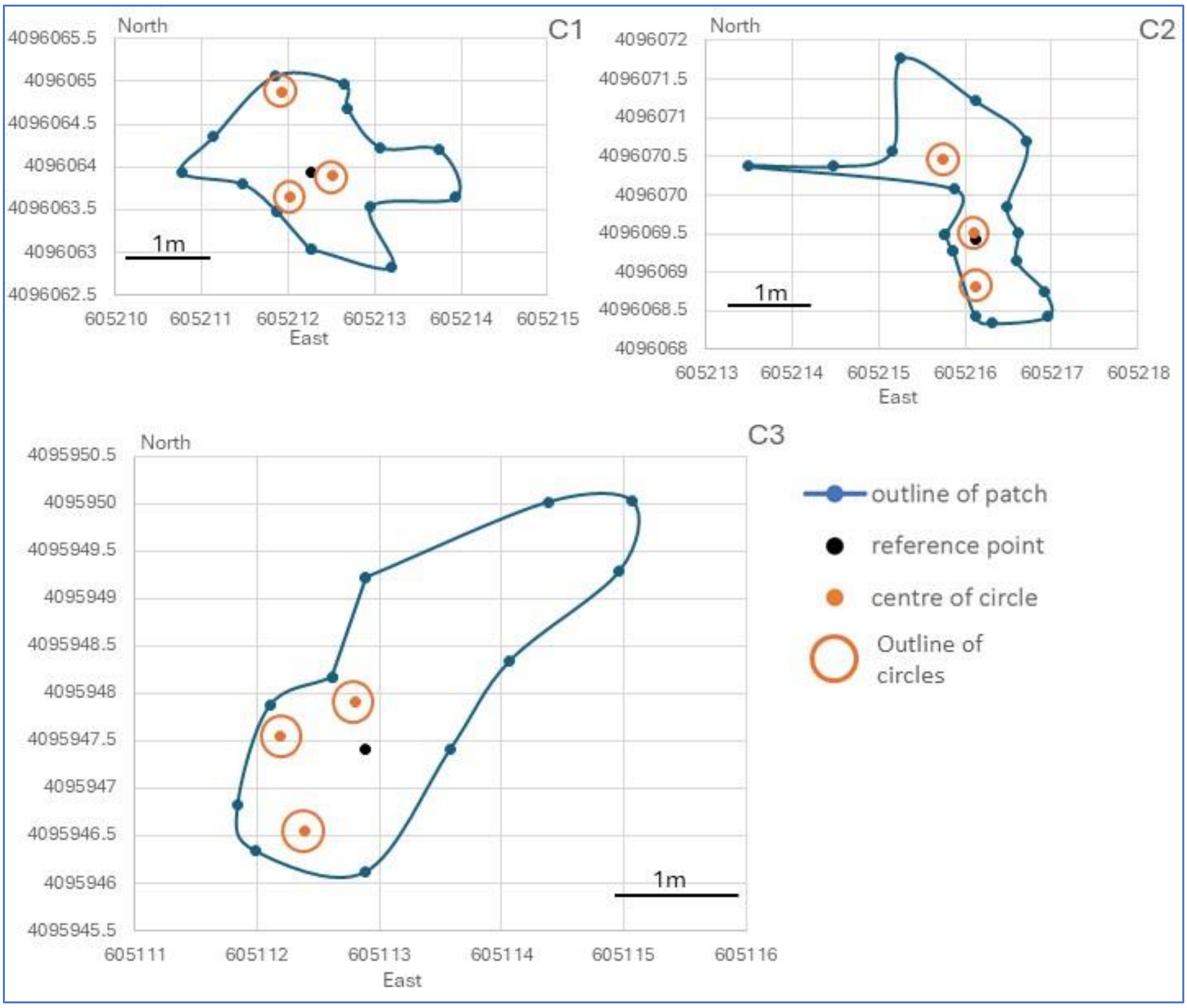

Figure 8: Shape and location of patches invaded by Caulerpa prolifera *and assigned with the short term C1, C2 and C3, as indicated at the right top of each patch. The y-axis represents the east coordinate and the x-axis the north coordinate in UTM system.*

The covered area of the sea urchin patches varied from 2.01 square meters to over 270 square meters. The *Caulerpa*-invaded patches ranged from 3.31 square meters to 5.66 square meters (Table 2). Within this range a great difference in the means of the sea urchin patches and the *Caulerpa*-invaded patches can be observed (Annex Table A 8). The invaded patches show smaller mean values of covered area than the non-invaded patches, although the values are statistically not significant (p-value = 0.13; Annex Table A 8).

Table 2: Area covered by each of the sampled ten patches.

Patch nr.	Area covered by patch [m²]
S1	23.4
S2	7.74
S3	2.01
S4	4.53
S5	26.3
S6	276
S7	11.3
C1	3.45
C2	3.31
C3	5.66

4.3. Flora samples

The sampled flora in sea urchin patches S1 to S7 primarily consisted of the invasive algae *Rugulopteryx okamurae* across all three circles. The brown macroalgae was identified (Figure 9) by examining the transverse section of the thallus with multiple layers of medullary cells in the margin (Hwang *et al.* 2009).

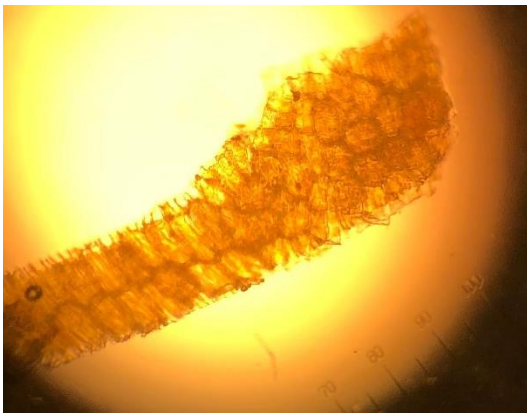

Figure 9: Transverse section, at a magnification of 100, of the thallus with multiple layers of medullary cells in the margin, allows the identification of Rugulopteryx okamurae. *The photo was taken by Javier Jimenz (UAlg).*

Additionally, a significant amount of dead seagrass was observed. As listed in Table A 6, *R. okamurae* was always scaled together with dead sea grass. Separating it from the brown algae would have required more time than available for this task during the on-board analysis. Smaller quantities of *C. prolifera*, *Ulva sp.*, and *Codium sp.* were also found in some of the circles (Figure 10 and Annex Table A 5). In the *Caulerpa*-invaded patches C1, C2, and C3, the flora mainly consisted of *C. prolifera*, as indicated by the names of the patches. Small amounts of *R. okamurae* were also present across the three patches (Figure 10 and Annex Table A 5). Circle B of patch C1 exhibited instead of *C. prolifera* mostly *R. okamurae* (Figure 10 and Annex Table A 5).

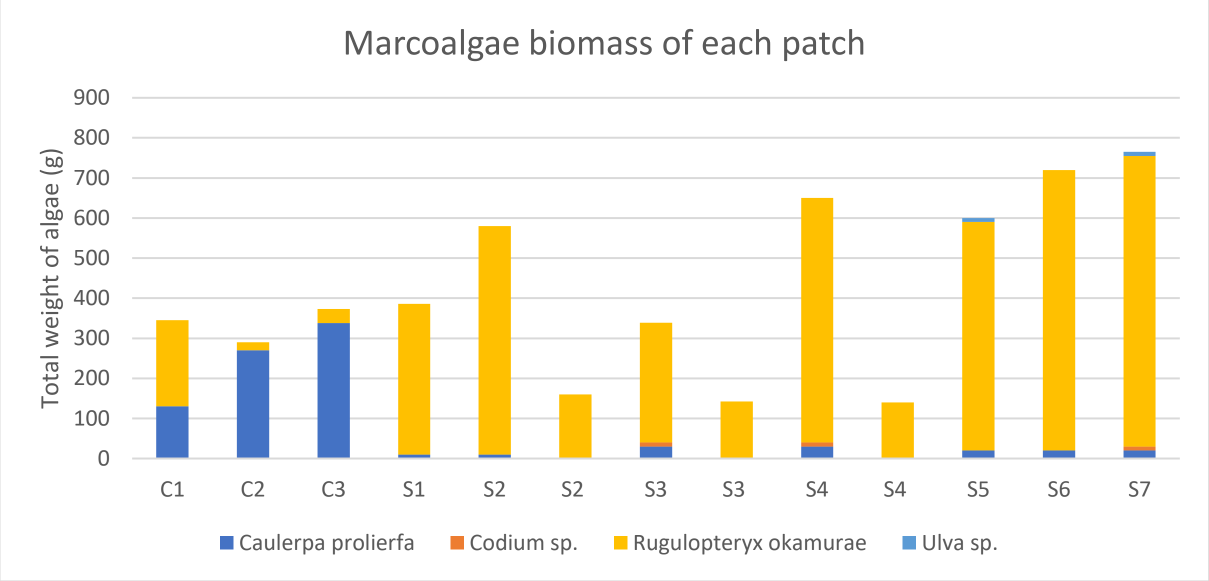

Figure 10: The total weight of algae sampled in each patch (in an area of 0.2 m²), and of each algae taxon: Caulerpa prolifera, Codium sp., Ulva sp. *and* Rugulopteryx okamurae*. The weight of* R. okamurae *includes the dead sea grass, which was not separated from the living flora samples.*

Furthermore, the average weight of the sampled flora per patch was calculated and a significant smaller amount of weight was noticeable for two of the *Caulerpa*-invaded patches (Figure 11). C1 was not included due to its half-invaded state, allowing a clearer comparison.

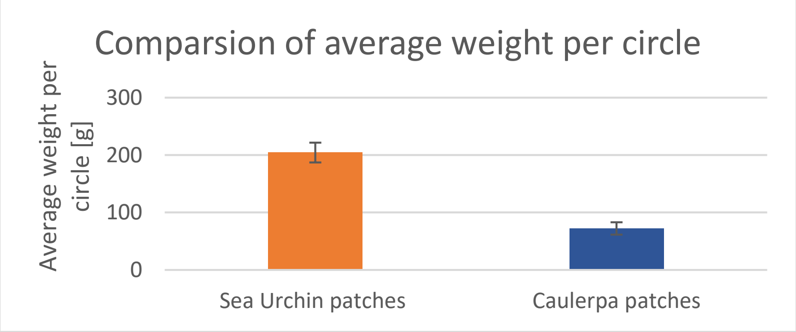

Figure 11: Comparison of the average weight of the predominant flora in each circle (0.07m²) for the sea urchin patches and two of the Caulerpa-invaded patches.

4.4. Fauna samples

The fauna samples included four different species of Annelida, totally over 20 organisms. Except for one case, Annelida were exclusively found in the *Caulerpa*-invaded patches (Figure 12 and Annex, Table 7). Arthropods, including various orders of crustaceans, were sampled more than 200 times. Notably, out of 213 samples, only six were found in the three *Caulerpa*-invaded patches (Figure 12 and Annex Table A 6). A total of 70 organisms from the Chordata phylum, including Actinopterygii, Ascidians, and Batoidea, were counted, with the majority being found in the sea urchin patches. At this point it is important to mention, that Ascidians can accumulate together, grow on top of each other or fully merch with each other, as described earlier on. In this case, when the individual organisms were not separatable, the accumulated organism was counted as one. Only five, out of the 70 organisms, were found in the *Caulerpa*-invaded patches (Figure 12 and Annex Table A 6). One Cnidarian was observed in one of the *Caulerpa*-invaded patches (Figure 12 and Annex Table A 6). Over 900 Mollusc samples, including Gastropods, Bivalves, Cephalopods, and Polyplacophora, were observed across all ten patches, without a clear pattern evident from the raw data (Figure 12 and Annex Table A 6). Lastly, over 60 Echinodermata, including sea stars, sea urchins, and sea cucumbers, were counted. Similar to the Arthropoda pattern, almost all Echinodermata were found in the sea urchin patches, with only one exception in the *Caulerpa*-invaded patches (Figure 12 and Annex Table A 6). The morphological measures of the sea urchins ranged in weight from 23 to 240 grams, with diameters varying from 36 to nearly 90 millimetres and heights from 20 to 56 millimetres (Annex Table A 7).

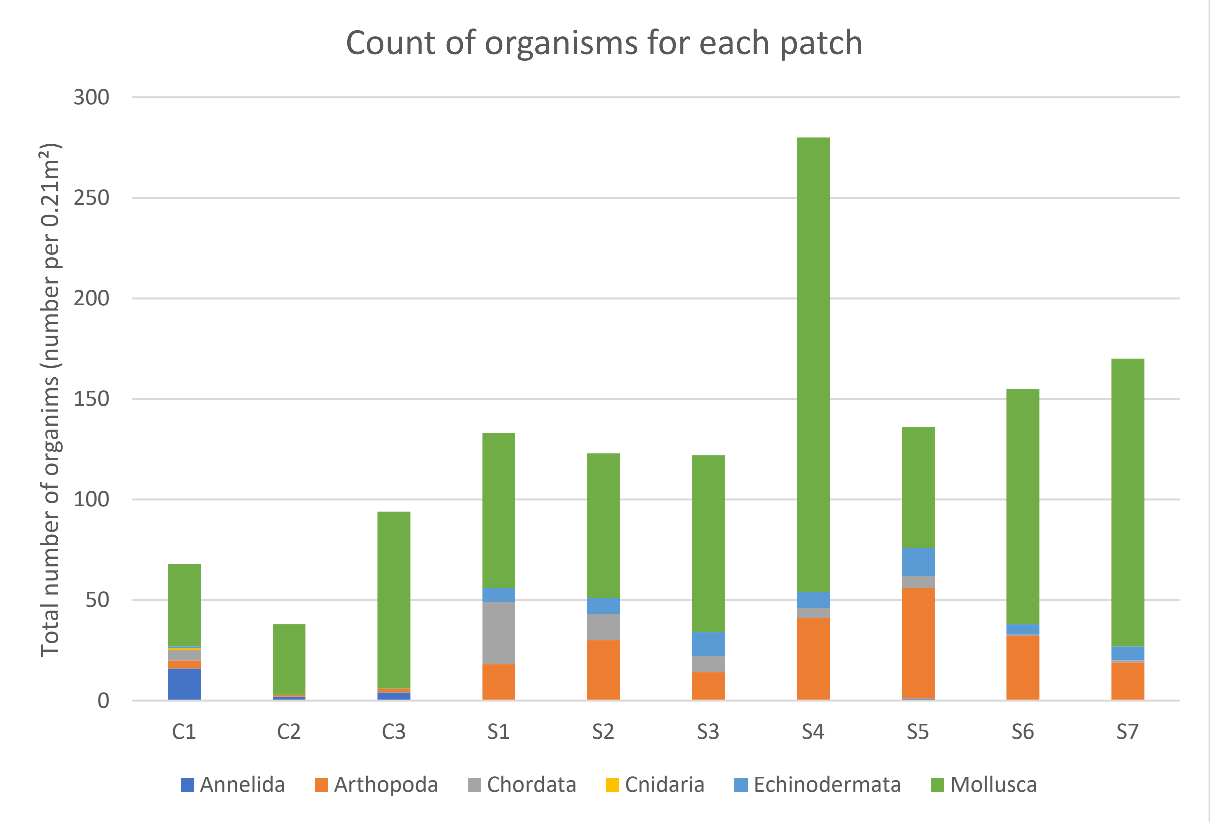

Figure 12: Organism composition for each patch includes fauna form the phyla Annelida, Arthropoda, Chordata, Cnidaria Echinodermata and Mollusca. A one-sided t-test indicates a significant lower number of taxa per sampled patch (p-value = 0.012), highlighting the reduced richness of the Caulerpa-invaded patches (Annex Table A 10).

Using the total number of taxa for each patch, the relative composition of taxa was calculated and visualized in Figure 13. This figure presents a comparison of the distribution of different taxa across the patches, showing the dominance of Annelida in the *Caulerpa*-

invaded patches. Additionally, the mentioned absence of Chordata and Echinodermata in these patches becomes more evident.

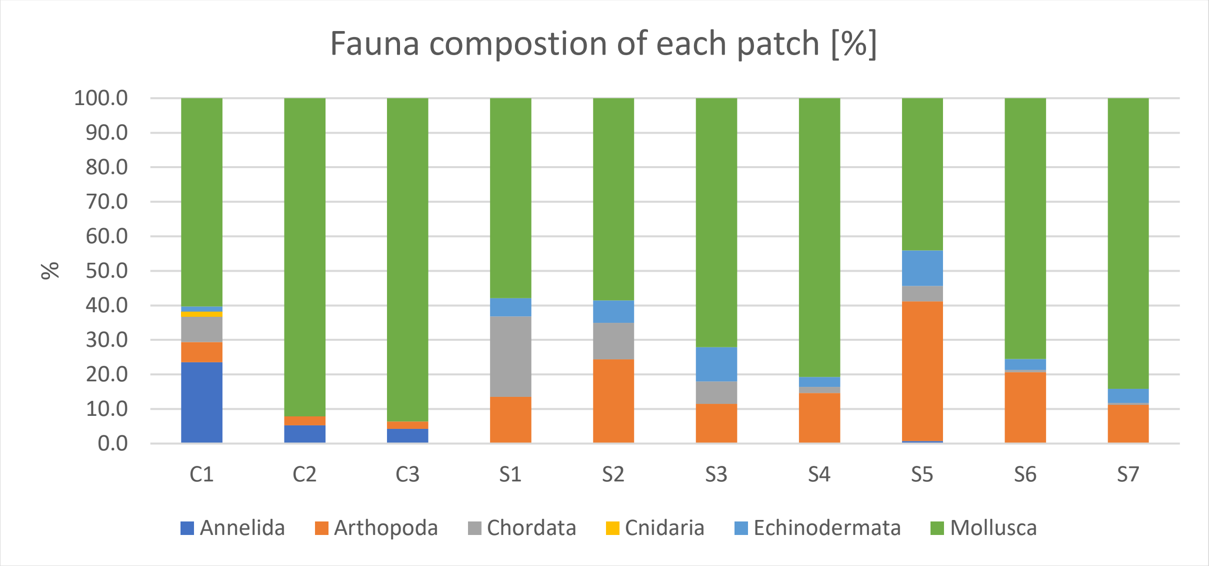

Figure 13: Relative composition of the fauna for each patch is shown.

The difference in composition of the patch types was mirrored as well in Chi-squared tests. Both, the average phylum composition of each patch type and the sum of each patch type showed significant differences between the invaded patches and the sea urchin patches (for both chi tests: p-values < 0.01; Annex Table A 9). The notably reduced richness is particularly indicated when comparing the number of taxa per patch, with counts ranging from 7 to 12 different taxa in the sea urchin patches and only 4 to 7 different taxa in the *Caulerpa*-invaded patches (Figure 14). A one-sided t-test indicating a significant lower number of taxa per sampled patch in the invaded patches (p-value = 0.012), highlighting the reduced richness of the *Caulerpa*-invaded patches (Annex Table A 10). This absence of Echinodermata in the *Caulerpa*-invaded patches indicates again the different distribution of taxa, when comparing the patches of the two states. The density of sea urchins ranges from 0 to 1 per 0.2m^2 in the invaded patches, whereas it ranges from 1 to 7 sea urchins per 0.2m^2 in the sea urchin patches (Figure 15). When examining the density distribution of the two sea urchin species closer, a clear pattern emerges. *P. lividus* is the more abundant sea urchin species in only three of the patches, while the other five patches, inhabited by sea urchins, are dominated by *S. granularis* (Figure 15).

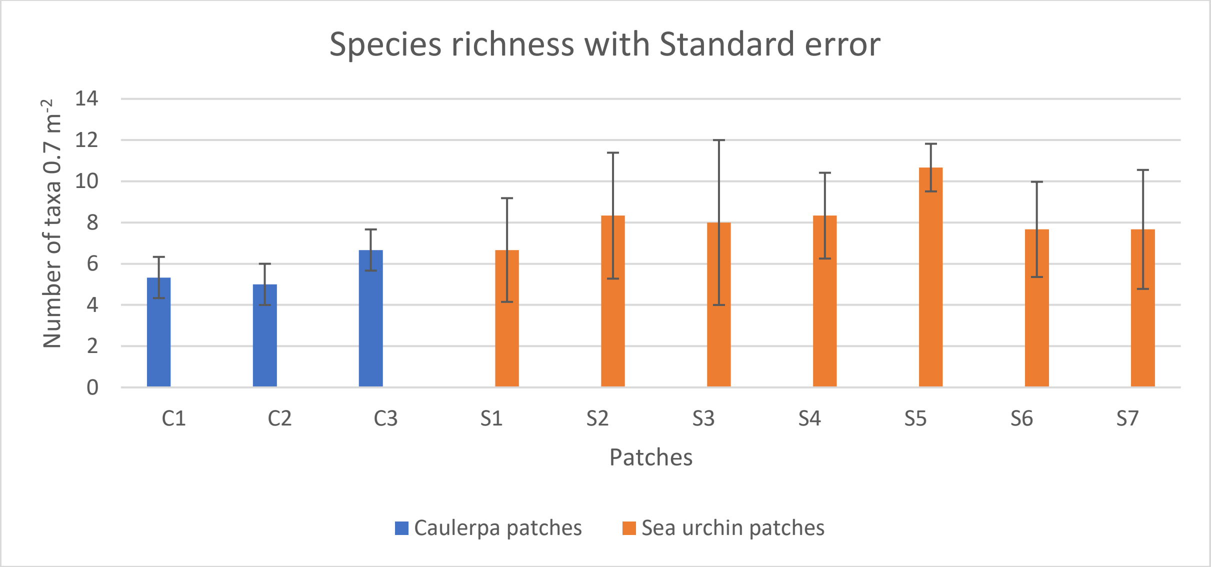

Figure 14: Average values with standard error of species richness for each patch is represented.

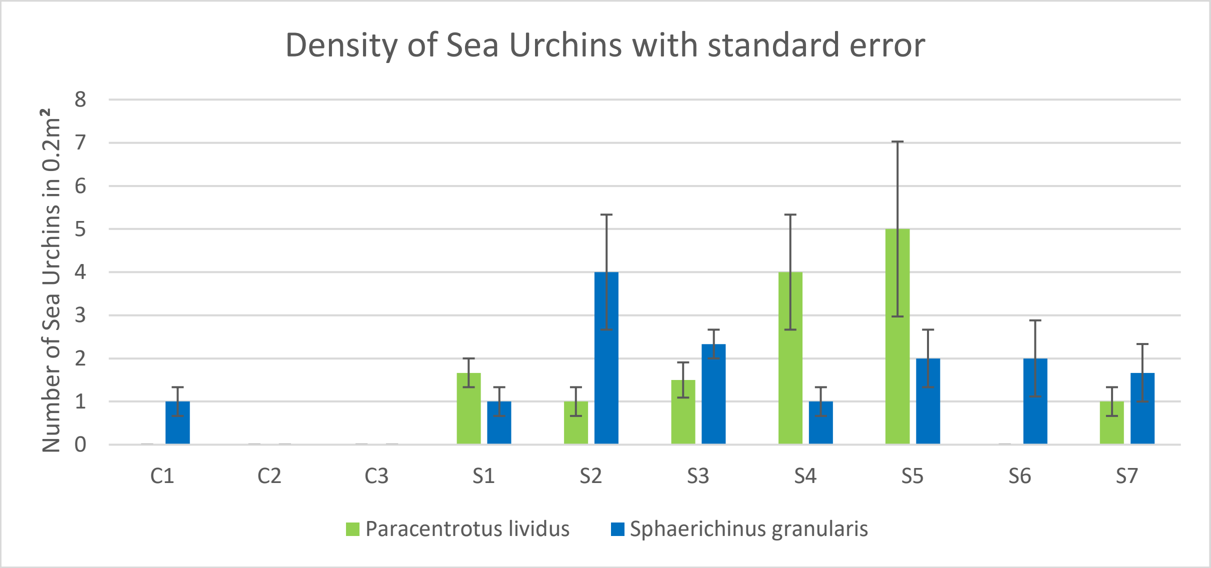

Figure 15: Average density and standard error of the two sea urchin species S. granularis and P. lividus are shown for each patch.

For further demonstration of the difference between the *Caulerpa*-invaded patches and the sea urchin patches a MDS analysis was performed. Notably, both, comparison of only the patches and comparison of the circles in each patch showed reliable patterns under low stress values (0.14 and 0.05, respectively, Figure 16 A and B). The clustering of the sea urchin patches replicates in the left side of the diagram, in opposition to the clustering of the *Caulerpa*-invaded patches replicates in the right side (Figure 16 A) clearly highlights the differences between fauna composition, which is clearly seen when only the patches are considered (Figure 16 B).

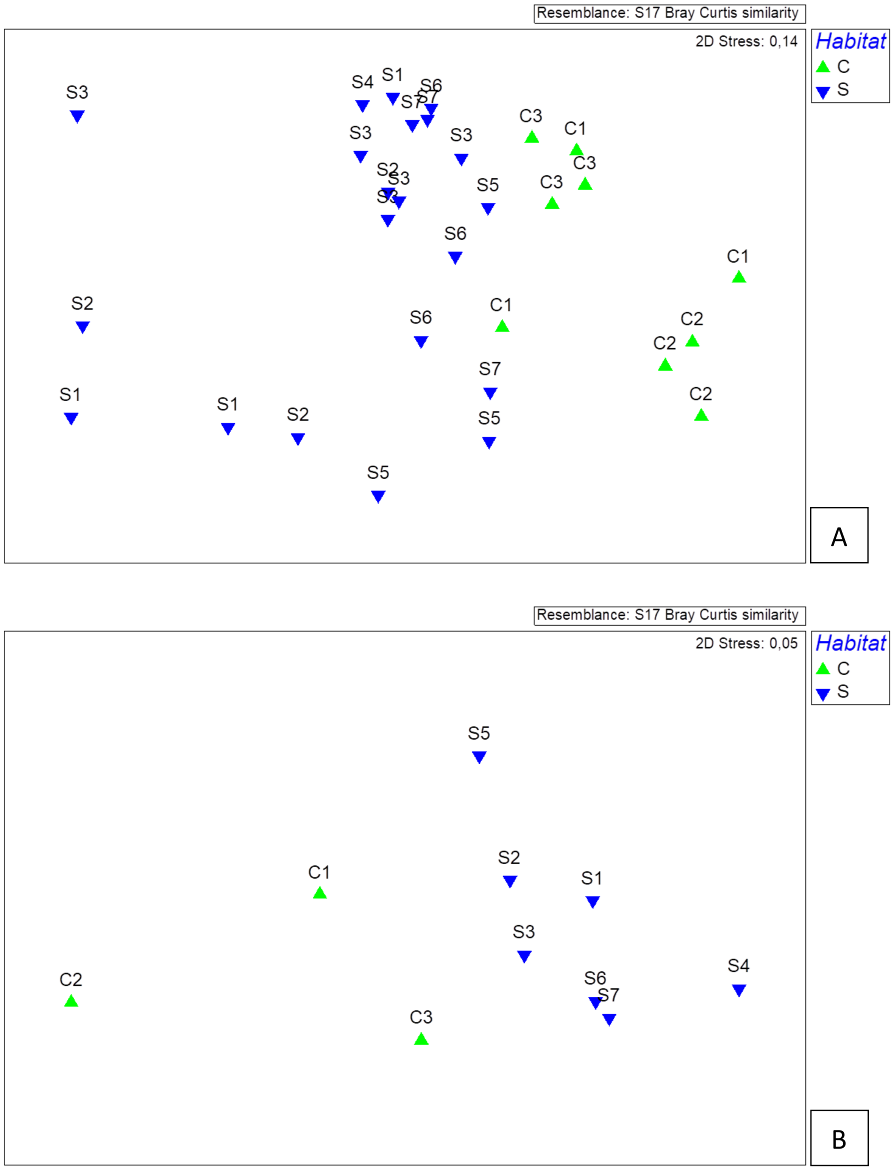

Figure 16: Non-metric Multi-Dimensional Scaling (nMDS) plots of different patch replicates obtained from Bray Curtis dissimilarities in abundance of organisms in each circle (A) and abundance of whole sampled patch (B).

Lastly, the three indices, Shannon-Wiener-, Evenness- and Simpson-Index, were estimated, based on the total number of species and relative abundance per patch type. The values of the indices are not significantly different between the two patch types (Table 3).

Table 3: Values of each patch type for the three established indices, Shannon-Wiener-, Evenness- and Simpson-Index. The values were estimated considering the total number of species sampled in each patch type.

Indices	*Caulerpa* -invaded patches	Sea urchin patches
Shannon Index H' (logarithmic base of 2):	2.68	2.90
Evenness:	0.60	0.60
Simpson Index (1-D):	0.69	0.66

4.5. Sediment samples

A distinct layer of shell, on which the flora grows, as seen in previous years by the team, was not found in any of the patches. All samples of each circle of all the patches showed the same sediment colour. The observed colour, dark brown/ black, matched 3/5 PB in the Munsell Soil colour charts (Munsell Color, 1994). The pH values varied between 7.35 and 7.92 across all the patches. The grain size analyses revealed that the sediment varied from muddy sand to sandy mud, with mean grain sizes ranging from 17.5 to 78.6 micrometres (Figure 17; Annex Table A 11). Statistically, the mean grain sizes do not differ from each other (p-value=40.7; Annex Table A 12).

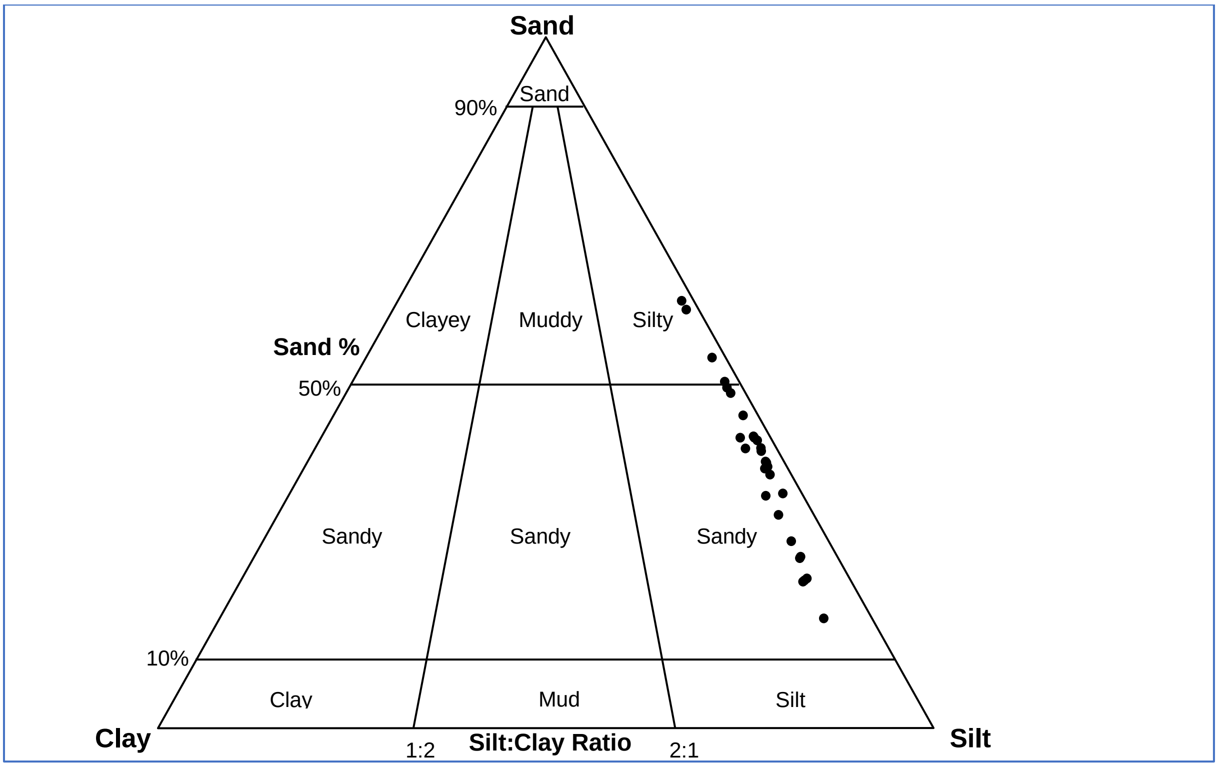

Figure 17: Results from the polydisperse Laser Granulometer are shown in the soil texture triangle and revel the different grain sizes from all patches, ranging from muddy sand to sandy mud.

5. Discussion

Overall, the presented results show differences between the *Caulerpa*-invaded and sea urchin patches on multiple levels. The difference in fauna, flora and the actual size and shape of the patch are broken down in the following sections to reveal a change due to the invasion of this habitat.

5.1. Differences in size of *Caulerpa*-invaded patches and Sea urchin patches

The calculated area covered by each patch ranges from barely over two square meters to over 270 square meters. As mentioned, a great difference in the means of the sea urchin patches and the *Caulerpa*-invaded patches can be observed. The invaded patches show smaller mean values, although not significant statistically (p-value = 0.13; Annex Table A 8). The high p-value can be explained by an insufficient amount of measured *Caulerpa*-invaded patches, altering the result of the t-test. Beyond the test results, during the diving campaigns locating *Caulerpa*-invaded patch, with an area large enough for georeferencing, presented a challenge, since most of the invaded patches were too small for measuring. This issue arises from the fact that *C. prolifera* can grow on unvegetated soft bottom (Parreira. 2018, Parreira *et al.* 2021). Therefore, *C. prolifera* must not only rely on taking over already existing vegetation but can grow in between the sea urchin patches and form those small patches. Despite the t-test results, the great difference in means and the first observation during the dives can still indicate a change or shift in size of the patches, due to the invasion.

5.2. Distinguishing between *Caulerpa*-invaded and sea urchin patches

Already during the diving campaigns, when collecting the samples and without any further analysis of the raw data, the sampled patches could be distinguished based on the vegetation. As anticipated, the presence of invasive algae was documented, regarding its known occurrence in the Algarve (Cunha et al., 2013) and its previous identification in Ria Formosa (Vital et al., 2022; Alexandre & Santos, 2020). The patches were accordingly classified as either *Caulerpa*-invaded patches or sea urchin patches. Closer examination of the first *Caulerpa*-invaded patches (C1) showed a noticeable dominance of the brown algae *R. okamurae* and instead of *Caulerpa prolifera* (Figure 11 and Annex Table A 5). The patch had not been fully invaded at the time of the campaigns, so one of the circles was placed in an area that had not yet been affected by the invasion. This explains the predominant presence of *R. okamurae* in circle B of patch C1. Due to the lack of separating *R. okamurae* from the dead seagrass, the brown algae covered 56% of the overall weight of C1, even though it is only predominantly present in one of the three circles sampled. When comparing two circles, one with is fully covered with *C. prolifera* and the other with *R. okamurae*, the difference in weight is noticeable (Figure 11). This shows the much denser biomass of *R. okamurae*, that contributes to the overall weight shift in patch C1. Besides this shift, a clear difference between the *Caulerpa*-invaded patches and the sea urchin patches is noticeable not only in the composition of the flora, but also in the distribution of the fauna.

5.3. Differences in macrofauna between *Caulerpa*-invaded patches and Sea urchin patches

The changes in composition become evident as well, when examining the fauna data (Annex Table A 6). As previously mentioned, the four different species from the taxa Annelida were, aside from one exception, exclusively found in the *Caulerpa*-invaded patches. In contrast Chordata, Echinodermata, except for one case, and significantly higher number of Arthropoda were predominantly present in the sea urchin patches. The already mentioned Chi-squared test mirrored this difference in composition, where the average phylum composition of each patch type and the sum of each patch type showed significant differences between the two patch types (Annex Table A 9). A noticeable difference in fauna amongst the *Caulerpa*-invaded patches was observed. Over 90% of the fauna consisted of Mollusca in patch C2 and 3, but only 60% in patch C1. Instead C1 exhibits a larger amount Arthropoda, Chordata and Echinodermata (Figure 13). This inconsistency is due to the explained issue of C1 not being fully invaded at the time of sampling. Therefore, one of the circles was placed in a non-invaded area, which drastically altered the faunal structure. Parreira *et al.* (2021) found similar patterns of significant differences between *Caulerpa prolifera* habitats and non-invaded habitats in Ria Formosa. Although the authors revealed and described a similar pattern, the amount of counted organisms differed from those found in this study. The season, when sampling was conducted and the use of different sampling methods, such as beam trawl tows (Parriera *et al.* 2018), might be important factors for such variation in the observation. Other studies reported an introduction of different *Caulerpa* species in the Mediterranean Sea in the early 1990s (Verlaque *et al.* 2000; Piazzi *et al.* 2005). Simultaneously a comparable shift in the fauna composition was described and shows a similar pattern of significant differences between the non-invaded and the invaded state (Santini-Bellan *et al*. 1996).

Unexpectedly, the three different diversity indices, Shannon-Wiener-, Evenness- and Simpson-Index, do not vary significantly from each other. Therefore, they cannot be used to provide any evidence for a clear difference and separation based on the biodiversity of the two patch types. The Shannon Index might be affected by the sampled area. The more is sampled the more taxa are found, due to the wide range of biodiversity in the sea urchin patches. Parreira *et al.* 2021, researching the invasive behaviour of *Caulerpa prolifera* in Ria Formosa, showcased significant differences in the indices. The same authors proved, based on that, a higher biodiversity, richness and evenness in a non-invaded habitat compared to a *C. prolifera* habitat. Despite the result of the indices, the only evidence for a change in biodiversity is the difference in species richness between the *Caulerpa*-invaded patches and the sea urchin patches (Figure 14). This is shown by the mentioned one-sided t-test, indicating a significantly lower number of taxa in the *Caulerpa*-invaded patches (p-value = 0.012). This highlights the reduced richness of the invaded patches.

The MDS results, based on the macrofauna abundance, can provide further evidence for the described shift in the patches due to the invasion. Notably, the comparison of only the patches and of the circles in each patch had reliable patterns under low stress values (0.14 and 0.5, respectively, Figure 16). A visible similarity in the macroinvertebrate community of the sea urchin patches can be observed, clustering together on the right side of the diagram. The *Caulerpa*-invaded patches are separated on the right side of the diagram, although visible differences amongst the *Caulerpa*-invaded patches (Figure 16 A). Patch C2 shows great displacement, hence differentiates from the two remaining (Figure 16 B). The reason for the difference becomes clear when comparing the quantitative, sampled amount (Figure 12). The total number of sampled organisms in C2 is noticeably lower than in C1 and C3, creating the most dissimilarity amongst the invaded patches. Besides that, the similarity between C1 and the sea urchin patches is noticeable. As already mentioned, C1 has not been fully invaded at the time of sampling, resulting in a circle showing a non-invaded state. Therefore, this circle shares more similarities with the sea urchin patches (Figure 16 A). Consequently, the dissimilarities of C1 are altered from a fully invaded patch. A comparable MDS analysis done in another study in Ria Formosa revealed a similar pattern, in which *Caulerpa prolifera* habitats showed significant dissimilarities to non-invaded habitats. However, that study did not involve sample areas where both invaded and non-invaded states were present simultaneously (Parreira. 2018; Parreira et al. 2021). Nevertheless, that study, as well as the present study, allowed to infer that these dissimilarities highlight the alternation in composition and abundance of macrofauna due to the *C. prolifera* invasion. Foundation species, such as *C. prolifera*, *C. nodosa*, and *Codium sp.*, are commonly accepted to play a significant role in shaping animal and plant communities, often contributing to high diversity and abundance of macroinvertebrates and Chordata (Heck & Orth. 1980; Lloret *et al*. 2005; Parreira *et al.* 2021; Tuya *et al.* 2013). However, there is an ongoing debate whether the specific effects of *C. prolifera* decrease or increase abundance and biodiversity. Some studies have found that the invasive seaweed supports greater abundance and diversity than seagrass (Sánchez-Moyano *et al.* 2007; Png-Gonzalez *et al*. 2014), while others have reported the opposite (Parreira *et al.* 2021; Piazzi *et al*. 2005). In this study, the analysis of the fauna and flora showed differences and a shift in the composition, richness, and abundance of the patches. Still, the question remains, whether a loss or alternation of biodiversity in the sea urchin patches due to an impact of *C. prolifera* is present. The applied indices did not reveal any further inside about any change in biodiversity. Multiple studies have shown negative effects on biodiversity of the *Caulerpa sp.* invasion in the same environment Ria Formosa (Alexandre & Santos. 2020; Parreira *et al.* 2021; Vital. 2022). However, the applied diversity indices did not reveal any further inside about any change in biodiversity, although a significant reduction on the number of invertebrate taxa was noticed in the *Caulerpa-invaded* patches. The reason for the lack of evidence to prove a loss of biodiversity might be the predominant abundance of *R. okamurae*. As briefly mentioned above, the brown macroalgae is in invasive species as well, but its presence was only reported more recently than the appearance of *C. prolifera* (Liuela *et al.* 2023).

5.4. Poor biodiversity even on the sea urchin patches?

R. okamurae was first described in Europe in the early 2000s and proliferating since 2016 in the Mediterranean Sea (Borriglione *et al.* 2024; García-Gómez *et al.* 2021). Liulea *et al.* 2023 reported the first appearance of the brown algae in the Algarve, in southern Portugal. The same authors state that due to its fast spread rate, both, species abundance and richness of flora, strongly decreased in the invaded areas. The origin of the impact of *R. okamurae* stems from the direct competition for space, light and nutrients (García-Gómez *et al.* 2021). The effect on flora influences the fauna, living in the same habitat. Due to the very recent spreading of the macroalgae, a lack of knowledge about the effects of the invasion exists. The impact on fauna due to the flora change was only studied in Mediterranean Sea (Borriglione *et al.* 2024;

García-Gómez *et al.* 2021), but not in Portugal. The abundance of different invertebrate species was significantly altered, in comparison to a non-invaded state (Borriglione *et al.* 2024). This decline of most invertebrate species could be explained by the dense growth of the algae, which might lead to a reduction in dissolved oxygen due to its respiration (Borriglione *et al.* 2024; Gribben *et al*. 2009). Furthermore, changes in sedimentation and water column flow can influence environmental parameters. Hence, this shift of flora can lead to reduced oxygen levels and increased sulphide concentrations, which subsequently affects the invertebrate species (Gribben *et al*. 2009). Given these factors, the similarities in biodiversity indices of the *Caulerpa*-invaded patches and sea urchin patches are understandable, as both habitats are affected by invasions that influence the fauna drastically. This means that not only *C. prolifera* has a negative effect on the ecosystem, but *R. okamurae* potentially as well. The team's experience and knowledge of previous years described, as mentioned before, a dominant layer of shells, that is thought of to form a base for the sea urchin patches. In this year's study, no shells were found in any of the patches, which could be directly related to the shift in flora. The impact of the absence of the shell layer remains unknown, due to the gap of knowledge about the sea urchin patches. Additionally, no nudibranchs were sampled in one of the circles nor spotted in a patch during the diving campaigns, but the dives were not directed to find nudibranchs. The absence of nudibranchs can be directly related to the shift in flora, as nudibranchs were commonly found within the patches, according to the observations in diving campaigns of the team's previous research (Hoett. 2019). Another potential consequence of this invasion could be a change in abundance of the two sea urchin species *P. lividus* and *S. granularis*. Reis (2018) and Fragkopoulou (2016) found exclusively *P. lividus* in sea urchin patches during previous diving surveys, in the same area in Ria Formosa. Whereas this study showed an average equal distribution of *P. lividus* and *S. granularis* within the patches (Table A 6). The grazing behaviour of *S. granularis* may explain this change. Trenzado *et al.* (2012) proofed that *S. granularis* has a greater ability to graze on a wider range of different algae, compared to *P. lividus* and hence, *S. granularis* is better equipped for a change in flora composition. Given the increasing competition of *S. granularis*, a decline in the growth of *P. lividus* might be anticipated. Brundu (2017) describes how population density can affect the somatic growth of *P. lividus*. According to the same author, a reduction in growth would be apparent in a decrease of the sea urchin's diameter and wet weight, when population density increases. However, a data comparison does not show a comparable reduction in diameter or height. Measurements of height and diameter for *P. lividus* in this study (Annex Table A 7) align closely with those reported by Fragkopoulou (2016) (Annex Table A 13), showing no significant difference (p-values > 0.05 for both measurements; Annex Table A 14; Figure 18). Although the wet weight data reveals a significant difference, while showing similar average values in diameter and height (p-value < 0.05, Table A 15; Figure 18), the results are inconclusive. These ambiguous results prevent a clear conclusion on whether competitive pressure from *S. granularis* is affecting *P. lividus* morphology based on the morphological measurements provided.

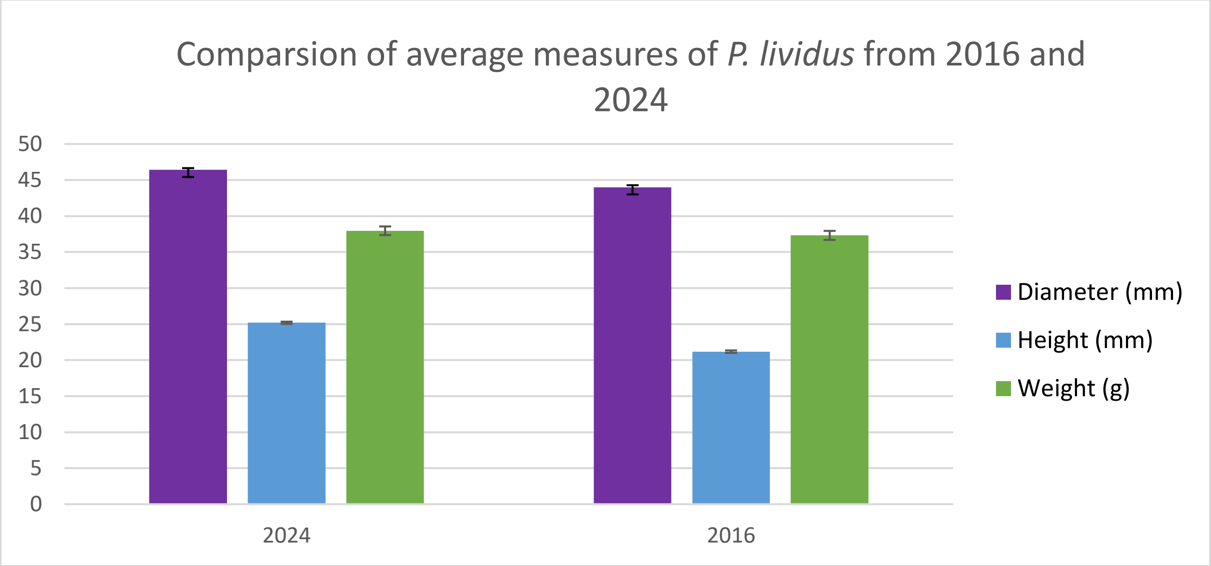

Figure 18: Compensation of the average diameter in milli meters, average height in milli meters and average wet weight in grams measures of P. lividus *samples. The sea urchin data from 2016 is based on the team's previous work, Fragkopoulou (2016).*

Noteworthy is

Insufficient information about the fauna composition in the sea urchin patches before the invasion, and the subsequent ambiguous results imply greater difficulties researching the impact on biodiversity.

5.5. Spatial variation and similarities of the patches

Despite the differences and changes due to the invasion of the two macroalgae, similarities and differences of the patches can be directly related to their spatial proximity or distance. The MDS analysis clearly indicates a clustering of all the sea urchin patches (S1-S7). Within this clustering, specific patches form distinct groups. Patch S6 and S7 group together in the MDS plot (Figure 16 B), which corresponds to their geological proximity (Figure 6). Similarly, patches S1, S2, and S3 form a group in the plot (Figure 16 B), matching their spatial distribution (Figure

6). This grouping in the MDS plot, and the resulting similarities, can be explained with the exposure to similar environmental factors. Ria Formosa experiences physical and chemical impacts, that can rapidly alter this environment (Carrasco & Matias 2019; Duarte *et al.* 2020). These factors can change over short distances (Duarte *et al*. 2020), which exposes each grouping of the patches to unique influences and shapes them accordingly in a way that groupings vary from each other. The mentioned distribution pattern of *S. granularis* in Figure 15, where *S. granularis* is more abundant in five patches, shows the overall dominance of this species. In two of the patches *S. granularis* is present, while *P. lividus* is fully absent. This distribution pattern might again be explained by the grazing behaviour of *S. granularis*. Its broader feeding range on different algae, compared to *P. lividus* (Trenzado *et al.* 2012), equips this species better to adapt to the invasion, and is directly related to the shift in fauna.

5.6. *Caulerpa* influence on Sediment composition

In previous years, the diving team reported a distinct shell layer, forming a foundation for the flora and fauna of the sea urchin patches. The absence of a shell layer could directly be linked to the invasion, not only to *C. profilera* but also to *R. okamurae*. Another potential reason for the lack of that layer could be due to a different position of the observed patches in Ria Formosa. Comparing the location of the patches from this study to the location of sea urchin patches from previous years (Annex Table A 16), a greater distance to shore can be observed (Figure 19). These sea urchin patches, analysed in 2019 to 2021, exhibited a distinct layer of shells, according to the team's observations in previous years (Annex Table A 16). Physical factors, such as hydrodynamics, can alter over short distances inside Ria Formosa (Duarte *et al.* 2020) and expose the patches to different currents or extents of currents. Given the location of the patches from this study, positioned in deeper waters and further away from shore, they might be more likely to experience stronger currents, resulting in the lack of shells. The exact reasons for the absence of the shell layer or its impacts remain unknown, due to the gap of knowledge about the sea urchin patches in Ria Formosa.

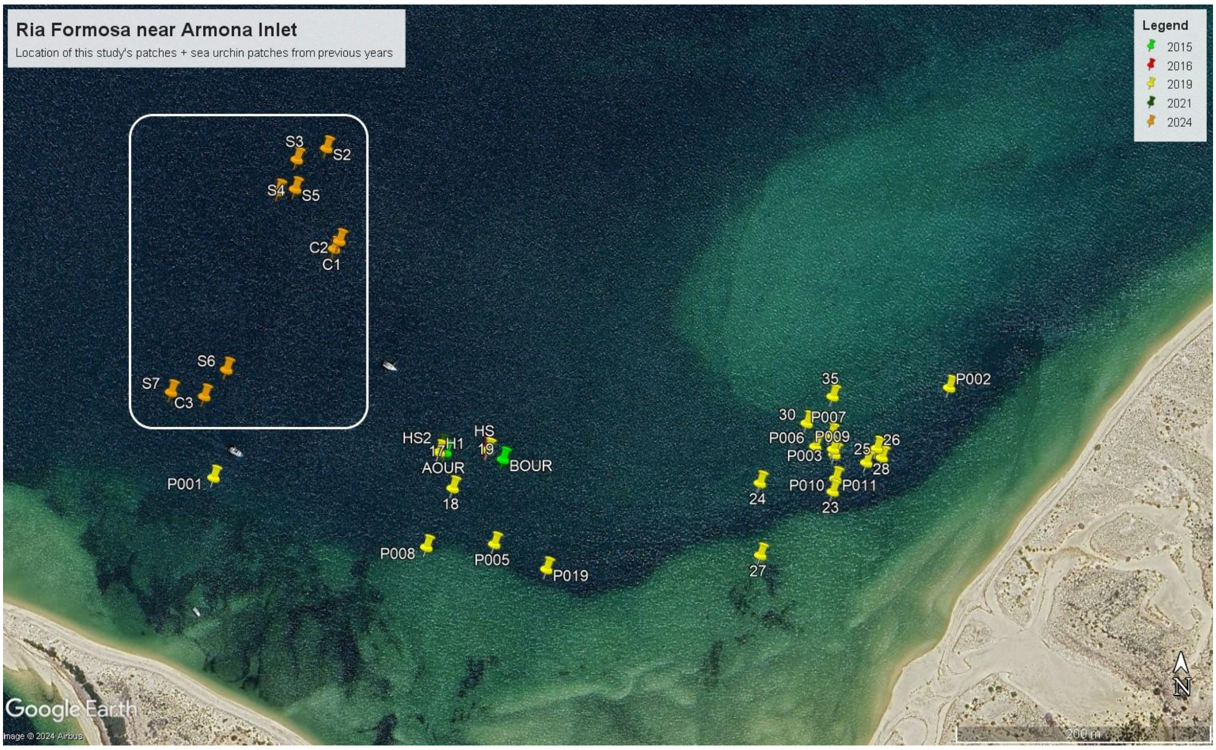

Figure 19: Location of the patches from this study and sea urchin patches from previous years are shown. The patches marked with the orange pin within the white rectangle represent the ones analysed in this study. The remaining ones, outside of the rectangle and closer to shore, were analysed in previous studies. The green pins represent a sea urchin patches from 2016, the red pins from 2015, the yellow pins from 2019 and the dark blue pin resembles a sea urchin patch from 2021.(Image courtesy of Google Earth Pro; Google Earth Pro Version 10.40.0.2, Portugal; 37° 00'11''N, 7°48'40''W (WGS 84)/ 29S 605218m N, 4095984m E (UTM); 2D map; reviewed 12.09.2024 (http://www.google.com/earth/index.html).

Parreira *et al.* 2021 and Pérez-Ruzafa *et al.* 2020 reported that macroalgae like *C. prolifera* can create anoxic sediment conditions with the production of sulphides and herbivory deterrent metabolites. These anoxic conditions were expected to show a change in the Redox potential and differentiation between the two habitats. However, the Redox potential, assessed from the colour, using the Munsell colour soil chart (Munsell Color, 1994), revealed no significant differences between induvial patches or different patch types and therefore disables a distinction between the *Caulerpa*-invaded patches and the sea urchin patches. The lack of differentiation between the two patch types based on the Redox state of the sediment, might be due to the ongoing invasion of *R. okamurae*. The impact on the redox potential of sediment of *R. okamurae* remains unknown. The grain size analysis did not reveal any differences between the habitat types either and presents a similar issue with distinguishing the two patch types. Previous studies showed courser sediment sampled from the patches, with mean grain sizes of 134 micrometre, on which the patches form (Correira. 2015). The difference in grain size between the patches of this study and the previous years could be attributed to the exposure to different hydrodynamics, similar to the lack of shells. Another potential reason might be the invasive algae species, trapping fine sediment and changing the bottom composition. Insufficient information about the sediment conditions before the invasions of the macroalgae creates difficulties in assessing the impact, especially under such condition of overlapping invasions and needs further research on this habitat.

6. Conclusion

This study highlights the impact of the invasive seaweed *Caulerpa prolifera* on the lagoonal biodiversity within the sea urchin patches in Ria Formosa. Through extensive fieldwork and sample analysis, it has been demonstrated that an invasion of *C. prolifera* can lead to significant alterations in the composition and abundance of the patch's inhabitants. The comparison between two patch types, the *Caulerpa*-invaded and sea urchin patches, reveals clear distinctions in faunal assemblages. Annelids were predominantly found in the Caulerpa-invaded patches, while arthropods and chordates were more abundant in sea urchin patches. Molluscs showed a more even distribution across all patches, though no clear pattern emerged. Echinoderms were restricted to sea urchin patches, with only a single exception in the *Caulerpa*-invaded areas. In addition to *C. prolifera*, the presence of another invasive species, *Rugulopteryx okamurae*, in the sea urchin patches complicates the assessment of biodiversity shifts, as overlapping of two invasions makes it difficult to isolate the impact of any single invasive species. The lack of data about flora and fauna prior to the invasions makes it challenging to fully assess the impact of the invasive macroalgae. Additionally, a shell layer was not observed in any of the patches. The reason for its absence remains unclear but could be attributed to the invasions of the macroalgae or the location of the patches exposing them to different hydrodynamics, compared to patches from previous years. In September 2024, new observations from diving surveys conducted by the same team for a new project revealed that the patches had shifted location. According to their observations, the patches shifted their location or disappeared. The sea urchin patches were found, using the labelled metal sticks of this survey as a reference. Only in two sea urchin patches the tagged metal stick still remained inside them, in the other case the patches shifted to such a degree, that the sticks were found in close proximity to the patch on the unvegetated bottom, or the patch disappeared. This opens further questions about whether the lack of shells is a result of macroalgal invasion, making the patches more vulnerable to hydrodynamic and resulting in shifts, or if the patches had never formed on a shell layer. The shell layer might have not accumulated due to the hydrodynamics and the patches started on the unvegetated bottom. In this case, the shell absence would not be linked to the invasions. A possible explanation for the disappearance of some patches is the increased boat traffic during the summer season in the lagoon. Small channels in the Sediment, created by anchors dragging along the bottom were frequently observed during the September survey. If an anchor were to be dragged across a patch, it could potentially destroy the entire habitat. The results and their analysis demonstrate a larger issue, the limited data for this specific habitat in Ria Formosa, making it very challenging to fully understand the long-term impact of these invasions. Furthermore, this highlights the urgent need for continued monitoring and further research to fully grasp the extent of the ecological changes caused by invasive species. Future research should consider sessional changes in the patches and imply both spatial and temporal sampling strategies, as the patches are not only influenced by a changing ecosystem dynamic, but also by varying abiotic factors. Studies from other regions have shown the importance of temporal observation, due to abiotic factors, such as epidemic storms and seasonal change in nutrients (Turon *et al.* 1995). The sea urchin patches exhibit a highly reduced activity during winter and a reassembly of fauna and flora in spring, according to the team's experiences and observations from previous dives. The impact of this seasonal fluctuation on biodiversity remains unclear, as does the combined long-term effect of the impact of invasive algae and seasonal change. Understanding the impact of invasive species in native ecosystems is most important for the conservation and management of Ria Formosa, as the presence of species like *C. prolifera* and *R. okamurae* can have severe consequences on local biodiversity, ecosystem function, and community structure.

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8. Annex

Table A 1: Location of each sea urchin patch (S1 to S7) and Caulerpa invaded patches (C1 to C3) corresponds to a measured coordinate. The coordinates are provided in the WGS 84 system and in the UTM system.

Patch	WGS 84		UTM (zone: 29S)	
	North (Latitude)	West (Longitude)	East	North
S1	37°00'20.13516	7°49'05.85295	605138.102	4096145.458
S2	37°00'19.97844	7°49'03.14042	605205.159	4096141.52
S3	37°00'19.69186	7°49'04.05366	605182.67	4096132.364
S4	37°00'18.90090	7°49'04.63985	605168.47	4096107.777
S5	37°00'18.95870	7°49'04.12793	605181.171	4096109.71
S6	37°00'14.40277	7°49'06.28817	605129.528	4095968.708
S7	37°00'13.81819	7°49'08.05093	605086.154	4095950.084
C1	37°00'17.45981	7°49'02.89077	605212.263	4096063.938
C2	37°00'17.63804	7°49'02.73411	605216.111	4096069.423
C3	37°00'13.71841	7°49'06.97062	605112.884	4095947.42

Table A 2:Underwater measures used to recreate the shape of the sea urchin patches (S1 to S4).

S1 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	230	2.3	10
2	218	2.18	40
3	245	2.45	80
4	216	2.16	120
5	311	3.11	130
6	310	3.1	145
7	358	3.58	160
8	278	2.78	165
9	287	2.87	220
10	295	2.95	240
11	315	3.15	260
12	340	3.4	280
13	372	3.72	320
14	315	3.15	323
15	300	3	325
16	220	2.2	0
Circle A	65	0.65	340
Circle B	295	2.95	150
Circle C	220	2.2	140

S2 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	110	1.1	150
2	130	1.3	110
3	150	1.5	90
4	190	1.9	80
5	171	1.71	60
6	216	2.16	50
7	208	2.08	45
8	161	1.61	15
9	182	1.82	0
10	193	1.93	310
11	169	1.69	300
12	199	1.99	270
13	146	1.46	250
14	154	1.54	230
15	130	1.3	210
16	131	1.31	185
Circle A	160	1.6	45
Circle B	102	1.02	300
Circle C	25	0.25	0

S3 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	100	1	0
2	120	1.2	40
3	90	0.9	70
4	80	0.8	110
5	80	0.8	170
6	90	0.9	180
7	92	0.92	200
8	80	0.8	225
9	60	0.6	290
10	80	0.8	340
11	95	0.95	350
Circle A	65	0.65	230
Circle B	51	0.51	55
Circle C	25	0.25	140

S4 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	130	1.3	0
2	170	1.7	40
3	205	2.05	52
4	220	2.2	65
5	170	1.7	100
6	102	1.02	120
7	110	1.1	220
8	150	1.5	225
9	90	0.9	230
10	90	0.9	320
11	130	1.3	325
12	106	1.06	350
Circle A	75	0.75	220
Circle B	80	0.8	120
Circle C	115	1.15	65

Table A 3: Underwater measures used to recreate the shape of the sea urchin patches (S5 to S7).

S5 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	240	2.4	0
2	260	2.6	20
3	240	2.4	25
4	220	2.2	40
5	190	1.9	80
6	150	1.5	130
7	230	2.3	150
8	280	2.8	155
9	290	2.9	160
10	370	3.7	165
11	460	4.6	180
12	400	4	200
13	390	3.9	210
14	430	4.3	220
15	370	3.7	240
16	390	3.9	270
17	410	4.1	280
18	320	3.2	285
19	200	2	290
20	240	2.4	320
21	280	2.8	340
Circle A	180	1.8	240
Circle B	160	1.6	300
Circle C	180	1.8	320

S7 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	230	2.3	0
2	220	2.2	50
3	190	1.9	90
4	130	1.3	120
5	170	1.7	190
6	280	2.8	220
7	260	2.6	250
8	380	3.8	260
9	100	1	310
10	210	2.1	350
Circle A	308	3.08	260
Circle B	160	1.6	50
Circle C	30	0.3	100

S6 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	200	2	0
2	210	2.1	13
3	255	2.55	30
4	240	2.4	55
5	215	2.15	88
6	190	1.9	130
7	160	1.6	170
8	190	1.9	190
9	200	2	220
10	370	3.7	230
11	400	4	232
12	400	4	255
13	620	6.2	258
14	770	7.7	260
15	1040	10.4	262
16	1130	11.3	272
17	1730	17.3	274
18	1920	19.2	284
19	2200	22	286
20	2640	26.4	290
21	2830	28.3	310
22	2060	20.6	318
23	1710	17.1	320
24	1390	13.9	324
25	800	8	330
Circle A	308	3.08	330
Circle B	160	1.6	160
Circle C	30	0.3	230

Table A 4: Underwater measures used to recreate the shape of the Caulerpa invaded patches (C1 to C3).

C1 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	90	0.9	180
2	145	1.45	140
3	80	0.8	120
4	170	1.7	100
5	150	1.5	80
6	85	0.85	70
7	85	0.85	30
8	110	1.1	20
9	120	1.2	340
10	120	1.2	290
11	150	1.5	270
12	80	0.8	260
13	60	0.6	220
Circle A	100	1	340
Circle B	38	0.38	220
Circle C	25	0.25	100

C3 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	180	1.8	0
2	300	3	30
3	340	3.4	40
4	280	2.8	48
5	150	1.5	52
6	70	0.7	90
7	130	1.3	180
8	140	1.4	220
9	120	1.2	240
10	90	0.9	300
11	80	0.8	340
Circle A	100	1	210
Circle B	70	0.7	280
Circle C	50	0.5	350

C2 underwater measures			
Number	Distance [cm]	Distance [m]	Compass angle [°]
1	30	0.3	240
2	100	1	180
3	110	1.1	170
4	130	1.3	140
5	105	1.05	130
6	55	0.55	120
7	50	0.5	80
8	57	0.57	40
9	140	1.4	25
10	180	1.8	0
11	250	2.5	340
12	150	1.5	320
13	190	1.9	300
14	280	2.8	290
15	70	0.7	340
16	35	0.35	280
Circle A	110	1.1	340
Circle B	60	0.6	180
Circle C	10	0.1	350

Table A 5: Sampled flora for the sea urchin patches S1 to S7 and for the Caulerpa invaded patches C1 to C3 are listed

Patch	Circle	Species name	Weight (g)
S1	A	*Rugulopteryx okamurae*	106
S1	A	dead sea grass	
S1	B	*Rugulopteryx okamurae*	146
S2	B	dead sea grass	
S1	B	*Caulerpa prolierfa*	10
S1	C	*Rugulopteryx okamurae*	124
S1	C	dead sea grass	
S2	A	*Rugulopteryx okamurae*	160
S2	A	dead sea grass	
S2	B	*Rugulopteryx okamurae*	260
S2	B	dead sea grass	
S2	B	*Caulerpa prolierfa*	10
S2	C	*Rugulopteryx okamurae*	310
S2	C	*dead sea grass*	
S3	A	*Rugulopteryx okamurae*	142
S3	A	dead sea grass	
S3	A	*Caulerpa prolierfa*	10
S3	B	*Rugulopteryx okamurae*	85
S3	B	dead sea grass	
S3	B	*Caulerpa prolierfa*	10
S3	C	*Rugulopteryx okamurae*	214
S3	C	dead sea grass	
S3	C	*Codium sp.*	10
S3	C	*Caulerpa prolierfa*	10
S4	A	*Rugulopteryx okamurae*	140
S4	A	dead sea grass	
S4	A	*Caulerpa prolierfa*	10
S4	B	*Rugulopteryx okamurae*	220
S4	B	dead sea grass	
S4	B	*Caulerpa prolierfa*	10
S4	B	*Codium sp.*	10
S4	C	*Rugulopteryx okamurae*	390
S4	C	dead sea grass	
S4	C	*Caulerpa prolierfa*	10
S5	A	*Rugulopteryx okamurae*	155
S5	A	dead sea grass	
S5	A	*Caulerpa prolierfa*	10
S5	A	*Ulva sp.*	10
S5	B	*Rugulopteryx okamurae*	210
S5	B	dead sea grass	
S5	C	*Rugulopteryx okamurae*	205
S5	C	dead sea grass	
S5	C	*Caulerpa prolierfa*	10
S6	A	*Rugulopteryx okamurae*	240
S6	A	dead sea grass	
S6	B	*Rugulopteryx okamurae*	230
S6	B	dead sea grass	
S6	B	*Caulerpa prolifera*	<10
S6	C	*Rugulopteryx okamurae*	230
S6	C	dead sea grass	
S6	C	*Caulerpa prolifera*	<10
S7	A	*Rugulopteryx okamurae*	240
S7	A	dead sea grass	
S7	A	*Caulerpa profilera*	<10
S7	A	*Codium sp.*	<10
S7	B	*Rugulopteryx okamurae*	135
S7	B	dead sea grass	
S7	B	*Caulerpa prolifera*	<10
S7	C	*Rugulopteryx okamurae*	350
S7	C	dead sea grass	
S7	C	*Ulva sp*	<5
C1	A	*Rugulopteryx okamurae*	40
C1	A	dead sea grass	
C1	A	*Caulerpa prolierfa*	50
C1	B	*Rugulopteryx okamurae*	185
C1	B	dead sea grass	
C1	B	*Caulerpa prolierfa*	<20
C1	C	*Rugulopteryx okamurae*	35
C1	C	dead sea grass	
C1	C	*Caulerpa prolifera*	35
C2	A	*Caulerpa prolierfa*	45
C2	A	*Rugulopteryx okamurae*	<10
C2	A	dead sea grass	
C2	B	*Caulerpa prolifera*	85
C2	B	*Rugulopteryx okamurae*	<10
C2	B	dead sea grass	
C2	C	*Caulerpa prolifera*	140
C2	C	dead sea grass	
C3	A	*Caulerpa*	60
C3	A	*Rugulopteryx okamurae*	60
C3	A	dead sea grass	
C3	B	*Caulerpa prolifera*	78
C3	C	*Caulerpa prolifera*	169

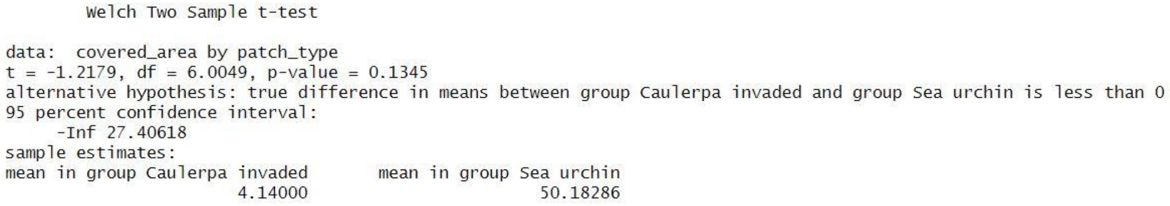
Table A 6: The Pivot table, based on the data set from the diving surveys, with the total number of sampled organisms for each phylum, class and lower taxonomic level is presented.

Sum of Number	Column Labels																														
	C1			C2			C3			S1			S2			S3			S4			S5			S6			S7			Gra
Row Labels	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Annelida	**6**		**10**	**1**		**1**	**2**	**1**	**1**													**1**									
Polychaeta	**6**		**10**	**1**		**1**	**2**	**1**	**1**													**1**									
Aphroditidae																						1									
Euploymnia nebulosa									1																						
Nepthys hombergii				1		1																									
Notomastus latericeus	6		10				2	1																							
Arthropoda		**4**		**1**			**2**			**1**		**17**	**13**		**17**	**14**			**8**	**16**	**17**	**2**	**21**	**32**	**19**	**2**	**11**	**8**	**7**	**4**	
Crustacean		**3**		**1**			**2**					**17**	**13**		**16**	**14**			**8**	**16**	**17**	**2**	**21**	**32**	**19**	**2**	**11**	**8**	**7**	**4**	
Amphipod		3										10	10		15	12			5	15	15			10	15		10	5	5		
Brachyura				1								7	3		1	2			3	1	2	1		7	1	2		2			
Carcinus maenas																							1		3		1	1	2	4	
Caridea																							20	15							
Majidae							1																								
Stenorhynchus lanceolatus							1															1									
Crustacean/Hermit crab		**1**								**1**					**1**																
Clibanarius erythropus										1																					
Pagurus sp.		1													1																
Chordata		**5**								**3**	**16**	**12**	**4**	**5**	**4**	**7**	**1**		**3**	**1**	**1**	**4**	**2**		**1**			**1**			
Actinopterygii		**1**										**1**					**1**			**1**		**1**									
Gobidae		1																		1											
Hippocampus guttulatus												1					1					1									
Ascidian		**3**								**3**	**16**	**11**	**4**	**5**	**4**	**7**			**2**		**1**	**3**	**2**		**1**			**1**			
Microcosmus squamiger		2								2	6	7	3	3	2	4			1			3	2		1			1			
Phallusia mammilata		1								1	10	2	1			1			1		1										
Styela plicata												2		2	2	2															
Batoidea		**1**																	**1**												
ray egg		1																	1												
Cnidaria		**1**																													
Actiniaria		**1**																													
Actiniaria n.id.		1																													
Echinodermata		**1**								**3**	**2**	**2**	**4**	**2**	**2**	**4**	**6**	**2**	**6**		**2**	**5**	**8**	**1**	**3**	**1**	**1**	**2**	**1**	**4**	
Echinodermata																										**1**					
Asterias rubens																										1					
Holothuroidea (Sea cucumber)												**1**		**2**	**1**	**1**	**1**		**2**		**1**		**1**	**1**						**1**	
Holothuria mammata												1		2	1	1	1		2		1		1	1						1	
Sea urchin		**1**								**3**	**2**	**1**	**4**		**1**	**3**	**5**	**2**	**4**		**1**	**5**	**7**		**3**		**1**	**2**	**1**	**3**	
Paracentrotus lividus										2	2	1			1	1	2		4			3	7					1			
S. granularis		1								1			4			2	3	2			1	2			3		1	1	1	3	
Mollusca	**20**	**12**	**9**	**11**	**12**	**12**	**31**	**24**	**33**	**73**	**2**	**2**	**62**	**4**	**6**	**45**	**39**	**4**	**96**	**43**	**87**	**33**	**19**	**8**	**24**	**68**	**25**	**20**	**71**	**52**	
Bivalve				**2**	**1**		**4**	**5**	**4**				**2**				**1**	**2**			**2**	**1**	**2**	**2**	**3**		**2**		**1**		
Cerastoderma edule																		1			2	1			3		1		1		
Loripes lacteus				2	1			3	2																						
R. decussata																	1						2								
Tellina sp.																								1							
Venerupis corrugata							4	2	2				2					1						1			1				
Cephalopod																			**15**												
Sepia egg																			15												
Gastropod	**20**	**12**	**9**	**9**	**11**	**12**	**27**	**19**	**29**	**73**	**2**	**2**	**60**	**4**	**6**	**44**	**38**	**2**	**81**	**43**	**85**	**32**	**17**	**6**	**21**	**68**	**23**	**20**	**70**	**52**	
Bittium reticulatum	20	10	5	5	3	5	25	15	20	70			50			40	35		80	40	80	25	10	2	10	60	20	5	60	50	
Calliostoma sp.							1		3																						
Cerithium vulgatum								4	1				4	2	2			2				1			1	1	1	1			
Gibbula sp.		2		1	4								4			3	2			2			2	1			1	2			
Hexaplex trunculus										3	2	2	1	2					1	1	1		1							1	
Jujubinus sp.			1	2	4	3	1		4													3		1	10	7	1	10	10	1	
Tritia reticulata			3	1		4			1				1		4	1	1				4	3	4	2				2			
Polyplacophora																**1**															
Chaetopleura angulata																1															
Grand Total	**26**	**23**	**19**	**13**	**12**	**13**	**35**	**25**	**34**	**80**	**20**	**33**	**83**	**11**	**29**	**70**	**46**	**6**	**113**	**60**	**107**	**45**	**50**	**41**	**47**	**71**	**37**	**31**	**79**	**60**	

Table A 7: The table represents the morphological measures of each sampled sea urchin. Each sea urchin is referenced with the corresponding patch and circle number.

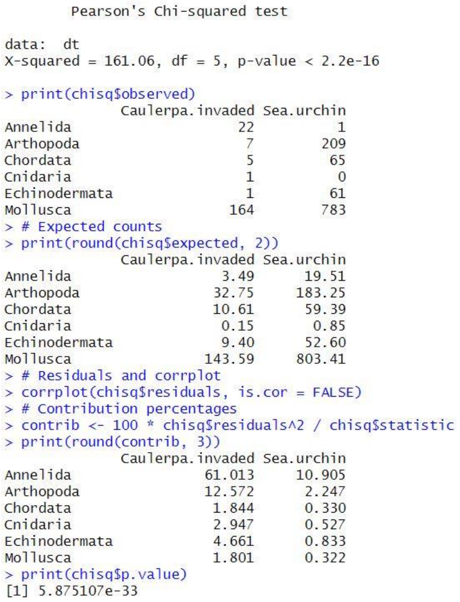
Patch	Circle	Species	Diameter (mm)	Height (mm)	Weight (g)
S1	A	Paracentrotus lividus	52.55	26.8	48
S1	A	Paracentrotus lividus	38.5	21.3	18
S1	B	Paracentrotus lividus	44.85	26.26	36
S1	B	Paracentrotus lividus	36.69	20.34	23
S1	C	Paracentrotus lividus	49.8	26.5	45
S2	C	Paracentrotus lividus	48.1	23.05	36
S3	A	Paracentrotus lividus	47.79	22.47	43
S3	B	Paracentrotus lividus	39.7	29.9	22
S3	B	Paracentrotus lividus	40.19	24.46	25
S4	A	Paracentrotus lividus	59.16	28.78	76
S4	A	Paracentrotus lividus	50.59	25.6	55
S4	A	Paracentrotus lividus	46.49	24.21	38
S4	A	Paracentrotus lividus	41.76	21.87	29
S5	A	Paracentrotus lividus	54.84	32.22	66
S5	A	Paracentrotus lividus	51.88	23.04	32
S5	A	Paracentrotus lividus	45.11	24.97	23
S5	B	Paracentrotus lividus	47.42	27.02	36
S5	B	Paracentrotus lividus	44.64	30.02	35
S5	B	Paracentrotus lividus	48.31	23.34	38
S5	B	Paracentrotus lividus	52.65	32.63	55
S5	B	Paracentrotus lividus	42.76	20.61	28
S5	B	Paracentrotus lividus	46.93	23.81	41
S5	B	Paracentrotus lividus	38.29	18.74	23
S7	A	Paracentrotus lividus	45.02	26.52	40
C1	B	S. granularis	76.5	46.07	
S1	A	S. granularis	67.96	14.22	125
S2	A	S. granularis	74.51	51.44	170
S2	A	S. granularis	66.2	42.02	42.02
S2	A	S. granularis	63.46	42.16	42.16
S2	A	S. granularis	61.77	38	38
S3	A	S. granularis	79.62	47.43	195
S3	A	S. granularis	70.64	47.83	153
S3	B	S. granularis	84.48	54.38	208
S3	B	S. granularis	60.13	38.65	96
S3	B	S. granularis	55.23	36.96	65
S3	C	S. granularis	69.94	42.66	112
S3	C	S. granularis	68.68	39.89	102
S4	C	S. granularis	78.78	51.11	144
S5	A	S. granularis	67.23	39.3	125
S5	A	S. granularis	71.89	48.26	139
S6	A	S. granularis	81.54	48.07	205
S6	A	S. granularis	88.58	56.76	240
S6	A	S. granularis	84.29	78.66	170
S6	C	S. granularis	80.97	56.77	208
S7	A	S. granularis	69.61	40.67	115
S7	B	S. granularis	70.91	45.77	120
S7	C	S. granularis	80.86	49.16	148
S7	C	S. granularis	70.26	42.47	95
S7	C	S. granularis	58.65	36.43	63

Table A 8: A one-sided T-Test was preformed (R Core Team. 2024). The covered areas of the two patch types, Caulerpa-*invaded patches and sea urchin patches were tested for statistical differences.*

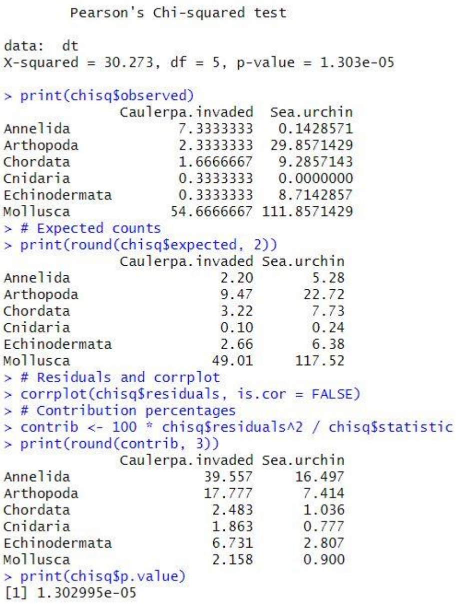


```
        Welch Two Sample t-test

data:  covered_area by patch_type
t = -1.2179, df = 6.0049, p-value = 0.1345
alternative hypothesis: true difference in means between group Caulerpa invaded and group Sea urchin is less than 0
95 percent confidence interval:
      -Inf 27.40618
sample estimates:
mean in group Caulerpa invaded       mean in group Sea urchin
                       4.14000                       50.18286
```

Table A 9: Chi-squared tests were performed (R Core Team. 2024). The right table represents the contrast between the average phylum count of the Caulerpa-*invaded patches and the sea urchin patches. The left table shows the difference of the sum of the phylum count for both patch types.*

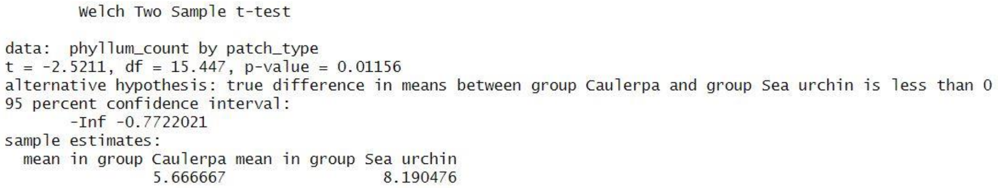


```
        Pearson's Chi-squared test

data:  dt
X-squared = 161.06, df = 5, p-value < 2.2e-16

> print(chisq$observed)
              Caulerpa.invaded Sea.urchin
Annelida                    22          1
Arthopoda                    7        209
Chordata                     5         65
Cnidaria                     1          0
Echinodermata                1         61
Mollusca                   164        783
> # Expected counts
> print(round(chisq$expected, 2))
              Caulerpa.invaded Sea.urchin
Annelida                  3.49      19.51
Arthopoda                32.75     183.25
Chordata                 10.61      59.39
Cnidaria                  0.15       0.85
Echinodermata             9.40      52.60
Mollusca                143.59     803.41
> # Residuals and corrplot
> corrplot(chisq$residuals, is.cor = FALSE)
> # Contribution percentages
> contrib <- 100 * chisq$residuals^2 / chisq$statistic
> print(round(contrib, 3))
              Caulerpa.invaded Sea.urchin
Annelida                61.013     10.905
Arthopoda               12.572      2.247
Chordata                 1.844      0.330
Cnidaria                 2.947      0.527
Echinodermata            4.661      0.833
Mollusca                 1.801      0.322
> print(chisq$p.value)
[1] 5.875107e-33
```



```
        Pearson's Chi-squared test

data:  dt
X-squared = 30.273, df = 5, p-value = 1.303e-05

> print(chisq$observed)
              Caulerpa.invaded  Sea.urchin
Annelida             7.3333333   0.1428571
Arthopoda            2.3333333  29.8571429
Chordata             1.6666667   9.2857143
Cnidaria             0.3333333   0.0000000
Echinodermata        0.3333333   8.7142857
Mollusca            54.6666667 111.8571429
> # Expected counts
> print(round(chisq$expected, 2))
              Caulerpa.invaded Sea.urchin
Annelida                  2.20       5.28
Arthopoda                 9.47      22.72
Chordata                  3.22       7.73
Cnidaria                  0.10       0.24
Echinodermata             2.66       6.38
Mollusca                 49.01     117.52
> # Residuals and corrplot
> corrplot(chisq$residuals, is.cor = FALSE)
> # Contribution percentages
> contrib <- 100 * chisq$residuals^2 / chisq$statistic
> print(round(contrib, 3))
              Caulerpa.invaded Sea.urchin
Annelida                39.557     16.497
Arthopoda               17.777      7.414
Chordata                 2.483      1.036
Cnidaria                 1.863      0.777
Echinodermata            6.731      2.807
Mollusca                 2.158      0.900
> print(chisq$p.value)
[1] 1.302995e-05
```

Table A 10: A one-sided T-Test was preformed (R Core Team. 2024). The test analysed a significant difference between the specie richness of the Caulerpa-*invaded and the sea urchin patches.*



```
        Welch Two Sample t-test

data:  phyllum_count by patch_type
t = -2.5211, df = 15.447, p-value = 0.01156
alternative hypothesis: true difference in means between group Caulerpa and group Sea urchin is less than 0
95 percent confidence interval:
       -Inf -0.7722021
sample estimates:
  mean in group Caulerpa mean in group Sea urchin
                5.666667                 8.190476
```

Table A 11: The results of the analysis from the grain size measurements are present, using the Folk and Ward Method (Folk & Ward, 1957) in the Excel extension developed by Blott (2010). Grain size measurements were gathered using the Malvern Instrument Mastersizer Mirco Version 2.18.

		C1-A	C1-B	C1-C	C3-A	C3-B	C3-C
	SAMPLE TYPE:	Unimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted
	TEXTURAL GROUP:	Sandy Mud	Sandy Mud	Sandy Mud	Muddy Sand	Muddy Sand	Sandy Mud
	SEDIMENT NAME:	Very Fine Sandy Very Coarse Silt	Very Fine Sandy Coarse Silt	Very Fine Sandy Coarse Silt	Very Coarse Silty Fine Sand	Very Coarse Silty Fine Sand	Fine Sandy Coarse Silt
FOLK AND WARD METHOD (μm)	MEAN ($\bar{x}_a$):	46.05	40.93	36.51	57.58	55.61	38.23
	SORTING (σ_G):	3.274	3.559	3.482	3.504	3.608	3.930
	SKEWNESS (Sk_G):	-0.141	-0.082	-0.033	-0.234	-0.228	-0.062
	KURTOSIS (K_G):	0.911	0.868	0.893	0.822	0.826	0.836
FOLK AND WARD METHOD (Description)	MEAN: (M_Z):	Very Coarse Silt	Very Coarse Silt	Very Coarse Silt	Very Coarse Silt	Very Coarse Silt	Very Coarse Silt
	SORTING:	Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted
	SKEWNESS:	Fine Skewed	Symmetrical	Symmetrical	Fine Skewed	Fine Skewed	Symmetrical
	KURTOSIS:	Mesokurtic	Platykurtic	Platykurtic	Platykurtic	Platykurtic	Platykurtic
	MODE 1 (μm):	96.24	130.6	24.35	177.3	177.3	177.3
	MODE 2 (μm):		24.35	96.24	33.04	33.04	20.90
	MODE 3 (μm):						
	% GRAVEL:	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	% SAND:	43.1%	39.8%	35.3%	51.7%	50.8%	38.9%
	% MUD:	56.9%	60.2%	64.7%	48.3%	49.2%	61.1%

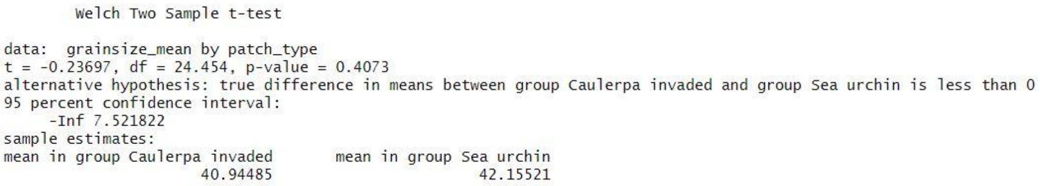
S4-A	S4-B	S4-C	S7-A	S7-B	S7-C
Bimodal, Poorly Sorted	Unimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted
Sandy Mud	Sandy Mud	Sandy Mud	Sandy Mud	Sandy Mud	Muddy Sand
Very Fine Sandy Coarse Silt	Very Fine Sandy Very Coarse Silt	Very Fine Sandy Very Coarse Silt	Very Fine Sandy Very Coarse Silt	Fine Sandy Very Coarse Silt	Coarse Silty Fine Sand
40.94	39.85	45.59	43.52	45.32	78.69
3.579	3.560	3.340	3.472	3.544	3.502
-0.093	-0.128	-0.075	-0.097	-0.124	-0.483
0.873	0.917	0.864	0.867	0.851	0.814
Very Coarse Silt	Very Coarse Silt	Very Coarse Silt	Very Coarse Silt	Very Coarse Silt	Very Fine Sand
Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted
Symmetrical	Fine Skewed	Symmetrical	Symmetrical	Fine Skewed	Very Fine Skewed
Platykurtic	Mesokurtic	Platykurtic	Platykurtic	Platykurtic	Platykurtic
130.6	96.24	130.6	130.6	152.2	240.6
28.36		28.36	28.36	28.36	28.36
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
39.9%	39.2%	41.9%	41.5%	43.4%	62.2%
60.1%	60.8%	58.1%	58.5%	56.6%	37.8%

S6-A	S6-B	S6-C	S5-A	S5-B	S5-C
Bimodal, Poorly Sorted	Bimodal, Poorly Sorted	Unimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted	Bimodal, Poorly Sorted
Sandy Mud	Muddy Sand	Sandy Mud	Sandy Mud	Muddy Sand	Sandy Mud
Very Fine Sandy Very Coarse Silt	Very Coarse Silty Fine Sand	Very Fine Sandy Very Coarse Silt	Fine Sandy Coarse Silt	Very Coarse Silty Fine Sand	Fine Sandy Very Coarse Silt
38.00	64.89	45.53	54.00	76.83	50.19
3.709	3.631	3.527	3.543	3.279	3.404
-0.108	-0.297	-0.145	-0.218	-0.452	-0.162
0.906	0.807	0.877	0.818	0.860	0.852
Very Coarse Silt	Very Fine Sand	Very Coarse Silt	Very Coarse Silt	Very Fine Sand	Very Coarse Silt
Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted	Poorly Sorted
Fine Skewed	Fine Skewed	Fine Skewed	Fine Skewed	Very Fine Skewed	Fine Skewed
Mesokurtic	Platykurtic	Platykurtic	Platykurtic	Platykurtic	Platykurtic
28.36	240.6	130.6	177.3	206.5	152.2
112.1	38.50		28.36	28.36	33.04
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
38.0%	55.2%	43.6%	50.0%	63.5%	46.7%
62.0%	44.8%	56.4%	50.0%	36.5%	53.3%

S2-C	C2-A	C2-B	C2-C	S3-A	S3-B
Bimodal, Very Poorly Sorted	Unimodal, Very Poorly Sorted	Bimodal, Very Poorly Sorted	Unimodal, Very Poorly Sorted	Unimodal, Very Poorly Sorted	Bimodal, Very Poorly Sorted
Sandy Mud	Sandy Mud	Sandy Mud	Sandy Mud	Sandy Mud	Sandy Mud
Fine Sandy Coarse Silt	Very Fine Sandy Coarse Silt	Fine Sandy Coarse Silt	Very Fine Sandy Coarse Silt	Very Fine Sandy Coarse Silt	Very Fine Sandy Coarse Silt
31.18	21.73	29.70	25.16	24.29	19.96
4.628	4.077	4.497	4.103	4.121	4.441
-0.082	-0.078	-0.039	-0.067	-0.063	0.026
0.858	0.994	0.885	0.992	0.973	0.994
Coarse Silt	Coarse Silt	Coarse Silt	Coarse Silt	Coarse Silt	Coarse Silt
Very Poorly Sorted	Very Poorly Sorted	Very Poorly Sorted	Very Poorly Sorted	Very Poorly Sorted	Very Poorly Sorted
Symmetrical	Symmetrical	Symmetrical	Symmetrical	Symmetrical	Symmetrical
Platykurtic	Mesokurtic	Platykurtic	Mesokurtic	Mesokurtic	Mesokurtic
177.3	24.35	24.35	28.36	24.35	17.94
28.36		177.3			130.6
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
34.9%	22.8%	32.1%	26.0%	25.8%	22.3%
65.1%	77.2%	67.9%	74.0%	74.2%	77.7%

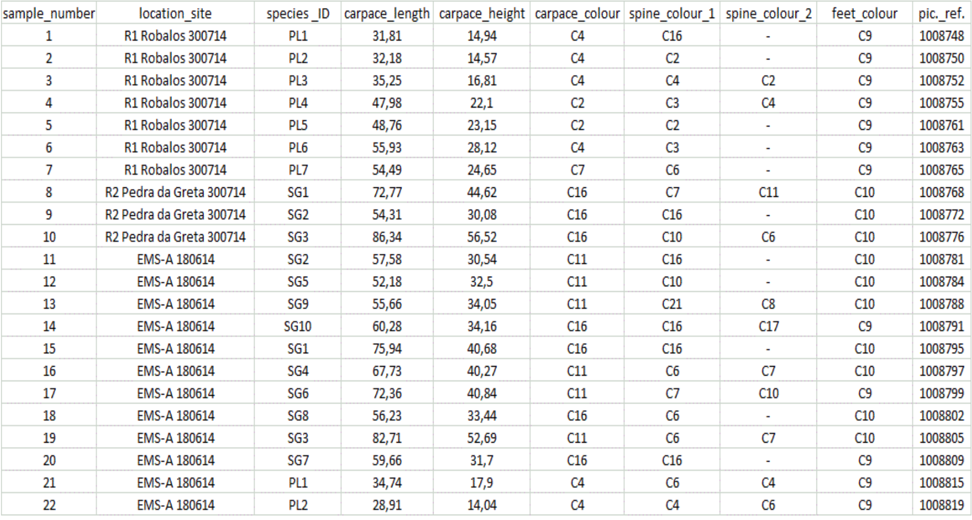
S1-A	S1-C	S1-B
Bimodal, Very Poorly Sorted	Bimodal, Very Poorly Sorted	Bimodal, Very Poorly Sorted
Sandy Mud	Sandy Mud	Sandy Mud
Very Fine Sandy Coarse Silt	Fine Sandy Coarse Silt	Fine Sandy Coarse Silt
25.70	39.53	38.61
4.283	4.564	4.395
-0.009	-0.142	-0.158
0.926	0.789	0.841
Coarse Silt	Very Coarse Silt	Very Coarse Silt
Very Poorly Sorted	Very Poorly Sorted	Very Poorly Sorted
Symmetrical	Fine Skewed	Fine Skewed
Mesokurtic	Platykurtic	Platykurtic
20.90	206.5	177.3
130.6	24.35	28.36
0.0%	0.0%	0.0%
28.3%	43.4%	41.9%
71.7%	56.6%	58.1%

Table A 12: A one-sided T-Test was preformed (R Core Team. 2024). The test analysed a significant difference between the mean grain size values of the Caulerpa*-invaded and the sea urchin patches.*



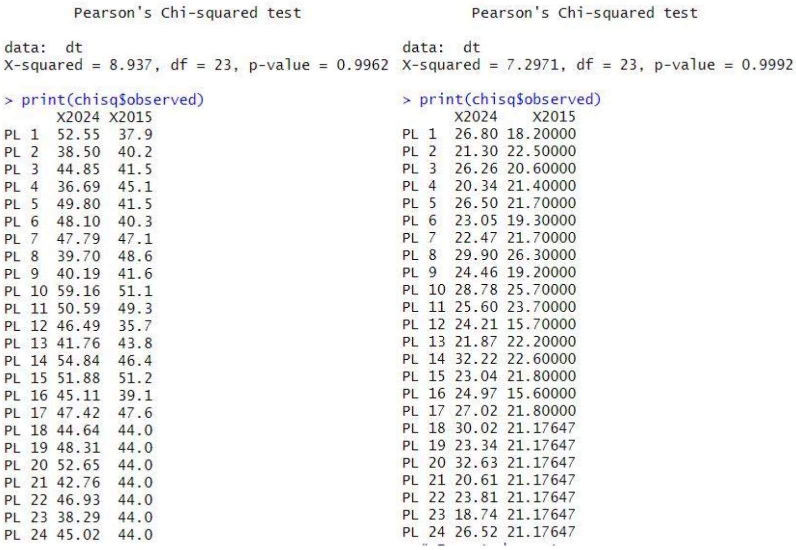
```
	Welch Two Sample t-test

data:  grainsize_mean by patch_type
t = -0.23697, df = 24.454, p-value = 0.4073
alternative hypothesis: true difference in means between group Caulerpa invaded and group Sea urchin is less than 0
95 percent confidence interval:
     -Inf 7.521822
sample estimates:
mean in group Caulerpa invaded       mean in group Sea urchin
                      40.94485                       42.15521
```

Table A 13: The different biometric measures of the sea urchin species P. lividus *are displayed in the following, along the location site and colour. The table is based on the team's work of previous years, from Fragkopoulou 2016. The table is used for a comparison of the data from this year's work and the data from 2016.*



sample_number	location_site	species _ID	carpace_length	carpace_height	carpace_colour	spine_colour_1	spine_colour_2	feet_colour	pic._ref.
1	R1 Robalos 300714	PL1	31,81	14,94	C4	C16	-	C9	1008748
2	R1 Robalos 300714	PL2	32,18	14,57	C4	C2	-	C9	1008750
3	R1 Robalos 300714	PL3	35,25	16,81	C4	C4	C2	C9	1008752
4	R1 Robalos 300714	PL4	47,98	22,1	C2	C3	C4	C9	1008755
5	R1 Robalos 300714	PL5	48,76	23,15	C2	C2	-	C9	1008761
6	R1 Robalos 300714	PL6	55,93	28,12	C4	C3	-	C9	1008763
7	R1 Robalos 300714	PL7	54,49	24,65	C7	C6	-	C9	1008765
8	R2 Pedra da Greta 300714	SG1	72,77	44,62	C16	C7	C11	C10	1008768
9	R2 Pedra da Greta 300714	SG2	54,31	30,08	C16	C16	-	C10	1008772
10	R2 Pedra da Greta 300714	SG3	86,34	56,52	C16	C10	C6	C10	1008776
11	EMS-A 180614	SG2	57,58	30,54	C11	C16	-	C10	1008781
12	EMS-A 180614	SG5	52,18	32,5	C11	C10	-	C10	1008784
13	EMS-A 180614	SG9	55,66	34,05	C11	C21	C8	C10	1008788
14	EMS-A 180614	SG10	60,28	34,16	C16	C16	C17	C9	1008791
15	EMS-A 180614	SG1	75,94	40,68	C16	C16	-	C10	1008795
16	EMS-A 180614	SG4	67,73	40,27	C11	C6	C7	C10	1008797
17	EMS-A 180614	SG6	72,36	40,84	C11	C7	C10	C9	1008799
18	EMS-A 180614	SG8	56,23	33,44	C16	C6	-	C10	1008802
19	EMS-A 180614	SG3	82,71	52,69	C11	C6	C7	C10	1008805
20	EMS-A 180614	SG7	59,66	31,7	C16	C16	-	C9	1008809
21	EMS-A 180614	PL1	34,74	17,9	C4	C6	C4	C9	1008815
22	EMS-A 180614	PL2	28,91	14,04	C4	C4	C6	C9	1008819

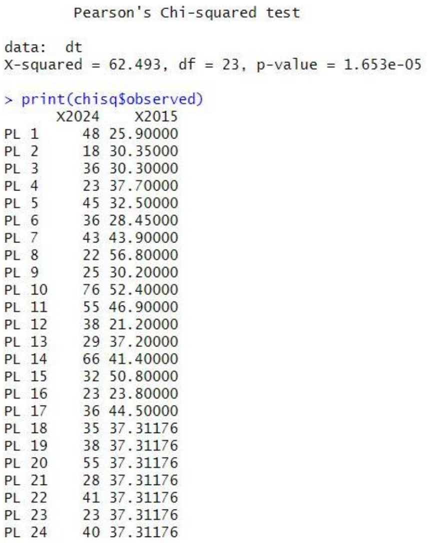
Table A 14: Chi-squared tests were performed (R Core Team. 2024). The measurements of P. lividus *data from Fragkopoulou 2016 and this work, 2024, were tested. The tests analysed significant differences in the diameter (left) and height (right) of the sea urchins samples.*



```
	Pearson's Chi-squared test

data:  dt
X-squared = 8.937, df = 23, p-value = 0.9962

> print(chisq$observed)
      X2024 X2015
PL 1  52.55  37.9
PL 2  38.50  40.2
PL 3  44.85  41.5
PL 4  36.69  45.1
PL 5  49.80  41.5
PL 6  48.10  40.3
PL 7  47.79  47.1
PL 8  39.70  48.6
PL 9  40.19  41.6
PL 10 59.16  51.1
PL 11 50.59  49.3
PL 12 46.49  35.7
PL 13 41.76  43.8
PL 14 54.84  46.4
PL 15 51.88  51.2
PL 16 45.11  39.1
PL 17 47.42  47.6
PL 18 44.64  44.0
PL 19 48.31  44.0
PL 20 52.65  44.0
PL 21 42.76  44.0
PL 22 46.93  44.0
PL 23 38.29  44.0
PL 24 45.02  44.0
```

```
	Pearson's Chi-squared test

data:  dt
X-squared = 7.2971, df = 23, p-value = 0.9992

> print(chisq$observed)
      X2024    X2015
PL 1  26.80 18.20000
PL 2  21.30 22.50000
PL 3  26.26 20.60000
PL 4  20.34 21.40000
PL 5  26.50 21.70000
PL 6  23.05 19.30000
PL 7  22.47 21.70000
PL 8  29.90 26.30000
PL 9  24.46 19.20000
PL 10 28.78 25.70000
PL 11 25.60 23.70000
PL 12 24.21 15.70000
PL 13 21.87 22.20000
PL 14 32.22 22.60000
PL 15 23.04 21.80000
PL 16 24.97 15.60000
PL 17 27.02 21.80000
PL 18 30.02 21.17647
PL 19 23.34 21.17647
PL 20 32.63 21.17647
PL 21 20.61 21.17647
PL 22 23.81 21.17647
PL 23 18.74 21.17647
PL 24 26.52 21.17647
```

Table A 15: Chi-squared test was performed (R Core Team. 2024). The weight measurements of P. lividus *data from Fragkopoulou 2016 and this work, 2024, were tested.*



```
	Pearson's Chi-squared test

data:  dt
X-squared = 62.493, df = 23, p-value = 1.653e-05

> print(chisq$observed)
      X2024    X2015
PL 1     48 25.90000
PL 2     18 30.35000
PL 3     36 30.30000
PL 4     23 37.70000
PL 5     45 32.50000
PL 6     36 28.45000
PL 7     43 43.90000
PL 8     22 56.80000
PL 9     25 30.20000
PL 10    76 52.40000
PL 11    55 46.90000
PL 12    38 21.20000
PL 13    29 37.20000
PL 14    66 41.40000
PL 15    32 50.80000
PL 16    23 23.80000
PL 17    36 44.50000
PL 18    35 37.31176
PL 19    38 37.31176
PL 20    55 37.31176
PL 21    28 37.31176
PL 22    41 37.31176
PL 23    23 37.31176
PL 24    40 37.31176
```

Table A 16: The sea urchin patches with their specific short term and the year of observation are listed. Each sea urchin patch corresponds to a specific coordinate, listed in both WGS 84 and UTM system.

		WGS 84		UTM (zone: 29S)	
Patch name	year	North (Latitude)	West (Longitude)	East	North
47BA HS2	2021	37.00347118	7.816527793	605298	4095912
47BA HS1	2020	37.00341733	7.816551105	605296	4095906
47BA HS	2020	37.00344022	7.816134914	605333	4095909
47BA AOUR	2020	37.00341677	7.81649492	605301	4095906
47BA BOUR	2020	37.0033848	7.816000908	605345	4095903
17	2019	37.00341733	7.816551105	605296	4095906
18	2019	37.00316375	7.81643141	605307	4095878
19	2019	37.00343109	7.816123817	605334	4095908
23	2019	37.00313943	7.813093872	605604	4095879
24	2019	37.00319991	7.813733542	605547	4095885
25	2019	37.00337849	7.812663081	605642	4095906
26	2019	37.00344203	7.812707048	605638	4095913
27	2019	37.00269509	7.813730148	605548	4095829
28	2019	37.00334379	7.812798486	605630	4095902
30	2019	37.00361945	7.813322424	605583	4095932
35	2019	37.00380646	7.81309474	605603	4095953
seaurchinspatch	2019	37.00289708	7.814097887	605515	4095851
ben001	2019	37.00339787	7.812797645	605630	4095908
P001	2019	37.00323876	7.8185319	605120	4095884
P002	2019	37.00386833	7.81207104	605694	4095961
P003	2019	37.00343684	7.813089248	605604	4095912
P005	2019	37.00277251	7.816066598	605340	4095835
P006	2019	37.00345644	7.813246287	605590	4095914
P007	2019	37.0035362	7.813110181	605602	4095923
P009	2019	37.00340068	7.813078571	605605	4095908
P010	2019	37.00322933	7.813069997	605606	4095889
P011	2019	37.00322933	7.813069997	605606	4095889
P019	2019	37.00259668	7.815608538	605381	4095816
P008	2019	37.00366464	7.817850978	605180	4095932