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Mestrado em Biologia Marinha

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

Nufar Yadlin

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Resumo

Lagoas costeiras são habitats muito importantes para várias espécies de peixe, pois elas atuam como ambiente de desenvolvimento de juvenis de peixes de diversas espécies, incluindo muitas com valor comercial. Por isso, a preservação das lagoas costeiras é importante para a manutenção da diversidade de comunidades de peixes. Apesar disso, muitos destes ecossistemas foram destruídos em todo o mundo devido à atividade humana.

As ervas marinhas crescem em fundos arenosos ou lamacentos, sendo ecossistemas altamente produtivos, fornecendo alimento e proteção para diversos peixes e invertebrados. Porém, as pradarias de ervas marínhas têm sofrido um declínio, uma vez que são extremamente sensíveis a fatores estressantes, sendo já sugeridos como os indicadores biológicos da qualidade ecológica dos sistemas costeiros. Sabe-se pouco sobre o impacto deste declínio nas comunidades de peixe que utilizam este tipo de ambiente para viver. Por isso, é importante analisar a importância relativa das ervas marinhas como berçário.

No Mediterrâneo, houve um declinio de pradarias de ervas marinhas que foram substituídos por algas verdes, como *Caulerpa*. Estudos recentes mostraram a expansão da *Caulerpa prolifera*, do sul da Espanha para os canais da Ria Formosa. Se os campos de ervas marinhas forem substituídos por *C. prolifera*, a quantidade e qualidade das espécies de peixe poderá sofrer alterações. Alguns estudos descobriram que as áreas de *C. prolifera* são habitats altamente ricas que suportam juvenis de várias espécies de peixes e que deveriam ser considerados um viveiro tão importante quanto as pradarias de ervas marinhas. As comunidades de peixes nos campos de *C .prolifera* não eram tão diferentes das pradarias de plantas marinhas. Porém, não há muitos estudos acerca deste assunto e por isso é necassário analisar os impactos ecológicos da expansão de *C. prolifera* na Ria Formosa.

Áreas sem vegetação são consideradas habitats menos importantes comparados com áreas cobertas por ervas marinhas, e há vários estudos que apoiam este argumento ao reportarem uma diversidade de peixe mais elevada em áreas com vegetação do que em áreas sem vegetação. Porém, também foi reportado de que os fundos arenosos são habitats de alta importância, tal como os fundos com vegetação. Adicionalmente, em algumas casos descobriu-se que alguns peixes vivem exclusivamente em áreas sem

vegetação. Investigar a escolha de habitat pelas espécies peixe é uma maneira de determinar a importância relativa dos diferentes habitats.

Habitats variam de acordo com sua complexidade estrutural, nível de competição interespecífica e risco de predação, e estes fatores influenciam a escolha habitacional do peixe. Diferentes adaptações podem modificar a escolha de habitação. Consequentemente, a preferência habitacional do peixe varia entre espécies e diferentes agrupamentos são típicos de diferentes habitats. Por exemplo, áreas vegetativas como as ervas marinhas e macroalgas estão associadas a uma abundância dos recursos alimentares e baixas probabilidades de predação relativamente às áreas sem vegetação, ao fornecer um refúgio. Todavia, algumas espécies têm adaptações específicas, como cor de camuflagem ou a habilidade de cavar, e não precisam da vegetação para esconder-se de predadores.

A estrutura e densidade das pradarias de ervas marinhas podem afetar a disponibilidade de comida e a eficiência do refúgio, o que também pode ser um fator na escolha de habitat. Por exemplo, os limites destes campos são a escolha preferida das marinhas (Syngnathidae) por terem uma disponibilidade maior de alimento. Enquanto isso, outras espécies podem considerar as áreas de pouca densidade pouco eficiente para proteção e alimentação. Adicionalmente, a escolha de habitat costuma variar de acordo com a fase de desenvolvimento do indivíduo. Por isso, a diferença de agrupamentos poderá ocorrer também entre indivíduos da mesma espécie, mas de tamanhos diferentes, já que algumas espécies apresentam comportamento de migração ontogenética ao longo do ciclo anual. Mudanças sazonais nos agrupamentos também podem ocorrer devido a mudanças em condições ambientais. Por exemplo, mudanças temporais nas estruturas cobertas por plantas e na densidade da vegetação. Por causa disto, é difícil fazer a avaliação do valor de diferentes habitats para diferentes espécies de peixe.

Comparações entre diferentes agrupamentos de peixe em diferentes tipos de habitat na lagoa costeira da Ria Formosa aumentará o nosso entendimento relativamente ao valor das áreas de ervas marinhas, *C. prolifera*, e fundos sem vegetação. Este estudo comparou comunidades de peixe nestes 3 habitats, assim como avaliou o efeito das mudanças sazonais e variações ontogenéticas dos agrupamentos, para além de aprofundar o entendimento sobre a importância relativa e a função de berçário de cada um destes habitats para as comunidades de peixe. Também, poderá aprofundar o que se sabe sobre os possíveis impactos da invasão da espécie *Caulerpa* em pradarias de plantas marinhas mundialmente.

Nossos resultados encontraram uma biodiversidade significativamente menor em habitats arenosos comparado com os dois outros tipos de habitat. O habitat de ervas marinhas tinha a maior abundância e era o mais rico quando se comparava com habitats de *C. prolifera* e areia, embora não hovesse diferença estatística significativa. É provável que isto seja devido a grande variabilidade entre cada estação sazonal em que foram coletadas as amostras e entre as duas réplicas dentre da estação, particularmente no habitat arenoso. Os resultados são parecidos com os de outras comparações entre áreas com e sem vegetação tanto na Ria Formosa quanto em outras partes do mundo.

Nossos resultados mostraram que mudanças sazonais na abundância de peixe e diversidade não são significativamente afetados pelo tipo de habitat, embora fossem encontrados variações sazonais em vários testes. Não houve diferença significativa em testes de semelhança, sugerindo que a maioria das espécies é capaz de utilizar todos os 3 tipos de habitat. Encontrou-se uma alta semelhança entre estações de *C. prolifera* de ervas marinhas, ao contrário da alta separação com o habitat arenoso.

Não houve uma diferença significativa entre os três habitats em relação à abundância de juvenis de peixes, mas sim uma grande semelhança entre os agrupamentos de juvenis, sugerindo que os 3 habitats poderão atuar como berçário para a maior parte das espécies de peixe. Apesar disso, os resultados não foram estaticamente significantes, fazendo com que a avaliação de cada habitat como berçário seja bastante limitado. Nas amostras coletadas durante o verão, houve um acréscimo na abundância de juvenis marítimos em todos os tipos de habitat, o que significa que esta estação é a mais importante para a Ria Formosa atuar como berçário em todos os tipos de habitat. O maior aumento de juvenis marinhos foi encontrado na areia durante o verão, com proporção de 88:12 juvenis comparado com espécies residents, sugerindo que a importancia relativa do habitat arenoso varia durante as estações.

Não encontramos nenhuma mudança ontogenética significativa entre as 8 espécies mais abundantes que coletamos. *C. prolifera* foi a pradaria mais densa e teve o maior comprimento médio dos peixes. Porém, os resultados de densidade foram altamente variados entre réplicas, provavelmente devido a grande irregularidade de distribuição de vegetação que caracteriza este tipo de habitat. Diferenças sazonais de comprimento de peixe foram significativas, provavelmente devido a preferência de certas espécies e não às funções de berçário, uma vez que a abundância de juvenis de peixes no verão era mais alto neste habitat.

Nossos resultados indicam de que a temporada de verão é altamente importante para um berçário e recrutamento em todos os habitats e a importância relativa do berçário varia ao longo do ano, especialmente no habitat arenoso. A invasão da espécie *C. prolifera* nos fundos sem vegetação na Ria pode afetar o recrutamento algumas espécies, sendo necessário investigar mais para obter resultados precisos que ajudarão nas decisões de gestão e conservação.

Abstract

Coastal lagoons support many fish species including of some valuable commercial fish species. Protecting coastal habitats is important to maintain the diversity of fish populations, thus it is important to asses relative important of the different habitats that those areas provide. Seagrasses are marine plants growing on sandy or muddy bottoms, they are highly productive habitat types, providing food and shelter for diverse invertebrates and fish assemblages. However, seagrass meadows have been experiencing widespread decline as they are sensitive to environmental stressors. Little is known about how these declines are affecting fish communities associated to seagrass habitats. *Caulerpa* seaweeds have expended in many areas outcompeting seagrass meadows. If seagrass meadows are replaced by seaweed the fish assemblages may be altered. In some studies fish assemblages in *Caulerpa* meadows were not significantly different then in seagrass beds. Thus, the role of macroalgae have received little attention and not many comparisons had been conducted. This study aims to investigate the relative importance of sand, Seagrass, and *Caulerpa prolifera* habitats, and provides insights regarding the effects of *C. prolifera* invasion over sand and seagrass habitats along the year, as habitats relative importance varied between seasons. Our results found Sand habitats significantly less diverse then the other two habitats, and seasonality changes in the fish abundance and diversity were not significantly affected by the habitat type. Summer had the highest juvenile abundance in all habitats. Although seagrass was the richest and most abundant habitat, differences were not significant. Moreover, our results showed that most species can utilize all 3 habitat types, and that nursery value changes along the year, especially in sand habitat. Our results showed high variability between replicates which probably affected the significance of the results. Therefore more research must be performed to obtain accurate results regarding nurseries and the relative importance of different habitats.

Keywords: habitat choice, *Caulerpa prolifera*, Seagrass, Nursery, Ria Formosa

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List of acronyms

Acronym	**Description**
Sa	Sand habitat
CP	*Caulerpa Prolifera* habitat
Sea	Seagrass habitat
Ju	Juvenile fish
NJ	Non-Juvenile fish
H	Shannon-Wiener Biodiversity index
J	Evenness index
S	Species richness
R	Resident species
MJ	Marine juveniles
D	Diadromous species
NI	Not Identified taxa

1 Introduction

1.1 Different habitat types

Estuaries and coastal lagoons are highly important habitats for fish and invertebrates as spawning, nursery, and feeding areas (da Silva et al., 2018). They support many fish species as they are an important nursery grounds for juveniles of diverse species, including of some valuable commercial fish species (Gray et al., 1996; Ribeiro et al., 2006; Ribeiro et al., 2012; McDevitt-Irwin et al., 2016). They are formed by many different habitats including deep channels, steep banks, muddy banks, sandy bottoms, inter tidal and subtidal seagrass beds (Sheaves, 2006) mangroves, and oyster beds (da Silva et al., 2018). The wide range of habitat types forms highly productive environment (Lloret et al., 2008) as they provides a variety of niches for colonization by diverse communities of organisms (Pérez-Ruzafa et al., 2006; Sheaves, 2006).

Coastal lagoons however, are highly exposed to human activities such as economic development accompanied by a high rate of degradation of their natural resources; they are extremely vulnerable to eutrophication caused by increase population densities along their coastlines and fertilizers use in their surrounding watershed (Lloret et al., 2008). Thus, in many parts of the world those ecosystems have been destroyed as a result of human activities (Ribeiro et al., 2012), which caused changes in fish assemblages and distribution (Verdiell-Cubedo et al., 2007b). Moreover, significant changes in coastal lagoons will probably occur from global environmental changes such as increase in global mean temperature (Lloret et al., 2008). Thus, the impact of anthropogenic disturbances and the value of the different habitats of those ecosystems have been of stakeholder interests (Gray et al., 1996). Identifying coastal nursery habitats and understanding the mechanisms by which they serve as nurseries is increasingly important as human populations and associated impacts continue to grow in these areas (McDevitt-Irwin et al., 2016).

However, the effects of these changes are difficult to infer and will depend on the particular characteristics of each area (Lloret et al., 2008). A better understanding of the functions of those coastal lagoons ecosystems is vital for protecting these vulnerable habitats (Ribeiro et al., 2012). And protecting coastal habitats is important to maintain the diversity of fish communities (Guidetti, 2000). Furthermore, information regarding the requirement of juveniles in those habitats will provide a valuable tool for management and conservation of the marine fish populations (Verdiell-Cubedo et al., 2007a); therefore, there is a clear need to carry out new studies (da Silva et al., 2018).

Seagrasses are marine plants growing on sandy or muddy bottoms (Bertelli and Unsworth, 2014). They form extensive meadows (Tuya et al., 2013) of intertidal to shallow subtidal beds (Nordlund et al., 2016). There are more than 50 known species of seagrass with a variety of different characteristics such as leaf shape, length and area, and shoot density (Orth et al., 1984). They modify their local environmental conditions and provide essential services to ocean ecosystems and human well-being (Tuya et al., 2014a) by supporting nutrient cycling, sediment stabilization, and sequestration of carbon (Nordlund et al., 2016).

Furthermore, due to their complex structured form and spatial variability (Malavasi et al., 2007), they are highly productive habitat types, providing food and shelter for diverse invertebrates and fish assemblages (Png-Gonzalez et al., 2014). Many species that reside in seagrass habitats have reduced predatory pressure, lower energy requirements and higher growth rates compared with those found in other coastal habitat types (Bertelli and Unsworth, 2014). For instance, Tuya et al. (2014a) reported that 82% of total fish individuals used *C. nodosa* as nursery grounds in the Gran Canaria Island, and McDevitt-Irwin et al. (2016) found that although juvenile survival was varied according to taxonomic groups, it tended to be higher in seagrass habitats.

Thus seagrass are a crucial nursery grounds for many fish juveniles including commercially exploited species (Bertelli and Unsworth, 2014; Tuya et al., 2014a). However, seagrass meadows have been experiencing widespread decline throughout their range for a variety of reasons such as reduced water quality, overgrazing, and physical damages due to development (Hughes et al., 2009). Hence, seagrass species are sensitive to environmental stressors and have been proposed as biological indicators of changing coastal conditions (Hughes et al., 2009). The worldwide decline in seagrass meadows are poorly recorded and there is no information on seagrass distribution patterns from most coasts around the world (Tuya et al., 2013). Moreover, little is known about how these declines in seagrass meadows are affecting fish communities associated to this habitat type (Hughes et al., 2009). Thus, it is important to determine the relative importance of seagrasses as nursery habitats (McDevitt-Irwin et al., 2016).

The genus *Caulerpa* comprise a group of green algae species with a worldwide distribution in tropical and subtropical shallow marine habitats (Cacabelos et al., 2019). These seaweeds usually form patchy meadows over mud and fine sand but can also grow on rocky substrates or areas colonized by algae (Cacabelos et al., 2019). *Caulerpa prolifera* species forms extensive beds on soft bottoms in ca. 5-50 m water depths (Png-Gonzalez et al., 2014). *C. prolifera* has a high sediment retention capacity which induces organic enrichment, potentially altering associated animal populations (Png-Gonzalez et al., 2014).

Recent surveys showed an expansion of *C. prolifera* into the Ria Formosa channels from its previous geographic limit in southern Spain (Cunha et al., 2013). This phenomena occurred in other areas as well, for instance, in a rockpool located in Mosteiros in one of the Azores islands a patch first found in October of 2013, rapidly colonized the area, and by 2017 it appeared to colonize several different spots within the pool and managed to thrive (Cacabelos et al., 2019). Similarly, in the Mediterranean, there has been a widespread decline of seagrass meadows that have often been replaced by green algae such as *Caulerpa*; this may occur from several reasons, some studies report that it happened as a result of anthropogenic activities that cause increase in nutrient loading and suspended sediments in the water column, resulting in reduced water transparency (Tuya et al., 2013). Other studies report that expansion of the *Caulerpa* species over the regression in *C. nodosa* seagrass abundance are caused by competition for nutrients in the sediments between the species (Tuya et al., 2013).

If seagrass meadows are replaced by seaweeds the quantity and quality of habitat associated with different fish assemblages may be altered, as well as flows of energy and materials through the ecosystem (Png-Gonzalez et al., 2014). Consequently, habitats that are characterized by sediment stability, high water clarity, an oxic water column, productivity and nursery grounds may be replaced by habitats characterized by sediment instability, turbid water, localized hypoxia and unpredictability in productivity and nursery function (Tuya et al., 2014a).

Seagrasses provide food for a wide array of fish and invertebrates (Hughes et al., 2009). The leaves of the seagrass are colonized by epiphytes that may provide food for associated herbivores; however, certain macrophytes developed mechanisms to minimize herbivory (Del Río et al., 2016). For instance, *Caulerpa* species contains different levels of Caulerpenyne, a chemical that is toxic to some herbivore fish (Marco-Méndez et al., 2017). However, Del Río et al. (2016) found significantly greater numbers of fish bites on *C. prolifera* than in *C. nodosa* plants, and a positive correlation between average number of bites per thallus and total herbivorous fish abundance. Thus, *C. prolifera* was more consumed by herbivores than *C. nodosa* (Del Río et al., 2016).

Furthermore, some studies found *C. prolifera* beds highly rich and diverse habitats that support many juvenile fish species and to be as important nursery ground as seagrass beds. Additionally, it has been reported that the fish assemblages in *Caulerpa* meadows were not significantly different from species in seagrass beds e.g (Jenkins and Wheatley, 1998; Verdiell-Cubedo et al., 2007b; Franco et al., 2012; Koulouri et al., 2016). Fish may seek shelter in the vegetated meadows without a specific preference for the species of the meadow (Jenkins and Wheatley, 1998). However, the role of macroalgal habitats have received very little attention compared with seagrass beds (Koulouri et al., 2016). Thus, there is a clear necessity to assess the ecological impacts of *C. prolifera* invasion (Png-Gonzalez et al., 2014).

And learning about the fish habitat choice is a way to determinate the relative importance of the different habitat types (McDevitt-Irwin et al., 2016).

Unvegetated areas such as bare sand attract adults of large mobile fish or species protected by schooling behavior that are seeking valuable foraging areas that provide the maximum potential for growth (Connolly, 1994; da Silva et al., 2018). In these habitats there are usually small size resident species that spend their entire life cycle there and large size marine migrants which use these habitats as nursery grounds (Schafer et al., 2002). These unvegetated habitats are considered less important habitat types compared with the seagrass beds; and indeed, many studies reported higher fish diversity in vegetated compared with unvegetated habitats (Gray et al., 1996; Jenkins and Wheatley, 1998; Guidetti, 2000; Ribeiro et al., 2006; Ribeiro et al., 2012). However, it also has been reported that sandy bottoms are extremely important habitat types as well as the vegetated ones mentioned above (Gray et al., 1996; da Silva et al., 2018). Moreover, in some comparisons there were certain fish species that were found solely in the unvegetated habitats, including some commercial species e.g (Gray et al., 1996; Jenkins et al., 1997; Travers and Potter, 2002; Ribeiro et al., 2006).

In general, habitat types vary in their structural complexity, level of interspecific competition, and predation risk and those factors influence fish habitat choice (Malavasi et al., 2007). Habitat type has an important role in defining fish assemblages in the Ria Formosa lagoon (Ribeiro et al., 2006). Thus, comparison of fish assemblages between different habitat types, in the Ria Formosa coastal lagoon will increase the understanding of the relative value of seagrass beds, *C. prolifera* meadows, and unvegetated bottoms in the lagoon, and provide better knowledge regarding habitat preferences between different fish species. Furthermore, information about nursery value of fish in their early life stage and about factors influence a successful recruitment is important to support the local fish community of the Ria Formosa lagoon.

1.2 Fish Habitat choice

Nursery habitats are often defined as habitats that provide greater than average number of individuals to the adult population of a certain species on a per-unit-area compared with other habitats used by juveniles (McDevitt-Irwin et al., 2016). However, this definition excludes habitats that may be critical to sustaining adult populations (Dahlgren et al., 2006). Therefore Dahlgren et al. (2006) developed the term effective juveniles habitat (EJH) which unlike the common definition, takes into account the juveniles proportion of contribution to the adult population of a certain species compared with all habitat occupied by that species regardless of the area coverage. In this case the overall contribution is considered rather than the per-unit-area comparison, and thus the areal coverage does not need to be known in EJH classification scheme (Dahlgren et al., 2006). Mortality rate during the fish early life stages are extremely high compared to a more mature life stages, mainly due to predation risks. Thus it is expected that they will choose nurseries providing the maximum potential for growth (Sogard, 1992). Different adaptations can modify the habitat selection (Sogard, 1992). Consequently, fish habitat preferences are different among species (da Silva et al., 2018), and different assemblages are typical of different habitats (Gray et al., 1996).

For instance, while unvegetated habitats are usually composed of highly diverse fish fauna, they tend to be dominated by a small number of species in high densities (Schafer et al., 2002). These high abundances are in part due to high densities of zooplankton and benthic macroinvertebrates which are a relatively important food source for many nearshore fish species (Schafer et al., 2002). Vegetated habitats such as seagrass and macroalgae not only are associated with enhanced food resources but also reduced predation rates relative to unvegetated substrates by providing a protective refuge (Sogard, 1992). However, Sogard (1992) found that *Pseudopleuronectes americanus* can exploit unvegetated habitat due to their cryptic coloration and burying abilities consequently they do not need the vegetated habitats as refuge from predators. Likewise, Bertelli and Unsworth (2014) found 3 juvenile species of flatfish, *Solea solea*, *Limanda limanda*, and *Platichthys flesus*, only in sand habitat and not in seagrass beds.

Structure and density of the meadow can affect food availability and shelter efficiency, which may also influence fish habitat choice (da Silva et al., 2018). For example, planktonic crustaceans are a primary food source for pipefish species; they are carried to seagrass patches by water currents, thus seagrass edges are a preferred habitat for pipefish due to greater food availability (Macreadie et al., 2009). Different values of pipefish abundance were observed between locations in the result of Malavasi et al. (2007), while the authors reported differences could be related to different structure and complexity of the meadows. However, this could also be related to other environmental factors characterizing the 3 different sites. In contrast, some species may find low bed density less efficient for shelter and foraging uses (da Silva et al., 2018). Anthropogenic pressures might effect as well the fish habitat choice. For instance, Henriques et al. (2013) found significant influence of fishing, sewage discharges and port activities on fish diversity, abundance, nursery function and trophic structure.

The Shannon Wiener diversity index is commonly used to compare fish communities and by that to evaluate different nursery grounds e.g (Heck et al., 1989; Connolly, 1994; Gray et al., 1996; Jenkins et al., 1997; Jenkins and Wheatley, 1998; Guidetti, 2000; Travers and Potter, 2002; Horinouchi, 2005; Ribeiro et al., 2006; York et al., 2006; Verdiell-Cubedo et al., 2007b; Ribeiro et al., 2012; Koulouri et al., 2016; da Silva et al., 2018). While seagrass habitats were found to be more diverse in many of these studies, results were not always significant, and the comparisons produced many different outcomes and conclusions. Therefore, many studies additionally used other methods to compare between the communities in the different habitats; such as fish density and abundance, multi-dimensional scaling (MDS), similarity percentage (SIMPER), and analysis of similarities (ANOSIM) e.g (Ribeiro et al., 2006; York et al., 2006; Ribeiro et al., 2012; Bertelli and Unsworth, 2014).

1.3 Variability in habitat choice

In addition to those variations in habitat choice between different fish species discussed above, the fish habitat choice often change within the species life cycle and seasonal influences (Gray et al., 1996; Jenkins et al., 1997; Jenkins and Wheatley, 1998; Guidetti, 2000; Kalogirou et al., 2012; Ribeiro et al., 2012; da Silva et al., 2018). Consequently, differences between the habitat types could occur as well between fish sizes of same species, and not only between the fish species (Gray et al., 1996; Jenkins et al., 1997; Jenkins and Wheatley, 1998; Guidetti, 2000; Kalogirou et al., 2012; Ribeiro et al., 2012; da Silva et al., 2018). For instance, Jenkins et al. (1997) found small juveniles of *Platycephalus laevigatus* mainly in unvegetated habitats, while the adults were only collected from seagrass habitats. A similar trend was found by Ribeiro et al. (2012) with *Atherina presbyter*, where only very early life stage items were found in the unvegetated habitats, and more advanced juvenile stages were dominant in the seagrass beds. Schafer et al. (2002) in a study on sandy bottoms found that there was a shift in the dominant size classes between seasons due to seasonal spawning life history of *S. bassensis* and *S. vittata* which dominated their samplings.

Seasonal changes in the fish assemblages may occur due to changes in environmental conditions. For example, temporal changes in the structure of the seagrass beds (Horinouchi, 2005), and in the meadows density (Orth et al., 1984; MacArthur and Hyndes, 2001). Changes in peak breeding times and short life cycle of the major prey taxa, caused those prey species to change composition during the year and may affect fish composition as well (Schafer et al., 2002). Assemblage changes through the yearly cycle may also be caused by sequential immigration of some of the species (Travers and Potter, 2002). For instance, Koulouri et al. (2016) found seasonal dissimilarity due to the high abundance of some species in a certain season and higher abundance of others in other seasons. Thus, changes in the fish assemblages occur as well from ontogenetic migration behavior of certain species (Travers and Potter, 2002; Ribeiro et al., 2012; Tuya et al., 2014a); during spawning or settlement periods the habitat preferences may change (Guidetti, 2000).

Overall, as presented above, fish habitat preferences are affected by many factors that may influence the results of comparisons between communities in different habitat types. Hence, judgments on the values of different habitats for different fish species are often difficult (Gray et al., 1996). Additionally, the use of different sampling methods complicates comparisons among studies and among habitats (de la Morinière et al., 2002). For example, sampling strategy has influenced the results of Santos et al. (2002), and there were significant differences in the fish taxonomy among sampling methods compared by Franco et al. (2012). da Silva et al. (2018) as well reported possible influence of the sampling methods on the number of individuals collected in vegetated areas.

Furthermore, comparisons from different regions may also reach different results as each ecosystem is different and comprises various complex interrelations and interactions among the different biological elements (Sheaves, 2006). For instance, comparisons of fish assemblages between 4 estuaries in July of 4 years made by Sheaves (2006) had different fish composition at each estuarine. Thus, the role of seagrass in supporting increased juvenile growth relative to other habitats varied between geographic regions (McDevitt-Irwin et al., 2016). For instance, seagrass meadows in the US have been found to be more important as fish nursery grounds than those in Australia. Therefore it is unreliable to make assumptions about the ecological value of this habitat in one location based on a study carried out in another location (Bertelli and Unsworth, 2014). Hence, there is a need to perform research in different geographic locations (Ribeiro et al., 2012), as more comparisons are necessary in order assess habitat ecological function (Tuya et al., 2014b).

1.4 Objectives

This study aims to investigate fish habitat preferences in the Ria Formosa lagoon by comparing fish assemblages, and biodiversity among 3 different habitat types in the Ria: seagrass bed, *C. prolifera* meadow, and unvegetated sediment. It will also asses the effects of seasonal changes and ontogenetic variation over those preferences. This study will give insights into the relative importance and nursery function of each of these habitat types to the fish community they support. Additionally, this research may increase the knowledge of the possible effects of *Caulerpa* species invasion on seagrass meadows around the world.

2 Material and Methods

2.1 Study area

This study took place in the Ria Formosa lagoon, a 55 km of large tidal lagoon located in the Algarve, south coast of Portugal, with a maximal width of 6 km (Ribeiro et al., 2012). A seaward belt of dunes protects a system of salt marshes, subtidal channels and tidal flats (Ribeiro et al., 2006). The lagoon surface area is approximately 170 km^2; six outlets to the Atlantic Ocean that are connecting a strong branched system of creeks and channels (Ribeiro et al., 2006; Ribeiro et al., 2012). The lagoon does not receive any permanent freshwater input, except of surface water coming from rainfalls in the winter period, salinity range of 35.5-36.9 % all year round (Ribeiro et al., 2006). The water temperature in the lagoon varies from 12 to 28^{o}C (Ribeiro et al., 2006).

The study area for this research was near the Culatra barrier-island in Ria Formosa (Figure 2.1). Sampling took place in 3 different habitats types: the macroalga *C. prolifera* (CP) 37.00122N, 7.82245W; subtidal seagrass mixed meadow *Cymodocea nodosa* and *Zostera noltii* (Sea) 37.00206N, 7.82998W; bare sand with no vegetation, (Sa) 37.00286N, 7.81874W. The distance between Sea and CP habitats was ~700m, between CP and Sa ~400m, and between Sea and Sa ~1000m (Figure 2.1). Salinity measured in all the area was ~36 %. Three sampling events were done along the year; 27.2.2019 (winter), 26.4.2019 (spring), and 25.6.2019 (summer). Water temperature was measured in the winter and the summer sampling and ranged between ~16^{o}C in the winter and ~23^{o}C in the summer. All samplings took place in low tide at 1.5m water depth.

Figure 2.1. Sampling locations with the 3 habitat types: Sand (Sa) in yellow, Seagrass (Sea) in green, and *Caulerpa prolifera* (CP) in red; Cularta island and the city Faro, are mentioned in black. Map was created using Arcmap 10.5 sotware.

2.2 Field work

2.2.1 Fish sampling

Fish were collected using a 25m beach seine, an encircling gear made of 11mm mesh, with a float line and a lead line that is effective in fishing the entire water column and catching juvenile fish (Erzini et al., 2002). In each habitat type, 2 replicates were used, where each replicate was estimated as a fishing area of 300m^2 (Figure 2.2a). Fish were collected into labelled plastic bags and kept frozen until farther analysis, except for fish from the Syngnathidae family that were identified and measured on the spot and then released back into the water (Figure 2.2b).

Figure 2.2. a) Sampling method used for fishing: beach seine b) *Syngnathus acus* measured right before being relased back to the water. Photos taken by Carl Robert Priester in the winter sampling, February 2019.

2.2.2 Sampling of plants

Plants were collected in the spring sampling assuming that plants species composition and their density does not significantly change along this period of 6 months. In each replicate 2 different random plots of $0.06m^2$ were collected by 2 snorkel divers with a ~10m distance between them; thus, overall 4 plots were collected from each vegetated habitat type (CP and Sea). The plants were stored in labelled plastic bags and kept frozen until farther analysis.

2.3 Lab work

2.3.1 Fish analysis

In the lab all the fish were identified to species level, except for *Pomatoschistus microps* and *Pomatoschistus minutus* that were unable to be distinguished from each other in most cases, therefore were recorded as *Pomatoschistus* spp (Figure 2.3). The total length (TL) of each fish was measured to the closest mm and recorded according to their habitat type, season, and replicate number.

Figure 2.3. Primary sorting in the lab of fish caught in spring sampling (April 2019). Photos taken by Nufar Yadlin.

2.3.2 Analysis of seagrasses and algae

The plants that were collected in the spring sampling were all washed from sediments leftovers and identified into the lowest taxonomic level possible (Figure 2.4). The different seagrass species and *C. prolifera* were identify to species level, and the other macroalgae found in the sample identified into species, genus, or not identified. All plants were dried at 100°C until constant weight of maximum 2% fluctuation and weighed, following the method of Heck et al. (1989). The dry weights were recorded once according to their species in order to estimate contribution of each plant species to the meadow, and once according to habitat type in order to estimate the average density of each of the 2 vegetated habitats type (Sea and CP).

Figure 2.4. a) plants being washed from sediment leftovers. b) primary sorting of the plants. photos taken by Vitor Dias (a) and Nufar Yadlin (b)

2.4 Data analysis

2.4.1 Fish abundance and Biodiversity indices

After all the fish were measured and identified, each species was counted and sorted according to their habitat types replicates and seasons; *Pomatoschistus* spp was considered as one species. Each fish was recorded as a juvenile (Ju) or not juvenile (NJ) based on what was found in the literature corresponding to their TL measurements; only individuals that were undisputedly Ju were recorded as Ju; all others were recorded as NJ.

To estimate how the diversity varied between the habitat types and the seasons, the Shannon-Wiener diversity index was calculated assuming that all species are represented in the sample. Biodiversity index (H) is calculated according to eq. 1.1 Where Pi is the number of individuals of one species divided by the total number of species in a given area (Magurran, 2004).

$$\text{Eq 2.1. } H = -\Sigma Pi \ \ln \mathrm{P}i$$

Pielou evenness index (J), is calculated according to eq. 2.2 Where S is the total amount of species counted in a given habitat (Magurran, 2004), and is defined as species richness (Verdiell-Cubedo et al., 2007b).

$$\text{Eq 2.2. } J = \frac{H}{lnS}$$

2.4.2 Species composition and habitat nursery value

In order to compare the fish assemblage functional structure, the ecological guild for each species was recorded according to Ribeiro et al. (2012): resident species (R), - present in the Ria all year round, marine juvenile migrates (MJ) - species that are present in the Ria only in their early life stages, and diadromous migrant species (D) - species that migrate from fresh water sources. The percentage of each ecological guild were calculated for each habitat type and season in order to compare relative guilds between habitats and seasons.

2.4.3 Habitat characterization

The contribution for each plant species in the 2 vegetated habitats (Sea and Sa) was estimated by dividing the dry weight of each plant type with the defined plot area of the plants collected in the field, followed the methodology of Heck et al. (1989), and Jenkins and Wheatley (1998). Density was estimated for each plant species found in each habitat to evaluate the relative contribution of each species. The habitat types mean densities were estimated by taking the average density of each species in each of the 4 replicate that has been taken from each habitat type.

2.5 Statistical analysis

2.5.1 Fish Biodiversity and habitat choice

The independent variables were habitat types (CP, Sea, and Sa), and seasons (winter, spring, and summer) both considered fixed. Since the data failed to meet normality according to Shapiro-Wilk test, a multi-variate analysis of 2 factors (2-way PERMANOVA) was performed in order to test differences in fish abundances and diversity indices between habitats and seasons. Two – way PERMANOVA was also performed on the fish TL in order to test difference in the fish sizes and their habitat type preference, and whether it is affected by the habitat characteristics: plants species composition, and density; and in order to examine if those preferences changes along the seasons. If significance of $p<0.05$ was found, a pairwise test was used in order to determine which means differed.

2.5.2 Species composition and habitat nursery value

In order to compare the fish communities of each of the 3 habitat types and the seasonality effect of it, an analysis of similarity (ANOSIM) was performed on the differences in species assemblages between habitats and seasons. The test ran on a square root transformed data for species abundance so that each species contribution will be fairly equal in the analysis, following Ribeiro et al. (2006), and by that allowing the intermediate abundance species to play a part in the results as well (Clarke et al., 2014). Species Presence\absence data between habitats and seasons were performed in order to allow contribution of the rarer species to the analysis (Clarke et al., 2014). The seasonality ANOSIM analysis, both on assemblage and on species presence/absence, were performed in each habitat separately following Ribeiro et al. (2006).

In order to evaluate the nursery value of each habitat and the seasonality effect on it, a 2-way PERMANOVA test was run for Ju fish abundance of the transformed data for habitats and seasons. Moreover, ANOSIM between habitat types and seasons was used on the transformed data of the Ju fish assemblage in order to test similarity between the factors. PERMANOVA pairwise test was used when significant differences were found. Additionally, ANOSIM test were performed to test similarity between transformed data of Ju compared with NJ assemblages for the 8 most abundant species caught. That was in order to evaluate habitat nursery value for those specific species and whether their habitat preferences changes along their life cycle.

All ANOSIM tests were based on Bray-Curtis similarity coefficient using 9999 random permutations of residuals. If significance of $p < 0.05$ were found, a pairwise test was run in order to determine which assemblages differed and to obtain p values. PERMANOVA tests were based on Bray-Curtis similarity coefficient in the abundance tests (fish abundance, Ju abundance, S index) and Euclidean similarity on other tested features (mean TL, H and J indexes) (Faith et al., 1987; Clarke, 1993). Shapiro-Wilk test for normality was applied using IBM SPSS Statistics 20 software. All ANOSIM and Two - ways PERMANOVA tests were run on past 3.26 software (Hammer et al., 2001); apart from two – way PERMANOVA for difference in fish TL that was run on R-studio software (Rstudio Team, 2018).

3 Results

3.1 Fish habitat choice, abundance, and Biodiversity indices

A total of 1243 fish were caught from 20 different species, with 16 species caught in Sea habitat and 14 species in both CP and Sa habitat. The Sea habitat had the highest abundance, with 534 individuals, followed by Sa with 424 and CP with 285 fish caught along the 3 sampling campaigns (Annex 1). However, differences in fish abundance were found not significant according to 2-way PERMANOVA results (Table 3.1). The mean value in all 3 diversity indices (H, J, and S) was lower in Sa compared with the 2 other habitat types (Figure 3.1). However, significant differences among habitats ($p<0.05$) were only found for H and J indices (Table 3.1). According to pairwise test results, biodiversity in Sa was significantly lower than both Sea and CP, whereas evenness was only significantly lower in Sa compared to CP habitat (Figure 3.1).

The highest number of fish were caught in the summer sampling, 593 fish, followed by winter and spring with 327 and 323 fish respectively, while summer sampling was the richest with S value of 17 species followed by 15 and 13 in the spring and winter respectively (Annex 1). Mean S and fish abundance were highest in the summer, but not significantly different according to two-way PERMANOVA results (Figure 3.1). No significant differences were found in the H and J indices as well between the 3 seasons in any of the 3 habitats. Moreover, no significant difference was found between habitat-season interaction in the fish abundance nor in any of the 3 diversity indices tested (Table 3.1).

Table 3.1. F-values and significance levels for two-way PERMANOVAs of fish abundance, Juveniles abundance (Ju), fish total length (TL), and the Shannon-Winner indices: biodiversity (H), richness (S), and evenness (J); between habitats and seasons and habitat-season interaction.

Effects	**df**	**H**		**S**		**J**		**fish abundance**		**Ju abundance**		**fish TL**	
		F	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Habitat	2	5.65	<0.05	3.45	n.s	4.82	<0.05	2.00	n.s	1.34	n.s	12.66	<0.001
Season	2	1.49	n.s	2.34	n.s	0.42	n.s	1.84	n.s	3.80	<0.01	23.67	<0.001
Habitat x Season	4	0.89	n.s	1.18	n.s	2.14	n.s	1.52	n.s	1.35	n.s	11.28	<0.001

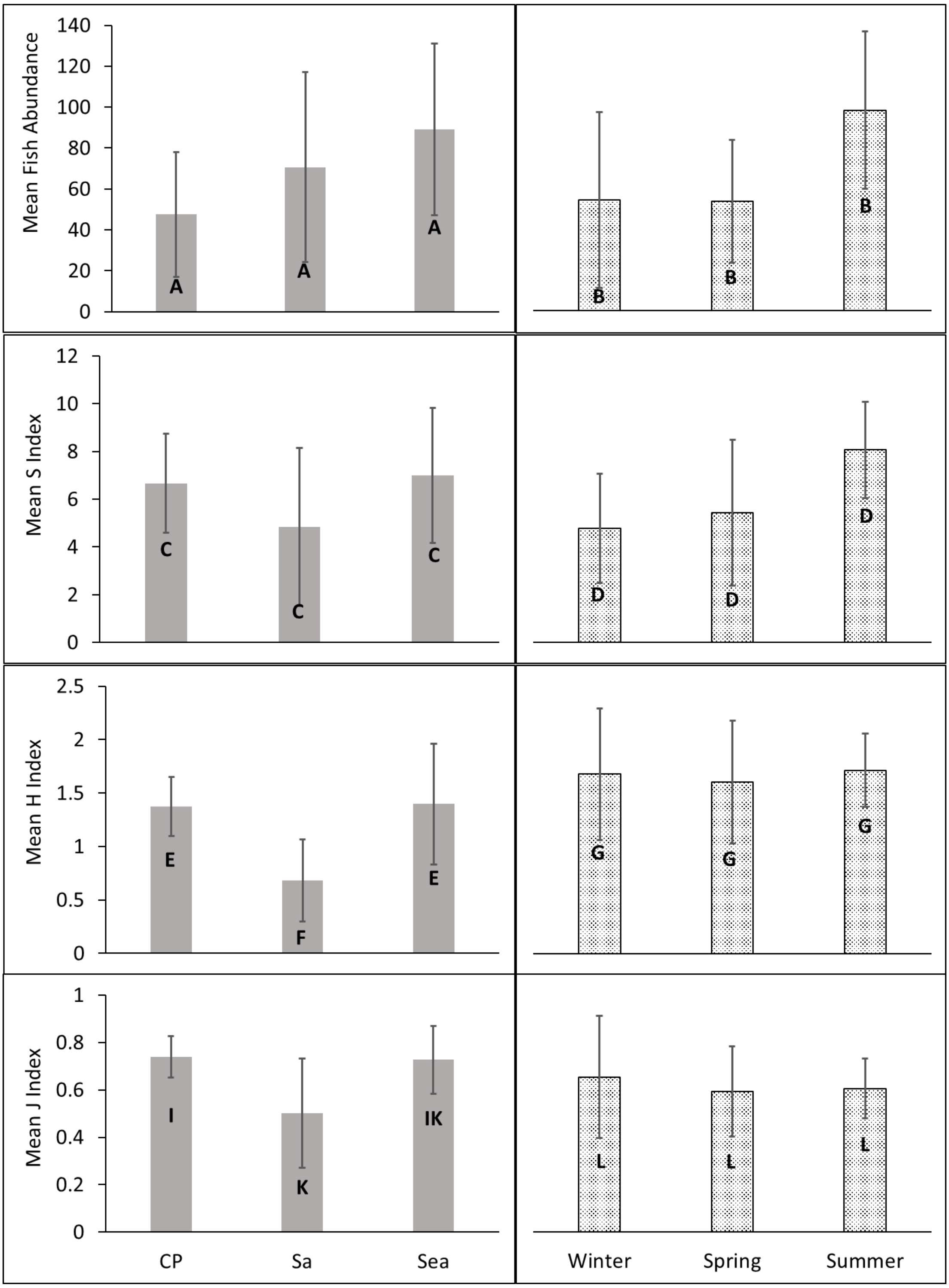

Figure 3.1. Average fish abundance and Shannon-Wiener indices: Richness (S), Biodiversity (H), and evenness (J) between the 3 habitats: Seagrass (Sea), Sand (Sa), and *Caulerpa prolifera* (CP); and between the 3 seasons: winter, spring, and summer. Error bars represents ±1 SD and unlike letters signify statistical differences within each factor in pairwise PERMANOVA test.

Two-way PERMANOVA for fish TL found significant differences between all the habitat types and all seasons (p<0.001), while the habitat-season interaction was also significant, p<0.001 (Table 3.1). Therefore, seasonal differences within habitats were investigated: in summer and spring TL was highest in CP than in the other habitats, while in the winter the highest TL was found in the Sea habitat. Significant differences within habitats however were found only in the Sa habitat between all 3 seasons, p<0.001; pairwise results revealed that summer-spring were significantly different (p<0.05), while summer-winter, and winter-spring were significantly different (p<0.001) (Figure 3.2).

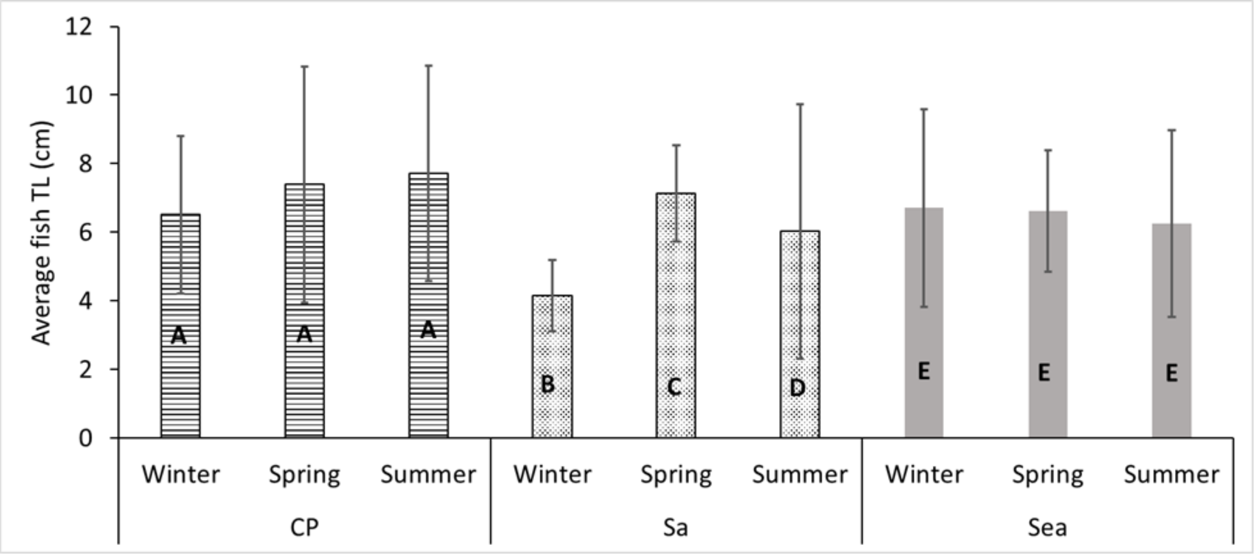

Figure 3.2. Average fish TL over Seagrass (Sea), Sand (Sa), and *Caulerpa prolifera* (CP) across the 3 seasons (winter, spring, and summer). Error bars represents +1 SD and unlike letters signify statistical differences in seasons within each habitat according to pairwise PERMANOVA test.

3.2 Species composition

Most of the fish caught in all habitat types were of the resident species guild; the ratio between resident species and marine juveniles was similar in the CP and Sea habitats of 62:37 and 63:37 respectively. In the Sa habitat however, the ratio was fairly even between resident and marine juveniles 49:51 (Figure 3.3). The 8 most abundant species caught and showed presence in all habitat types all year round were: *Atherina presbyter*, *Gobius niger*, *Pomatoschistus spp*, *Symphodus bailloni*, and *Symphodus cinereus* of the Resident species guild and *Diplodus annularis*, *Diplodus sargus, and Diplodus vulgaris* of the marine juvenile guild (Annex 1).

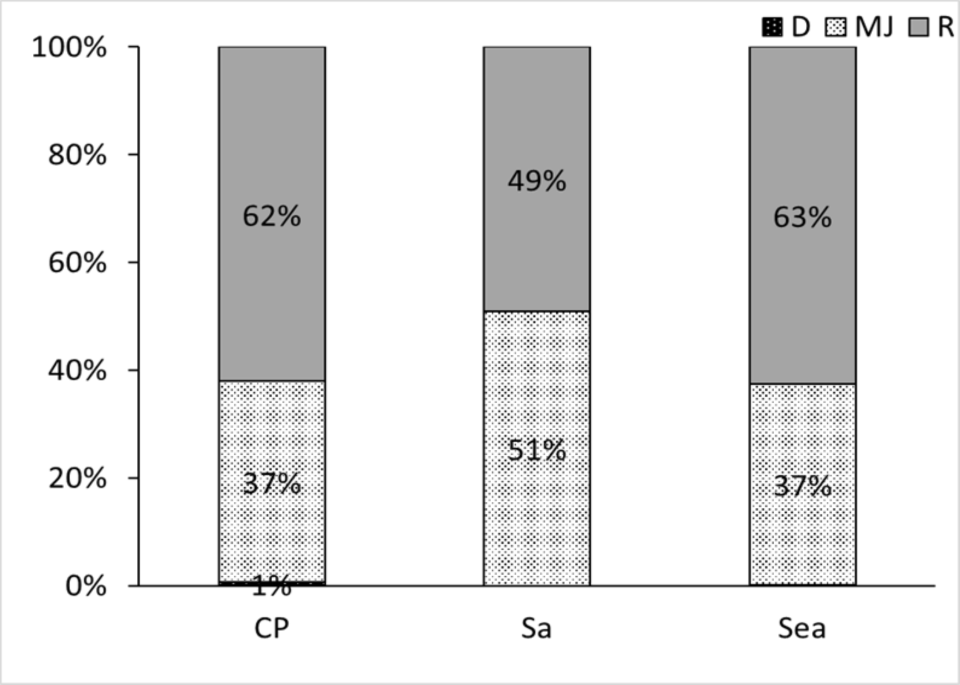

Figure 3.3. Percentage of fish caught in each ecological guild, over the 3 habitat types: *Caulerpa prolifera* (CP), Sand (Sa), and Seagrass (Sea): Diadromous species (D), marine juvenile (MJ), and residence species (R).

A. presbyter species had the highest abundance of 187 individuals in the Sea habitat followed by 72 and 32 items in Sa and CP habitats respectively. *Diplodus vulgaris* had the highest abundance in CP and Sa habitats with 96 and 147 fish respectively, and second highest abundance in the Sea habitat with a total of 110 fish (Annex 1). ANOSIM test for species assemblage between habitats showed significant similarity ($p<0.05$) with R-statistics= 0.203 (Table 3.2). According to pairwise analyses, the similarity was significant ($p<0.05$) only between Sa and CP habitats with a R-statistic = 0.272.

Out of the eight most abundant species that were caught (Table 3.4), four species were common between Sea and CP, and 4 species between Sa and Sea. Two species were present only in Sa, 1 species was present only in CP, and no species were present only in the Sea habitat. Presence/absence of species between habitat types were found highly similar, R-statistic = 0.193; however, the result was not significant (Table 3.2).

Table 3.2. Results of ANOSIM tests for differences among the habitats for Species assemblage, species presence/absence, and Juveniles (Ju) abundance. R – statistic and the significance level, n.s =not significant

Parameter Comparison	R-statistic	P-value
Species assemblage	0.203	<0.05
Species presence/absence	0.193	n.s
Ju abundance	0.097	n.s

A. presbyter species had the highest abundance among all species in the winter and spring with 104 fish in the winter and 174 in the spring. The summer sampling was dominated mostly from fish from the *Diplodus vulgaris* species, with a total amount of 297 fish (Annex 1). ANOSIM test for species assemblages between seasons found relatively high similarity in Sea and CP habitats with R – statistics of 0.167 and 0 respectively. In the Sa habitat the similarity was relatively low R – statistic = 0.833; the results however were not statistically significant (Table 3.3).

From the 8 most abundant species mentioned above (Table 3.4), only *Diplodus sargus* was absent from the spring sampling, others were present all year round. One species, *Hippocampus guttulatus*, was present only in the spring, and 3 species were present only in the summer: *Anguilla anguilla*, *Parablennius pilicornis*, and *Spondyliosoma cantharus*. There were no species that were present only in the winter sampling (Annex 1). Presence/absence of species between seasons was most different in the Sa habitat, R-statistic = 0.722, and quite similar in the CP and Sea habitats, R-statistics values of -0.028 and -0.167 respectively; results however were not statistically significant (Table 3.3).

Table 3.3. R – statistic results of ANOSIM tests for differences between the seasons in each habitat type *Caulerpa prolifera* (CP), Seagrass (Sea), and Sand (Sa); for Species assemblage, species presence/absence, and Species Juveniles (Ju) abundance. All the results were not significant.

Habitat Comparison	CP	Sea	Sa
Species assemblage	0	0.167	0.833
Species presence/absence	-0.028	-0.167	0.722
Species Ju abundance	0	0.056	0.778

In the summer sampling there was an increase in the abundance of marine juvenile guild in all the habitat types; ratio between marine juveniles and residence species in that season was relatively even, 51:48 in CP and 52:48 in Sea. In the Sa habitat however, the summer abundance of marine juvenile species was 88% as opposed to only 12% from the resident species. In the other 2 seasons there were mostly resident species present in the Sa habitat, 99% and 98% in winter and spring respectively. CP and Sea habitats had as well mostly resident species in the winter and spring, and similar ratios between their resident and marine juveniles in those 2 seasons; 76:24 in winter sampling of CP and 68:32 in the Sea; the ratio between resident and marine juveniles in spring sampling was 75:25 and 79:21 in CP and Sea respectively. Diadromous species had the lowest abundance, 0.24% of the total species caught, and were present only in the summer sampling in CP and Sea habitats (Figure 3.4).

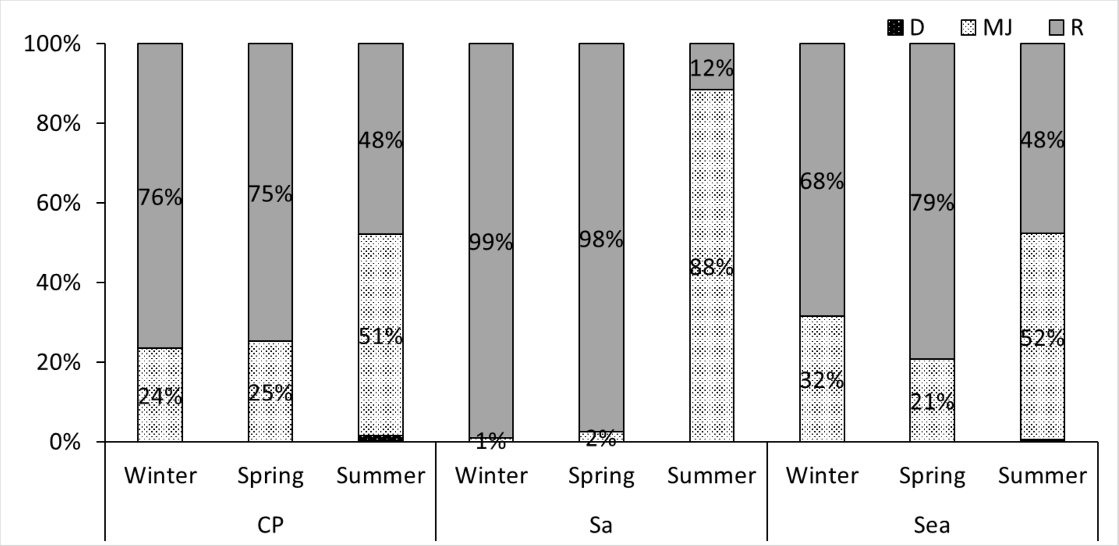

Figure 3.4. Percentage of fish number in each ecological guild, over the 3 seasons (winter, spring, summer) according to the 3 habitat types: *Caulerpa prolifera* (CP), Sand (Sa), and Seagrass (Sea); Diadromous species (D), marine juvenile (MJ), and resident species (R).

3.3 Nursery value

Ju abundance was highest in Sea habitat with an average of 95.0 fish per sample, followed by 79.3 and 41.7 in Sa and CP respectively. However, 2-way PERMANOVA test for Ju abundance found no significant difference between the 3 habitats nor between season-habitat interaction. ANOSIM test for Ju assemblage found high similarity between the 3 habitat types, R-statistic = 0.097; however, the result was not statistically significant (Table 3.2).

Differences in the Ju abundance between the seasons were found to be similar in the CP and Sea habitats with R-statistic values of 0 and 0.056 respectively; and relatively different in Sa habitat R – statistics = 0.778. However, these results were not statistically significant (Table 3.3). A significant difference ($p<0.01$) however was found between the 3 seasons in the Ju abundance (Table 3.1). Pair-wise test results revealed significant differences between summer and spring ($p<0.01$), and between winter and summer samplings ($p<0.05$) (Figure 3.5).

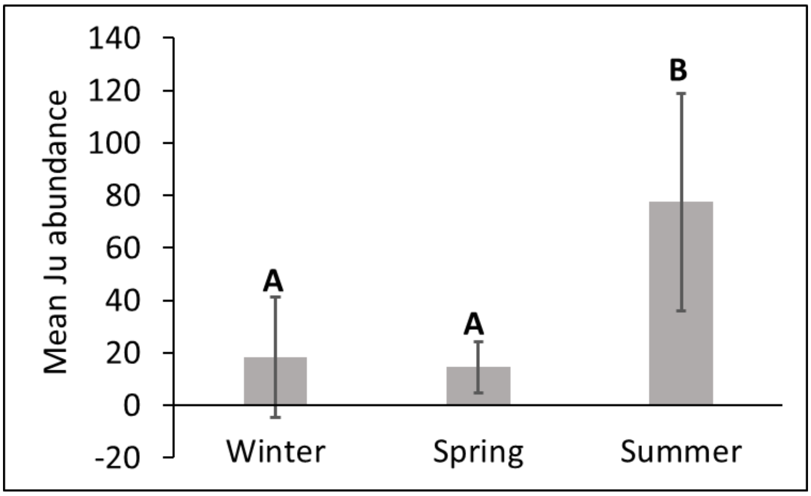

Figure 3.5. Average abundance of juveniles (Ju) caught in each of the 3 seasons (winter, spring, and summer). Error bars represents +1 SD and unlike letters signify statistical differences in seasons within each habitat in pair-wise PERMANOVA test.

3.4 Habitat characteristics and variations in fish habitat choice

Both vegetated habitats were structured with sporadic patches of vegetation which leads to high value of SD in the average estimated density, especially in the CP habitat with 147.49±117.44g/m^2 compared with a mean estimated density of 40.427±24.82g/m^2 in the Sea habitat. CP habitat was composed mainly of *Caulerpa prolifera* species, 176.12±145.66g/m^2, some seagrass species – *Z. marina*, 74.59± 68.1g/m^2, *C. nodosa*, 12.97±4.36g/m^2, and *Z. noltii*, 3.74±3.67g/m^2; and as well a small portion of other macroalgae species (Annex 2). Sea habitat was composed mainly of *Z. noltii*, with 38.61± 22.59g/m^2 mean estimated density, and some *C. nodosa*, 3.64± 1.83g/m^2 (Figure 3.6).

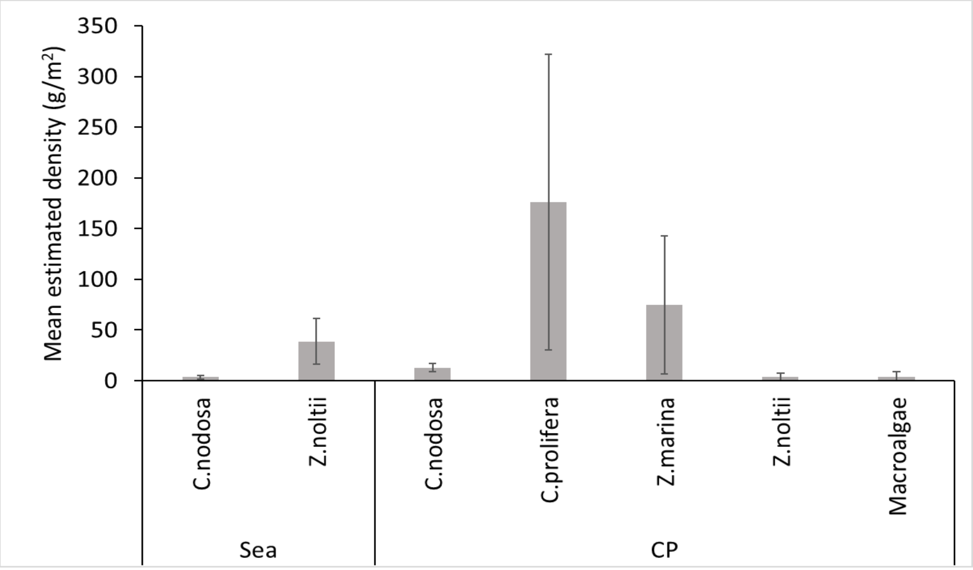

Figure 3.6. Mean estimated density of plants species (gDW m^{-2}) collected from the 2 vegetated habitats: *Caulerpa prolifera* (CP), and Seagrass (Sea) in the spring sampling (April 2019); error bars represent +1 SD

ANOSIM test for assemblages of NJ and Ju abundance for the 8 most abundant species in the samplings showed high similarity in most of the species. The R-statistic ranged from 0.24 for *Diplodus vulgaris* to -0.08 for *Diplodus sargus*. Statistical significance ($p<0.05$) however, was found only in *Diplodus vulgaris* (Table 3.4). Only Ju fish were caught from the *Diplodus vulgaris* species; according to pairwise results a significant difference ($p<0.01$) was found between Sa and CP habitats, by a mid-range R-statistic value of 0.5, and no significant difference ($p<0.05$) between Sa and Sea with R-statistic = 0.22.

A. presbyter had highest abundance of NJ fish compared with Ju in all the 3 habitat types, most of them were found in the Sea habitat, 162 NJ fish compared with 67 and 28 in Sa and CP respectively. This species however was found more abundant in the Sea habitat with 25 NJ fish compared with 5 and 4 in the Sa and CP respectively. Only Ju were collected from *Diplodus sargus* with highest abundance of 63 fish in the Sa followed by 55 in Sea and only 8 in CP habitat. Only Ju fish were collected as well from *Symphodus bailloni* and *Diplodus annularis* species; both with highest abundance in the Sea habitat. *Symphodus bailloni* had 51 Ju in the Sea while only 5 and 8 in Sa and CP respectively. *Diplodus annularis* had 25 Ju in the Sea, 2 in CP and no fish were collected from Sa habitat (Table 3.4).

Table 3.4. List of the 8 most abundant species caught along the 3 habitats and their nursery stage juveniles (Ju) or Not Juveniles (NJ) with the similarity results and significances level according to ANOSIM test; n.s = not significant.

Species	***Caulerpa prolifera***		**Sand**		**Seagrass**		**R - statistic**	**P - value**
	Ju	**NJ**	**Ju**	**NJ**	**Ju**	**NJ**		
Atherina presbyter	4	28	5	67	25	162	0.06	n.s
Diplodus annularis	2	0	0	0	25	0	0.03	n.s
Diplodus sargus	8	0	63	0	55	0	-0.08	n.s
Diplodus vulgaris	96	0	147	0	110	0	0.24	<0.05
Gobius niger	5	36	4	2	6	19	0.21	n.s
Pomatoschistus spp.	0	8	7	98	1	14	-0.02	n.s
Symphodus bailloni	8	0	5	0	51	0	0.00	n.s
Symphodus cinereus	0	76	0	11	2	34	0.01	n.s

4 Discussion

4.1 Differences in biodiversity and abundance

Our results found significantly lower biodiversity (H) in Sa habitat compared with the other 2 habitat types. Similarly, Ribeiro et al. (2012) as well got higher biodiversity value in the *C. nodosa* compared with the unvegetated habitat, in their study in the Ria Formosa. Sea habitat had the highest abundances and was the richest (S) habitat compared with CP and Sa habitats, although differences were not statistically significant. However, that is probably due to high variability between each sampling campaign (season) and even between the 2 replicates within season, especially in the Sa habitat since many studies did find significantly higher values in the seagrass meadows compared with unvegetated areas (Table 4.1).

For example, the comparison of Connolly (1994), between unvegetated and seagrass habitats, mainly of *Z. mulleri*, took place in Southern Australian Estuary, showed more fish in seagrass then the unvegetated area all through their sampling campaigns. Bertelli and Unsworth (2014) study from North Wales, found mean abundance and S values significantly higher in seagrass beds compared with sand, and both Jenkins et al. (1997) study from Victoria Australia, and Kalogirou et al. (2012) study from Rhodes Island Greece, reported significantly higher S in the seagrass habitats compared with unvegetated habitats. Travers and Potter (2002) study that took place in Western Australia found mean S values seven times higher in the seagrass habitats than in the bare sand.

Additionally, mean S in *Z. marina* beds was significantly higher than in the sandy habitats in the study took place by Horinouchi (2005) in central Japan. Similarly, *P. oceanica* seagrass had higher abundance in Guidetti (2000) study from the Adriatic Sea Italy. Higher S and fish abundance were found as well in the *Z. capricorni* bed compared to sand in the comparison of Gray et al. (1996) in several estuaries in Australia; and both abundance and S values were significantly higher in *Heterozostera tasmanica* compared to sand in the Jenkins and Wheatley (1998) study from Southern Australia. Moreover, seagrass beds of monospecific *Posidonia australis* or *Amphibolis antartica* species were compared to bare sand in Travers and Potter (2002) study; their results show that habitat mean fish density was 20 times higher in the seagrass habitats compared with bare sand.

Heck et al. (1989) study that performed in Cape Cod found nearly eight times higher fish abundance in the *Z. marina* habitats than the sandy unvegetated habitats. And fish abundance was 10 times higher in *C. nodosa* habitats in Ria Formosa than in sand in the study of Ribeiro et al. (2006). However, neither mean fish abundance nor biodiversity differed significantly between seagrass and sand habitats (Horinouchi, 2005). Additionally da Silva et al. (2018) comparison applied in Northern Brazil, found higher values in the unvegetated areas than the vegetated ones, and higher fish abundance were found in the unvegetated habitat in 2 of the 3 sites sampled by Ribeiro et al. (2012) in the Ria Formosa.

The J value of Sa habitat was significantly lower than CP habitat only, and not significantly different from Sea habitat in our results. Moreover, CP and Sea habitats in our study were significantly more diverse then Sa habitat and with no significant differences in H index between them. Correspondingly, Koulouri et al. (2016) authors reported that comparisons between seagrass meadows and macroalgae beds showed very few differences in abundance, growth and survival of juveniles in their comparison from Crete Island Greece. For example, their study of fish assemblages in *C. prolifera* meadow found a diverse fish community with a relatively high number of families compared with other studies that took place in different seagrass meadows (Koulouri et al., 2016). Moreover, Verdiell-Cubedo et al. (2007b) comparison in Mar Menor Spain, found no significant differences in H and J indexes between seagrass and *C. prolifera* meadows.

Not many comparisons were performed between algae and seagrass meadows in relative to comparisons of seagrass vs. sand (Table 4.1). CP meadow had the lowest fish abundance, however differences were not significant. Guidetti (2000) also showed no significant differences in the abundances of fishes between algae and seagrass meadows. York et al. (2006) also reported similar abundance between seagrasses and *C. taxifolia* habitats in 2 of their sampling sites in Southern Eastern Australia; the other site displayed more fish in the *Z. capricorni* seagrass meadows. Several studies however, did find seagrass beds more diverse habitat compared with algae habitats. For instance, the mean S was higher in seagrass meadows compared with the algae reef in the Guidetti (2000) study.

Similarly, *C. taxifolia* had significantly lower S in 2 out of the 3 sites in York et al. (2006) study. In their other sampling site, *P. australis* seagrass had more species than both seaweed and the other seagrass species, implying that differences can occur between different seagrass as well (York et al., 2006). S value was significantly higher in seagrass meadows compared with algae in the work of Jenkins and Wheatley (1998). Moreover, Tuya et al. (2014b) study from Gran Canaria Island, found total fish abundance and species density larger in *C. nodosa* than in *C. prolifera* consistently through time for the adult fish. However, for the juveniles significant results were detected only for higher values of fish abundance in *C. nodosa* compared to *C. prolifera* (Tuya et al., 2014b). Thus, some variations were found between diversity and abundances outcomes of the different studies due to differences in sampling locations (York et al., 2006; Ribeiro et al., 2012), different seagrass species (Travers and Potter, 2002; York et al., 2006) and meadow density (da Silva et al., 2018) (Table 4.1). Additionally, diversity and abundance values often varied along the yearly cycle.

Table 4.1. Significant differences in Biodiversity (H), species richness (S), species evenness (J), and fish abundance compared between habitats types, seagrass and sand or seagrass and seaweed meadows. in different studies from different geographic locations.

Reference	Habitats compared	Geographic location	Significant Outcomes
(Koulouri et al., 2016)	seagrass and algae	Crete Greece	few differences in abundance and S
(Verdiell-Cubedo et al., 2007b)	seagrass and algae	Mar Menor Spain	H and J not significantly different
(Tuya et al., 2014b)	seagrass and algae	Gran Canaria Spain	higher abundance in seagrass
(Jenkins and Wheatley, 1998)	seagrass and algae	Southern Australia	higher S in seagrass
(York et al., 2006)	seagrass and algae	Southern East Australia	higher S in seagrass in 2 out of 3 locations and abundance not significantly different in 2 out of 3 locations
(Guidetti, 2000)	seagrass and algae	Adriatic Sea Italy	mean S higher in seagrass and abundance not significantly different
(Jenkins and Wheatley, 1998)	seagrass and sand	Southern Australia	higher abundance and S in seagrass
(Connolly, 1994)	seagrass and sand	Southern Australia	higher abundance in seagrass

Reference	Habitats compared	Geographic location	Significant Outcomes
(Guidetti, 2000)	seagrass and sand	Adriatic Sea Italy	higher abundance in seagrass
(Heck et al., 1989)	seagrass and sand	Cape Cod	higher abundance in seagrass
(Ribeiro et al., 2006)	seagrass and sand	Ria Formosa Portugal	higher abundance in seagrass
(da Silva et al., 2018)	seagrass and sand	Northern Brazil	higher diversity and abundance in sand
(Ribeiro et al., 2012)	seagrass and sand	Ria Formosa Portugal	higher H in Seagrass and higher abundance in sand at 2 out of 3 locations
(Bertelli and Unsworth, 2014)	seagrass and sand	north Wales	higher mean abundance and S in seagrass
(Gray et al., 1996)	seagrass and sand	Australia	higher S and abundance in seagrass
(Kalogirou et al., 2012)	seagrass and sand	Rhodes Greece	higher S in seagrass
(Jenkins et al., 1997)	seagrass and sand	Victoria Australia	higher S in seagrass
(Travers and Potter, 2002)	seagrass and sand	Western Australia	mean S and abundance higher in seagrass. Different S value between the 2 seagrass species
(Horinouchi, 2005)	seagrass and sand	Central Japan	mean S higher in seagrass and mean abundance and H not significantly different

In warm seasons there is recruitment of juveniles of marine species to the Ria Formosa (Ribeiro et al., 2012). Suitably, our results show that summer was the richest, with highest abundances, and there were no species that were present only in the winter samplings in any of the habitat types. Suggesting that summer is the most important season for preserving fish populations in those habitat types in Ria Formosa. This matches findings from other studies such as Santos and Nash (1995) work from the Azores that reported the highest fish density and biomass in the summer, in general the majority of the fish they caught were juveniles. Jenkins et al. (1997) also found significantly higher S values in the warmer months of the year in the seagrass habitats. Moreover, Ribeiro et al. (2006) showed that mean fish abundance in seagrass was significantly higher in the spring and summer compared to autumn, and the autumn abundance value was significantly higher than in the winter. In their unvegetated habitat, significant differences were found only in the spring with higher mean abundance compared with the other seasons. Their mean S as well was significantly higher in spring and summer in both seagrass and unvegetated sand (Ribeiro et al., 2006).

In our study however, differences were not statistically significant, that is probably due to relatively high deviation between the 2 replicates, especially in the spring sampling in the Sa habitat and CP habitat in the winter sampling. Similarly, the Santos and Nash (1995) seasonal comparison, that took place in unvegetated bottoms indicated no clear patterns in S value. And their H values showed no trend and seemed to be stable among the seasons. Thus, no significant correlation was detected between water temperature and abundance or diversity indices (Santos and Nash, 1995). Additionally, in our study no significance difference was found in habitat-season interaction, thus seasonality changes in the fish abundance and diversity are not significantly affected by the habitat type. Similarly, no significance was found in the habitat-season interaction in the York et al. (2006) study.

However, seasonal variations did appear in the results of several studies, for instance Ribeiro et al. (2012) showed no significant differences in seasonality in the seagrass meadows but significantly higher values were found in the autumn and winter compared with the summer in the unvegetated habitats (Ribeiro et al., 2012). Verdiell-Cubedo et al. (2007b) found significantly higher S values in the spring at *C. nodosa* meadows, and as for the *C. prolifera* meadows, the highest S value was in the autumn. They found no seasonal difference in the H and J indices within both habitats though. A significantly lower abundance was found in the winter at the algae habitats compared with the other seasons by Guidetti (2000), and no significant differences in fish abundance over *P. oceanica* meadow among seasons, with their unvegetated habitat having significantly higher abundance only in the autumn compared with the other seasons (Guidetti, 2000). Additionally, Tuya et al. (2014b) found that species density and total biomass differences varied through time. Thus, the authors are stating that their results highlighted the fact that the relative importance of seagrass beds compared to other habitats such as *C. prolifera* beds may change seasonally (Tuya et al., 2014b).

4.2 Species composition differences

Our results found no significance differences between habitats in presence/absence test, suggesting most species can utilize all 3 habitat types. This is unlike Ribeiro et al. (2006) where significant separation was found mainly in their presence/absence comparisons between sand and seagrass habitats. Species assemblages were found similar between the 3 habitats; a significant result was found only between Sa and CP habitats. In contrast, Gray et al. (1996) found consistently great differences in fish assemblages between sand and seagrass habitats in all of their sampling locations and sampling times. Kalogirou et al. (2012) results also showed significant separation between seagrass and sandy habitats. Connolly (1994) found significant separation between unvegetated and seagrass habitats at all sampling periods; assemblage was most similar in October. Bertelli and Unsworth (2014) however, found significant similarity between seagrass and sand assemblages.

Jenkins and Wheatley (1998) found significant differences between the unvegetated and their 2 vegetated habitat types. Differences however varied with time, with significant differences detected between unvegetated and seagrass habitat all 6 sampling months and between the unvegetated and both vegetated habitats in 3 of the sampling months (Jenkins and Wheatley, 1998). This implies that differences between seagrass and unvegetated habitat are more consistent then between unvegetated and algae bed. Comparisons between vegetated habitats produced several different outcomes. For example, Comparison between *C. taxifolia* habitat and 2 seagrass meadows, *P. australis* and *Z. capricorni* showed significantly high separation between all habitat types at all sampling times (York et al., 2006). implying different seagrass species can influence fish assemblage as well. Similarly, Tuya et al. (2014b) had significant difference between assemblages both in juvenile and adult fish species between *C. prolifera* and seagrass meadows, according to Canonical Analysis of Principal coordinates (CAP) ordinations. In contrast, Jenkins and Wheatley (1998) results showed that differences between algae and seagrass habitat were not significant consistently through time.

The most abundant species collected are relatively similar to the ones collected in Ria Formosa by Ribeiro et al. (2012) and (Ribeiro et al., 2006). Out of the eight most abundant species that were caught no species were present only in the Sea, which imply that those species can exploit other habitat types as well. As reported as well by Bertelli and Unsworth (2014), the fish assemblage found in seagrass samplings are not obligate seagrass users and can probably utilize alternative nursery habitats (Bertelli and Unsworth, 2014). Most of the fish caught in all the habitat types were resident species. This is in agreement with the results of Ribeiro et al. (2006) who found that both seagrass and sandy habitat types were dominated by resident species of Ria Formosa. We found *A. presbyter* had highest abundance in Sea habitat and lowest in CP habitat, in contrast to Ribeiro et al. (2012) who found it more abundant in the sand in most of their samplings. This species had the highest abundance among all other species in the winter and spring in our study, in contrast to Ribeiro et al. (2012) who found the lowest abundance of that species the winter.

Our results found high similarity between season in CP and Sea habitat, and high separation between seasons in the Sa habitats, although results were not statistically significant. Ribeiro et al. (2006) however, found significant difference among most of the seasons apart from between autumn and winter in the sand, while Koulouri et al. (2016) found that fish assemblages were significantly different between the seasons in *C. prolifera* meadows. Similarly Kalogirou et al. (2012) found separation among seasons, mainly in summer sampling which was clearly separated from other seasons in both seagrass and sand habitats. Summer samples (January) was the most different between the 3 habitat types in York et al. (2006) work as well. With some variations over time with highest separation in October between the 2 seagrass species, and in January between *P. australis* and *C. taxifolia*, while those 2 latter species showed the lowest separation from each other in October.

4.3 Nursery function

Ju abundance values did not significantly differ between the 3 habitats, and Ju assemblage test found high similarity between the 3 habitat types, (R-statistic = 0.097), suggesting that the 3 habitat types could function as a nursery ground for most fish species. However, this ANOSIM result was not statistically significant, making judgment regarding relative importance of each habitat as a nursery function quite limited. Moreover, our results suggest some variations between seasons.

In the summer sampling there was an increase in the abundance of marine juvenile guild in all the habitat types. This implies that this season is the most important season for the Ria Formosa as a nursery ground in all the habitat types. Ribeiro et al. (2012) also found the highest abundance of MJ in the summer. However their spring sampling showed the greatest abundance of MJ species relative to R species. Moreover, they found higher MJ species abundance in the seagrass habitat than in unvegetated habitat suggesting that this habitat is more important as a nursery ground, especially in the warm seasons (Ribeiro et al., 2012). Moreover, Guidetti (2000) found no juveniles of any species over the sand habitat.

That is in contrast to our results that showed increases in MJ species in the summer season, 88% as opposed to only 12% from the R species, while in the other 2 seasons there were mostly resident species present in the Sa habitat, 99% and 98% in winter and spring respectively. This suggests that the relative important of nursery ground for Sa habitat varies through the seasons. That as well corresponds to the high differences in the Ju abundance between the seasons in that habitat, (R – statistics = 0.778), while the other 2 habitats showed high similarity between seasons. Moreover, Guidetti (2000) reported that the lack of juveniles in their work was partly due to sampling depth, as some species use shallow unvegetated habitats for settlement (Guidetti, 2000).

Our comparisons between Ju and NJ abundance of the 8 most abundant species suggesting no significant ontogenetic shifts between habitat types along the life cycle of those particular species. The results however, was statistically significant only for *D. vulgaris*, that is probably due to a small sample sizes of the other 7 species compared with *D. vulgaris*. Only Ju fish were caught from *D. vulgaris*, *D.*

annularis, and *D. sargus* species, with the statistically significant mid-range similarity in Ju and NJ abundance between Sa and CP habitats for *D. vulgaris* and the non-significant separation between Sa and Sea implying that for *D. vulgaris* Sa and Sea have the same nursery value and CP is less significant as a nursery ground for this species. Nursery function for fish from the Sparidae family showed different outcomes in several studies; for example Espino et al. (2011) comparison from Eastern Canary Islands, found that fish from this family were highly abundant in *C. nodosa* meadows and in mixed meadows of *C. nodosa* and *Z. noltii*. However, Verdiell-Cubedo et al. (2007b) found higher abundance and biomass of *Sparus aurata* species in the *C. prolifera* meadows compared with *C. nodosa*. Moreover, Verdiell-Cubedo et al. (2007a) study that performed in Mar Menor Spain, found high abundance of juveniles from that family in the mixed meadows of *C. nodosa* and *C. prolifera*, especially in areas with well-developed meadows of submerged vegetation regardless of the specific plant species.

4.4 Habitat choice

Meadows density was found to affect habitat choice in several studies. For example (Tuya et al., 2014b) found greater species density and biomass of juvenile fish in the seagrass meadows, particularly at periods with maximum seagrass biomass. However, their results found maximum seagrass biomass in July 2012 and May 2013, thus warm temperature might have also effected the juvenile abundance, as indicated from our results and some of the others that found greater fish abundance (Ribeiro et al., 2006), fish densities (Santos and Nash, 1995), or species richness (Jenkins et al., 1997; Verdiell-Cubedo et al., 2007b) in seagrass beds in the warm seasons.

CP habitat was the densest meadow and had significantly higher TL compared with the other 2 habitats. However, density results were highly variable between replicates, probably due to great patchiness that characterized this habitat type. Significant differences in fish TL between seasons were detected only in Sa habitat with highest fish TL in the spring sampling and lowest in the winter. This is probably related to certain species preferences and not to nursery function since juvenile abundance in this habitat was greatest in the summer. Thus, it is not possible to evaluate the relationship between habitat choice and habitat density based on our results as density was estimated only for spring samples.

5 Conclusions

Our results showed high variability between replicates which probably affected the significance of the results. Thus, although we found more species and higher fish abundance in the Sea habitat compared with the other two habitats, these results were not significant. Additionally, neither richness and abundance values nor differences between the 3 assemblages were statistically significant. Therefore, it is difficult to draw conclusions regarding relative importance of each habitat type and further comparison must be carried out for that. Moreover, although many comparisons found higher values in the seagrass habitats compared with unvegetated ones, not many comparisons have been made between seagrass and *Caulerpa* meadows.

Our results indicated that summer season is significantly more important for nursery and recruitment in all habitats and relative important of nursery varies along the year, specially within Sa habitat. Invasion of *C. prolifera* into unvegetated areas in the Ria might affect the recruitment for the next generations for some of the species, since there was a great increase in MJ in that habitat in the summer, but since Ju abundance was not significant different between habitats further investigation must be done in order to obtain accurate results that will assist management and conservation decisions.

Fish TL was significantly different between seasons and habitat. However, seems that it was related to different preferences of different species and not to interspecies ontogenetic changes. Thus, meadows density might affect habitat utilization for some species, as stated from several studies, and further research need to be obtained in order to learn about the correlation between habitat structure and fish habitat choice. Overall, as mentioned above many factors can affect the fish habitat choice such as meadows structure and species, seasonal change, human impact and specific adaptation for each species, and while biodiversity is highly important to maintain local fish community it is extremely important to carry out more research in order to obtain more accurate results regarding habitat choice and habitat relative importance.

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7 Annexes

Annex 1. List of fish families and species caught over seagrass, sand, and *Caulerpa prolifera* in the 3 sampling events (winter, spring, summer) total abundance, ecological guild (R, MJ, D), size and size at maturation (cm), and nursery (Ju, NJ)

Family	**Species**	***Caulerpa Prolifera***			**Sand**			**Seagrass**			**Ecological guild**	**Nursery**	**size (cm)**	**Maturation (cm)**	**Total no. of individuals**	**Maturation Reference**
		Winter	**Spring**	**Summer**	**Winter**	**Spring**	**Summer**	**Winter**	**Spring**	**Summer**						
Anguillidae	***Anguilla anguilla***	0	0	2	0	0	0	0	0	1	D	Ju	<40	40-62	**3**	(Vøllestad, 1992)
Atherinidae	***Atherina Presbyter***	0	4	0	1	4	0	17	0	8	R	Ju	<6.5	6.5-7.3	**34**	(Moreno et al., 2005)
	Atherina Presbyter	4	24	0	1	66	0	81	76	5	R	NJ	>6.5	6.5-7.3	**257**	(Moreno et al., 2005)
Blenniidae	***Parablennius pilicornis***	0	0	0	0	0	1	0	0	0	R	Ju	<8.6	8.6	**1**	(Henriques et al., 2013)
	Salaria pavo	0	0	0	0	0	1	0	3	7	R	NJ	>6	6-10	**11**	(Oliveira et al., 2001)
gobiidae	***Gobius niger***	2	1	2	4	0	0	0	0	6	R	Ju	<6	6	**15**	(Froese and Pauly, 2019)
	Gobius niger	2	19	15	0	0	2	0	6	13	R	NJ	>6	6	**57**	(Froese and Pauly, 2019)
	Gobius paganellus	0	1	2	0	0	0	2	0	0	R	NJ	>6	6-7	**5**	(Froese and Pauly, 2019)
	Pomatoschistus spp.	0	0	0	7	0	0	1	0	0	R	Ju	<3	3	**8**	(Froese and Pauly, 2019)
	Pomatoschistus spp.	3	4	1	84	11	3	4	9	1	R	NJ	>3	3	**120**	(Froese and Pauly, 2019)
Labridae	***Symphodus bailloni***	2	4	2	0	0	5	6	3	42	R	Ju	<12.9	12.9	**64**	(Henriques et al., 2013)
	Symphodus cinereus	0	0	0	0	0	0	0	0	2	R	Ju	<4	4	**2**	(Froese and Pauly, 2019)
	Symphodus cinereus	13	20	43	2	0	9	15	2	17	R	NJ	>4	4	**121**	(Froese and Pauly, 2019)
	Symphodus roissali	0	2	0	0	0	0	0	0	0	R	NJ	>5	5-7	**2**	(Froese and Pauly, 2019)
Sparidae	***Diplodus annularis***	0	1	1	0	0	0	0	9	16	MJ	Ju	<8	8 - 19.6	**27**	(Froese and Pauly, 2019)
	Diplodus bellottii	0	0	0	1	2	0	0	0	0	MJ	Ju	<11	11	**3**	(Santos et al., 1998)
	Diplodus puntazzo	0	0	0	0	0	2	0	1	0	MJ	Ju	<34.1	34.1	**3**	(Henriques et al., 2013)
	Diplodus sargus	5	0	3	0	0	63	52	0	3	MJ	Ju	<25	25	**126**	(Froese and Pauly, 2019)
	Diplodus vulgaris	3	28	65	0	0	147	9	16	85	MJ	Ju	<17	17	**353**	(Froese and Pauly, 2019)
	Spondyliosoma cantharus	0	0	0	0	0	1	0	0	8	MJ	Ju	<19.7	19.7	**9**	(Froese and Pauly, 2019)
Syngnathidae	***Hippocampus guttulatus***	0	1	0	0	0	0	0	0	0	R	NJ	>12.5	12.5	**1**	(Froese and Pauly, 2019)
	Syngnathus abaster	0	1	0	0	0	0	1	0	0	R	NJ	>6	6	**2**	(Malavasi et al., 2007)
	Syngnathus acus	0	5	0	0	0	6	1	0	1	R	NJ	>7	7- 25.6	**13**	(Froese and Pauly, 2019)

Family	Species	*Caulerpa Prolifera*			Sand			Seagrass			Ecological guild	Nursery	size (cm)	Maturation (cm)	Total no. of individuals	Maturation Reference
		Winter	Spring	Summer	Winter	Spring	Summer	Winter	Spring	Summer						
	Syngnathus typhle	0	0	0	0	0	1	4	0	1	R	NJ	>7	7-14.8	**6**	(Gurkan et al., 2015)
Total no. of individuals		**34**	**115**	**136**	**100**	**83**	**241**	**193**	**125**	**216**					**1243**	
Total no. of individuals per habitat		**285**			**424**			**534**								
Total no. of species per season		**7**	**12**	**9**	**5**	**3**	**12**	**10**	**9**	**13**						
Total no. of Residents individuals per season		**26**	**86**	**65**	**99**	**81**	**28**	**132**	**99**	**103**						
Total no. of Marine juvenile migrants individuals per season		**8**	**29**	**69**	**1**	**2**	**213**	**61**	**26**	**112**						
Total no. of diadromus migrant species individuals per season		**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**1**						

Annex 2. List of the plants taxa and their relative density collected from the 2 vegetated habitat types: *Caulerpa prolifera* (CP), and Seagrass (Sea), in Spring sampling (April 2019); according to habitat replicates and the plots replicates. NI = not identified taxa.

habitat	habitat replicate	replicate number of plot	species	density (g/m^2)	Density per replicate and sub replicate
Sea	Sea1	1	*Z. noltii*	24.247	24.247
Sea	Sea1	2	*Z. noltii*	25.812	25.812
Sea	Sea2	1	*Z. noltii*	72.09	77.025
Sea	Sea2	1	*C. nodosa*	4.935	
Sea	Sea2	2	*Z. noltii*	32.273	34.623
Sea	Sea2	2	*C. nodosa*	2.35	
CP	CP1	1	*Z. noltii*	9.092	308.14
CP	CP1	1	*C. prolifera*	279.117	
CP	CP1	1	*Dictyopteris polypodioides*	0.197	
CP	CP1	1	*Dictyota* sp	0.059	
CP	CP1	1	*Gelidium* sp	15.4	
CP	CP1	1	NI	4.277	
CP	CP1	2	*Dictyopteris polypodioides*	0.1	84.898
CP	CP1	2	*Z. noltii*	3.182	
CP	CP1	2	*Gelidium* sp	8.437	
CP	CP1	2	*C. prolifera*	73.125	
CP	CP1	2	*Dictyota* sp	0.055	
CP	CP2	1	*Dictyopteris polypodioides*	2.447	39.965
CP	CP2	1	*Z. marina*	26.433	
CP	CP2	1	*C. nodosa*	9.887	
CP	CP2	1	*Z. noltii*	1.198	
CP	CP2	2	*Gelidium* sp	5.035	156.943
CP	CP2	2	*Z. noltii*	1.483	
CP	CP2	2	*C. nodosa*	16.058	
CP	CP2	2	*Z. marina*	122.745	
CP	CP2	2	*Dictyopteris polypodioides*	0.223	
CP	CP2	2	NI2	0.188	
CP	CP2	2	NI	11.21	