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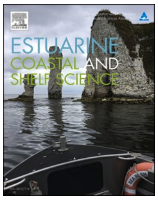

Sea urchins' patches in Ria Formosa coastal lagoon altered under invasion pressure of *Caulerpa prolifera* and *Rugulopteryx okamurae*

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ABSTRACT

This study investigates specific epibenthic community in sea urchin patches from Ria Formosa lagoon, near the Armona Inlet in southern Portugal. These patches are concentrated in distinct spatially isolated areas surrounded by unvegetated, bare sediment bottom and support diverse organisms, dominated by a high density and biomass of *Paracentrotus lividus* and ascidians. Field surveys conducted between 2014 and 2023 revealed a shift in flora composition, with the replacement of native seagrasses and seaweed by the invasive macroalgae *Caulerpa prolifera.* The main aim of this study was to evaluate the effects of this invasive alga in the epibenthic communities of the patches. However, in 2024 surveys, another invasive macroalgae, *Rugulopteryx okamurae,* was detected in the patches. *Sphaerechinus granularis* was the dominant sea urchin species in biomass, in *R. okamurae* patches. In the patches invaded by *Caulerpa prolifera*, sea urchins were generally absent, as were ascidians' species. Faunal analyses indicate a significant decline in macroinvertebrate taxa richness, abundance and biomass within *Caulerpa*-invaded patches, when compared with patches invaded by *R. okamurae*. Non-metric multidimensional scaling, confirm the observed differences between the two invaded patches. The invasion by *R. okamurae* complicates the ecological balance, potentially leading to species replacement and eventual biodiversity loss. Additionally, the disappearance of shell layers in these patches, observed in previous years, raises concerns about habitat stability and resilience. The findings highlight the urgent need for continuous monitoring and management strategies to mitigate the ecological consequences of macroalgal invasions in the Ria Formosa.

1. Introduction

Shallow costal lagoon systems can house extensive seagrass and macroalgae areas, acting as pivotal elements and hosting a variety of fish and invertebrates (Barnes, 1980; De Wit, 2011). Seagrass meadows are among the most productive coastal vegetation types, playing a vital role in sediment stabilization and enrichment and represent one of the most productive types of coastal vegetation (Ward et al., 2022). 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 and Chiscano, 1999; Duarte, 2000). Seagrass meadows are rated as highly valuable ecosystems, due to their great biodiversity and ecological services (Fourqurean et al., 2012; Duffy et al., 2019). These meadows are threatened by the invasive behaviour of some macroalgae species such as *Caulerpa prolifera*. The change towards a *C. prolifera* dominated environment can influence the composition of sediment, since anoxic conditions are created in the sediment that can lead to an increased accumulation of sulphides and herbivory deterrent metabolites (Duffy et al., 2019).

Seagrass beds support a rich community of heterotrophic organisms, including sea urchins. 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 (Fernandez et al., 2012). At moderate population densities, sea urchins can influence plant species composition and enhance species diversity through selective feeding (Boudouresque and Verlaque, 2013). 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). Consequently, a high abundance can result in the

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formation of "sea urchin barren grounds" (Pinna et al., 2012). Their preferred habitat predominantly consists of solid rock and boulders due to the unsuitability of the sandy bottom for locomotion (Boudouresque and Verlaque, 2013). In Mediterranean, the sea urchin *Paracentrotus lividus* is also found in seagrass meadows, although it might settle down first in hard substrates and then migrate to the seagrass meadows (Pinna et al., 2012).

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, characterized by high water renewal and salinities similar to those of the adjacent Atlantic Ocean (Falcão and Vale, 1990; Gamito, 2008; Cabaço et al., 2010; Rosa et al., 2019). The economic importance of the Ria Formosa depends on its rich biodiversity, making the preservation of its lagoon ecosystem vital for both environmental conservation and the continued production of fish and bivalves. Among the crucial activities, shellfish and finfish aquaculture stand out prominently (Ferreira et al., 2009). Around 10 km^2 of the lagoon's intertidal flats are utilized for clam cultivation, primarily of *Ruditapes decussatus*, with an annual estimated production of up to 7000 tons (Carvalho et al., 2006). The establishment of bivalve aquaculture has resolved in clearing *Zostera noltii* meadows, and the transport of sand from local beaches (Falcão and Vale, 1990). This traditional bivalve culture is now overlapped by oysters' culture, predominantly *Magallana gigas*.

Within the Ria Formosa shallow environment, seagrass and macroalgae are frequently found on muddy and sandy substrate (Cabaço et al., 2010). The lagoon houses one of the most important populations of seagrass, amongst the native seagrass species are *Cymodocea nodosa* and *Zostera marina* (Parreira et al., 2021). Additionally, *Zostera noltii* is describe as a commonly spread seagrass species in the lagoon, predominantly in the low intertidal zone (Guimarães et al., 2012). Recently, since 2011, *Caulerpa prolifera* is spreading unpredictably in the lagoon, but the extent of the impact on biodiversity remains largely uncertain (Cunha et al., 2013). *C. prolifera* has been first described in Ria Formosa 70 years ago but remained unnoticed until recently (Cunha et al., 2013). In the last decade, an exotic macroalgae, *Rugulopteryx okamurae*, have been invading the southern coast of Portugal (Herrero et al., 2023). In 2015 this species was detected in the Strait of Gibraltar, expanding subsequently in the south of the Iberian Peninsula, towards the Atlantic (2018) and the Mediterranean (2019) (García-Gómez et al., 2020).

Seagrass is found in Ria Formosa in both meadows and smaller accumulations within spatially restricted patches (Guimarães et al., 2012). Observations from previous campaigns showed that these spatially restricted patches exhibit subtidal seagrass and seaweed spreading, surrounding and interweaving accumulation of shells, at the beginning of each warm season. The flora promotes a wide range of species within those patches. Sea urchins stand as one of the most found organisms in these patches and are therefore referred to as sea urchin patches. These sea urchin patches are not very common in sandy bottoms of Portuguese lagoons or estuaries and were never been previously studied.

The primary objective of this study was to investigate the epibenthic communities within the sea urchin patches and those invaded by *Caulerpa prolifera* in Ria Formosa near the Armona Inlet (Fig. 1). The research involved assessing the impact of *Caulerpa prolifera*, and was later expanded to include *Rugulopteryx okamurae*, which was identified during subsequent field campaigns. To fully address this objective, spatially extended surveys were carried out, enabling comparative analyses of macroalgal impacts across different locations and in reference to previous years' field data. This work is particularly relevant given the ongoing spread of the non-indigenous macroalgae in Ria Formosa and contributes valuable insights into their effects on benthic communities in this Natura 2000 protected area.

2. Methodology

The field campaigns were deployed near the Armona inlet (Fig. 1), between June and July from 2014 to 2024, at depths of 3.5–5.5 m. The initial step in each field campaigns was the identification of the sea urchin patches and their location. A camera (F01S Waterproof Cable Night Vision Lights Underwater Fishing Camera), mounted on a pole, was used from the boat, allowing for an efficient observation of the

Fig. 1. Location of the patches (Coordinates in Annex Table A1), in southern Portugal near Armona inlet. The green pins represent sea urchin patches from 2014, the dark blue pins from 2016, the yellow pins from 2019, the red pin from 2021 and the brown pin from 2023. The orange pins represent the patches from 2024 (Identifier: S1-S7, C1-C3). (Image courtesy of Google Earth Pro (Google, 2022), Portugal; 37° 00′11′′N, 7°48′40′′W (WGS 84)/29S 605218 m N, 4095984 m E (UTM)). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

lagoon bottom, of the epibenthic organisms and an estimation of the patch locations. The boat navigated the inner channel near the Armona inlet, cruising along routes parallel to the barrier island. Additional patches were located during subsequent dives using underwater scooters (Diver propulsion vehicle Apollo AV-1) to expand the survey area. After the patch selection, they were georeferenced during the diving campaigns, which enabled their mapping afterwards. During the dives, a metal stick was positioned at the centre of each patch and labelled with a tag. This stick was used as a reference for the georeferencing and for deployment of a buoy. The surface maker buoy was used to extract the location of the centre of the patches.

In 2014, 2019, 2023 and 2024, the diving campaigns were carried out with a special focus on sea urchins and ascidians species density and biometry. In 2014 four squares, measuring 0.5m × 0.5m, were randomly placed on the patches for sample collection (Fig. 2 and Annex Figure A1 A). In 2019, and 2013, the four squares were replaced by four plastic circles, with a 25-cm diameter (Annex Figure A2 A). In 2024, a more detailed sampling was performed: seven sea-urchin patches and three *Caulerpa prolifera* invaded patches were selected. In each patch three 25-cm circles were randomly placed and all the biological material collected (Annex Figure A3 C, E). Since the research areas of the campaigns from 2014 to 2023 were completely covered by *Caulerpa prolifera*, the target area in 2024 was displaced to west and towards the tidal channel (Fig. 1).

Unlike in previous years, in 2024 all fauna and flora within these circles were collected without any preselection or focus on specific target organisms. This unselective sampling approach was unique to 2024, as the primary objective that year was to investigate the ecological impact of non-indigenous macroalgae. In contrast, campaigns from earlier years were more focused on particular taxa, which resulted in different approaches in field methodologies over the years (Fig. 2). In addition to flora and fauna, sediment samples for each circle were extracted afterwards. On board, the identification of the organisms and flora was performed by marine biologists with expertise in this habitat and beforehand prepared identification keys. Invertebrate organisms larger than 2 cm were identified, measured and scaled, while for smaller organisms up to around 1 cm, only their number and taxa was estimated. Sampled macroalgae and seagrass was identified and scaled. Macroalgae and trapped dead seagrasses amongst were scaled altogether. Separating them would have required more time than available for this task during the on-board analysis. Therefore, a relative percentage of each vegetation type per circle was estimated, which allowed an approximation of their individual biomass. Since the organisms were released in-situ after sampling, time was limited. On the boat, before sunlight and air exposure alters the properties of the sediment, the sediment samples were analysed for pH-values and for colour using the Munsell Soil colour charts (Munsell Color, 1994).

Water temperature, PH-values and salinitywere not considered, because diving campaigns were conducted during incoming as well as outgoing tide. The variation of the physical parameters, such as pH-value and temperature, of the lagoonal water can change within a tidal cycle, due to the water mixing of the acting tides (Rosa et al., 2019) and are therefore unsuitable to integrate into analysis. Nevertheless, the salinity is usually stable and around the values observed in the adjacent ocean waters, 36 PSU. Sediment samples were analysed in the laboratory for grain size using a Laser Granulometer, 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 and Ward, 1957) was applied using an Excel extension developed by Blott (2010).

Due to the unexpected discovery of a recently arrived and spread brown macroalga, its identification was carried out in the laboratory. The species was confirmed as *Rugulopteryx okamurae* based on the examination of a transverse section of the thallus, which revealed multiple layers of medullary cells at the margin, an identification feature described by Hwang et al. (2009).

A map of each patch was created in Microsoft Excel. To compute the maps simple trigonometric calculations were used, implementing the measured angle and distance from centre to a point at the edge of the patch. The organism counts per taxa were used to estimate diversity and density, while biomass estimates were based on the weights of individuum recorded on board. The diversity and the density or biomass of the main taxa within patches were established and possible differences tested. For an accurate description of difference in diversity of the patches the Shannon-Wiener Index (H'bits) (Shannon, 1948); and the Simpson Index (D-1) (Simpson, 1949) 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

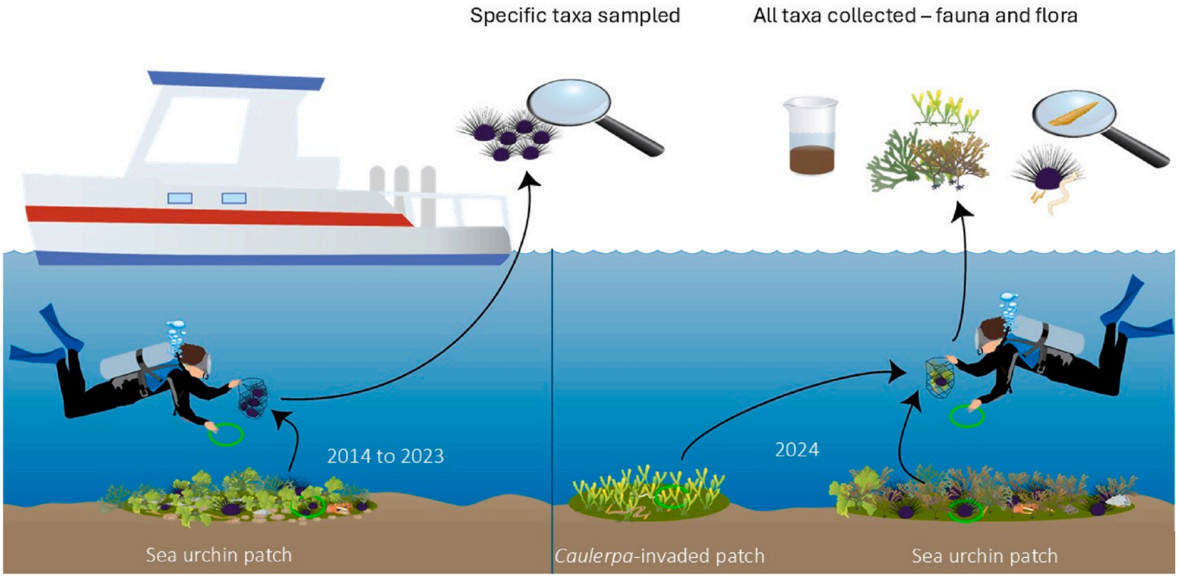

Fig. 2. Schematic representation of the organization of the field surveys and a comparison of 2024 and the previous years. In 2024, onboard analysis included the examination of unselected flora, fauna and sediment, whereas previous years focused on selected organisms (Image courtesy of Integration and Application Network (2025) (https://ian.umces.edu/media-library).

different habitats was based on the number of different taxa presented in the patches. Potential differences in the distribution of measurements in the dataset were calculated in the statistical computing software R (R Core Team, 2024). Depending on the distribution of the data, either the Welch two sampled T-test or the Wilcoxon-Rank Sum test was applied. The Shapiro-Wilk test was used to assess the normality of the data distributions. In addition, the Pearson's Chi-squared test of independence was employed where appropriate. Data manipulation and visualisation were performed using the R packages *dplyr* (Wickham et al., 2023a), *ggplot2* (Wickham, 2016; Wickham and Chang, 2025), and *tidyr* (Wickham and Girlich, 2023b). Using this methodology, the similarities or differences in the composition of the patches could be presented and discussed, and possible disturbances on the biodiversity inferred.

3. Results

3.1. Sea urchin patches from 2014 to 2024

A total number of 35 sea urchin patches and 3 *Caulerpa prolifera* invaded patches from 2014 to 2024 were located and georeferenced (Fig. 1; Coordinates for centre of each patch in Annex Table A1) with depths ranging from 3.5 m to 5.5 m and temperatures varying from 15 to 19 °C, as observed on the diving computers. Furthermore, all patches showed a well-defined flora layer regardless of flora type. From 2014 to 2023 the sea urchin patches showed a well-defined layer of shells, on which the flora could grow (Annex Figure A1). *Codium* sp. and *Ulva* spp., occasionally the seagrasses *Zostera* marina or *Cymodocea nodosa*, were the most found species of flora in these years and sea urchin, ascidians and nudibranchs were widely distributed within the patches (Annex Figure A1). In 2023, the patches were already invaded by *Caulerpa prolifera*, which spread in small tufts all over the area (Annex Figure A2). Within the patches, sea urchins were occasionally present in aggregates, with a very high individuum density (Annex Figure A2 B). Seashells accumulations were visible over large areas, with no vegetation neither macrofauna, probably as remains of old sea urchins' patches (Annex Figure A2 E). The impact of *C. prolifera* on the sea urchin patches could only be assessed in 2024, being the only time *Caulerpa prolifera* patches were sampled. In this year, different locations for the patches had been chosen, as previous ones had been fully invaded by *C. prolifera* and were not suitable for analysis of the its impact. In this new area sea urchins' patches were found together with *Caulerpa* patches. Therefore, the ten patches from 2024 (Fig. 1, orange pins) were either classified as sea urchin patches, assigned with a short identifier S1, S2, S3, etc., or as *Caulerpa*-invaded patches, labelled as C1, C2, C3. The circles, that were utilized for the sampling process, were labelled A, B and C for each patch individually. Noticeable was the absence of a defined shell layer underneath the flora layer in 2024 (Annex Figure A3 A, D). The georeferenced patches and its measurements from the underwater cartography from that year were used to calculate the covered area of each patch. The area of the sea urchin patches varied from 2.01 m^2 to over 270 m^2. The *Caulerpa*-invaded patches ranged from 3.31 m^2 to 5.66 m^2 (Annex Table A2). Within this range a great difference in the means of the sea urchin patches and the *Caulerpa*-invaded patches could be observed (Annex Table A2). The invaded patches showed smaller mean values of covered area than the non-invaded patches, although the values were statistically not significant (p-value = 0.13; Annex Table A4 [1]).

3.2. Sampled flora

From 2014 to 2023, observations have shown that the sea urchin patches experienced a shift in flora throughout the season towards an *Ulva* sp. dominated sea urchin patch. During the diving campaigns in 2024 the sampled patches exhibited mostly a different flora. The sampled flora in sea urchin patches S1 to S7 primarily consisted of another invasive algae *Rugulopteryx okamurae* (Annex Figure A3). Additionally, a significant amount of dead seagrass was observed. As listed in Table S3, *R. okamurae* was always scaled together with other macroalgae and dead seagrass. Therefore, a relative percentage of each vegetation type was estimated, which allowed an individual estimation of their biomass. Smaller quantities of *C. prolifera*, *Ulva* sp., and *Codium* sp. were also found in some of the circles of the sea urchin patches (Fig. 3 and Supplementary data Table S1). Notably, Circle B of patch C1 exhibited less quantities of *C. prolifera* and more of *R. okamurae* (Fig. 3 and Supplementary data Table S1). The average total biomass of the macroalgae per patch reaching 4.4 kg m^{-2} for the sea urchin patches and 2.3 kg m^{-2} for the ones invaded by *C. prolifera* (Fig. 5).

3.3. Sampled fauna

The fauna samples from 2024 included four different species of Annelida, totally over 20 organisms. Except for one case, Annelida were exclusively found in the *Caulerpa*-invaded patches (Fig. 4 and Supplementary data Table S2). More than 200 Arthropods, including various orders of crustaceans, were sampled. Notably, only six were found in the three *Caulerpa*-invaded patches (Fig. 4 and Supplementary data Table S2). A total of 70 organisms from the Chordata phylum, including Actinopterygii and ascidians 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 (Holland, 2016). In this case, when the individual organisms were not separatable, the accumulated organism were still counted individually. Only five, out of the 70 organisms, were found in the *Caulerpa*-invaded patches (Fig. 4; Supplementary data Table S2). One Cnidarian was observed in one of the *Caulerpa*-invaded patches (Fig. 4 and Supplementary data Table S2). Over 900 Mollusc specimens, including Gastropods, Bivalves, Cephalopods, and Polyplacophora, were observed across all ten patches, without a clear pattern evident from the data (Fig. 4 and Supplementary data Table S2). *Bittium reticulatum* was the dominant gastropod, being present in large numbers in all samples. 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 (Fig. 4 and Supplementary data Table S2). The average total biomass of macroinvertebrates per patch was calculated from the sum of the weights of all sampled organisms within each patch type. Biomass was substantially greater in the sea urchin patches, averaging approximately 6.7 kg m^{-2}, compared to just 0.3 kg m^{-2} in the *Caulerpa prolifera*-invaded patches (Fig. 5). The three diversity indices estimated, Shannon-Wiener-, Evenness- and Simpson, were not very different between the two patch types (Annex Table A3).

3.4. Sea urchin biometry

In 2024 the morphological measures of the sea urchin species *Sphaerechinus granularis* demonstrated an average weight of 130 g, average height of 47 mm (without spines), and average diameter of 72 mm. Samples from the same year of the species *Paracentrotus lividus* showed an average weight of 38 g, with an average diameter of 46 mm and height of 25 mm (Fig. 6; Supplementary data Table S3). In 2023 the average sea urchins' measurements of the species *S. granularis* presented an average weight of 140 g, a diameter of 72 mm and a height of 45 mm. Biometry measurements of the species *P. lividus* showed average values in weight of 58 g, in diameters of 55 mm and height of 32 mm (Fig. 6; Supplementary data Table S3). Samples of the same sea urchin species from 2019 exhibit an average in weight of 35 g, diameter of 23 mm and diameter of 43 mm (Fig. 6; Supplementary data Table S3). Morphological measures from the species *P. lividus* in 2014 present similar average values, indicating measurements in weight of 35 g, in height of 23 mm and in diameter of 44 mm (Fig. 6; Supplementary data Table S3).

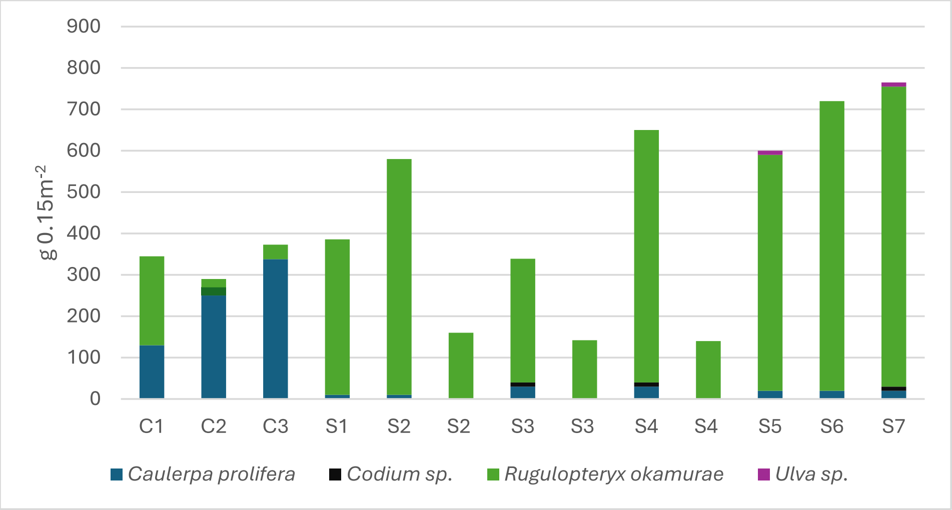

Fig. 3. Total weight of algae (g) sampled in each patch (*Caulerpa*-invaded patches: C1-3 and sea urchin patches: S1-7) from 2024 (sample area of 0.15 m^2), and of each algae taxon: *Caulerpa prolifera*, *Codium* sp., *Ulva* sp. and *Rugulopteryx okamurae*. The weight of *R. okamurae* includes the dead seagrass, which was not separated from the living flora samples.

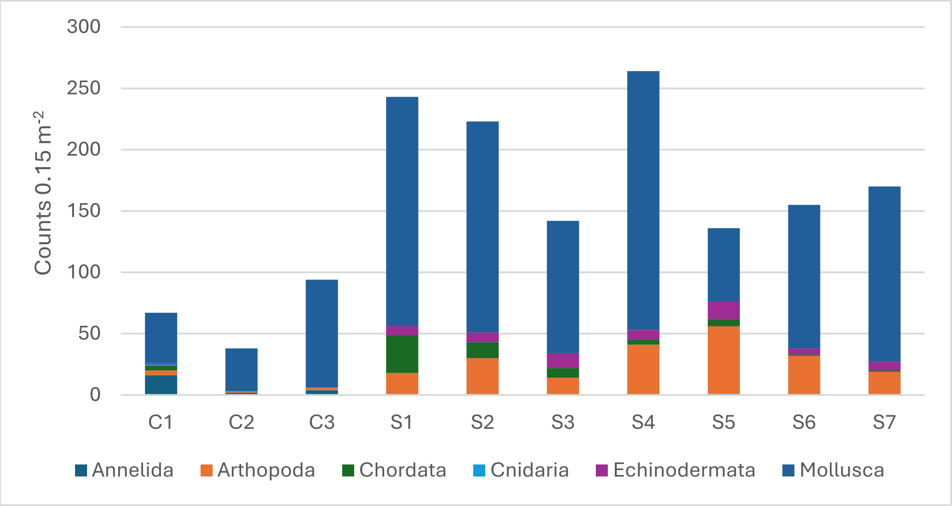

Fig. 4. Total counts of main macrofauna phyla (incl. Annelida, Arthropoda, Chordata, Cnidaria, Echinodermata and Mollusca) for each patch (*Caulerpa prolifera* invaded patches: C1-3 and sea urchin patches: S1-7) from 2024 (sample area of 0.15 m^2) sampling.

3.5. Sea urchin density and biomass

Sea urchin density and biomass estimates from 2023 were excluded from direct comparisons with the estimates from other years. The organisms in 2023 were found highly concentrated in small aggregates, disabling a random sampling methodology. It is important to remark, however, the presence of some *Sphaerechinus granularis* in 2023 asamples. A higher abundance of sea urchins in previous years becomes evident from the sea urchin density. The count of the species *P. lividus* in 2014 showed average values of 119 individuals per m^2 and in 2019 an average of 102 sea urchins per m^2 was documented. In 2024 the density of *P. lividus* and the other documented species *S. granularis* showed both average values of 23 individuals per m^2 (Fig. 7). A similar trend is reflected in biomass densities. In 2014, *P. lividus* biomass density was nearly 4.2 kg m^{-2}. In 2019 the biomass for *P. lividus* was approximately 3.5 kg m^{-2} and for *S. granularis* 264 gm^{-2}. By 2024, the biomass densities were 1.0 kg m^{-2} for *P. lividus* and 2.8 kg m^{-2} for *S. granularis* (Fig. 8). The biomass of both species together, 3.8 kg m^{-2}, approaches the observed biomass of *P. lividus* in the previous years, since *S. granularis* was usually larger and heavier.

3.6. Ascidians biometry

Although sea urchin 2023 densities and biomass were excluded from the analysis, due to the lack of random sampling, the ascidians measurements from 2023 were included. The reason is the assumption that the selective sampling was targeted towards denser sea urchin areas and should not affect the ascidian dynamic. In 2023 and 2024 three different ascidian species were sampled: *Phallusia mammilata, Microcosmus squamiger* and *Styela plicata*. No difference in size could be detected amongst the different species. In 2023, the length of ascidians varied from 10 to 97.4 mm and the width from 10 to 42.6 mm. The length of the ascidians in 2024 ranged from 20.8 to 73.9 mm and the width from 16.2 to 53.3 mm (Supplementary data Table S4). The individual weight was not considered, since ascidians can colonize. Colonial organisms can share tunic or can be attached at the base to each (Holland., 2016; Matos and Antunes, 2021), which disables a separation of the individual weight without damaging or killing the organisms. The biomass in 2023 revealed an average value of 1830.2 gm^{-2} and 1237.7 in 2024. The densities in 2023 showed an average value of 40.7 individuals per m^2 and 58.2 individuals per m^2 in 2024 (Supplementary data Table S4).

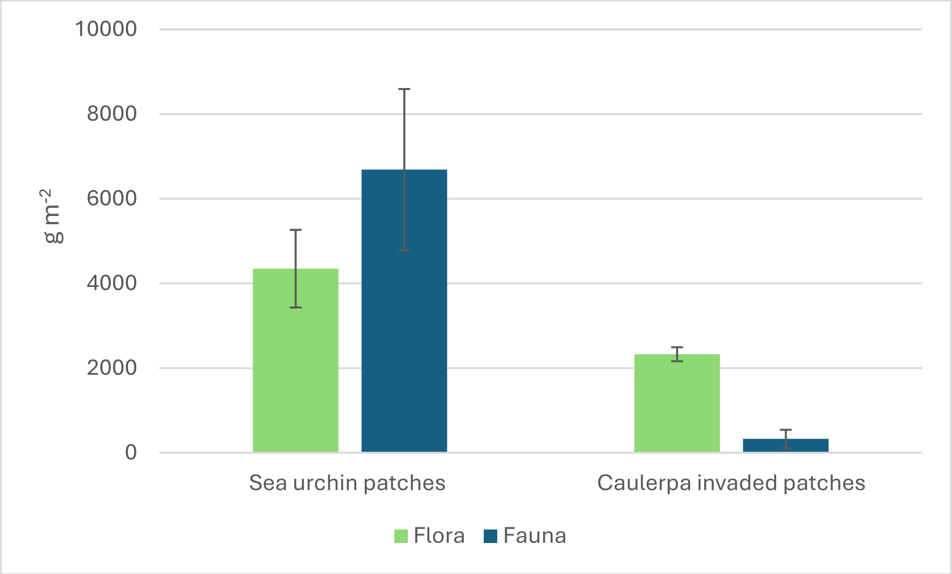

Fig. 5. Total biomass of flora and fauna in gm^{-2} for both sea urchin patches and *Caulerpa prolifera* invaded patches from 2024 with standard error bars.

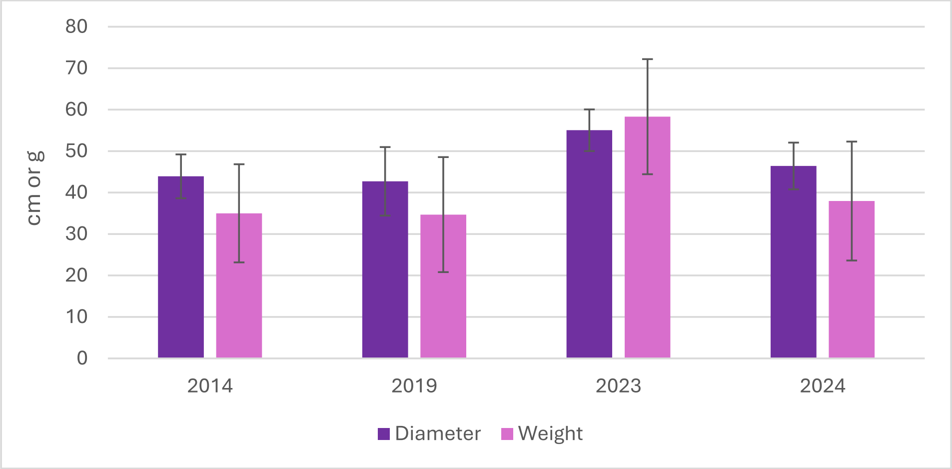

Fig. 6. Average measurements and standard deviation of *Paracentrotus lividus* diameter without spines (cm) and weight (g).

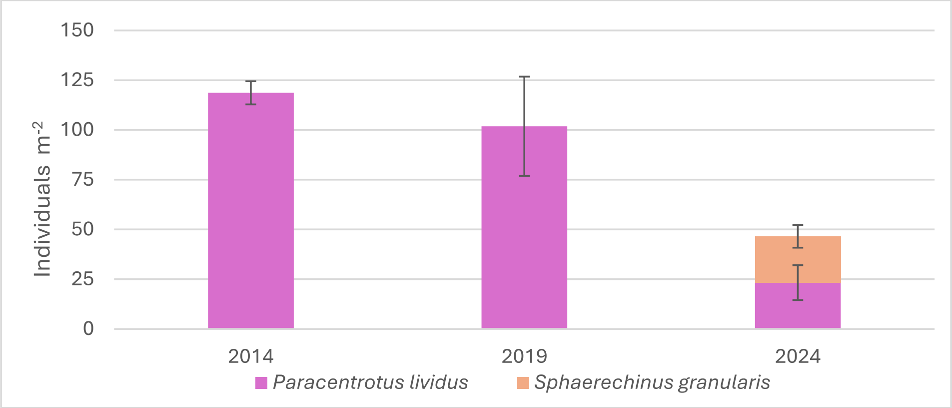

Fig. 7. *Paracentrotus lividus* and *Sphaerechinus granularis* densities [Individuals m^{-2} and standard error] for the years 2014, 2019 and 2024.

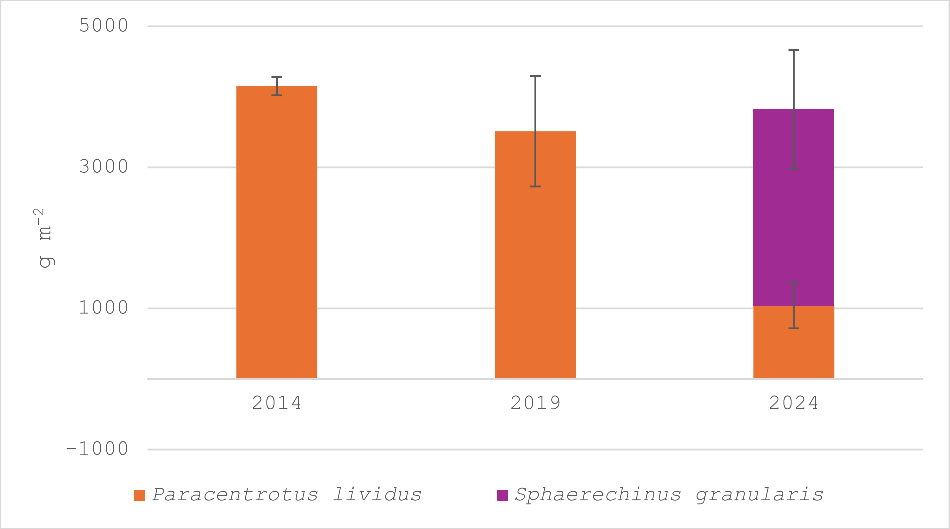

Fig. 8. *Paracentrotus lividus* and *Sphaerechinus granularis* biomasses [g m^{-2}] with standard error for the years 2014, 2019 and 2024.

3.7. Statistical tests

A difference in composition of the patch types in 2024 is highlighted in Chi-squared tests. Both, the average phylum composition of each patch type and the sum of the phyla for each patch type showed significant differences between the invaded *Caulerpa* patches and the sea urchin patches (for both chi tests: p-values <0.01; Annex Table A4 [2] and [3]), with *Caulerpa* patches presenting lower values of both indicators. Additionally, a 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. 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 A4 [4]). Table S7 The results of the Non-metric Multi-Dimensional Scaling (MDS) show that, both, comparison of only the patches and comparison of the circles in each patch, reliable patterns under low stress values (0.19 and 0.07, respectively, Fig. 9A and B). The clustering of the sea urchin patches replicates in the left side (Fig. 9 A) and right side of the diagram (Fig. 9 B), highlights the similarities among these patches (Fig. 9 A). Dissimilarities of the patch types are underscored by the clustering of the *Caulerpa*-invaded patches on the opposite side of the sea urchin patches, the right side (Fig. 9 A) and left side of the diagram (Fig. 9 B). Patch C1 shows great displacement, hence differentiates from the two remaining (Fig. 9 B).

3.8. Sediment samples

During the diving campaigns from 2014 to 2023 a distinct layer of shells was observed. In the campaigns of 2024, no shells were found in any of the patches. Sediment sampled from 2014 showed mean grain sizes from 14,1x10^3 to 20,6 × 10^3 μm across all the patches investigate (Supplementary data Table S5) and in 2019 the mean grain sizes ranged from 12,9x10^3 to 14,1 × 10^3 μm (Supplementary data Table S6). In 2023 the mean grain size varied from 41.5 to 72 μm (Supplementary data Table S7). The sediments samples from 2024 showed the same colour across all patches, a dark brown to black colour, matching 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, without any clear

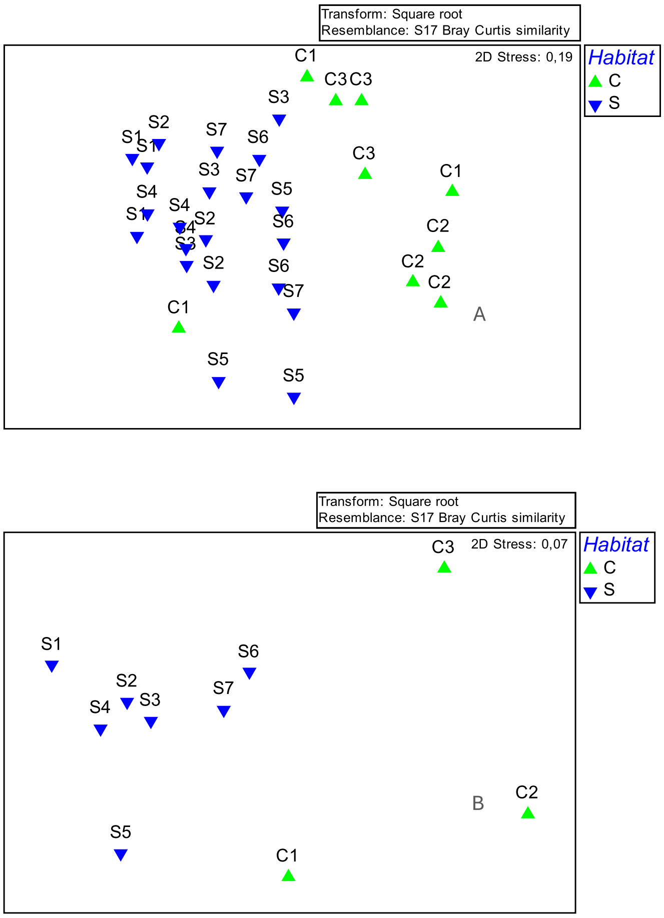

Fig. 9. Non-metric Multi-Dimensional Scaling (nMDS) plots of different patch replicates obtained from Bray Curtis dissimilarities in abundance of organisms in each circle (A) and abundance of whole sampled patch (B). Habitats: S – Sea urchin patches; C – Caulerpa patches.

distribution pattern between the different patch types. The grain size analyses for the samples from 2024 revealed that the sediment varied from muddy sand to sandy mud, with mean grain sizes ranging from 17.5 to 78.6 μm (Supplementary data Table S8). Statistically, the mean grain sizes do not differ from each other (p-value >0.05; Annex Table A4 [5]).

4. Discussion

4.1. Floral composition altered by invasive algae

From 2014 to 2023, sea urchin patches primarily exhibited *Ulva* sp. and *Codium* sp. Throughout a season the sea urchin patches experience a shift in flora, as observation have shown, caused by the sea urchins, being *Ulva* sp. their preferred food source (Prato et al., 2018). Hence *Ulva* sp. is trapped by the sea urchins, which induces the flora change towards an *Ulva* sp. dominated sea urchin patch (Annex Figure A1 (A-G)). In 2024, as anticipated, the presence of invasive algae, *C. prolifera*, was documented, with all the previous locations studied by the team completely invaded by *C. prolifera*. Its occurrence was known in the Ria Formosa since the 20th century (Cunha et al., 2013) and remained unnoticed till recently (Cunha et al., 2013; Alexandre and Santos, 2020). The sea urchin patches in 2024 exhibited only very small amounts of *Ulva* spp. or *Codium* sp., and mainly *Rugulopteryx okamurae*. These unexpected observations of the flora composition varied from the ones from previous year. The brown macroalgae *R. okamurae* was detected in the Algarve at least since 2021 (Herrero et al., 2023) and is known to have a decreasing effect on abundance and richness of flora (García-Gómez et al., 2021). This recent introduction of the macroalgae could explain the sudden shift in flora. 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. The patch C1 had not been fully invaded by *C. prolifera* at the time of the campaigns, so one of the circles was placed in an area that had not yet been affected by the spreading of *Caulerpa*. This explains the predominant presence of *R. okamurae* in that patch. Additionally, the total average biomass of flora per square meter shows higher values for the sea urchin patches.

4.2. Faunal analysis indicates difference among patch types

The described fauna composition highlights the Mollusca and Annelida dominated fauna in *Caulerpa*-invaded patches while the sea urchin patch fauna, mainly consist of Chordata, Crustaceans and Echinodermata besides Mollusca. C1 patch, not fully invaded by *Caulerpa* sp., exhibits a faunal composition more similar to the sea urchin patch's fauna. Beside the difference in composition, a clear reduction of fauna density is observable. A comparison between the two patch-types, *Caulerpa*-invaded and sea urchin patches, shows a reduced number of sampled organisms per patch for the *Caulerpa*-invaded patches. The density ranges from 38 to 94 individuals per 0.15 m^2 for the invaded patches and from 122 to 280 individuals per 0.15 m^2 for the sea urchin patches. The differences in densities between the two patch types is complemented by the average total biomass per patch. The comparison shows a significant reduction in average biomass per square meter of the *C. prolifera* invaded patches, when compared to the sea urchin patches. Additionally, to the noteworthy lower organism count for the invaded patches, the described difference in fauna composition, as well as the significant reduced taxa richness of the *Caulerpa*-invaded patches, highlight the shift in fauna between the two patch types. In this study, no invasive species other than the two macroalgae were encountered during sampling. Therefore, the observed faunal changes are most likely attributable to their presence. Parreira et al. (2021) found similar patterns of significant differences between *C. prolifera* habitats and non-invaded habitats in Ria Formosa, with a reduction on fauna richness. 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). 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).

4.3. Multivariate analysis highlights difference among patch types

Further evidence for a shift in the benthic community can be drawn from the dissimilarities emerging from the MDS analysis. 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 clearly highlights the differences between fauna composition of the two patch types, when comparing all the circles with each other. In the same way, similarities in the macroinvertebrate community of the sea urchin patches can be observed by the clustering of the sea urchin patches. The *Caulerpa*-invaded patches are separated on the right side of the diagram, although visible differences amongst the *Caulerpa*-invaded patches. Resemblance 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. Consequently, the dissimilarities of C1 are altered from a fully invaded patch. The comparison of the patches itself with each other brings up a similar pattern of dissimilarities between the patch types. Patch C1 shows great displacement, hence differentiates from the two remaining. The reason for the difference stems from the described half-invaded state. A comparable MDS analysis done in another study in Ria Formosa revealed a similar pattern, in which *C. prolifera* habitats showed significant dissimilarities to non-invaded habitats (Parreira et al., 2021). However, that study did not involve sample areas where both invaded and non-invaded states were present simultaneously (Parreira et al., 2021). Nevertheless, that study, as well as the present study, allowed to infer that these dissimilarities highlighting the alternation in composition and abundance of macrofauna due to the spreading of *C. prolifera*.

4.4. Epibenthic community in Ria Formosa altered by Caulerpa 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 and 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 change in diversity. 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 fauna composition, richness, and abundance of the patches. This study as well as others have shown negative effects on abundance and taxa diversity due of the *C. prolifera* invasion in the Ria Formosa (Alexandre and Santos, 2020; Parreira et al., 2021). Parreira et al. (2021), researching the invasive behaviour of *C. prolifera* in Ria Formosa, showcased significant differences in the diversity indices, pointing to a higher diversity, richness and evenness in a non-invaded habitat compared to a *C. prolifera* habitat. However, the applied diversity indices of this study did not reveal any further inside about any change in fauna composition, although a significant reduction on the number of taxa and a reduction of organism density was noticed in the *Caulerpa-invaded* patches. The reason for the similarities in the indices of 2024 might be the predominant abundance of *R. okamurae*. As briefly mentioned above, the brown macroalgae is a recent invasive species, since its presence was only reported more recently than the appearance of *C. prolifera. R. okamurae* was first described in Europe in the early 2000s in Thau lagoon (Verlaque et al., 2009) and proliferating since 2015 in the Mediterranean Sea and the Atlantic coast (Borriglione et al., 2024; García-Gómez et al., 2020). Herrero et al. (2023) reported the presence of the brown algae in the Algarve, in southern Portugal, at least since 2021. The impact of *R. okamurae* stems from the direct competition for space, light and nutrients (García-Gómez et al., 2021). 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 the three 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* can have a negative effect on the ecosystem, but *R. okamurae* potentially as well. Additionally, no nudibranchs were sampled nor spotted in any patch during the diving campaigns in 2024, which raises concerns as nudibranchs were commonly found within the patches from 2014 to 2021 (example Annex Figure A1 F).

4.5. *Rugulopteryx okamurae has potential of altering sea urchin populations*

A potential consequence of the invasion of *R.* okamurae could be a change in abundance of the two sea urchin species *P. lividus* and *S. granularis*. In 2014, 2019 and 2023, *P. lividus* was observed as the predominant sea urchin species in the sampled patches. Notably, when *Caulerpa prolifera* was first recorded within a sampled sea urchin patch, *S. granularis* was also documented for the first time in 2023. By 2024, however, *P. lividus* and *S. granularis* were found in equal proportions. This shift in 2024 is further supported by density and biomass measurements. In both 2014 and 2019, *P. lividus* was the only sea urchin species recorded within the patches, with densities of 102 and 119 individuals per m^2, respectively. In 2014, its biomass density reached nearly 4153 gm^{-2}. Even though the sea urchin measurements of 2023, were excluded from the data comparison, a shift in the species distribution of sea urchins is evident. That year, *S. granularis* was describe within the sea urchin patches for the first time while *P. lividus* remained the dominant species in the patches. This trend continued into 2024, where *S. granularis* becomes as dominant as *P. lividus*. The density measurements of 2014 and 2019 not only exceeded the 2024 values for *P. lividus*, declining to 32 individuals per m^2, but also surpassed the combined densities of *P. lividus* and *S. granularis* in 2024. While the decline in sea urchin densities was observed over the years, the biomass densities of the sea urchins did not experience the same decline and remained within a range from 3512 to 4153 gm^{-2}. This consistency is likely due to the larger body size of *S. granularis*, which causes the overall biomass to maintain within similar levels despite a reduction in individual numbers. The grazing behaviour of *S. granularis* may explain this shift of sea urchin composition, towards a *S. granularis* dominated habitat. 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 less affected by a change in flora composition. Given the increasing competition of *S. granularis*, a decline in the growth of *P. lividus* might be anticipated. Brundu et al. (2016) describes how population density can affect the somatic growth of *P. lividus*. A reduction in growth would be apparent in a decrease of the sea urchin's diameter and wet weight, when population density increases (Brundu et al., 2016). However, the measurements from 2024 align closely with those reported in 2014 and 2019, showing no significant difference. The reason for the increase of biometric measurements in 2023 for *P. lividus* remains unclear. In that year the sea urchins were accumulated in high density aggregates within the patches, including larger and heavier specimens, possibly older ones from the previous year. This incoherence prevents a clear conclusion at this time that the competitive pressure from *S. granularis* is affecting *P. lividus* population based on the morphological measurements provided. It is possible that competitive pressure exerted by *S. granularis* may only become apparent over a longer timescale, highlighting the need for continued monitoring and analysis to fully understand potential long-term effects.

4.6. *Ascidian populations*

Unlike the sea urchin populations, the ascidian density was not significantly affected by the spread of *C. prolifera*. The density increased from an average of 40.8 in 2023 to 58.2 individuals per m^2 in 2024, while the biomass decreased from 1830 gm^{-2} in 2023 to 1238 in 2024. This insignificant variation in population density and biomass can be explained by sampling errors. A reduction in growth is not present, as the biometry measurements of 2023 align closely with the ones of 2024. The alignments of the biometry, the similar densities, as well as biomasses points to the lack of change in the ascidian population, since the arrival of the invasive macroalgae.

4.7. *Changes in sediments and shell layer*

During the diving campaigns from 2014 to 2023 a distinct layer of shells, that is thought of to form a base for the sea urchin patches, was observed. In the campaigns of 2024, no shells were found in any of the patches, which could be directly related to the shift in flora. Another potential reason for the lack of that layer could be due to a different position of the patches in 2024. Comparing the location of these patches to the location of sea urchin patches from 2014 to 2021, a greater distance to shore can be observed (Fig. 1). Physical factors, such as hydrodynamics, can alter over short distances inside Ria Formosa (Duarte et al., 2020) and expose the patches to different hydrodynamics. All sediment samples of each circle of all the patches showed the same dark brown/black sediment colour, hence the same redox potential. The pH values varied between 7.35 and 7.92 across all the patches, lacking a clear distribution pattern to indicate any difference between the patch types. Pérez-Ruzafa et al. (2012) 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 differentiate the two patch types. However, the pH-values and the Redox potential, revealed no significant differences between patch types and therefore disables a distinction between the *Caulerpa*-invaded patches and the sea urchin patches. The grain size analyses for the samples from 2024 revealed that mean grain sizes range from 17.5 to 78.6 μm, but do not present statistical difference among the patch types. Sediment sampled from 2014 showed the highest values for the mean grain sizes with 14068–202592 μm. From 2014 to 2023 the mean grain size of the sediment gradually decreased and reach values from 41.5 to 72,4 μm in 2023. These values from that year align closely with the ones from 2024. Therefore, the finer sediment sampled from underneath the patches cannot be attributed to the change in flora, since changes already occurred previously to any spreading of *R. okamurae*. The gradual decrease in grain sizes from the patches in 2014–2024 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 sea urchin patches 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.

4.8. Future research

In September 2024, new observations from diving surveys conducted by the same team for a new project revealed that the patches had shifted location or disappeared. Some of the sea urchin patches from July 2024 were found, using the labelled metal sticks of this survey as a reference. Only in two sea urchin patches the tagged metal stick was found within the patches, the other patches shifted to such a degree, that the sticks were found near 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 survey in 2024. If an anchor were to be dragged across a patch, it could potentially destroy the entire habitat. These external effects make it very challenging to fully understand the long-term impact of these macroalgae 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. The impact of this seasonal fluctuation on diversity of the benthic communities remains unclear, as does the combined long-term effect 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.

5. Conclusion

This study highlights the impact of the invasive seaweed *Caulerpa prolifera* on the benthic community within the sea urchin patches in Ria Formosa. Through extensive fieldwork and sample analysis from 2014 to 2024, it has been demonstrated that an invasion of *C. prolifera* can lead to significant alterations in the composition and abundance of benthic community structure of the patches. In addition to the spreading green macroalgae, the fieldwork of 2024 showed the presence of another invasive species, *Rugulopteryx okamurae*. The presence of this second spreading macroalgae in the sea urchin patches complicated the assessment of diversity and shifts in the benthic communities, as the overlapping of two invasions created difficulties to isolate the impact of a single invasive species. Still, faunal analysis showed distinction between the two patch types. The *Caulerpa*-invaded patches show a different fauna composition, including mainly molluscs and annelids and lacking Chordata and Echinodermata, when compared to the sea urchin patches (invaded by *R. okamurae*). Additionally, a lower taxa richness was found for the patches invaded by *C. prolifera* and a significant reduction of density and biomass of sampled organisms. The spread of *R. okamurae* was associated with a decline in *Paracentrotus lividus* sea compared to records from 2014 to 2023, and an increase of another sea urchin species, the *Sphaerechinus granularis,* both in density and biomass. Furthermore, it led to a complete shift in flora composition, replacing the previously dominant *Ulva* sp. and *Codium* sp. with *R. okamurae*. Additionally, a shell layer, that was found across all patches in 2014–2023, was not observed in the campaigns of 2024. The reason for its absence remains unclear but could be attributed to a different location of the patches in 2024, exposing them to different hydrodynamics.

CRediT authorship contribution statement

Tim Felix Hartmann: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Inês Ribeiro:** Writing – review & editing, Methodology, Investigation, Data curation. **Duarte Duarte:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Sofia Gamito:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sofia Gamito reports equipment, drugs, or supplies was provided by University of Algarve Centre of Marine Sciences. Duarte Nuno Duarte reports equipment, drugs, or supplies was provided by University of Algarve Centre for Marine and Environmental Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2025.109449.

Annex.

Table A1
Coordiantes of the measured sea urchin and Caulerpa-invaded patches from 2014 to 2024. The coordinates are presented in UTM and WGS 84.

Patch name	Identifier in Map	year	WGS 84		UTM (zone: 29S)	
			North (Latitude)	West (Longitude)	East	North
47BA HS1	3	2014	37,00341733	7,816551105	605296,000	4095906,000
47BA HS	4	2014	37,00344022	7,816134914	605333,000	4095909,000
47BA AOUR	1	2016	37,00341677	7,81649492	605301,000	4095906,000
47BA BOUR	2	2016	37,0033848	7,816000908	605345,000	4095903,000
17	5	2019	37,00341733	7,816551105	605296,000	4095906,000
18	6	2019	37,00316375	7,81643141	605307,000	4095878,000
19	7	2019	37,00343109	7,816123817	605334,000	4095908,000
23	8	2019	37,00313943	7,813093872	605604,000	4095879,000
24	9	2019	37,00319991	7,813733542	605547,000	4095885,000
25	10	2019	37,00337849	7,812663081	605642,000	4095906,000
26	11	2019	37,00344203	7,812707048	605638,000	4095913,000
27	12	2019	37,00269509	7,813730148	605548,000	4095829,000
28	13	2019	37,00334379	7,812798486	605630,000	4095902,000
30	14	2019	37,00361945	7,813322424	605583,000	4095932,000
35	15	2019	37,00380646	7,81309474	605603,000	4095953,000
P001	16	2019	37,00323876	7,8185319	605120,000	4095884,000
P002	17	2019	37,00386833	7,81207104	605694,000	4095961,000
P003	18	2019	37,00343684	7,813089248	605604,000	4095912,000
P005	19	2019	37,00277251	7,816066598	605340,000	4095835,000
P006	20	2019	37,00345644	7,813246287	605590,000	4095914,000
P007	21	2019	37,0035362	7,813110181	605602,000	4095923,000
P008	26	2019	37,00366464	7,817850978	605180,000	4095932,000
P009	22	2019	37,00340068	7,813078571	605605,000	4095908,000
P010	23	2019	37,00322933	7,813069997	605606,000	4095889,000
P011	24	2019	37,00322933	7,813069997	605606,000	4095889,000
P019	25	2019	37,00259668	7,815608538	605381,000	4095816,000
47BA HS2	27	2021	37,00347118	7,816527793	605298,000	4095912,000
23_01	28	2023	37,00351213	7,812351013	605669,582	4095921,172
24_S1	S1	2024	37,00559312	7,818292011	605138,102	4096145,458
24_S2	S2	2024	37,00555013	7,817539014	605205,159	4096141,520
24_S3	S3	2024	37,00547013	7,81779301	605182,670	4096132,364
24_S4	S4	2024	37,00525013	7,817956012	605168,470	4096107,777
24_S5	S5	2024	37,00526612	7,817813016	605181,171	4096109,710
24_S6	S6	2024	37,00400112	7,818413017	605129,528	4095968,708
24_S7	S7	2024	37,00383813	7,818903015	605086,154	4095950,084
24_C1	C1	2024	37,00485012	7,817470015	605212,263	4096063,938
24_C2	C2	2024	37,00489913	7,817426008	605216,111	4096069,423
24_C3	C3	2024	37,00381113	7,818603018	605112,884	4095947,420

Table A2
Covered area in m^2 of each patch from 2024

Patch reference	Area covered of patch [m^2]
24_S1	23.4
24_S2	7.74
24_S3	2.01
24_S4	4.53
24_S5	26.3
24_S6	276
24_S7	11.3
24_C1	3.45
24_C2	3.31
24_C3	5.66

Table A3
Average values per patch type, of the three indices, Shannon-, Simpson and Evenness, for *Caulerpa*-invaded and sea urchin patches.

	Caulerpa	Sea-urchins
Shannon-Winer (bits)	2,23	2,02
Evenness (j)	0,69	0,52
Simpson (1-D)	0,68	0,57

Table A4
Results of the applied statistical tests and the corresponding context.

Nr.	Content of statistical test:		Applied test	Results
[1]	Comparison of area covered by Caulerpa patches and Sea urchin patches		**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
[2]	Comparison of fauna composion based on average phyllum count per patch type		**Pearson's Chi-squared test**	data: fauna composition based on average phylum count X-squared = 30.273, df = 5, p-value = 1.303e-05
[3]	Comparison of fauna composion based on sum of phyllum count per patch type		**Pearson's Chi-squared test**	data: fauna composition based on sum of phylum count X-squared = 161.06, df = 5, p-value = < 2.02e-16
[4]	Comparsion of taxa richness between patch types		**Welch Two Sample T-test**	data: taxa_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 15 less than 0 95 percent confidence interval: Inf −0.7722021
[5]	Comparsion of grain size between different patch types		**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
[6]	Biometry comparison of *P. lividus* data from 2014 & 2024 for:	Height measurements	**Welch Two Sample T-test**	data: *P.lividus* comparsion 2014/2024 for height df = 34.742, p-value = 0.9706
[7]		Diameter measurements	**Welch Two Sample T-test**	data: *P.lividus* comparsion 2014/2024 for diameter W = 963, p-value = 0.7707
[8]		Wet weight measurements	**Wilcoxon Rank Sum Test**	data: *P.lividus* comparsion 2014/2024 for weight df = 35.346, p-value = 0.9965
[9]	Biometry comparison of *P. lividus* data from 2019 & 2024 for:	Height measurements	**Welch Two Sample T-test**	data: *P.lividus* comparsion 2019/2024 for height df = 29.741, p-value = 0.9066
[10]		Diameter measurements	**Welch Two Sample T-test**	data: *P.lividus* comparsion 2019/2024 for diameter df = 30.443, p-value = 0.9476
[11]		Wet weight measurements	**Welch Two Sample T-test**	data: *P.lividus* comparsion 2019/2024 for weight df = 39.318, p-value = 0.7733
[12]	Biometry comparison of *P. lividus* data from 2023 & 2024 for:	Height measurements	**Welch Two Sample T-test**	data: *P.lividus* comparsion 2023/2024 for height df = 43.028, p-value = 5.321e-08
[13]		Diameter measurements	**Wilcoxon Rank Sum Test**	data: *P.lividus* comparsion 2023/2024 for diameter W = 467, p-value = 4.235e-06
[14]		Wet weight measurements	**Welch Two Sample T-test**	data: *P.lividus* comparsion 2023/2024 for weight df = 43.861, p-value = 6.997e-06
[15]	Biometry comparsion of ascidian data from 2023 & 2024		**Wilcoxon Rank Sum Test**	data: Ascidian comparsion 2023/2024 for length

W = 139, p-value = 0.6725.

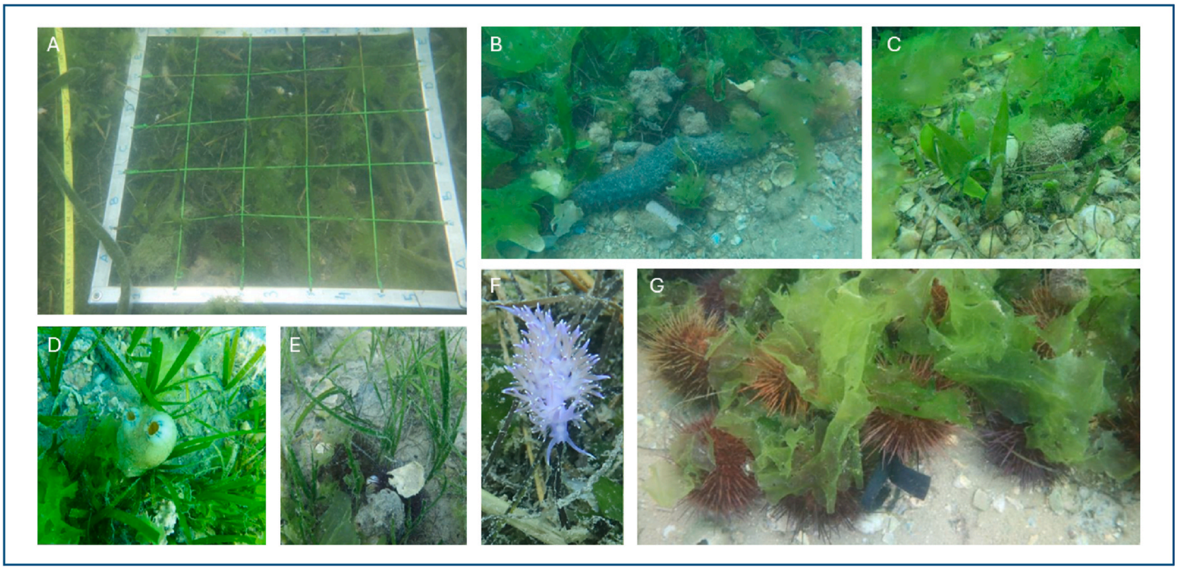

Fig. A1. The pictures present important aspects of the sea urchin patches in 2014 and 2019 prior to the macroalgae invasion. A: The square used for the sampling in 2014 lying on top of the main flora, Codium sp. and Ulva spp.; B: Main fauna of the patches, including sea urchin, ascidians and in here a sea cucumber (2019); C: Shell layer with Ulva spp. and Codium sp. growing on it, as well as a sea squirt (2019); D: Sea squirt as part of the main fauna amongst seagrass shoots and Ulva spp.; E: Ascidian and sea urchins amongst seagrass shoots and Ulva spp. on shell layer (2014); F: Nudibranch within the flora on dead seagrass (2019); G: Sea urchins and Ulva spp. on shell layer.

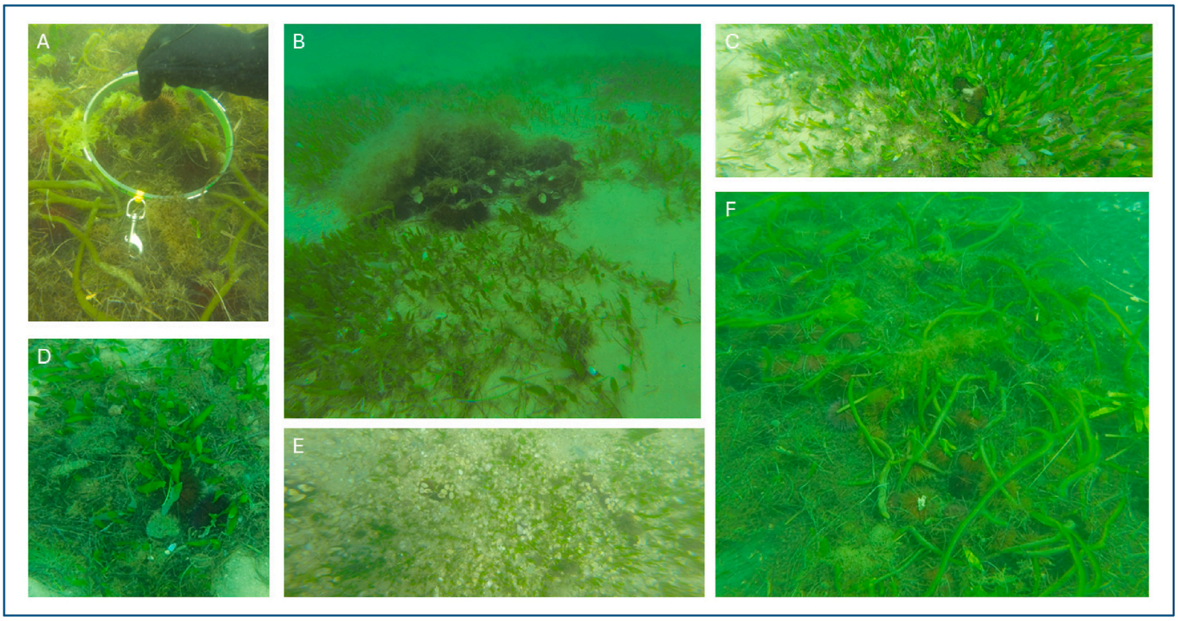

Fig. A2. The pictures present important aspects of the sea urchin patches in 2023 prior to the invasion of Rugulopteryx okamurae. A: The circle, used for sampling in 2019, 2023 and 2024, placed in a sea urchin patch showing sea urchin and the main flora Codium sp. and Ulva spp.; B: The small aggregates of sea urchins, with a very high individuum density, surrounded by Caulerpa prolifera; C: Single individuals of P. lividus amongst C. prolifera; D: Partial picture of sea urchin patch exhibiting sea urchins and smaller quantities of C. prolifera; E: C. prolifera starting to cover otherwise unvegetated, empty seashell bed; E: Overview of Sea urchin patch in non-invaded state, displaying numerous sea urchins amongst the main flora Codium sp. and Ulva spp. and exposing the shell layer on its border (top left corner).

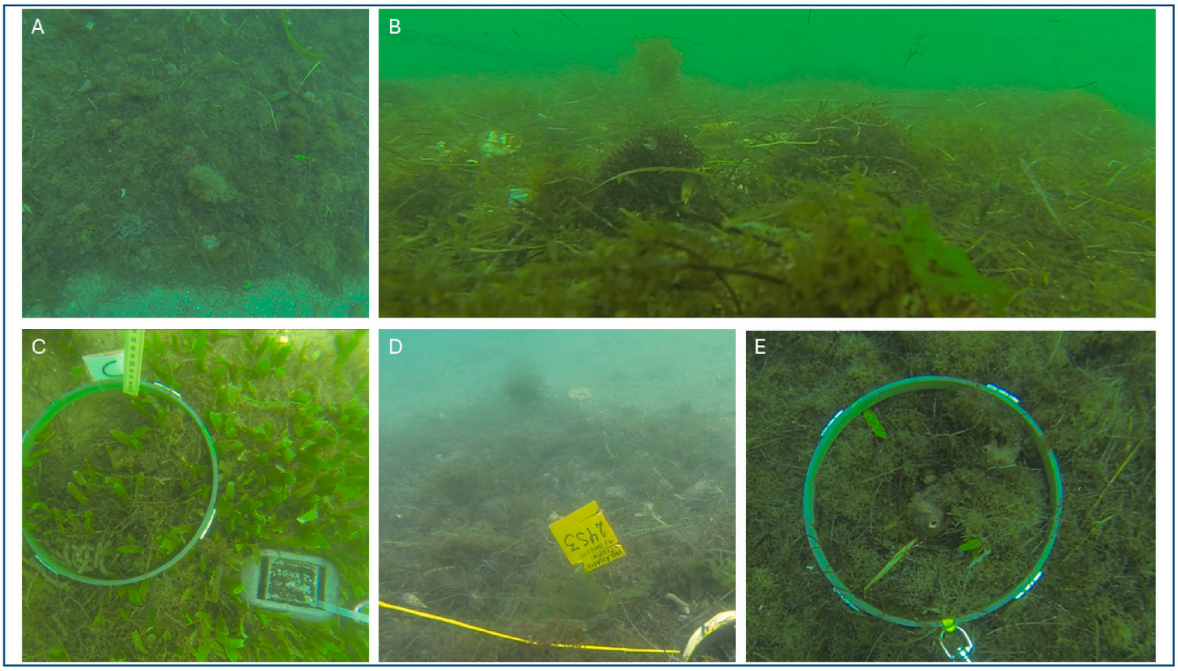

Fig. A3. The pictures present important aspects of the sea urchin patches and Caulerpa invaded patches in 2024. A: Border of a sea urchin patch exhibiting only Rugulopteryx okamurae and dead seagrass, Ulva spp. and Codium sp. are largely absent as well as the shell layer; B: Sea urchin amongst R. okamurae within a sea urchin patch; C: The circle used for sampling in 2019, 2023 and 2024 on a Caulerpa-invaded patch, which does not present a shell layer; D: Sea urchin patch 3 (S3) showing R. okamurae and dead seagrass; E: The circle for sampling on top of a sea urchin patch, which presents Ascidians amongst the macroalgae.

Data availability

Data is supplied in the Annex and in the supplementary file

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