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Resumo

Caulerpa prolifera (Forsskål) J. V. Lamouroux é uma alga verde oportunista que se está a estabelecer nas áreas não vegetadas da Ria Formosa, por vezes em áreas previamente colonizadas por comunidades de ervas marinhas. Esta alga tem uma grande capacidade de propagação e vantagem competitiva relativamente a outras macrófitas proveniente da sua reprodução através do alongamento do estolho e de fragmentação, da produção de metabolitos que impedem a herbivoria e da sua capacidade de prevalecer em áreas enriquecidas em nutrientes, fraca renovação de água e elevada turvação. A Ria Formosa é um sistema lagunar costeiro que cobre 55km ao largo da costa sul de Portugal, lugar para um vasto conjunto de atividades comerciais e de lazer. Atividades como estas, são suportadas na zona subtidal por importantes comunidades de ervas marinhas que se colocam entre os ecossistemas mais valiosos na Terra. Os fundos deste sistema lagunar são maioritariamente caracterizados por áreas não vegetadas, pradarias de ervas marinhas e de *C. prolifera*. Ao longo de mais de 170 anos, desde que a existência desta alga foi primeiro relatada na Ria Formosa, os seus efeitos na biodiversidade nunca foram estudados. Plantas hospedeiras (e.g. ervas e algas marinhas) são conhecidas pela sua capacidade de providenciar habitat para muitas espécies e assim influenciar a agregação de fauna e a biodiversidade. Têm sido feitas associações entre a cobertura vegetal e a fauna associada onde os habitats não vegetados estão regularmente associados a uma menor abundância e diversidade. Neste trabalho pretendemos compreender de que forma o estabelecimento de *C. prolifera* em zonas de sedimento afetam a biodiversidade da Ria Formosa. Para tal, comparamos aqui a associação de fauna e plantas entre os três habitats, um dominado por uma pradaria contínua de *C. prolifera*, um dominado por uma pradaria mista de *Cymodocea nodosa* e *Zostera marina* e uma área de fundo não vegetado. Avaliamos também a provisão de berçário e de espécies de interesse comercial de cada habitat. Abordamos aqui três questões principais: 1) A associação da fauna e plantas difere na abundância, biomassa, riqueza específica e diversidade entre habitats? 2) Os habitats diferem na associação da fauna de espécies de interesse comercial? E 3) diferem nas funções de berçário? Definimos três habitats-tipo (Sediment, Caulerpa e Seagrass) e três unidades de habitat (SED 1 – 3; CAUL 1 - 3; SG 1 - 3) para cada habitat-tipo. Foram utilizadas duas técnicas de recolha diferentes com espécies alvo específicas. Uma técnica (cores de PVC com um saco de rede) dedicada à coleta de material vegetal e macrofauna de mobilidade reduzida e fraca capacidade de dispersão (i.e. Gastropoda) e outra (arrasto de vara sem corrente) dedicada à coleta de macrofauna de maior mobilidade e capacidade de dispersão (i.e. Pisces). A abundância e biomassa resultantes das colheitas e triagens foram transformadas em valores por unidade de área e os dados das duas técnicas foram unificados. Os organismos foram separados em classes de tamanho e classificados em juvenis e não juvenis com base numa extensiva pesquisa bibliográfica e na opinião de especialistas. O interesse comercial foi também atribuído com base na publicação da DGRM (2018). Várias análises estatísticas foram executadas com o fim de entender as relações entre habitats e a contribuição da composição faunística para essas relações. Para a diversidade foi utilizado um dos índices mais comuns (Shannon & Wiener Index) que tem em conta não só a riqueza específica, mas também a abundância relativa de cada espécie. Foram encontradas diferenças diferenças estatísticas significativas na associação de fauna e plantas, funções de berçário e nas espécies de valor comercial. Os moluscos *Bittium reticulatum* e *Loripes orbiculatus* revelaram-se bons indicadores para as diferenças na associação de fauna e nas funções de berçário. O habitat Seagrass revelou os valores mais altos de riqueza específica e de funções de berçário (praticamente 50% da fauna coletada eram juvenis), enquanto que o Sedimento mostrou a maior abundância. A alta provisão de habitat para espécies de interesse comercial encontrada na Caulerpa deve-se maioritariamente ao abundante crustáceo *Upogebia spp.*, (“rallo”) que é comummente utilizado como isco para a pesca. O habitat dominado pela alga oportunista revelou o menor número de associações específicas com apenas 20% do total de espécies com associações a um único habitat. Os restantes 80% estavam similarmente divididos entre Sediment e Seagrass. Os resultados foram consistentes a nível espacial sugerindo que a composição dos fundos tem um papel importante na agregação de fauna associada. Concluímos que o estabelecimento de *C. prolifera* em fundos não vegetados tem um efeito na abundância e diversidade da Ria Formosa. O habitat dominado por *C. prolifera* revelou-se o mais instável, sendo mais heterogéneo do que os restantes e com afinidades específicas muito inferiores que advêm de um habitat recente e de rápida propagação, limitando assim o tempo para as espécies se adaptarem. A rápida proliferação desta alga oportunista em áreas de sedimento não só tem o potencial para alterar as comunidades aí existentes, mas também inibe a progressão ou mesmo o repovoamento de comunidades de ervas marinhas. Comunidades estas largamente estudadas e caracterizadas como de grande importância para a biodiversidade e com importantes funções de berçário. Assim, o estabelecimento de *C.*

prolifera tem o potencial para aumentar a diversidade numa área que foi previamente não vegetada, mas este aumento pode ter efeito negativo na medida em que põe em causa as associações específicas de espécies de áreas não vegetadas podendo oprimir importantes comunidades de ervas marinhas com potenciais consequências a longo prazo. Este estudo cobriu um intervalo de tempo curto deixando em aberto questões que devem ser abordadas em estudos futuros para uma melhor compreensão de como a proliferação desta alga oportunista está a afetar os ecossistemas em que se estabelece, em particular a Ria Formosa: Quais são as variações sazonais e anuais na estrutura das comunidades entre os três habitats? Quais são os efeitos a longo prazo que estas alterações na cobertura dos fundos terão na estrutura ecológica da Ria Formosa? Que efeitos terá limitação da progressão das comunidades de ervas marinhas, provocada por esta alga? A que taxa está *C. prolifera* a expandir-se na Ria Formosa?

Palavras-chave: Biodiversidade; *Caulerpa prolifera*; ervas marinhas; fauna associada; funções de berçário; espécies comerciais.

Abstract

Caulerpa prolifera (Forsskål) J. V. Lamouroux is an opportunistic green seaweed that is establishing on the unvegetated bottoms of Ria Formosa, often where seagrass meadows took place in the past. Ria Formosa is a coastal lagoon that extends along the southern Portuguese coast, place for a wealth of commercial and leisure activities. Such activities are supported, in the subtidal, by the important dominant seagrass communities which rank among the most valuable ecosystems on earth. The bottom of the lagoon is mainly constituted by unvegetated areas, seagrass meadows and *C. prolifera* meadows. For over than 170 years since the first report of this seaweed in Ria Formosa, its effect on the biodiversity has never been studied. In this study we aimed to understand how the establishment of *C. prolifera* on bare sediment in Ria Formosa affect the lagoon's biodiversity. To do so we compare the plant and fauna assemblage between three habitats, one dominated by a continuous *C. prolifera* meadow, one dominated by a *Cymodocea nodosa* and *Zostera marina* mix meadow and one unvegetated bed. We address three main questions: 1) Does fauna and plant assemblages differ in abundance, biomass, species richness and diversity between habitats?, 2) Do the three habitats differ in the fauna assemblages of commercial valued species?, and 3) do they differ in nursery functions? Similar fauna diversity was found within vegetated habitats (*C. prolifera* and *C. nodosa/Z. Marina* dominated habitats), followed by unvegetated sediments. *Bittium reticulatum* and *Loripes orbiculatus* revealed to be good indicators for the differences found in the fauna assemblage and nursery function, where seagrass showed the highest nursery role. Differences in the assemblage of commercial valued species were marked by *Upogebia spp*. ("ralos") presence in *C. prolifera* meadow, where the highest provision of habitat for commercial species was found.

Keywords: Biodiversity; *Caulerpa prolifera*; seagrass; associated fauna; nursery functions; commercial species.

Contents

1. Introduction

1.1. Theme justification

Ria Formosa is a coastal lagoon place for seagrass populations, among the most productive ecosystems in the world, that are severely decaying at a global scale, under threat mostly by human interactions (Thomsen et al., 2012; Tuya et al., 2013). Opportunistic green seaweeds often replace areas previously populated by seagrass communities in decline (Martínez-Samper, 2011). The bottom of Ria Formosa lagoon is mainly constituted by unvegetated areas and seagrass meadows (Cristina et al., 2006; Duarte et al., 2007; Guimarães et al., 2012). Currently, the green seaweed *Caulerpa prolifera* is occupying extensive areas of the Ria Formosa bottom (Fig 1.1).

Caulerpa prolifera is an opportunist green seaweed that has been reported in Ria Formosa (Cunha et al., 2013), settling on unvegetated bottoms where seagrass took place in the past (de la Rosa et al., 2011; Cunha et al., 2013). After *C. prolifera* had been first reported in Ria Formosa in the 19th century it "disappeared" for over 60 years until being reported again in 2011, always with a patchy distribution and never reported as well established on the lagoon (Cunha et al., 2013). Recently has been observed that this seaweed is now expanding and establishing on continuous meadows for the first time.

Foundation species, such as seagrasses and macroalgae, play an important role in determining the biodiversity of the entire ecosystem, since they provide not only substrate for organism fixation, but also food, shelter against predators, or nursery areas for several animal species (Heck & Orth, 1980; Heck et al., 1989; Guerao e Ribera, 2000; Beck et al., 2001; Beaumont et al., 2007). They influence as well the physical environment of the habitat, for instance, by retaining fine sediment and stabilizing the bottom and the hydrodynamic properties of the habitat (Lloret et al., 2005; Ceccherelli and Cinelli, 1997; Cunha et al., 2013; Tuya et al., 2013; Png-Gonzalez et al., 2014). The identity of the foundation species has a relevant effect on the modification of the physical environment, the identity of the associated-organisms, and on the resulting overall biodiversity (Sánchez-Jerez et al., 2000; Png-Gonzalez et al., 2014; Tuya et al., 201). The presence of vegetation allows a greater species diversity and abundance than unvegetated bottoms (Heck & Orth 1980; Peterson et al. 1984; Irlandi 1994; Heck et al. 1995) and vegetated habitats dominated by *Caulerpa* species are important for the biodiversity in coastal areas (Sánchez-Moyano et al., 2001a).

Fig. 1.1. Main subtidal habitats of Ria Formosa. **Top left**: seagrass meadow; **Top right**: unvegetated bottom; **Bottom**: *C. prolifera* meadow. (**Photo credits**: André Silva).

The effects of *C. prolifera* spread on biodiversity are yet unknown in Ria Formosa. Such effects are also poorly studied worldwide particularly entailing vegetation and fauna of above or belowground habitats. Previous studies compared the biodiversity on *C. prolifera* and *Cymodocea nodosa* meadows (Monterroso et al., 2012; Png-Gonzalez et al., 2014), and others studied single habitats such as *Zostera marina* meadows (Heck et al., 1989) or unvegetated sandy bottoms (Heck et al., 1989; Vázquez-Luis et al., 2009). Such studies focused on a particular part of the associated diversity, entailing only below-ground fauna (i.e. infauna) or small crustaceans as indicators for changes in the foundation species.

In this study, we compare the biodiversity in *C. prolifera* meadows to the two most common subtidal habitats in Ria Formosa lagoon: seagrass meadows (mixed *C. nodosa* and *Z. marina*) and unvegetated bottoms (bare sediment) (Fig. 1.1).

1.2. Taxonomy, physiology, morphology and life cycle of *Caulerpa*

Caulerpa prolifera (Forsskål) J. V. Lamouroux (Chlorophyta, Caulerpaceae) is a green macroalga that inhabits subtidal environments (Cunha et al., 2013). This rhizophytic seaweed have a siphonous thallus structure composed of a series of stolons that constitutes a complex net from which thin rhyzoids develop bellow ground and erect flat fronds emerge above ground (Sánchez Moyano et al., 2001b; de la Rosa et al., 2011). As other Caulerpa species, it can uptake nutrients from the sediment pore water with its thin rhyzoids (Williams, 1984) and can synthesize caulerpenyne as a herbivore deterrent (Amade and Lemée, 1998).

Its life cycle is intermediate between annual and perennial (Sánchez Moyano et al., 2001b) reaching the maximum vegetative development in muddy bottoms (de la Rosa et a., 2011). It has a subtropical affinity and it is strongly affected by natural seasonal disturbances, as its life cycle is dependent on water temperature, with a maximum growth on higher temperatures (Sánchez-Moyano et al., 2007). This seaweed can reproduce by stolon elongation and fragmentation, what leads to its ability to rapidly colonize unvegetated habitats (Stafford and Bell, 2006).

Overall, the morphology and propagation mechanisms of *Caulerpa* makes it an opportunistic species that successfully outcompete other species (Sánchez Moyano et al., 2001b). The biochemistry of *C. prolifera* can also have an impact to its advantage towards seagrass. Allelochemicals, such as caulerpenyne a major secondary metabolite synthesized by Caulerpaceae species, can have a relevant role on the colonization success of *C. prolifera* over marine macrophytes like *C. nodosa* (Mozzachiodi et al., 2001; Raniello et al., 2009; Tuya et al., 2013).

1.3. *Caulerpa* distribution and historical path in the Ria Formosa lagoon

C. prolifera is mainly distributed across the subtropical and tropical Atlantic Ocean, including the Macaronesian oceanic archipelagos of Madeira and the Canaries, and the Mediterranean Sea (Cunha et al., 2013; Tuya et al., 2013). In the Macaronesia islands, it is the only native species of the genus *Caulerpa* (Cunha et al., 2013).

Although the western limit of *C. prolifera* distribution in the European Atlantic coast has been reported in Cádiz (Sánchez-Moyano, 2004) until 2011 or lately in Huelva (de la Rosa et al., 2011), the green seaweed was already reported in Ria Formosa lagoon by several authors. The first to do so was the naturalist Frederick Welwitsh in 1845-47 (Welwitsch collection, LISU herbarium, Cunha et al., 2013) in Tavira, followed by Mendonça (1931) and Lami (1932 & 1933) (COI herbarium, Cunha et al., 2013). In 1951, Palminha reported "*important meadows of C. prolifera*" on the main channels of Ria Formosa, always as a subtidal species, "*never uncovered by the tides*" (Palminha, 1951). In 2013, Cunha et al., (2013) reported a small patch of *C. prolifera* (12 m^2 in size, at 1-4 m depth) found in April 2011 in Fuseta.

Since 1951 (Palminha, 1951) up until 2011 (Cunha et al., 2013), *C. prolifera* was never again reported in Ria Formosa, except by Ardré (1970 & 1971) with a report based on previous literature, in spite of the extensive surveys and mapping performed by the University of the Algarve throughout the lagoon (Cunha et al., 2013). It is not likely that the species remained unnoticed during this period. Its recent presence and enormous spread throughout the lagoon was probably originated from the introduction of a new clone well adapted to the lagoon environmental conditions or from the response of a cryptic population to recent alterations of environmental conditions. Since its life cycle is very temperature dependent (Sánchez Moyano et al., 2001b) the recent spread may be due to sea surface temperature rise.

In any case, *C. prolifera* in Ria Formosa, is genetically similar to the Mediterranean clones (Cunha et al., 2013).

C. prolifera often inhabits well sheltered areas with nutrient enrichment and poor water renovation (Lloret et al., 2005; de la Rosa et al., 2011), these areas are usually under a high anthropogenic influence like coastal lagoons (Lloret et al., 2005) or harbors (Sánchez-Moyano et al, 2001b). It forms large dense meadows typically on soft and hard bottoms (Sánchez Moyano et al., 2001a, 2001b; de la Rosa et al., 2011; Cunha et al., 2013) at a wide depth range between 1 to 30 m (Sánchez Moyano et al., 2001b; Lloret et al., 2005; de la Rosa et al., 2011; Cunha et al., 2013).

This seaweed has been widely reported to occupy bare sediments where seagrass loss took place in the past (Ceccherelli and Cinelli, 1997; Martínez-Samper, 2011; Thomsen et al., 2012; Cunha et al., 2013; Tuya et al., 2013; Del Río et al., 2016), but the exact reasons, by direct or indirect competition, are not clear yet (Tuya et al., 2013). Its tolerance to decreased irradiance, increased nutrients and to retain sediment with its stolons favor its establishment as well as influence the granulometry of the sediment stabilizing the bottom, also because it requires less irradiance, it can outcompete seagrass in suspended sediment situations where the water column transparency is compromised (Sánchez Moyano et al., 2001b; Lloret et al., 2005; de la Rosa et al., 2011; Cunha et al., 2013). Among the seagrass species present in the Ria Formosa lagoon, *C. nodosa* is a seagrass species distributed across the Mediterranean and the adjacent Atlantic coasts, from the southern Iberian Peninsula to Senegal, including the Macaronesia islands of Madeira and the Canary (Cunha and Araujo, 2009) and it is known to form mixed meadows with green rhizophytic seaweeds like *C. prolifera* (Tuya et al., 2013; Del Río et al., 2016). *Z. marina* is the most widely spread seagrass species in the northern hemisphere (Dahl et al., 2016), present in the northern Pacific and northern Atlantic (Duarte et al., 2002), in Europe from the southern Black Sea and the Gulf of Cádiz (southern Portugal) to Iceland and the northern parts of Norway (Green and Short, 2003), also known to form mixed meadows with *C. prolifera* (Rueda and Salas, 2003).

As seagrass meadows regress direct and/or indirectly, due to anthropogenic causes like dredging (Cunha et al., 2013), induced nutrient loading (Lloret et al., 2005; Morris et al., 2009; Cunha et al., 2013; Tuya et al., 2013) or due to natural competition for nutrients in the sediment and reduction of water transparency (Ceccherelli and Cinelli, 1997; Lloret et al., 2005; Cunha et al., 2013; Tuya et al., 2013), rhizophytic opportunistic seaweed species like *C. prolifera* may take advantage and rapidly settle in the sediment leaving no room for the re-establishment of seagrass species. In the Ria Formosa lagoon Cunha et al., (2013) has witness that the small patch of *C. prolifera* discovered in 2011, increased and settled in an area where a meadow dominated by *Z. marina* had disappeared.

1.4. Importance of species commercial value and nursery function to biodiversity

According to Worm et al. (2006), the reality that marine ecosystems are experiencing on the loss of populations and species "*is increasingly impairing the ocean's capacity to provide food"* as he connects the rates of resource collapse with the declining of diversity. Higher species richness lead to a more stable system where the rates of collapse and extinction of commercial species are lower (Worm et al., 2006).

Commercial valued species are more vulnerable to be exploited but the fact that they are registered as of commercial interest, also give us the opportunity to manage its exploitation and apply a more sustainable and not an undiscriminating use. An understating and evaluation on the species of commercial interest for an ecosystem under exploitation gives not only the possibility to better manage the resources in an ecological point of view for the balance of the ecosystem but also in a profitable way for the markets.

Species and population extinction not only debilitate marine ecosystems to provide food to the human population but also, in a changing environment, compromises their stability and recovery (Worm et al., 2006). "*Biodiversity is a direct source of ecosystem goods*" (Daily et al., 1999). There is a positive correlation between the biodiversity of an ecosystem and the availability of its resources (Palumbi et al., 2009; Worm et al., 2006).

According to Beck et al. (2001) a habitat is consider a nursery only if the average of individuals that recruit to adults is greater than in other habitats that juveniles also occur. It is not clear though, among the scientific community, whether nursery is valued as an ecosystem service or not. One of the main reasons for this is that as an ecosystem service, nursery can be correlated with other services like fisheries as they are directly connected, but the nursery function is an ecosystem function and a biodiversity component (Liquete et al., 2016), as a habitat that serve as a nursery ground will have characteristics (e.g. food and shelter provision) (Espino et al., 2011; Duarte & Chiscano, 1999) that influence biodiversity (Ceccherelli and Cinelli, 1997; Lloret et al., 2005; Png-Gonzalez et al., 2014; Tuya et al., 2013). Liquete et al. (2016) concludes that nursery function can be consider an ecosystem service when it is beneficial for humans but not if it is only representing an indicator of biodiversity. This is clarifying for when the presence of species with commercial value is involved and is most certainly according to the ecosystem services definition that links the services provided by the ecosystem to the welfare of human society.

1.5. Expected *Caulerpa* importance to the biodiversity

It has been shown that the three habitats being studied here (seagrass meadows, *C. prolifera* meadows and unvegetated bottoms) had different levels of diversity among them (Heck et al., 1989; Vázquez-Luis et al., 2009; Monterroso et al., 2012; Png-Gonzalez et al., 2014), therefore, changes in the habitat type may influence the biodiversity. As *C. prolifera* settles on bare sediment, that may or may not been populated by seagrass, changes on the animal and plant assemblage are expected.

Previous studies regarding the effects on biodiversity are not uniform. *C. prolifera* dominated bottoms were more diverse and abundant when compared with *C. nodosa* according to Png-Gonzalez et al. (2014) study in Gran Canaria (Canary Islands), where species-specific affinity was also detected when comparing the two habitats. In contrast, Vázquez-Luis et al., (2009) study in the Mediterranean shows that *C. prolifera* habitats were less diverse than *C. nodosa* habitats. This supports that coastal habitats and their biodiversity can differ from one region to another.

Vegetated habitats dominated by *Caulerpa* species are important for the maintenance of biodiversity in coastal areas (Sánchez-Moyano et al., 2001a) as they may benefit the assemblage of crustaceans (Sánchez-Moyano et al., 2007, Png-Gonzalez et al., 2014).

Seagrass play an important role in providing habitat for nearshore fish assemblages and a nursery ground for early stages of numerous fish species (Espino et al., 2011). Contrary to the epifaunal assemblage, fish abundance may be larger in *C. nodosa* than in *C. prolifera* meadows (Tuya et al., 2013; Png-Gonzalez et al., 2014). As suggested by Png-Gonzalez et al. (2014), this contrasting abundance patterns of epifaunal and fish assemblages between *C. nodosa* and *C. prolifera* meadows might be explained by the large abundance of fish predating, therefore removing, large quantities of epifauna (prey). As seagrass is known to be a suitable habitat for fish providing shelter and food resources (Espino et al., 2011; Duarte & Chiscano, 1999), *C. prolifera* dominated beds may not express the same structural qualities, manifesting this way a lack of predators (fish), when in comparison, allowing larger abundances of prey (epifauna) to assemble. The plant structure might be the reason why fish would prefer *C. nodosa* over *C. prolifera* but green seaweeds can also represent an additional food source for herbivores (Del Río et al., 2016).

The presence of vegetation allows a greater species diversity and abundance than unvegetated bottoms (Heck & Orth 1980; Peterson et al. 1984; Irlandi 1994; Heck et al. 1995).

1.6. Objectives

In this study, we aim to understand how the establishment of *C. prolifera* on the bare sediment of the Ria Formosa is affecting the biodiversity and the assemblage and nursery of commercial species of the lagoon. We compare the diversity, species richness and abundance on *C. prolifera* meadows to that on the two main subtidal habitats of the lagoon (unvegetated areas and seagrass meadows), and describe the assemblage of commercial valued species and the nursery function of the habitats.

Here we address the following questions:

1 – Does fauna and plant assemblages of the three habitats differ in abundance, biomass, species richness and diversity?

If yes, which species contribute the most?

2 – Do the three habitats differ in the fauna assemblage of commercial valued species?

If yes, which species contribute the most?

3 – Do the three habitats differ in nursery functions?

If yes, which species contribute the most?

2. Material & Methods

2.1. Study area

This study was carried out in Ria Formosa (south of Portugal) (Fig. 2.1), a highly dynamic lagoon system with important seagrass populations that offer a wide range of ecosystem services (Cristina et al., 2006; Duarte et al., 2007; Guimarães et al., 2012). The lagoon is classified as a "coastal system" instead as a "transitional water", since it only has one permanent river flow, the Gilão river, which cause a relatively low freshwater input and salinity gradient (Cristina et al., 2006). A series of barrier islands (i.e. Barreta or Deserta, Culatra, Armona, Tavira and Cabanas) restrict the water exchange between the lagoon and the Atlantic Ocean. Most of the water is replaced during the tidal cycle through its natural inlets (i.e. Faro-Olhão, Armona, Fuzeta, Cacela and Tavira) that separate the barrier islands (Águas, 1986; Cristina et al., 2006, Ribeiro et al., 2006). Ria Formosa covers a wet area of 10 500 ha (Falcão, 1996) (55 km long, 6 km wide, Ribeiro et al., 2006), of which one third is intertidal (Cristina et al., 2006), with minimum and maximum wet area coverage, during spring tides, of 14.1 km^2 and 63.1 km^2 respectively, where tidal elevations are 1.30 m at mean neap tide and 2.80 m at spring tide (Águas, 1986). With an average depth of 1.5 m (3.5 m in the main channels) and a tidal range that varies between 1.3 m at the neap tides and 2.8 m at the spring tides, 14% of the lagoon is permanently submerged and most of the water of the lagoon (50 – 75%) is replaced during the tidal cycle.

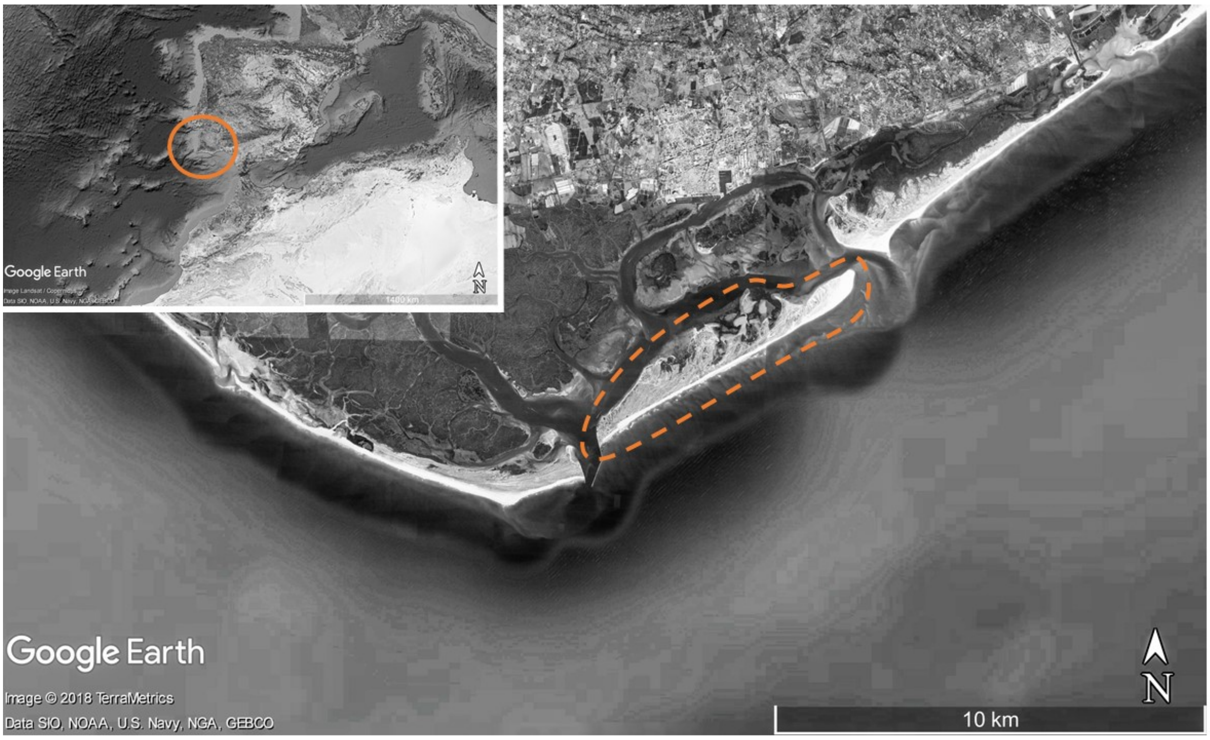

Fig. 2.1. Map of Culatra island (dashed orange line) in Ria Formosa Natural Park, Portugal. Map adapted from Google Earth Pro.

Our study area was defined along the inside of Culatra barrier-island (Fig. 2.1), an area (~400 000 m2, Fig. 2.2) where *C. prolifera* develops extensive meadows. Three habitat-types were chosen within the study area (ca. 400 000 m^2, Fig. 2.2) representing the three main habitats that can be found in Ria Formosa, *Caulerpa prolifera* meadow (Caulerpa - CAUL, ~5 000 m2, 37.002920°N, 007.831070°W, Fig. 2.2 and 2.3), seagrass meadow (*Cymodocea nodosa* mixed with *Zostera marina*) (Seagrass - SG, ca. 12 000 m2, 37.004070°N, 007.824700°W, Fig. 2.2 and 2.3) and unvegetated bottoms (bare sediment) (Sediment - SED, ca. 4 000 m2, 37.000500°N, 007.834040°W, Fig. 2.2 and 2.3). All sites were at similar depths (ca. 1,6 m at the low tide and ca. 3,4 m at the high tide) and exposed to similar hydrodynamic conditions.

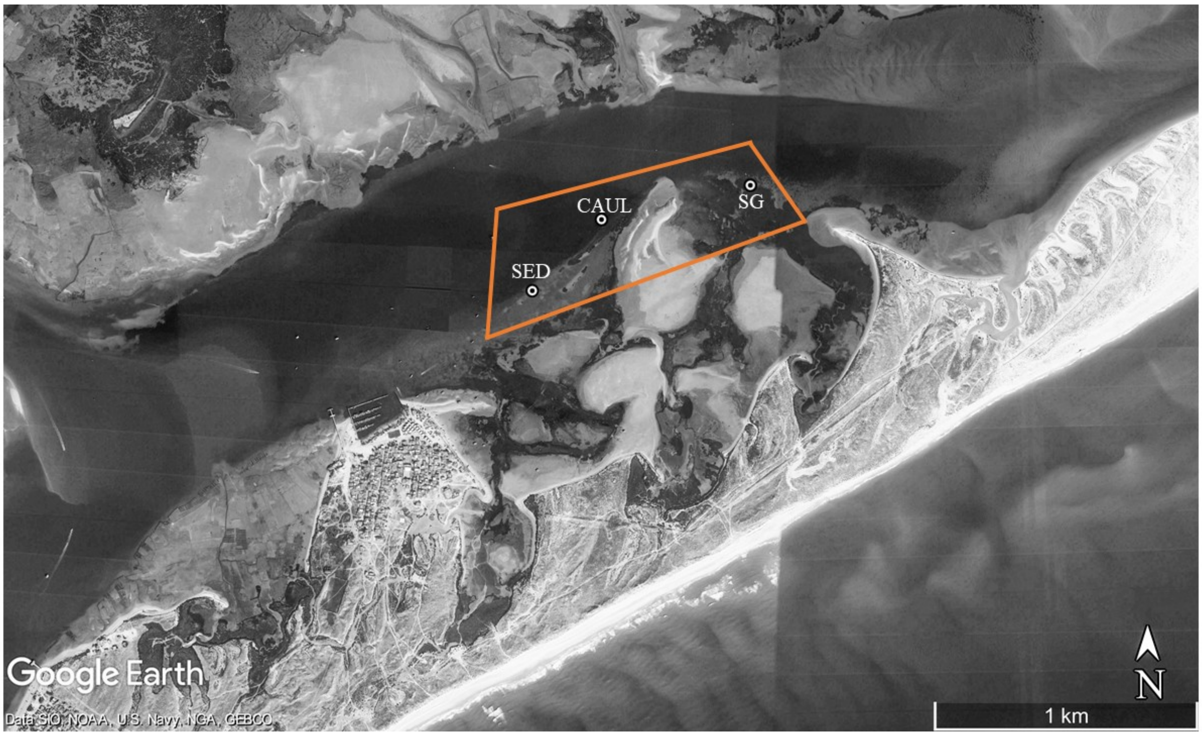

Fig. 2.2. Map of the study area (orange line, 400 000 m^{-2}) showing the three habitat-types, Sediment (SED), Caulerpa (CAUL) and Seagrass (SG), in Culatra island, Ria Formosa. Map adapted from Google Earth Pro.

2.2. Sampling design

For each habitat-type (Caulerpa, Seagrass and Sediment), three habitat-units (Fig. 2.3) were defined. To do so, we dove with snorkeling in the area and visually assess the bottom's coverage, the layout of the meadows and unvegetated areas. After a careful look we used a buoy tied to a scuba diving weigh to mark each site and by boat took the GPS coordinates. By the same procedure, the limits of the meadows and the unvegetated area were marked. In total, nine habitat-units (CAUL 1 - 3; SG 1 - 3; SED 1 - 3) were defined by two scuba divers creating a 50 x 10 m (ca. 500 m^2, Fig. 2.3) underwater rectangle with the help of two measuring tapes (30 m and 50 m long), a compass and four buoys to mark each extremity of the tapes.

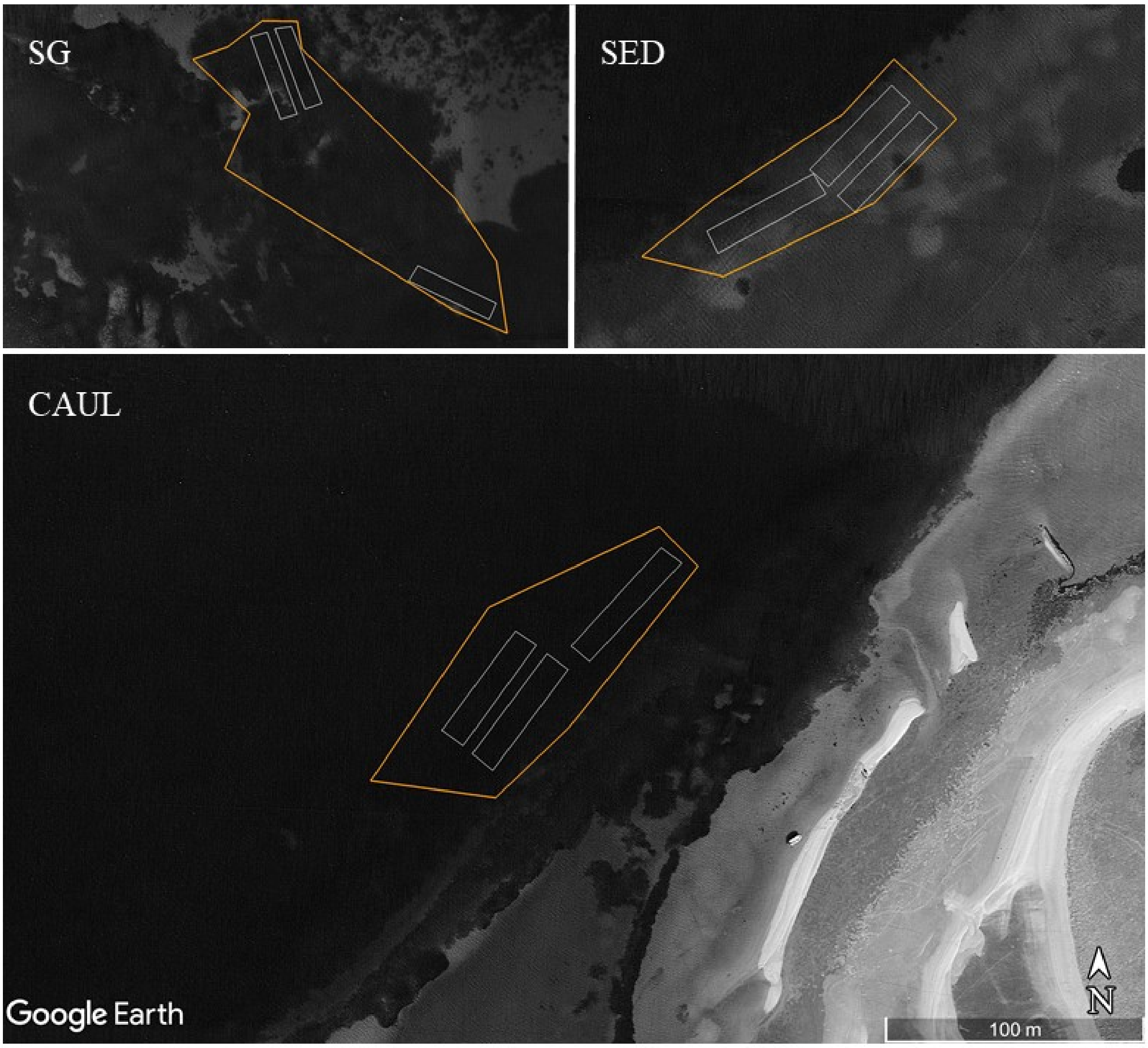

Fig. 2.3. Map showing the three habitat-units (white rectangles, 50 x 10 m, ~500 m^2) within each habitat-type (orange lines). **Top left**: Seagrass (SG); **Top right**: Sediment (SED); **Bottom**: Caulerpa (CAUL). Map adapted from Google Earth Pro.

In order to cover the highest range of animal and plant sizes possible (excluding microfauna), we combined two sampling methods: **(a) PVC cores with net bags** (adapt from Sánches-Moyano et al., 2001), to sample macrofauna with reduce mobility, seagrass and macroalgae (Fig. 2.4) and a **(b) beam trawl net** (Ribeiro et al., 2006) to sample macrofauna with higher mobility (Fig. 2.4). Three field campaigns were conducted, two to sample macrofauna and plant material using method **(a)** (13 to 16 of November and 11 to 14 of December, 2017) and one to sample macrofauna using method **(b)** (19 of January, 2018).

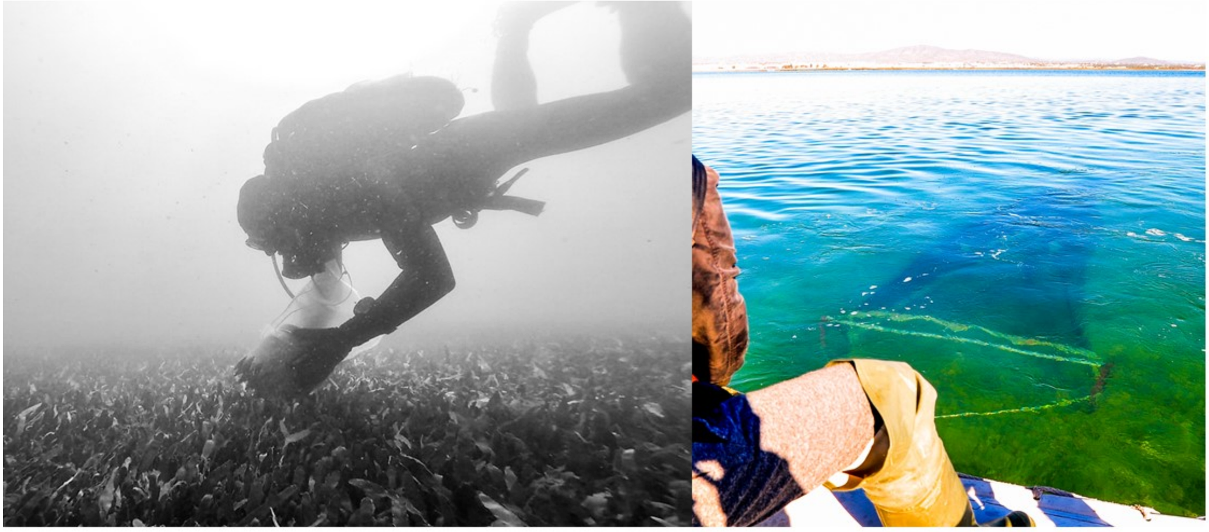

Fig. 2.4. Sampling methods used to collect fauna and plant material. **Left**: **(a)** PVC core with net bag; **Right**: **(b)** beam trawl net (**Photo credits**: André Silva (left) and Rui Santos (right)).

2.2.1. PVC cores with net bags

Field work

A total of 27 samples were collected with the **(a) PVC cores with net bags** by scuba diving. For each of the nine habitat-units, three samples were collected at the 10, 25 and 40 m mark of the 50 m transect by pushing 7.5 cm bellow ground a PVC core (22 cm of inner diameter, adapted from a 15 cm x15 cm x 5cm bore, Sánches-Moyano et al., 2001) with a nylon net bag (500 µm mesh size, Sánches-Moyano et al., 2001), with the help of a sledgehammer and a shovel. The net bags were dis-attached from the cores still underwater and closed with a zip-tie and once on the boat, carefully washed of thin sediment and kept in saltwater (Fig. 2.5).

Lab work

Once at the lab, the samples collected with the **(a) PVC cores with net bags** were kept fresh on a fitoclima (17ºC, 12h by 12h light cycle) with direct oxygenation until processed. For each of the 27 samples, the content from the net bags was carefully cleared to a tray, always with salt water, and all animal and plant material sorted (Fig. 2.5). With the help of a 500µm sieve, the material on the tray was subjected to two rinses with salt water and two with fresh water to help clean the samples off thin sediment and identify any left organisms, as some dead invertebrates float and are easier to spot. All the fauna was classified in size classes (e.g. S, M and L), adults were separated from juveniles and identified in major groups (e.g. Gastropoda, Bivalvia and Decapoda) with the help of a binocular microscope (Zeiss Stemi 2000-C), properly labeled and preserved in ethanol (96%) or a KEW solution (40% of salt water, 40% of Ethanol at 70%, 10% of glycerine and 10% of formaldehyde), depending on the organism degradation and intended storage period, for a future and more careful identification to the species level.

The remaining material on the tray was properly labeled and frozen for a further closer look of potentially left organisms with a binocular microscope. Because it was not possible to identify the entire remains of the samples, for this study, a small equal amount of each sample was analyzed.

All the plant material (i.e. seagrass and macroalgae) was cleared of potentially attached organisms, identified to the species level, separated in bellow and above ground (Fig 2.5) and dehydrated in a drying oven (VWR-VL115) at 60ºC during 48h, for dry weight calculation. Once the drying process was over, the plant material was weighted with an electronic scale (±0,01, Shimadzu-EL 200), smaller pieces were weighted on a more precise electronic scale (±0,0001, VWR precision-balance).

Fig. 2.5. **(a) PVC cores with net** bags campaign. **Top left**: Plant material triage; **Right**: net bag washed of thin sediment; **Bottom left**: Fauna triage (**Photo credits**: (Begoña Martínez-Crego).

Finally, all the organisms stored in Ethanol and KEW were identified to the species level with the help of taxonomic keys, guide books, papers and websites (Fauchald, 1977; Chícharo, 1996; Muzavor et al., 1999; Borges, 2007; Gamito, 2008; Conway, 2015; Cruz et al., 2015; Albuquerque, 2016; Oliver et al., 2016; Marine Species Identification Portal, 2018; Megabenthos, 2018; WORMS, 2018) and with the collaboration of my supervisor (Dr. Begoña Martinez-Crego) and taxonomy specialists (Dr. Jorge Gonçalves, Carlos Afonso, Frederico Oliveira, and Pedro Monteiro). After identified the organisms were dehydrated on the drying oven (60ºC, 48h) and weighted separately by size classes. Three different scales were used for different organism sizes, a precision electronic scale (±0,01g, Shimadzu-EL 200), an analytical electronic scale (±0,0001g, VWR precision-balance) and a micro electronic scale (±0,001mg, Sartorius micro). The identification of small crustaceans (Amphipoda, Tanaidacea, Cumacea, etc.) and polychaetes was not possible within the time framework of this master thesis and they were not consider in the study. Small epiphytic species were excluded from the study.

2.2.2. Beam trawl net

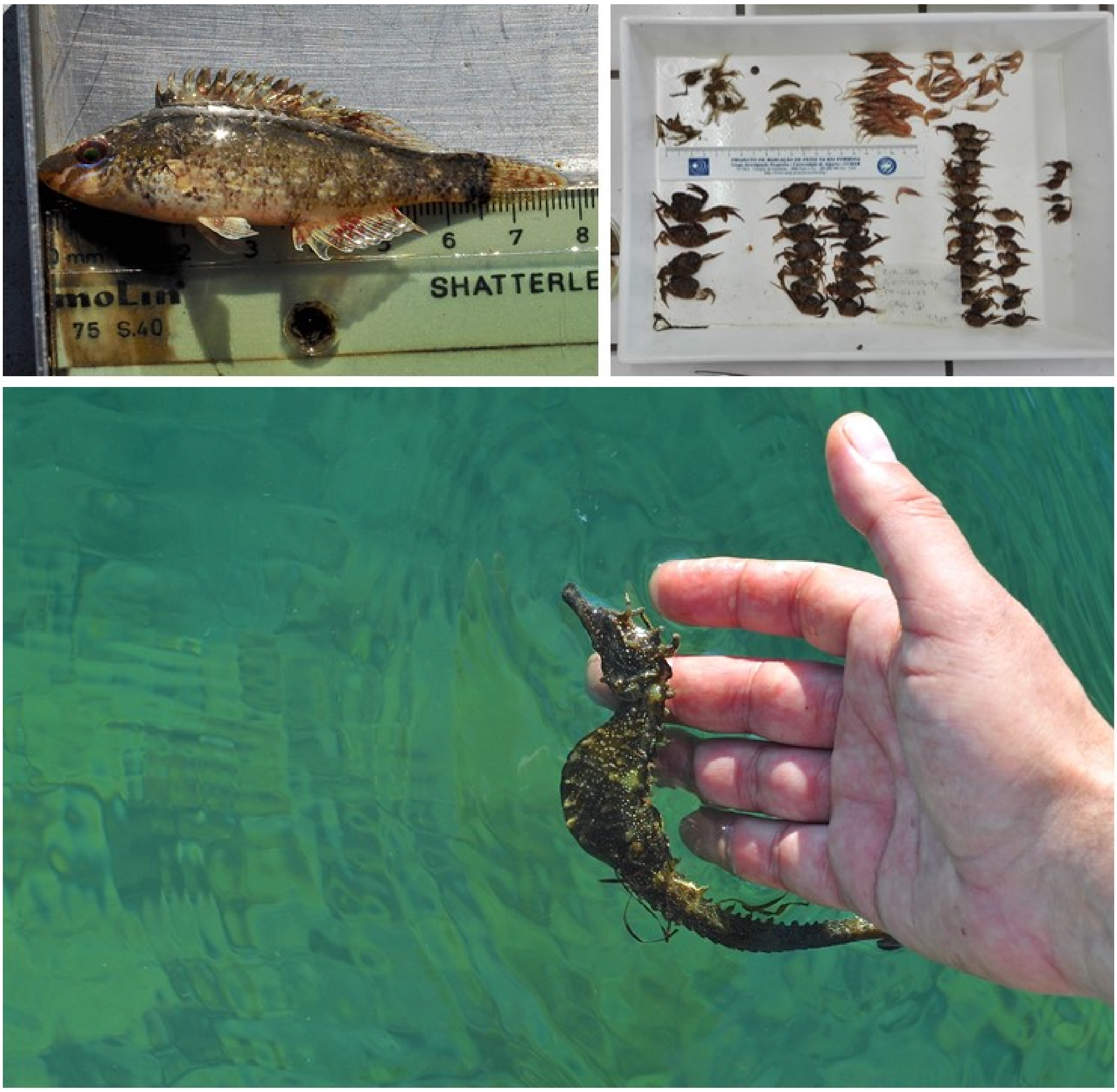

Fig. 2.6. **(b) Beam trawl net** campaign. **Top left**: *Symphodus cinereus* being measured with an ichtyometer; **Top right**: Fauna triage; **Bottom**: *Hippocampus guttulatus* being released. (**Photo credits**: Rui Santos (top left and bottom) and Filipe Parreira (top right)).

Field work

To sample macrofauna, a total of nine 50 m tows were done, one for each habitat-unit, with a **(b) beam trawl net** (2.6 m wide, 0.45 m high at the mouth and a mesh size of 9 mm in the cod end, Ribeiro et al., 2006), where the net was dragged by a boat at an average speed of 3 knots. Whenever possible, the organisms were identified to the species level *in situ*, with the help of a specialist (Dr. Jorge Gonçalves), and released (i.e. seahorses, pipefishes, cuttlefish and sea urchins, Fig. 2.6).

Lab work

When unfrozen, all the organisms collected with the beam trawl net were classified in size classes and identified to the species level as adults or juveniles with the help of literature and with the collaboration of taxonomic specialists. After identified, the organisms were dehydrated in a drying oven at 60ºC during 48h, for dry weight calculation.

2.3. Data analysis

2.3.1. Data matrix

From the campaigns, resulted 5 main data sets: plant biomass, fauna abundance and biomass from the method **(a)** (**PVC cores with net bags)** and fauna abundance and biomass from the method **(b) (beam trawl net)**. At this point there is a total of 3 samples *per* habitat-unit collected with **(a)** and 1 sample *per* habitat-unit collected with **(b)**.

In order to be possible to put together data from the two different sampling methods, first the number of samples from each technique must be the same, so the 3 samples of each habitat-unit collected with **(a)** were averaged. At last because the areas covered by the two techniques were different, the absolute values of abundance (number of individuals) and biomass in grams of dry weight (gDW) had to be transformed in densities, number of indv.m^{-2} and gDW.m^{-2} respectively. To do so, data collected with the method **(a)** was divided by the area covered by the core circumference (A_{core} = 0.038 m^2) and the method **(b)** data divided by the area covered by the trawl (A_{trawl} = 130 m^2).

An important consideration to take when working with two different sampling techniques is the target species for each one. Although the technique's targets were well defined from the beginning, accidental catches led to some targets of one technique be caught with the other, this is an issue since the choice of the two different techniques is based on organisms with different sizes, mobility and dispersion capabilities. For example, by collecting a relatively rare and big sized organism like *Cymbium olla* with the method **(a)**, the density of one individual would be 26.32 indv.m^{-2}, this result would be very far from the reality as in any locale of Ria Formosa is not possible to observe 26 *C. olla* in one square meter. On the other hand, if this specimen had been caught with the method **(b)**, its density would be 0.008 indv.m^{-2}, a much more credible result.

For this reason, before merging the data sets from different techniques: I) every organism that belonged to a different technique from the one it was caught on and was exclusively on that technique, it was discarded; II) every organism that was caught by both techniques, was discarded from the one it did not belong to.

To the point at which no species could be found at both techniques and every organism was attributed to the right one, fauna abundance **(a)** and fauna abundance **(b)** were merged as well fauna biomass **(a)** and fauna biomass **(b)**. From here, plant biomass and fauna abundance were both transformed in species richness by attributing every occurrence to 1 and 0 to no occurrences. Also, two additional matrices were built, one containing only juvenile individuals and other containing only individuals of commercial value. So at the end, the 5 initial data sets were transformed into 8 matrices (plant biomass, plant richness, fauna abundance, fauna richness, juvenile abundance, juvenile richness, commercial abundance and commercial richness). Matrices manipulation, summaries, means, standard deviations and table contents (except analysis results) were done using R studio open source software.

2.3.2. Classification as juveniles and commercial species

In order to classify individuals as juveniles or adults, an extensive literature research was done to find the exact size at which each species reach a mature stage (Annex V) and based on the class size, a classification was made, in some cases, juvenile attribution was done by specialists based on morphometric characters (Dr. Begoña Martinez-Crego, Dr. Jorge

Gonçalves, Carlos Afonso and Frederico Oliveira). The ones for which it was not possible to find literature about, as well as the adults, were consider non juveniles.

Regarding the classification as commercial species, this was done based on the most recent publication of the national entity responsible for such matters, *Direção-Geral de Recursos Naturais, Segurança e Recursos Marinhos* (DGRM, 2018) that by the terms of the article 37.º of the (UE) Regulation nº 1379/2013 of the European Parliament and of the Councile, of 11th December 2013, Portugal as established a list of the commercial denominations authorized on National Territory for the fishing and aquiculture's products.

2.3.3. Dry weight calculations

For some individuals, it was not possible to weight, to measure the length or both because some of the organisms were released into the wild and others due to technical failures. In these cases the following strategies were taken:

I) When an individual was not weighted but was measured, a length-weight relation was used from the literature (Annex V) and the weight was mathematically calculated.

II) When an individual was not weighted but was measured, a length-weight relation was not found and there was a consistent number of occurrences of the same species with measurements, then the weight for that individual was calculated by finding the gDW/indiv. for that species and multiplied by the number of individuals that were not measured.

III) When and individual was not measured by any means (weighted or measured), than the length of the individual was extrapolated from a photograph (in every of these cases, individuals were photographed) and strategy I) applied.

IV) For fish, when only the biomass in grams of wet weight (gWW) was possible to calculate via a length-weight relation but not the gDW, then the percentage of water for fish that were measured both for gDW and gWW was calculated and multiplied to obtain a gDW.

Although samples collected with the method **(a)** had an underground coverage of 7.5 cm deep and the bellow ground plant material was also collected and sieved, for this study we only account for the above ground plant material.

2.3.4. Diversity index

The definition of biodiversity is controversial and subjected to considerable discussion (Magurran, 1988; Heywood, 1995; Henderson, 2009). Magurran (1988) says that there is a simple explanation for why ecological diversity is sometimes so hard to define and that is because diversity consists in two components, variety (species richness) and species relative abundance. In ecology, species richness is defined as a measure of the number of species in an area of habitat, while species diversity referred to both species richness and abundance (Henderson, 2009). Several indices have been proposed to measure species diversity, such as the Shannon & Wiener Index (Shannon, 1948) or the Simpson index (Simpson, 1948). There are many other measures of diversity, but in this study we use the Shannon & Wiener Index as it takes into account the evenness of the samples and not the dominance of species.

Species richness gives a value but limited information on the Biodiversity of a habitat as it only presents the number of species present on a sample regardless the abundance of a certain species, in other words in a scenario where a habitat *A* have 100 organisms belonging to one species and habitat *B* only have 1 organism belonging to the same species, species richness will be the same for both habitats (S = 1) even though that same species plays a bigger role for the structure of habitat *A* than *B*.

The Shannon & Wiener Index for diversity allows a better understanding of how diversity is distributed between habitat-types, because it takes evenness into account. This Index (Shannon, 1948) was used to measure the fauna and plant diversity of the three habitat-types. H’ is a measure for diversity that takes into account the proportion of the total count as well as the evenness (how evenly the number of individuals *per* species is distributed among samples). Where p_i is the proportion of the total count, i the number of species and K a positive constant (Clarke and Warwick, 2001).

$$H' = -K \sum_{i=1}^{n} pi \log pi$$

2.3.5. Statistical analysis

Statistical analyses were conducted in order to understand how variables for plant (Species and Foundation species) and fauna (Species, Value and Nursery) assemblage differed among the habitat-types (Sediment, Caulerpa and Seagrass) and the homogeneity withing groups (habitat-types).

Multivariate non-parametric analyses (PERMANOVA, PERMDISP; Hierarchical cluster and SIMPER) were run using untransformed data because our data failed to meet normality (Shapiro-Wilk test). Analyses were run using the PRIMER 6.0 + PERMANOVA software. When our data met a normal distribution and only one variable was being analyzed, Univariate parametric analysis (One-way ANOVA and Tukey-test) were run using untransformed data with SygmaPlot 12.0 software.

The homogeneity of the habitat-types, for every variable, was tested using a permutational test of multivariate within-group (habitat-unit nested in habitat-type) dispersion and pair-wise comparisons (PERMDISP, Anderson et al., 2008) based on a Bray-Curtis similarity resemblance matrix testing for the distances to the centroids. By focusing only on the dispersion effects, if the null hypothesis is not rejected, PERMDISP teases out the hypothesis that the groups are not homogeneous (Anderson et al., 2008) for a viable comparison between groups with PERMANOVA.

A Hierarchical cluster analysis was run in order to visually access and evaluate the homogeneity within habitat-types (between habitat-units) for plant species biomass and richness and for fauna species abundance and richness, as it arranges samples based on a Bray-Curtis similarity resemblance matrix. Hierarchical clustering is a visual representation of the similarity network among samples and works as a complement to the non-parametric statistical tests and never as a replacement.

In order to identify which species contributed more (i.e. those with a higher % contribution) and more consistently (i.e. defined as those with a mean dissimilarity to standard deviation ratio equal or higher than 1.5, which means that mean contribution is higher than variation) to the dissimilarities between habitat-types, Similarity percentage analyses (SIMPER, Clarke and Warwick, 2001) were run on the plant species biomass and richness, fauna species abundance and richness and on the fauna abundance for Nursery (species as juveniles and non juveniles) and Value (species as commercial or non commercial). According to the author Clarke and Warwick (2001), performing a SIMPER analysis on the within-group similarities for species is irrelevant as the more abundant a species is within a group, the more it will contribute to the intra-group similarities. On the other hand, a SIMPER analysis on the within-group similarities for factors (juveniles *vs* non juveniles and commercial *vs* non commercial) was run, identifying which factor contributed more to the similarities of each habitat-type.

Following the PERMDISP analysis, for every variable a Permutational multivariate analysis of variance (PERMANOVA, Anderson et al., 2008) based on a Bray-Curtis similarity resemblance matrix with two factors: habitat-type (fixed), habitat-unit (random, nested in habitat-type).

To verify the differences on the variances of the mean for the fauna and plant diversity (Shannon & Wiener Index) between habitat-types, a One-way analysis of variance (ANOVA) was run together with a Tukey-test to evaluate pair-wise comparisons.

3. Results

3.1. Fauna and plant assemblage

A total of 2545 organisms across 101 different species were counted and identified with a total faunal biomass of 880gDW, where *Bittium reticulatum* and *Loripes orbiculatus* were the most abundant species with 814 and 367 absolute values of abundance, respectively. Regarding the plant material, 14 different species were identified, 2 of which were seagrass species (*Zostera marina* and *Cymodocea nodosa*) and the 12 remaining were macroalgae.

Table 3.1. Summary of similarity percentage (SIMPER) analysis showing the most important species responsible for the plant biomass dissimilarities between habitat-types.

Species	Av. Dissimilarity	Av. Abund	Av. Abund	Diss/SD	Contrib %	Cum.%
Plant biomass						
Sediment *vs* **Caulerpa**	100					
Caulerpa prolifera		0.00	223.95	93.39	99.13	99.13
Cystoseira spp.		0.00	1.04	0.73	0.74	99.87
Lithophyllum incrustans		0.20	0.00	0.61	0.10	99.97
Colpomenia spp.		0.04	0.00	0.61	0.02	99.99
Sediment *vs* **Seagrass**	100					
Cymodocea nodosa		0.00	83.01	3.93	61.42	61.42
Zostera marina		0.00	34.50	1.25	19.67	81.09
Cystoseira spp.		0.00	18.57	2.87	13.33	94.41
Cladophora spp.		0.00	6.07	3.09	3.90	98.32
Caulerpa *vs* **Seagrass**	98.35					
Caulerpa prolifera		223.95	1.56	4.81	59.93	59.93
Cymodocea nodosa		0.00	83.01	3.76	24.15	84.08
Zostera marina		0.00	34.50	8.99	9.14	93.22
Cystoseira spp.		1.04	18.57	2.36	4.94	98.17

Plant abundance (species biomass) significantly differed between habitat-types for Species and Foundation species (PERMANOVA results in Annex I). Although the PERMDISP main test in (Annex II) revealed that the habitat-types were not homogeneous (significant differences within groups), the pair-wise comparisons for the same analysis revealed homogeneity between replicates of the same habitat for each comparison (no significant differences within groups). The Hierarchical cluster analysis (Fig. 3.4) reveals why PERMDISP main test resulted in an overall no homogeneity within groups, Sediment samples had zero similarities among them or to any other samples from any other habitat. Both vegetated habitats (Caulerpa and Seagrass) were equally similar within groups. Both the Hierarchical cluster and the SIMPER analysis (Table 3.1) revealed that, although by a very low margin, vegetated habitats were the less dissimilar. These results indicate that Sediment did not share the same species between its samples (every sample was different). When comparing the Sediment with the other habitats, the foundation species from vegetated habitats (*C. prolifera* and *C.* nodosa/*Z. marina*) were consistently (higher Diss/SD) the top contributors (higher Contrib. %) for the dissimilarities between habitats. *Cystoseira spp.* and *Cladophora spp.* were among the top contributors for the dissimilarities between Sediment and Seagrass. C. prolifera was the plant species that contributed more and more consistently for the dissimilarities between the two vegetated habitats holding nearly 60% of contribution.

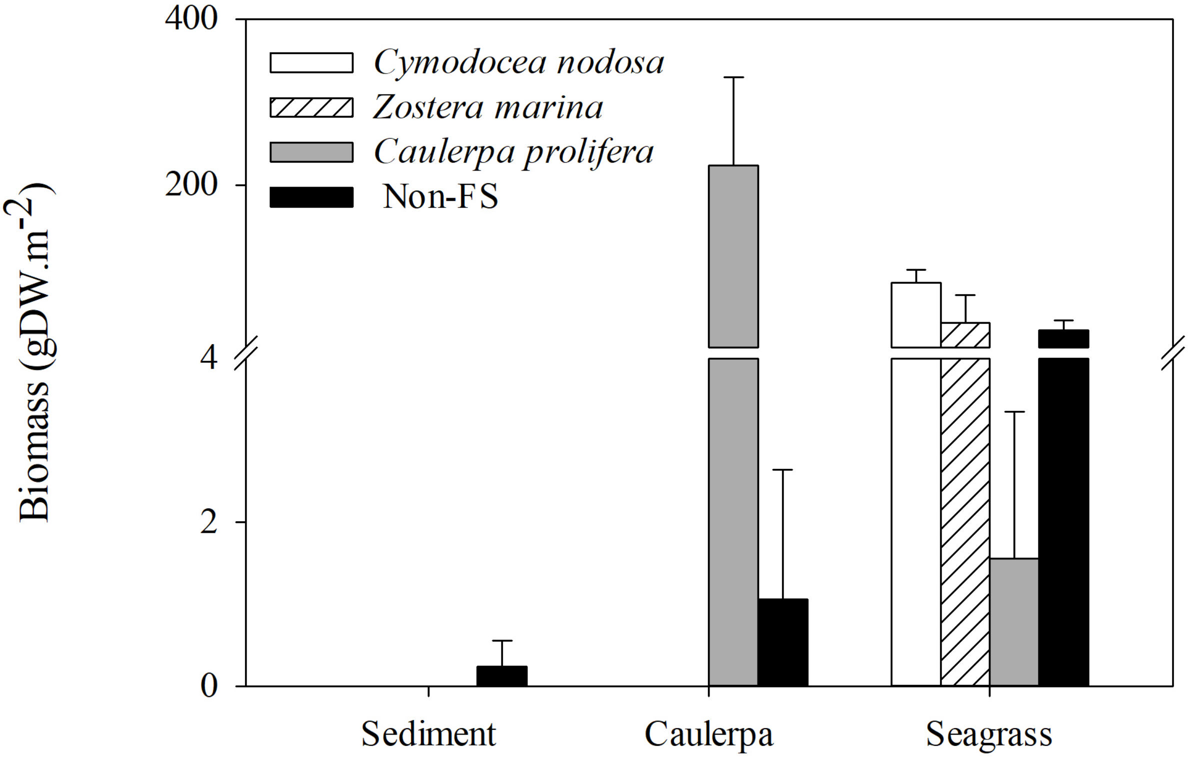

Fig. 3.1. Mean (±SD) of the plant biomass (gDW m^{-2}) *per* habitat-type for *Cymodocea nodosa*, *Zostera marina*, *Caulerpa prolifera* and Non-foundation species (Non-FS).

Those results were coherent with the clear dominance of each foundation species in each respective habitat-type, where Caulerpa habitat-type is largely dominated by *C. prolifera* and Seagrass dominated by *C. nodosa* and *Z. marina*, Sediment on the other hand has no dominant vegetation (Fig. 3.1). Non-foundation species were largely more present in Seagrass, with very low biomass (< 2 gDW m^{-2}, Fig. 3.1) in the other habitats. Overall, Caulerpa habitat had the highest plant biomass, more than 1.5 times denser than Seagrass (Annex IV).

Table 3.2. Summary of similarity percentage (SIMPER) analysis showing the most important species responsible for the plant species richness dissimilarities between habitat-types.

Species	Av. Dissimilarity	Av. Abund	Av. Abund	Diss/SD	Contrib %	Cum.%
Plant species richness						
Sediment *vs* **Caulerpa**	100					
Caulerpa prolifera		0.00	1.00	93.39	31.30	31.30
Cystoseira spp.		0.00	0.67	2.53	19.81	51.11
Dyctiota spp.		0.00	0.67	1.12	19.81	70.93
Lithophyllum incrustans		0.33	0.00	1.12	10.19	81.11
Sediment *vs* **Seagrass**	98.29					
Cymodocea nodosa		0.00	1.00	3.21	13.14	13.34
Cladophora spp.		0.00	1.00	3.21	13.14	26.68
Cystoseira spp.		0.00	1.00	3.21	13.14	40.02
Dyctiota spp.		0.00	1.00	3.21	13.14	53.36
Caulerpa *vs* **Seagrass**	53.33					
Cymodocea nodosa		0.00	1.00	4.39	21.16	21.16
Cladophora spp.		0.00	1.00	4.39	21.16	42.31
Zostera marina		0.00	0.67	1.26	12.60	54.91
Cystoseira spp.		0.00	1.00	0.65	7.32	62.23

The same results (heterogeneity between habitats and homogeneity within habitats) were obtained with PERMANOVA and PERMDISP analysis (Annex I and II, respectively) for plant composition (species richness). In accordance with plant biomass results, the Hierarchical cluster for species composition (Fig. 3.4) revealed Sediment to have zero similarity among samples and vegetated habitats to be similar, although Seagrass was less similar among samples than Caulerpa. The SIMPER analysis (Table 3.2) on the plant compostion confirms that Sediment did not have any species shared among all its samples, as the average of abundance was always lower than 1 for every species. Foundation species were again consistently among the top contributors except for *Z. marina* that was only present in one sample of the Seagrass habitat (mixed with *C. nodosa*). The analysis also indicate that all species in Seagrass habitat were present among all its samples (Av. Abund. > 1) except for *Z. marina*. A higher species richness was found in Sediment rather than in Caulerpa habitat (S = 4 and S = 3, respectively, Annex IV). On the other hand, Seagrass had the highest species richness, 2 times more than Sediment (S = 8, Annex IV).

Table 3.3. Summary of similarity percentage (SIMPER) analysis showing the most important species responsible for the fauna abundance dissimilarities between habitat-types.

Species	Av. Dissimilarity	Av. Abund	Av. Abund	Diss/SD	Contrib %	Cum.%
Fauna abundance						
Sediment *vs* **Caulerpa**	85.47					
Bittium reticulatum		2052.63	166.67	3.12	66.53	66.53
Loripes orbiculatus		55.56	415.20	1.10	12.23	78.77
Diogenes pugilator		67.25	0.00	0.76	3.52	82.29
Aplidium punctum		0.00	55.56	2.45	2.45	84.74
Sediment *vs* **Seagrass**	83.01					
Bittium reticulatum		2052.63	160.82	4.25	60.2	60.2
Loripes orbiculatus		55.56	602.34	3.19	19.45	79.65
Cerithium vulgatum		5.85	125.73	3.14	4.22	83.87
Diogenes pugilator		67.25	32.16	0.85	2.58	86.45
Caulerpa *vs* **Seagrass**	58.2					
Loripes orbiculatus		415.20	602.34	1.69	35.37	35.37
Bittium reticulatum		166.67	160.82	5.10	16.58	51.94
Cerithium vulgatum		5.85	125.73	3.32	11.17	63.11
Aplidium punctum		55.56	14.62	1.32	5.4	68.52

The associated fauna abundance significantly differed between habitats (PERMANOVA, Annex I) and according to the PERMDISP analysis the habitat-types were homogeneous within groups. Hierarchical cluster analysis (Fig. 3.5) revealed that Seagrass is the most homogeneous habitat with the highest similarity among habitat-units, followed by Sediment where all replicates within the groups (habitat-types) are more similar to each other than any replicates from different groups, whereas Caulerpa fall short with the lowest similarity within group. As expected, the analysis in Fig. 3.2 also reveals that of the three habitats, Seagrass and Caulerpa are the most similar. In accordance, the SIMPER analysis (Table 3.3) revealed that between the three habitat-types, Caulerpa and Seagrass are the less dissimilar and Sediment and Caulerpa the more dissimilar. When analyzing the species contribution for those dissimilarities, *B. reticulatum* and *L. orbiculatus* are the two species first to appear, with a cumulative percentage always over than 50%. A closer look reveals that *B. reticulatum* is the species that contributes more (higher Contrib. %) and more consistently (higher Diss/SD) to the dissimilarities between Sediment and the other habitats, whereas *L. orbiculatus* has a higher contribution between the two vegetated habitats, although not as consistently. A higher cumulative abundance was found in Sediment habitat followed by Seagrass and Caulerpa (2483, 1127 and 814 indiv.m^{-2}, respectively, Annex IV and Fig. 3.2). Sediment has largely the highest density of individuals, mainly due to the large amount of small gastropods (i.e. *B. reticulatum*, Table 3.3).

Table 3.4. Summary of similarity percentage (SIMPER) analysis showing the most important species responsible for the fauna species richness dissimilarities between habitat-types.

Species	Av. Dissimilarity	Av. Abund	Av. Abund	Diss/SD	Contrib %	Cum.%
Fauna species richness						
Sediment *vs* **Caulerpa**	71.5					
Liocarcinus navigator		0.00	1.00	2.65	2.65	2.65
Macropodia rostrata		0.00	1.00	2.65	2.65	5.31
Hippolyte inermis		0.00	1.00	2.65	2.65	7.96
Palaemon adspersus		0.00	1.00	2.65	2.65	10.62
Sediment *vs* **Seagrass**	68.18					
Macropodia rostrata		0.00	1.00	9.83	2.39	2.39
Hippolyte inermis		0.00	1.00	9.83	2.39	4.77
Palaemon adspersus		0.00	1.00	9.83	2.39	7.16
Rissoa membranacea		0.00	1.00	9.83	2.39	9.55
Caulerpa *vs* **Seagrass**	54.33					
Tritia pygmaea		0.00	1.00	9.17	3.13	3.13
Diogenes pugilator		0.00	1.00	9.17	3.13	6.27
Cerithium vulgatum		0.33	1.00	1.32	2.20	8.47
Athanas sp.		0.67	0.00	1.31	2.12	10.56

The associated fauna biomass significantly differed between habitat-types (PERMANOVA, Annex I) but the groups were not homogeneous according to the

PERMDISP analysis (*P-value* = 0.038, Annex II). Fauna biomass in Seagrass habitat was nearly 4 and 8 times higher than in Sediment and Caulerpa, respectively (Annex IV). These differences in the fauna biomass is due to the wide range on the sizes of the individuals collected. Biomass data for fauna was not statistically consistent, this way it was not consider in further comparisons.

With similar results to the associated fauna abundance, PERMANOVA and PERMDISP analysis on the fauna composition (species richness) (Annex I and II, respectively) also revealed significant differences between habitats and homogeneity within groups. The Hierarchical cluster for species composition (Fig. 3.5) confirms the homogeneity of Seagrass habitat with the highest similarity among habitat-units where Caulerpa was not far behind with high similarities among its replicates, on the other hand, one of Sediment's habitat-units (SED1) was more similar to replicates from other habitats that to the rest of its own. According to the fauna abundance and to both Hierarchical clusters (fauna abundance and composition), the SIMPER analysis for fauna composition (Table 3.4) revealed both vegetated habitats to be less dissimilar to each other than to Sediment. Here, the species with the highest percentages of contribution are the ones that manifest presence in all samples of one habitat and no presences for the other habitat. In this way, Sediment habitat had no species that were present in all of its samples and non of the other habitat's samples. The crustaceans *M. rostrata*, *L. navigator* and *H. inermis* were the species that most contributed, and more consistently, for the dissimilarities between vegetated and unvegetated habitats since they are in all samples of Caulerpa and Seagrass and non existent in Sediment. When comparing the two vegetated habitats, the small gastropod *T. pygmaea* and the small hermit crab *D. pugilator* are equally the species that most contributed, and more consistently, for the dissimilarity as they are the only species that are present in all samples of one habitat (i.e. Seagrass) and non existent on the other (i.e. Caulerpa). It is fair to say that *B. reticulatum*, *L. orbiculatus*, *L. navigator*, *M. rostrata*, *H. inermis*, *T. pygmaea* and *D. pugilator* play an important role to the differences between habitats (for species abundance and composition). A high Species richness was found in the unvegetated habitat-type (S = 55, Annex IV and Fig. 3.2), which was very close to that found in the Seagrass (S = 58) and higher than in the Caulerpa (S = 48, Annex IV and Fig. 3.2), which place vegetated habitats on opposite ends of the spectrum in terms of species composition.

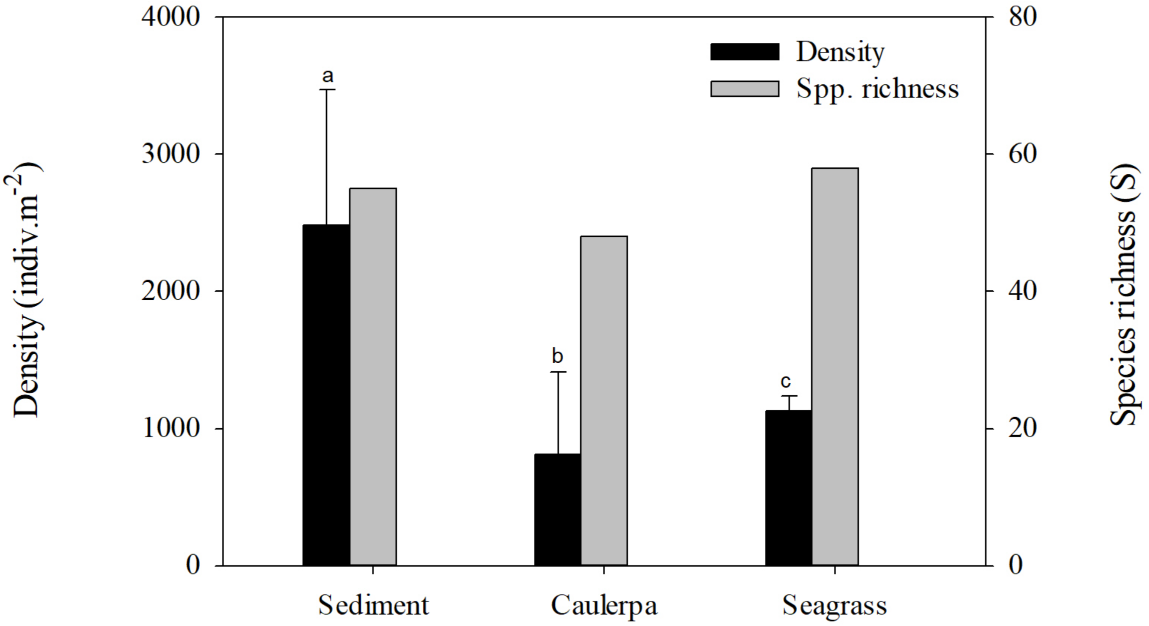

Fig. 3.2. Mean (+SD) fauna density (indiv.m^{-2}) (left) and species richness (S) (right) *per* habitat-type. Image created with SigmaPlot 12.0 software.

A one-way ANOVA revealed differences between habitat-types for plant diversity (Shannon & Wiener Index) (Annex III) but a closer look to the pairwise comparisons with a Tukey-test (Annex III) revealed that of the three possible comparisons, Sediment *vs* Caulerpa was not significantly different. The Shannon & Wiener Index (Fig. 3.3) showed that diversity was 9 times higher in Seagrass (H'= 0.969) than in Caulerpa (H'= 0.038) and 30 times higher than in Sediment (H'= 0.103). The unvegetated habitat had a higher species richness than Caulerpa but results on diversity showed that species in Caulerpa were more evenly abundant than in Sediment.

Similar to plant diversity, the one-way ANOVA and the Tukey test for fauna diversity revealed significant differences between habitat-types, except for Caulerpa *vs* Seagrass comparison. Results on fauna diversity (Shannon & Wiener Index) showed that Caulerpa and Seagrass hold the highest diversity when taking into account how the abundances are distributed among species (H'= 1.789 and 1.776, respectively), followed by Sediment (H'=0.987). Similar to what was found for plant diversity, Sediment had indeed a very large number of species but most species had very few individuals (with some exceptions as *B. reticulatum*) and were not evenly abundant, whereas Caulerpa and Seagrass had similar richness but the abundances were more evenly distributed among species, resulting in a higher H’.

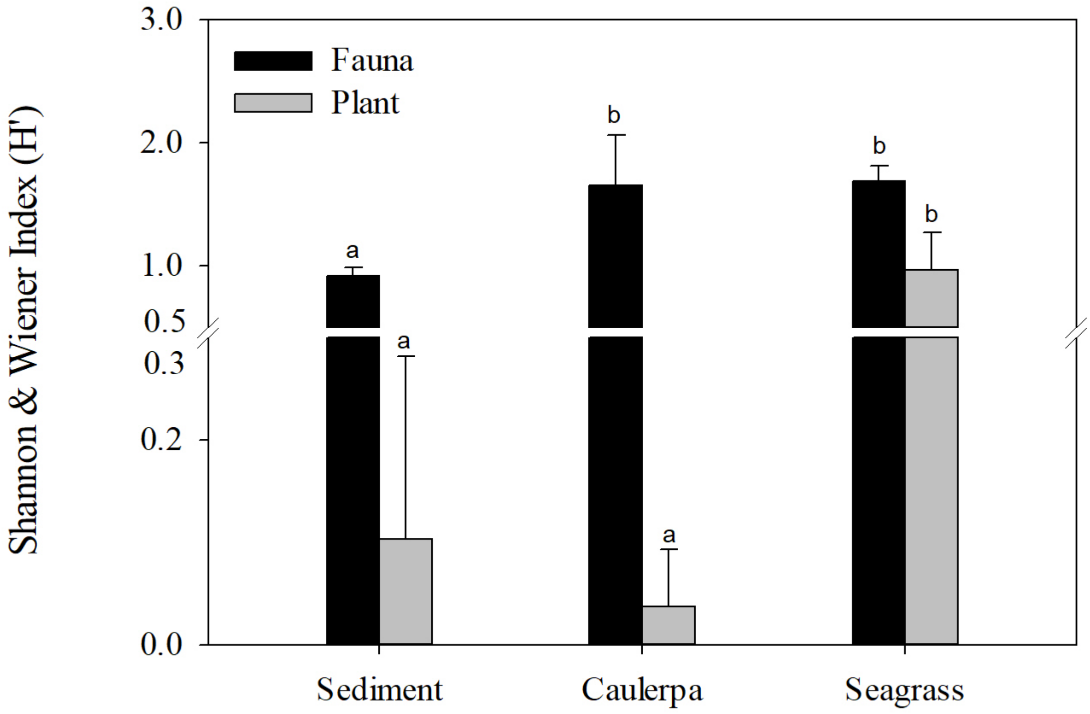

Fig. 3.3. Shannon & Wiener Index for fauna and plant diversity (H’) *per* habitat-type.

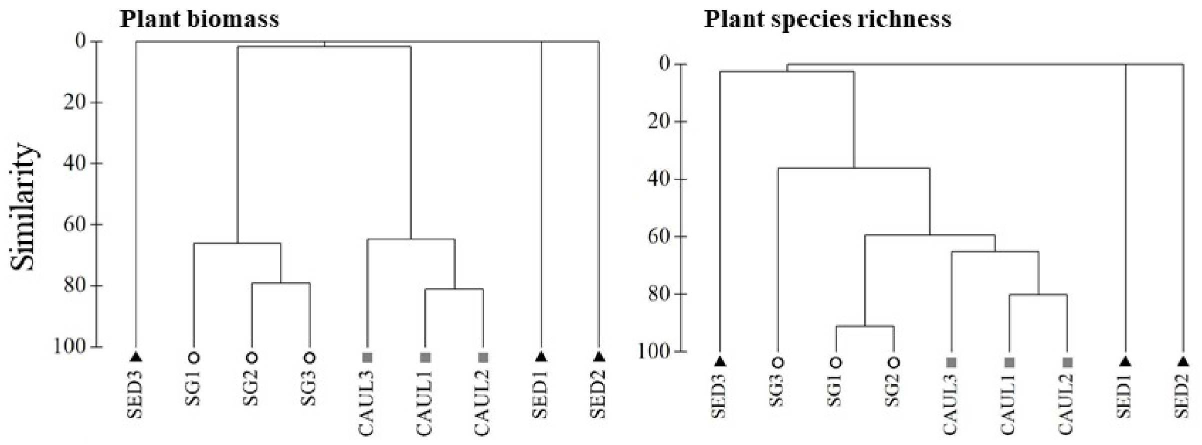

Fig. 3.4. Hierarchical Cluster analysis (Bray Curtis similarity) of similarities among habitat-units for plant biomass (**left**) and species richness (**right**). Sediment – black triangles, Caulerpa – grey squares and Seagrass – white circles.

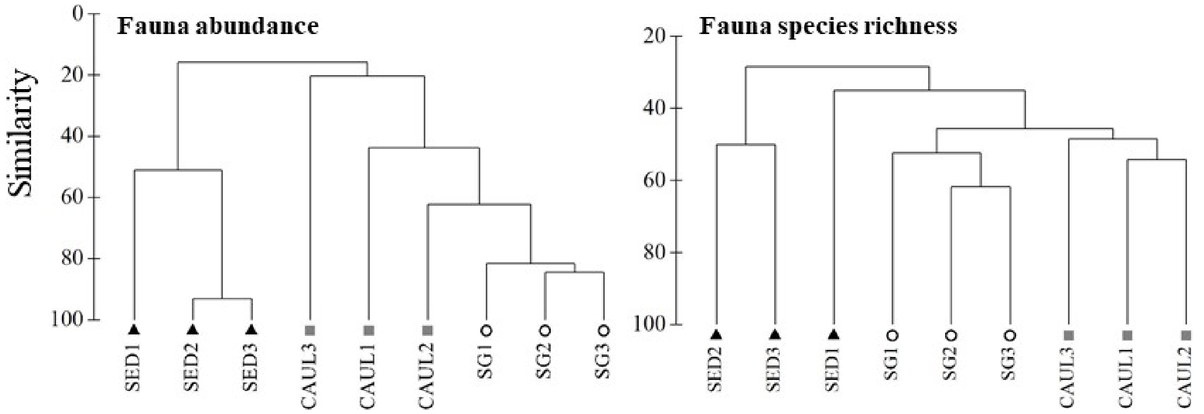

Fig. 3.5. Hierarchical Cluster analysis (Bray Curtis similarity) of similarities among habitat-units for fauna abundance (**left**) and species and richness (**right**). Sediment – black triangles, Caulerpa – grey squares and Seagrass – white circles.

3.2. Nursery function

Of the total 101 different fauna species collected, 32 had juveniles and 15 were only present as juveniles. Sediment and Caulerpa had a total 13 different species with juveniles associated each and Seagrass 17 (Annex IV).

The nursery function (fauna species as juveniles and non juveniles) was significantly different between habitat-types in terms of fauna abundance (PERMANOVA, Annex I) and, according to the PERMDISP analysis (Annex II), the habitats were homogeneous as there was no differences in the within-group multivariate dispersion (no differences among samples of the same habitat).

Table 3.5. Summary of similarity percentage (SIMPER) analysis showing the importance of juveniles and non juveniles for the similarities within habitat-types.

Factors	Av. Similarity	Av. Abund	Av. Abund	Sim/SD	Contrib%	Cum.%
Sediment	65.53					
Non Juvenile		1652.16	44.11	4.73	67.32	67.32
Juvenile		830.44	21.42	13.14	32.68	100.00
Caulerpa	54.61					
Non Juvenile		488.92	44.65	2.63	81.76	81.76
Juvenile		324.88	9.96	0.81	18.24	100.00
Seagrass	90.20					
Non Juvenile		571.32	43.11	16.40	47.79	100.00
Juvenile		555.75	47.09	12.01	52.21	52.21

A SIMPER analysis on the nursery function similarities within habitats (Table 3.5) indicated the importance of juvenile and non juvenile individual abundance to those similarities. The analysis showed that Seagrass was the habitat-type with the highest similarity (90.2%, Table 3.5) followed by Sediment and Caulerpa (65.53% and 54.61 respectively). On the Seagrass habitat juveniles had consistently (higher Sim/SD) a higher percentage of contribution (52.21%) for the similarities than non juveniles, showing the importance of this habitat as a nursery. In other words, in the Seagrass habitat-type, juveniles contribute even more than non juveniles to the similarity of the group. In Caulerpa, juveniles contribute less than 20% for the similarities. A second SIMPER analysis was run (Table 3.6) to indicate the most important species to the nursery function dissimilarities between habitats. Dissimilarities between habitat-types are consistent, the two vegetated habitats are the less dissimilar and Sediment and Caulerpa the more (51.47% and 89.14% respectively, Table 3.6). SIMPER analysis showed that the juveniles species responsible for the dissimilarities are also consistent with the dissimilarities found in Table 3.6 as *B. reticulatum* and *L. orbiculatus* continue to be the top contributors (Table 3.6). These results reinforces the importance of vegetated habitats as nurseries, specially Seagrass. Although clearly important for the assemblage of juveniles, the small gastropod and bivalve are not of commercial interest to our knowledge. But when accessing the entire set of juveniles, of the total of 32 juvenile associated species 15 are of commercial interest making almost 50%, with 60% of those being fish.

Table 3.6. Summary of similarity percentage (SIMPER) analysis showing the most important juvenile associated species responsible for the dissimilarity between habitat-types.

Species	Av. Dissimilarity	Av. Abund	Av. Abund	Diss/SD	Contrib%	Cum.%
Sediment *vs* **Caulerpa**	89.14					
Bittium reticulatum		760.23	17.54	3.15	73.98	73.98
Loripes orbiculatus		55.56	298.25	1.15	23.90	97.87
Mangelia sp1		5.85	0.00	0.99	0.65	98.52
Alpheus dentipes		2.92	2.92	0.75	0.47	98.99
Sediment *vs* **Seagrass**	81.45					
Bittium reticulatum		760.23	67.25	3.63	57.21	57.21
Loripes orbiculatus		55.56	482.46	3.33	41.12	98.34
Mangelia sp1		5.85	0.00	1.18	0.53	98.86
Scrobicularia plana		2.92	2.92	0.80	0.33	99.20
Caulerpa *vs* **Seagrass**	51.47					
Loripes orbiculatus		298.25	482.46	1.44	83.71	83.71
Bittium reticulatum		17.54	67.25	1.19	12.89	96.60
Tritia reticulata		0.00	2.92	0.62	0.75	97.36
Alpheus dentipes		2.92	0.00	0.67	0.70	98.06

Of the three habitat-types, Sediment is the one with the highest density of juveniles, mainly due to the presence of juvenile small gastropods (i.e. *B. reticulatum*), followed by Seagrass and Caulerpa (Fig. 3.6), but when looking to the total densities (Fig. 3.2), it also follows the same order, in other words, to look for the impact that juveniles have on one habitat we need to observe the differences between the non juveniles and the juveniles. This way, Seagrass is the habitat-type where the difference between the two is the lowest (Fig. 3.6) with a percentage of juveniles almost reaching the 50% (49.3%), followed by Caulerpa (39.9%) and Sediment (33.5%). As expected, Seagrass is the habitat-type with the highest percentage of juveniles *per* area, followed by Caulerpa with a 10% difference.

The nursery function was not significantly different in terms of species richness (PERMANOVA, Annex I) but according to the PERMDISP analysis (Annex II), habitats were homogeneous with no differences among samples of the same habitat.

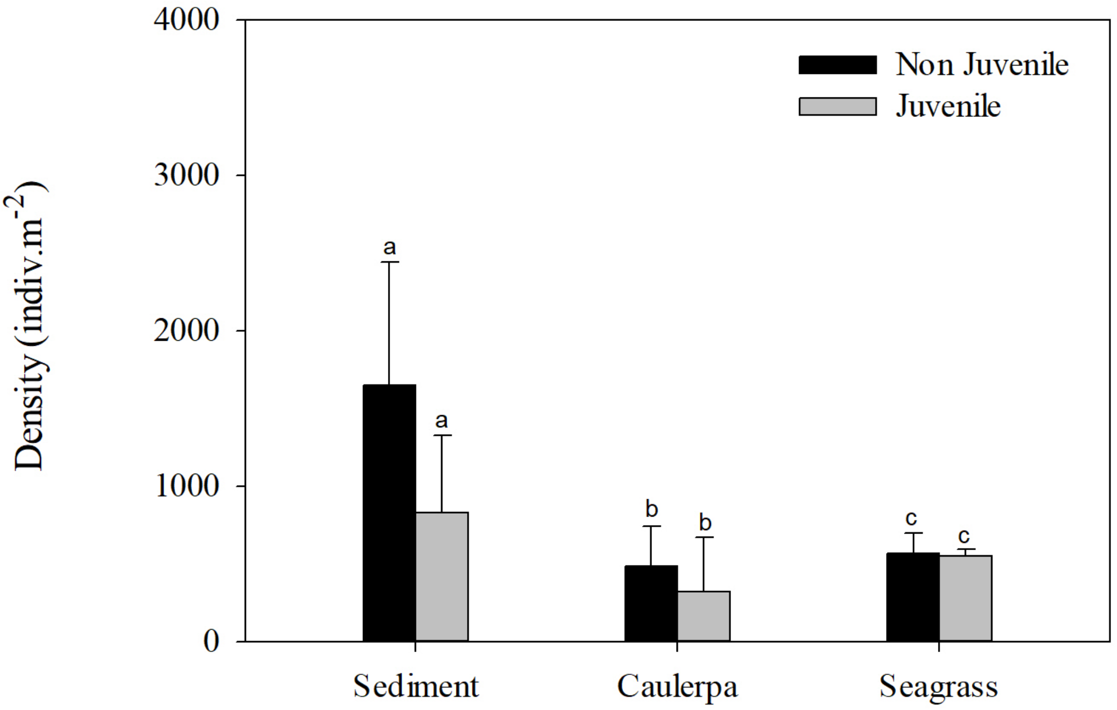

Fig. 3.6. Mean (1+SD) of the juveniles (grey) and non juveniles (black) density (indiv.m^{-2}) *per* habitat-type. Image created with SigmaPlot 12.0 software.

3.3. Commercial species

Of the total 101 different species collected, 21 were of commercial interest. Out of the 21 commercial species, 4 were bivalves, 9 were fish, 2 were gastropods and 2 were Caridea, Brachyura, Cephalopoda, Echinodermata and Gebiidea only had 1 species each associated.

PERMANOVA results (Annex I) revealed that habitat-types are significantly different concerning the fauna abundance and are homogeneous (no significant differences were found between samples of the same habitat, PERMIDSP, Annex II). A SIMPER analysis on the commercial value (fauna species abundance as commercial and non commercial) similarities within habitats was run and revealed, as in (Table 3.5), that Seagrass was the habitat with higher similarities (92.95%), followed by Sediment and Caulerpa (73.77% and 53.23%, respectively). As the differences between commercial and non commercial species abundance was so great, percentage at which non commercial species contributed to those similarities was above the 98% for all three habitats. A second SIMPER analysis on the commercial value (fauna species abundance as commercial and non commercial) dissimilarities between habitats (Table 3.7), in agreement with Fig. 3.7, showed that the vegetated habitats were the more dissimilar (95.80%, Table 3.7) and that Sediment and Caulerpa the less (78.45%). The small decapod, *Upogebia sp.*, commonly used as bait by local fisherman's (Carvalho et al., 2013), was consistently a top contributor between the three habitat-types with near 50% contribution in every comparison. The two bivalves *P. aureus* and *R. decussatus* were also among the big contributors with percentages around the 30% when Sediment was on the 'equation', although not as consistent (low Diss/SD). As it was only found on Seagrass, the highly demanded sea urchin *S. granularis* was also a top contributor when comparing Seagrass with other habitats.

Table 3.7. Summary of similarity percentage (SIMPER) analysis showing the most important commercial and non commercial species species responsible for the dissimilarity between habitat-types.

Species	Av. Dissimilarity	Av. Abund	Av. Abund	Diss/SD	Contrib%	Cum.%
Sediment *vs* **Caulerpa**	78.45					
Upogebia sp.		8.77	23.39	1.26	54.86	54.86
Politapes aureus		5.85	0.00	0.76	28.18	83.04
Ruditapes decussatus		2.92	2.92	0.76	14.18	97.22
Liocarcinus navigator		0.00	0.16	0.64	1.13	98.35
Sediment *vs* **Seagrass**	83.69					
Upogebia sp.		8.77	0.00	0.99	41.18	41.18
Politapes aureus		5.85	2.92	0.81	31.29	72.47
Sphaerechinus granularis		0.00	2.92	0.63	14.12	86.59
Ruditapes decussatus		2.92	0.00	0.66	10.92	97.52
Caulerpa *vs* **Seagrass**	95.80					
Upogebia sp.		23.39	0.00	1.07	44.82	44.82
Sphaerechinus granularis		0.00	2.92	0.50	15.52	60.34
Politapes aureus		0.00	2.92	0.50	15.17	75.51
Ruditapes decussatus		2.92	0.00	0.65	13.21	88.72

Commercial species were not significantly different between habitats in terms of species richness (PERMANOVA, Annex I) but according to the PERMDISP analysis (Annex II), habitats were homogeneous with no differences among samples of the same habitat.

Of the three habitat-types, Caulerpa is the one with the highest density of commercial species, mainly due to the presence of *Upogebia spp.*, followed by Sediment and Seagrass (Fig. 3.7) and when looking for the differences between commercial and non commercial species, Caulerpa is the one with lowest difference, being the only habitat-type with a percentage of commercial species over 1% (3.29%, Fig. 3.7), Sediment and Seagrass, both had very low percentage (0.71% and 0.55% respectively).

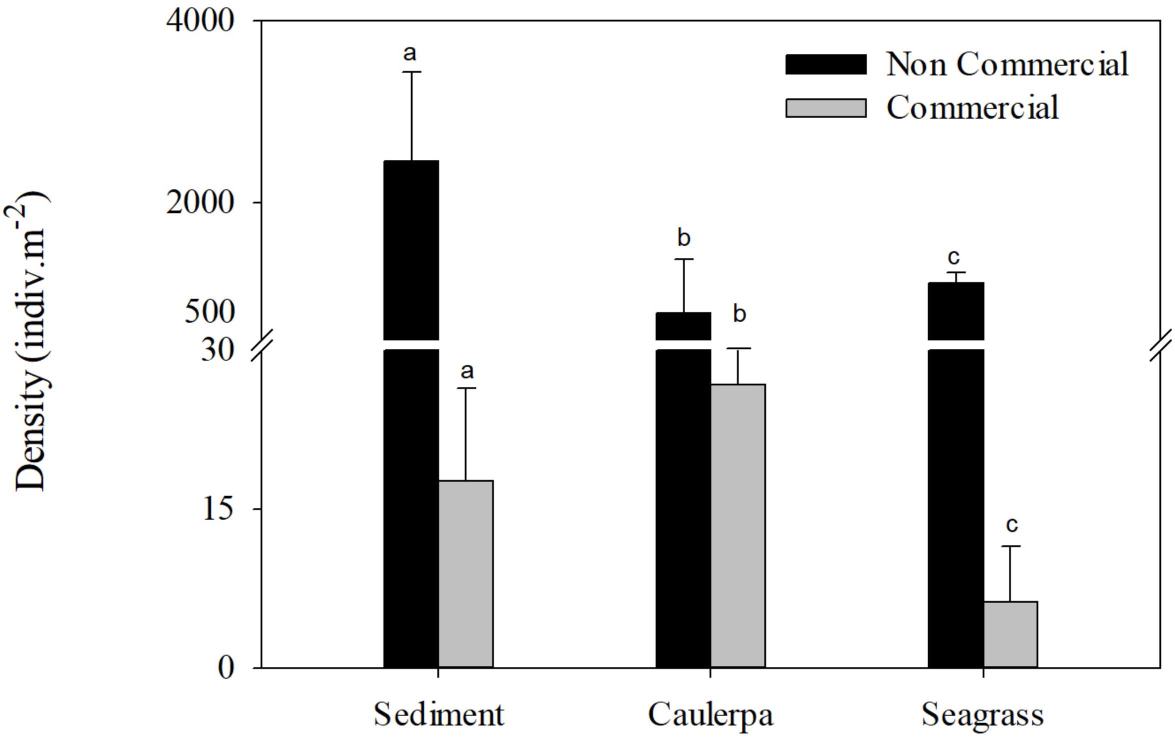

Fig. 3.7. Mean (1+SD) of the commercial (grey) and non commercial species (black) density (indiv.m^{-2}) *per* habitat-type. Image created with SigmaPlot 12.0 software.

4. Discussion

Overall, our results show differences between habitats dominated by seagrass (*C. nodosa* and *Z. marina* mix), *C. prolifera* and unvegetated bottom in terms of plant and fauna diversity and in terms of plant (species biomass and richness) and fauna (species abundance and richness) species assemblage. The three habitat-types (Sediment, Caulerpa and Seagrass) differed in the provision of habitat as a nursery and for species of commercial interest.

It is widely accepted that foundations species (i.e. *C. prolifera, C. nodosa* and *Z. marina*) influence the animal and plant assemblage and often associated with high diversity and abundance of fish and invertebrates (Heck & Orth 1980; Ceccherelli and Cinelli, 1997; Lloret et al., 2005; Tuya et al., 2013, 2014), but there seems to be a lack of consensus regarding the effects that *C. prolifera* and *C. nodosa* have on the fauna abundance and diversity, as studies have pointed out the green seaweed to be more abundant and diverse than the seagrass (Png-Gonzalez et al., 2014) as well as the opposite (Vázquez-Luis et al., 2009). We found, however, that *C. prolifera* had lower fauna species abundance and richness but higher diversity than unvegetated habitats but not than habitats dominated by *C. nodosa* and *Z. marina*. The importance *C. prolifera* in maintaining the biodiversity has been point by Sánchez-Moyano et al. (2001b). Monterroso et al. (2012) confirms that vegetated habitats are indeed more diverse than unvegetated. The authors also compares green seaweed with seagrass meadows and bare sediment beds and they found that diversity was greater when *C. prolifera* was present. In our study, the diversity in the *Caulerpa* habitat was so close to Seagrass, despite the low abundance and richness, because the species were more equally common than in the two other habitats where species composition was more uneven.

C. prolifera biomass revealed a higher biomass than seagrasses and than in any other reviewed paper. With over than 200 gDW m^{-2} and a wide extent that remains unknown to the date, this meadow of *C. prolifera* shows how Ria Formosa might offer great conditions for this opportunistic species to thrive. As the subtidal bottom of the lagoon is mainly sediment, it is hard for vegetation like many macroalgae species to attach. It is then more common to find vegetation that relay on a bellow ground structure like seagrass and *C. prolifera*. Seagrass showed a very high biomass (25 gDW.m^{-2}) as well as a higher species richness (S = 9) of non-foundation vegetation and a higher overall plant diversity, than the other two habitats. The architecture of seagrass plants may be one of the factors that contribute for the high non-foundation species density and richness. Most of these macroalgae (non-foundation species) (e.g. *Cystoseira sp.* and *Dictyota sp.*), can survive without being fixed by its basal disks, thus small fragments drift with the currents (in this case, strong currents created by the tides) and may be trapped by the long and thin leafs of seagrass plants. Macroalgae within Caulerpa habitat had a very low density (1 gDW m^{-2}) and richness (S=2) and the lowest overall plant diversity. The lack of other vegetation might be due to the shallow structure of the host plant as its short and wide lamina may not form an architecture at which drifting macroalgae fragments would easily hold to. Because it was free of any plant population like seagrass or *C. prolifera*, free floating macroalgae had no support except for sessile organisms (i.e. Tunicata) at which they fixate. Apart for the scarce presence of small macroalgae pieces, small encrustant algae were also found. Even with the unvegetated name and almost no plant density, Sediment had higher plant richness than Caulerpa (S=4).

The differences among habitats can be reflected in its species-specific affinities, that is, by the species that occur uniquely in one habitat and not on the others. In total, of the 101 different fauna species collected, 54 had specific affinities to one habitat. Seagrass and Sediment together hold more than 80% of those affinities (22 and 23 species, respectively) whereas Caulerpa completes the remaining with less than 20% (9 species). These results indicate that in the seagrass and unvegetated beds of Ria Formosa, species had time to adapt and develop specific affinities, whereas with *C. prolifera* dominated beds, the fast spreading and occupation of unvegetated areas not only makes it more difficult for species to adapt as it creates a drastic change on the landscape. The lower abundance and richness of the Caulerpa habitat reflects a higher instability (Worm et al., 2006). Lower abundance and richness of the associated fauna might be associated to the large extent of the continuous *C. prolifera* meadow here under study. According to (Vázquez-Luis et al., 2009), faunal abundance is higher in patchy habitats when comparing with continuous meadows. It is uncommon to an unvegetated bottom to hold the larger abundance and such high richness when comparing with vegetated habitats, there are many studies showing higher abundance and richness on vegetation compared to bare sediment (Heck et al., 1989; Sanchez-Jerez et al., 1999; Vázquez-Luis et al., 2009; Monterroso et al., 2012).

When comparing the associated fauna assemblage among habitat-types, *B. reticulatum* and *L. orbiculatus* were, respectively, the species that highly and more consistently contributed the dissimilarities between habitats. *B. reticulatum* had the higher density, averaging over 2000 indiv.m^{-2} in Sediment and ca. 160 indiv.m^{-2} in the vegetated habitats. This small gastropod marked the differences in the fauna composition between the unvegetated habitat and the two vegetated ones. Although with a low abundance on Sediment (55.6 indiv.m^{-2}), for Seagrass and Caulerpa, *L. orbiculatus*, was the species with the highest abundances, averaging over than 600 and 400 indiv.m^{-2}, respectively. Thus, *L. orbiculatus* not only marked the differences between Sediment and vegetated habitats but also between Seagrass and Caulerpa. We find these two mollusks to be the best indicators of between-habitat differences in the community structure

Coastal ecosystems, such as the Ria Formosa lagoon, provide goods and services to the human populations, being seafood one of these goods (Daily et al., 1999). Species of commercial interest are the main targets to be exploited and they are the first to manifest the anthropocentric pressure caused by fisheries. This pressure causes the collapse of species and populations what have a direct negative impact on the diversity (Worm et al., 2006). In this study, we found that the *Caulerpa* habitat held a higher percentage of commercial species (3.29%) than the Sediment and Seagrass (0.71% and 0.55%, respectively). This was not expected since not only Seagrass overall commercial value is well documented (Cristina et al., 2006; Duarte et al., 2007; Guimarães et al., 2012) but also its capability of hosting species of commercial interest (Vasconcelos et al., 2010; 2011; Plummer et al., 2013). Despite the lowest overall density of all species, Caulerpa also had the highest density of commercial species. The species of commercial interest that most contribute for the differences between habitats was the *Upogebia sp.*, a crustacean commonly used as fish bait by the local fisherman but not directly appreciated for human consumption (Carvalho et al., 2013). This crustacean was very common on Caulerpa (23.4 ± 33.2 indiv m^{-2}), less common in the Sediment (8.8 ± 8.8 indiv m^{-2}), and absent in Seagrass. In contrast, the commercial bivalves *Politapes aureus* and *Ruditapes decussatus*, were more common on Sediment (and also present in Seagrass and

Sediment, respectively) and the sea urchin *Sphaerechinus granularis* unique in Seagrass. It is known that seagrass beds host many important species (Vasconcelos et al., 2010; 2011; Plummer et al., 2013), but it was not easy to find in the literature association with *C. prolifera* dominated beds.

The nursery value of seagrass beds is widely recognized (e.g. Heck & Orth, 1980; Heck et al., 1989; Guerao e Ribera, 2000; Beck et al., 2001; Beaumont et al., 2007; Barbier et al., 2011), as they offer food and shelter to juveniles. On the other hand, we had difficulty to find *C. prolifera* beds and unvegetated bottoms associated with nursery function in the literature. Our results show indeed that of the three habitats, Seagrass is the one with higher nursery function where almost 50% of the fauna collected were juveniles and where they contribute more than 50% for the similarities within the habitat-type. Caulerpa was not far behind with about 40% and Sediment with 30% of juveniles. The juveniles of *B. reticulatum* (common in Sediment) and *L. orbiculatus* (common in vegetated habitats) were the species that most contribute for the differences between habitats as nursery grounds. Furthermore, when only species of commercial interest were considered Seagrass had the highest percentage of juveniles (77%) followed by Caulerpa (62%), and Sediment (45%). This supports previous studies suggesting that seagrass beds play a nursery role for several commercially important species (Heck et al. 1989). Overall, out of the 21 species with commercial interest, 15 had juveniles (71.4%). Our results are only partially in agreement with previous studies that suggest/report that biodiversity can be a good indicator of the nursery function as nurseries tend to be more diverse (Liquete et al., 2016).

The seagrass architecture is known to influence the fauna assemblage (Hacker & Steneck, 1990; Bologna & Heck, 2000; Chemello & Milazzo, 2002; Gartner et al., 2012), as well the provision of nursery for juveniles (Espino et al., 2011). Our results suggest that the larger biomass of *C. prolifera* together with the “shorter” architecture than the seagrass may equally influence diversity, while reduce the species richness or abundances and increase the affinity of the commercial species *Upogebia spp*. Other explanations alternative to the structural complexity are the production of toxic compounds produced by Caulerpa species as herbivore deterrents (Amade and Lemée, 1998).

In the end, *C. prolifera* establishment on bare sediment might have an effect on the biodiversity of Ria Formosa. As *C. prolifera* settles rapidly creating extensive meadows, it does not give time for species to adapt, creating a more unstable habitat and altering drastically the landscape, this is reflected in the low abundance and richness recorded as well as the low number of specific affinities. *C. prolifera* is well adapted and has a big spread potential in Ria Formosa, forming extensive and continuous meadows, that were proven to be associated to low numbers of abundance and richness. The replacement of unvegetated areas by *C. prolifera*, not only might diminish the probability for seagrass to spread, by reducing the space available, but at the same time compromises the existence of the species that once inhabited the sediment beds, some of the organisms will be able to move to other sandy bottoms but others will have to adapt to the new environment or will simply disappear. Although capable of hosting great numbers of species with commercial interest, it is limited to a lower capacity of juveniles comparing with seagrass beds and by replacing unvegetated areas and reducing the spreading potential of seagrass meadows, it might eventually reduce the nursery functions that these habitats are know to have. This changes not only have an effect on the biodiversity of the area that once was unvegetated but on the biodiversity of the entire ecosystem, with changes in the landscapes and species assemblages.

5. Conclusions

Overall, we found similar faunal diversity (Shannon & Wiener Index) within seagrass, and Caulerpa followed by unvegetated sediments. Concerning macroflora, it was highest in Seagrass and lowest in Caulerpa. Seagrass habitat also showed the highest species richness and nursery role (almost 50% of the fauna collected were juveniles), while Sediment habitat showed the highest individual faunal organism abundance (mostly due to the very common small gastropod *Bittium reticulatum*). The highest provision of habitat for commercial species that we found in Caulerpa beds was due to common and abundant crustacean *Upogebia spp.*, which is used as fish bait but not appreciated for human consumption.

Finally we conclude that *C. prolifera* establishment on unvegetated bottoms have an effect on the abundance and diversity of Ria Formosa lagoon as its rapid settlement change the communities that were previously assembled and prevent further progression and reestablishment of seagrass meadows, important for diversity and as nursery grounds. It is yet unknown the rate at which *C. prolifera* progress in Ria Formosa, but with this study we can predict that a rapid settlement of this opportunist seaweed will change the associated communities assemblage of unvegetated bottoms within the lagoon which may thus prevent the progression or even possible re-population of important seagrass beds.

This study was conducted in a short temporal frame leaving open questions that should be addressed in further studies to better understand how the proliferation of this opportunistic seaweed is affecting the ecosystems in which it settles: 1) What are the seasonal and annual variations of the communities structure between these three habitats? 2) What are the long term effects that these changes on the bottom coverage will have in the ecological structure of Ria Formosa? 3) What are the effects on the under threat seagrass communities by limiting its progression? 4) At what rate is *C. prolifera* progressing in Ria Formosa?

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Annexes

Annex I. PERMANOVA results for differences between habitat-types (Habitat-t) for the plant and fauna abundance, biomass and richness.

PERMANOVA	**Species abundance**					**Species biomass**				**Species richness**			
	df	**MS**	**F**	***P***	**Perm**	**MS**	**F**	***P***	**Perm**	**MS**	**F**	***P***	**Perm**
Plant													
Species													
Habitat-t	-	-	-	-	-	10862.00	5.4649	0.003**	130	7351.00	3.5794	0.004**	82
Residual	-	-				1987.60				2053.70			
Total	-												
Foundation species													
Habitat-t	-	-	-	-	-	7446.80	4.5971	0.003**	269	3572.60	2.4829	0.009**	149
Residual	-	-				1619.90				1438.90			
Total	-												
Fauna													
Species													
Habitat-t	2	6661.20	5.6363	0.003**	272	7510.30	3.8762	0.004**	270	3642.70	2.639	0.006**	270
Residual	6	1181.80				1937.60				1380.30			
Total	8												
Nursery													
Habitat-t	2	1869.50	3.1368	0.041*	274	4186.80	5.3157	0.28	269	205.58	1.8062	0.122	268
Residual	6	595.98				787.63				113.81			
Total	8												
Value													
Habitat-t	2	1993.80	3.49	0.037*	275	3693.40	3.5839	0.370	273	182.57	1.342	0.329	255
Residual	6	571.28				1030.60				136.06			
Total	8												

*Significance level: 0***; 0.001**; 0.01*; 0.05.; 0.1*

Annex II. PERMDISP results for the within-group (habitat-unit nested in habitat-type) dispersion and pair-wise comparisons for the plant and fauna abundance, biomass and richness.

PERMDISP		**Species abundance**					**Species biomass**			**Species richness**		
		df 1	**df 2**	**F**	**t**	***P***	**F**	**t**	***P***	**F**	**t**	***P***
Plant												
Species												
	Habitat-u (H-t)	-	-	-	-	-	46.0930	-	0.031*	16.9680	-	0.031*
	SED x CAUL	-	-	-	-	-	-	7.4828	0.101	-	5.0711	0.130
	SED x SG	-	-	-	-	-	-	16.6540	0.092.	-	7.2586	0.118
	CAUL x SG	-	-	-	-	-	-	0.1097	1.000	-	0.2420	0.698
Foundation species												
	Habitat-u (H-t)	-	-	-	-	-	14.9610	-	0.045*	16.4270	-	0.038*
	SED x CAUL	-	-	-	-	-	-	4.1618	0.096.	-	4.8504	0.114
	SED x SG	-	-	-	-	-	-	4.9720	0.108	-	3.7098	0.099.
	CAUL x SG	-	-	-	-	-	-	0.3190	1.000	-	1.6216	0.513
Fauna												
Species												
	Habitat-u (H-t)	2	6	11.5420	-	0.055.	13.3990	-	0.038*	3.1074	-	0.225
	SED x CAUL	-	-	-	2.3938	0.206	-	5.3189	0.110	-	1.6645	0.118
	SED x SG	-	-	-	2.2177	0.203	-	4.3455	0.099.	-	2.0444	0.093.
	CAUL x SG	-	-	-	5.2610	0.114	-	1.1623	0.511	-	1.0159	0.507
Nursery												
	Habitat-u (H-t)	2	6	5.5935	-	0.171	3.4147	-	0.298	4.2440	-	0.170
	SED x CAUL	-	-	-	0.7426	0.805	-	0.9773	0.616	-	0.0625	0.924
	SED x SG	-	-	-	5.6583	0.096	-	2.5812	0.103	-	2.6873	0.093.
	CAUL x SG	-	-	-	2.7760	0.096	-	1.8801	0.307	-	3.0365	0.093.
Value												
	Habitat-u (H-t)	2	6	6.6369	-	0.187	0.5951	-	0.750	3.2962	-	0.240
	SED x CAUL	-	-	-	1.2311	0.412	-	1.0042	0.488	-	0.0495	1.000
	SED x SG	-	-	-	2.9470	0.104	-	0.6843	0.701	-	0.3055	0.114
	CAUL x SG	-	-	-	3.5664	0.094	-	0.4101	0.792	-	2.2479	0.105

*Significance level: 0***; 0.001**; 0.01*; 0.05.; 0.1*

Annex III. One-way ANOVA results for differences between habitat-types (Habitat-t) for the plant and fauna diversity (Shannon & Wiener Index) and Tukey-test for the pair-wise comparisons between habitat-types.

	ANOVA & Tukey-test	**df**	**MS**	**F**	**q**	***P***
Plant						
ANOVA	Habitat-t	2	0.81	19.2200	-	0.002**
	Residual	6	0.0421			
	Total	8				
Tukey-test	SED x CAUL	-	-	-	0.5520	0.921
	SED x SG	-	-	-	7.3020	0.005**
	CAUL x SG	-	-	-	7.8540	0.004**
Fauna						
ANOVA	Habitat-t	2	0.562	8.845	-	0.016*
	Residual	6	0.0635			
	Total	8				
Tukey-test	SED x CAUL	-	-	-	5.037	0.028*
	SED x SG	-	-	-	5.259	0.023*
	CAUL x SG	-	-	-	0.222	0.987

*Significance level: 0***; 0.001**; 0.01*; 0.05.; 0.1*

Annex IV. Mean (±SD) of the habitat-units cumulative abundance (indiv.m^{-2}), biomass (gDW.m^{-2}) and species richness (presence/absence) for each habitat-type.

	Sediment					**Caulerpa**					**Seagrass**				
	Abund.	SD ±	Biom.	SD ±	SR	Abund.	SD ±	Biom.	SD ±	SR	Abund.	SD ±	Biom.	SD ±	SR
Plant															
Species	-	-	0.24	0.31	4	-	-	225.01		3	-	-	144.31	50.28	11
Foundation species	-	-	0.00		0	-	-	223.95	105.98	1	-	-	119.06	41.71	3
Non foundation species	-	-	0.24	0.31	4	-	-	1.06	1.58	2	-	-	25.25	11.94	8
Fauna															
Species	2482.60	987.93	131.68	103.43	55	813.81	599.64	73.69	35.45	48	1127.07	110.35	498.66	120.78	58
Nursery															
Juveniles	830.44	499.09	2.52	0.83	13	324.88	347.21	5.77	4.20	17	555.75	39.67	33.58	15.46	17
Non Juveniles	1652.16	789.76	129.16	102.74	47	488.92	255.68	67.93	32.29	40	571.32	129.09	465.08	105.33	47
Value															
Commercial	17.63	8.76	2.85	1.30	11	26.71	31.40	2.43	2.71	13	6.25	5.20	100.11	166.04	13
Non commercial	2464.97	980.21	128.82	102.96	44	787.10	600.22	71.26	33.12	35	1120.82	114.47	398.55	171.29	45

Annex V. Size classes (U=unique) and sizes at maturation for each species. Nursery (J=juvenile; NJ=non juvenile); (C=commercial; NC=non commercial).

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Axiidea	*Pleocyemata sp.*	U	-	NJ *n.d.	n.d.	NC	-	-	
Bivalvia	*Abra sp.*	L	0.7 - 1.4	NJ *n.d.	n.d.	NC	-	-	
		S	< 0.6	NJ *n.d.					
	Anomia ephippium	L	4 – 5	NJ *	n.d.	NC	0.0625	3.097	Palomares & Pauly, 2018
		M	2.1 - 3.9	NJ *					
		S	1.1 – 2	NJ *					
		XS	< 1	J *					
	Lasaeidae n.i.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Loripes orbiculatus	L	≥ 1.5	NJ	0.75	NC	-	-	Van der Geest, 2018
		M	1.1 - 1.4	NJ					
		S	0.6 - 1	J					
		XS	< 0.6	J					
	Lucinidae n.i.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Macoma sp.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Mactridae n.i.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Magallana angulata	L	> 1gDW*3	NJ *n.d.	n.d.	C	-	-	
		S	≤ 1gDW*3	NJ *n.d.					
	Nucula sp.	U	-	NJ *n.d.	n.d.	NC	-	-	

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**3 Size class was attributed based on the weight (gDW)*

Annex V. (Continuation)

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Bivalvia	*Nuculana sp.*	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
	Ostrea stentina	L	> 7gDW*^{3}	NJ *$^{\text{n.d.}}$	n.d.	C	-	-	
		S	≤ 7gDW*^{3}	NJ *$^{\text{n.d.}}$					
	Parvicardium exiguum	U	-	NJ *	n.d.	NC	-	-	
	Polititapes aureus	U	-	NJ *$^{\text{n.d.}}$	n.d.	C	-	-	
	Ruditapes decussatus	L	-	NJ *	n.d.	C	-	-	
		S	-	J *					
	Scrobicularia plana	U	-	NJ *	n.d.	NC	-	-	
		U	-	J *					
	Semelidae sp1	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
	Veneridae sp1	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
	Veneridae sp2	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
Brachiata	*Sicyonia carinata*	U	-	NJ *	n.d.	NC	-	-	
Brachyura	*Liocarcinus navigator*	L	≥ 2	J	2.1 – 2.2	C	-	-	Freire et al., 1991
		M	1.6 - 1.9	J					
		S	1.1 - 1.5	J					
		XS	≤ 1	J					
	Macropodia rostrata	L	≥ 1.5	NJ *$^{\text{n.d.}}$	n.d.	NC	0.8072	2.629	Palomares & Pauly, 2018

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Annex V. (Continuation)

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Brachyura	*Macropodia rostrata*	M	1.1 - 1.4	NJ *n.d.					
		S	0.7 - 1	NJ *n.d.					
		XS	≤ 0.6	NJ *n.d.					
	Pilumnus sp.	U	-	NJ *	n.d.	NC	-	-	
	Pinnotheres pisum	U	-	NJ *	n.d.	NC	-	-	
	Pisidia longicornis	L	> 0.3	NJ *	n.d.	NC	-	-	
		S	0.2 – 0.25	NJ *n.d.					
	Xantho sp.	L	≥ 0.4	NJ *	n.d.	NC	-	-	
		S	0.2 - 0.3	NJ *					
Bryozoa	*Amathia sp.*	U	-	NJ *n.d.	n.d.	NC	-	-	
	Bugula sp.	U	-	NJ *n.d.	n.d.	NC	-	-	
Caridea	*Alpheus dentipes*	L	> 0.6	NJ *	n.d.	NC	-	-	
		S	≤ 0.6	J *					
	Athanas sp.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Caridea n.i.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Hippolyte inermis	L	> 2.5	NJ	0.65	NC	-	-	Zupo, 1994
		M	2 - 2.5	NJ					
		S	≤ 2	J					

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Annex V. (Continuation)

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Caridea	*Palaemon adspersus*	L	> 4.5	NJ	3.8	C	-	-	Guerao & Ribera, 1995
		M	3.5 - 4.5	NJ					
		S	< 3.5	J					
		XS	< 1	J					
	Palaemon serratus	L	> 3.3	J	5.5	C	-	-	Forster, 1951
		S	0.9 – 3.3	J					
		XS	≤ 0.8	J					
	Philocheras trispinosus	U	> 1	NJ	0.37	NC	-	-	Oh & Hartnoll, 2002
	Processa sp.	L	> 1.1	NJ *					
		S	≤ 1.1	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
Cephalopoda	*Sepia officinalis*	U	≥ 8.1	NJ	8.1 - 14.1	C	0.347	2.574	Rossetti et al., 2003; Palomares & Pauly, 2018
Cirripedia	*Amphibalanus sp.*	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
	Cirripedia sp2	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
	Cirripedia sp3	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
Echinodermata	*Coscinasterias tenuispina*	U	-	NJ *	n.d.	NC	-	-	
	Echinocardium cordatum	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	3.449	3.011	Robinson et al., 2010
	Ophiuroidea n.i.	U	-	NJ *$^{\text{n.d.}}$	n.d.	NC	-	-	
	Sphaerechinus granularis	U	> 5	NJ	5.1-5.5	C	-	-	Guillou & Lumingas, 1998

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Annex V. (Continuation)

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Pisces	*Arnoglossus imperialis*	U	< 12	J	12 *2	C	0.00531	3.138	
	Arnoglossus thori	U	< 12	J	12	C	-	-	Froese & Pauly, 2018
	Buglossidium luteum	U	< 8	J	8	C	0.0026	3.648	Froese & Pauly, 2018
	Diplodus annularis	U	< 12.9	J	12.9 – 13.4	C	0.0132	3.096	Froese & Pauly, 2018
	Diplodus puntazzo	U	< 16	J	16 *1	C	0.0229	2.841	Froese & Pauly, 2018
	Gobius niger	L	≥ 6	NJ	6	C	0.0082	3.19	Froese & Pauly, 2018
		S	< 6	J					
	Gobius paganellus	U	≥ 6	NJ	6 – 7	NC	0.0081	3.22	Vieira et al., 2013
	Halobatrachus didactylus	U	< 16	J	16 – 30	C	0.0416	2.71	Palazón-Fernández et al., 2001
	Hippocampus guttulatus	U	≥ 12.5	NJ	12.5 – 12.9	NC	0.0007	3.243	Froese & Pauly, 2018
	Parablennius pilicornis	U	< 9	J	9 – 12	NC	0.007	3.24	Santos, 1995
	Pomatoschistus microps	U	≥ 2.7	NJ	2.7	NC	0.008	3.06	Vieira et al., 2013
	Pomatoschistus minutus	U	≥ 2.5	NJ	2.5	NC	0.0076	3.14	Froese & Pauly, 2018
	Scorpaena porcus	U	< 8.5	J	8.5 - 10	C	0.0183	3.02	Froese & Pauly, 2018
	Serranus hepatus	U	< 7.8	J	7.8	C	0.0142	3.11	Froese & Pauly, 2018
	Symphodus bailloni	L	≥ 4	NJ	4	NC	0.01	3.21	Froese & Pauly, 2018
		S	< 4	J					
	Symphodus cinereus	L	≥ 4	NJ	4	NC	0.01195	3.07	Froese & Pauly, 2018

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Annex V. (Continuation)

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Pisces	*Symphodus cinereus*	S	< 4	J	4				Froese & Pauly, 2018
	Symphodus roissali	U	≥ 5	NJ	5 – 7	NC	0.035	2.67	Froese & Pauly, 2018
	Syngnathus abaster	L	≥ 8	NJ	8	NC	0.00015	3.53	Froese & Pauly, 2018
		S	< 8	J					Franzoi et al., 1993
	Syngnathus acus	U	≥ 9	NJ	9	NC	0.0002	3.33	Froese & Pauly, 2018
	Syngnathus typhle	U	> 6	NJ	n.d.	NC	0.00024	3.172	Froese & Pauly, 2018
Gastropoda	*Bittium reticulatum*	L	≥ 0.7	NJ	0.13	NC	-	-	Lebour, 1936 in Russo et al., 2002
		M	0.5 - 0.6	NJ					
		S	0.3 - 0.4	NJ					
		XS	< 0.3	J					
	Bolinus brandaris	U	-	J *	n.d.	C	-	-	
	Bulla striata (eggs)	U	-	J	n.d.	NC	-	-	
	Calliostoma virescens	U	-	NJ *	n.d.	NC	-	-	
	Calyptraea chinensis	U	-	NJ *	n.d.	NC	-	-	
	Cerithium vulgatum	L	> 4	NJ *n.d.	n.d.	NC	-	-	
		M	3 – 4	NJ *n.d.					
		S	< 3	NJ *n.d.					
	Columbella rustica	U	-	J *	n.d.	NC	-	-	

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Annex V. (Continuation)

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(cm)				*a*	*b*	
Gastropoda	*Hydrobia sp.*	U	-	NJ *n.d.	n.d.	NC	-	-	
	Jujubinus striatus	L	0.6 – 1.1	NJ	n.d.	NC	-	-	
		S	< 0.6	NJ *n.d.					
	Mangelia sp1	L	-	NJ *	n.d.	NC	-	-	
		S	0.3 – 0.4	J *					
	Mangelia sp2	U	-	NJ *n.d.	n.d.	NC	-	-	
	Mesalia sp.	U	-	NJ *n.d.		NC	-	-	
	Ocenebra erinaceus	U	-	J *	n.d.	NC	-	-	
	Ocinebrina aciculata	L	-	NJ *	n.d.	NC	-	-	
		S	-	J *					
	Ringicula sp.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Rissoa membranacea	U	-	NJ *	n.d.	NC	-	-	
	Rissoa parva	U	-	NJ *	n.d.	NC	-	-	
	Steromphala umbilicalis	L	> 1.5	NJ	1	C	-	-	Bode & Andón, 1986
		M	1.3 - 1.5	NJ					
		S	1 - 1.2	NJ					
		XS	< 1	J					
	Tritia corniculum	U	-	NJ *	n.d.	NC	-	-	

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Annex V. (Continuation)

Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Gastropoda	*Tritia incrassata*	L	-	NJ *	n.d.	NC	-	-	
		S	-	J *					
	Tritia pygmaea	L	> 0.4	NJ *n.d.	n.d.	NC	-	-	
		S	0.3 – 0.4	NJ *n.d.					
	Tritia reticulata	L	-	NJ *	n.d.	NC	-	-	
		S	-	J *					
	Turbonilla sp.	U	-	NJ *	n.d.	NC	-	-	
		U	-	J *					
Gebiidea	*Upogebia sp.*	L	≥ 2	NJ *n.d.	n.d.	C	-	-	
		S	< 2	NJ *n.d.					
Nudibranchia	Nudibranchia n.i.	U	-	NJ *n.d.	n.d.	NC	-	-	
Paguridae	*Calcinus tubularis*	U	-	NJ *	n.d.	NC	-	-	
	Diogenes pugilator	L	≥ 1	NJ *	n.d.	NC	-	-	
		S	< 1	NJ *n.d.					
	Diogenidae sp.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Paguridae n.i.	U	-	NJ *n.d.	n.d.	NC	-	-	
	Paguristes eremita	U	-	NJ *	n.d.	NC	-	-	
	Pagurus anachoretus	U	-	NJ *	n.d.	NC	-	-	

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Taxa	Species	Size		Nursery	Maturation (cm)	Value	*Length – weight*		References
		Class	(*cm*)				*a*	*b*	
Polyplacophora	*Chiton olivaceus*	U	-	NJ *	n.d.	NC	-	-	
Polyplacophora	Polyplacophora n.i.	U	-	NJ *n.d.	n.d.	NC	-	-	
Tunicata	*Aplidium punctum*	U	-	NJ *	n.d.	NC	-	-	
	Microcosmus squamiger	U	-	NJ *	n.d.	NC	-	-	
	Phallusia mammillata	L	-	NJ *	n.d.	NC	-	-	
		S	-	J *					
	Styela plicata	U	-	NJ *	n.d.	NC	-	-	

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