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**An ecological assessment of fish recruitment in
Lagos through the monitoring of Biohuts**



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An ecological assessment of fish recruitment in Lagos through the monitoring of Biohuts

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Abstract

Marine ecosystems are crucial hubs of biodiversity and face increasing threats from human activities, particularly in coastal areas serving as nurseries for fish recruitment, a pivotal factor influencing the abundance and diversity of fish populations. This study focuses on evaluating the abundance, biodiversity and recruitment dynamics through an underwater visual census, with a specific focus on the innovative Biohut structures installed in Marina de Lagos and their potential contribution to marine biodiversity conservation. This study focused on ecological processes such as habitat association and the impact of environmental and biological factors, and assessed the Biohuts potential to enhance abundance, biodiversity and recruitment. The methodology employed underwater visual censuses and environmental data collection for a comprehensive assessment from September 2023 to June 2024. The period between spring and early summer had the highest values in terms of abundance of recruits. The results indicate that the Biohuts significantly increased fish abundance, biodiversity and recruitment when compared with the control areas inside and outside the marina. In conclusion, Biohuts are effective tools for enhancing fish abundance, biodiversity, and recruitment in urbanized coastal areas. They provide a promising solution for habitat restoration, marine biodiversity conservation and can also serve as platforms to study key processes for sustainable management of fisheries as it is the case of fish recruitment.

Keywords: Biohuts, Recruitment, Ecology, Biodiversity, Fisheries.

Resumo

O aumento da urbanização costeira e a consequente degradação dos habitats marinhos têm sido fatores significativos na perda de biodiversidade e na diminuição do recrutamento de peixes. Para mitigar esses efeitos, soluções inovadoras, como as Biohuts (berçários artificiais), têm vindo a ser implementadas com o objetivo de restaurar e preservar principalmente zonas que tenham sido modificações por ações antropogénicas. Este estudo foi conduzido na Marina de Lagos, Algarve, e teve como principais objetivos a avaliação da influência das Biohuts na abundância de peixes, bem como a nível da biodiversidade e do recrutamento, avaliando o mesmo tendo em conta a presença de diferentes espécies e a relação com as restantes variáveis.

As Biohuts são estruturas artificiais desenvolvidas pela empresa francesa Ecocean, projetadas para agir como viveiros artificiais, fornecendo abrigo e proteção para peixes juvenis, promovendo, dessa forma, um ambiente adequado para o recrutamento. Estas estruturas foram instaladas em diversas zonas da Marina de Lagos, abrangendo diferentes níveis de exposição a fatores ambientais e antropogénicos. Os dados foram recolhidos através de censos visuais subaquáticos realizados ao longo de dez meses, de setembro de 2023 a junho de 2024. De modo a perceber de que maneira as Biohuts influenciavam a abundância, a biodiversidade e o recrutamento, os censos foram realizados em três áreas distintas com Biohuts, mais especificamente, uma área de controlo dentro da marina, duas áreas de controlo fora da marina e uma área natural. A nível da recolha dos dados, foram realizados em todas as zonas vários transectos de 10 metros cada.

Os resultados indicaram que a instalação das Biohuts teve um impacto positivo na abundância de peixes, quando comparada às zonas de controlo. Em particular, nas áreas com Biohuts, foi observada uma densidade significativamente maior de indivíduos, especialmente em peixes com algum interesse comercial como Mugilidae e *Diplodus sargus*. Essa maior abundância pode ser atribuída ao facto das Biohuts proporcionarem um habitat protegido que favorece a sobrevivência de juvenis durante as fases críticas do recrutamento.

A análise estatística revelou diferenças significativas na abundância de peixes entre as áreas com Biohuts e as áreas de controlo, tanto dentro quanto fora da marina. A área natural obteve o maior valor em termos de abundância, no entanto as áreas com Biohuts dentro da marina superaram as áreas de controlo dentro e fora da marina em densidade de peixes por metro cúbico, demonstrando a eficácia das Biohuts em restaurar ambientes urbanos costeiros.

No que diz respeito à biodiversidade, as Biohuts também desempenharam um papel crucial no aumento e preservação de uma comunidade diversificada de peixes. De forma a analisar a biodiversidade, recorreu-se aos índices de biodiversidade de Shannon e Simpson. Os resultados permitiram verificar que as áreas com Biohuts apresentaram uma diversidade de espécies maior e uma dominância menor quando comparadas com as zonas de controlo dentro da marina. No entanto, quando comparadas com áreas naturais fora da marina, o valor é menor. Este resultado semelhante ao da abundância sugere que, embora as Biohuts aumentem a biodiversidade em ambientes altamente modificados, elas não substituem completamente os habitats naturais.

A presença de diferentes espécies em zonas próximas das Biohuts foi notável, com destaque para a observação de várias espécies de peixes juvenis de várias famílias e géneros, nomeadamente Mugilidae, *Atherina* spp. e *Diplodus* spp. Além disso, foram também observadas algumas espécies em menor abundância como é o caso do *Parablennius gattorugine* e *Trachurus trachurus*. Os resultados provenientes dos censos subaquáticos em relação à biodiversidade demonstraram a importância das estruturas artificiais para o suporte da

biodiversidade em ambientes urbanizados, consequentemente criando um habitat mais resiliente e com complexidade suficiente para albergar recrutamento.

O recrutamento de peixes foi um dos fatores mais influenciados pela instalação das Biohuts. Durante os censos, verificou-se que as Biohuts atuaram como viveiros eficientes, oferecendo abrigo e proteção às espécies, promovendo a sobrevivência de juvenis durante as suas fases mais vulneráveis. Espécies como *Diplodus sargus*, *Sarpa salpa*, *Sardina pilchardus* e Mugilidae apresentaram picos significativos de recrutamento nas zonas com Biohuts, principalmente nos meses de primavera e início de verão, coincidindo com a época de maior recrutamento. Também foi possível verificar que uma grande parte das espécies que apresentaram recrutamento, têm possivelmente o período de desova compreendido entre o final do inverno e o início da primavera.

Os resultados demonstraram uma variação temporal no recrutamento, com um aumento acentuado durante os meses de maio e junho de 2024. Este padrão sazonal reflete a influência das diversas condições ambientais na dinâmica de recrutamento e demonstra que as Biohuts são particularmente eficazes em fixar os peixes juvenis durante esses períodos críticos.

A análise do recrutamento em função das zonas permitiu concluir que o recrutamento foi maior nas áreas com Biohuts dentro da marina. Os dados indicam que as Biohuts recrutaram uma quantidade considerável de indivíduos, o que sugere que estas estruturas podem mitigar os impactos negativos da urbanização costeira. Relativamente à zona natural apresentar um recrutamento menor do que as Biohuts, poderá estar associado à presença de uma alga invasora (*Rugulopterix okamurae*).

Em termos de recrutamento específico, as Biohuts demonstraram-se eficazes no aumento do recrutamento de várias espécies, incluindo aquelas com maior importância ecológica e potencialmente económica. Entre os taxa com maior recrutamento destacaram-se Mugilidae, *Diplodus sargus*, *Sarpa salpa* e *Atherina* spp. A Mugilidae, em particular, apresentou uma densidade de recrutamento substancialmente maior nas áreas com Biohuts em comparação com as áreas de controlo e área natural. O recrutamento de *Diplodus sargus* foi também considerável, demonstrando que estas estruturas podem auxiliar na recuperação de populações, sendo assim, um benefício para as comunidades costeiras.

Espécies como *Sarpa salpa* e *Diplodus puntazzo* também beneficiaram das Biohuts, embora em menor número, o que pode estar relacionado com as preferências específicas por outro tipo de habitat ou também com o seu ciclo de vida. O recrutamento de várias espécies nas áreas com Biohuts indica que estas estruturas proporcionam um ambiente propício para o desenvolvimento de peixes juvenis, contribuindo, assim, para a reposição das populações adultas.

Este estudo fornece evidências robustas de que as Biohuts desempenham um papel essencial na melhoria da abundância, biodiversidade e recrutamento de peixes em áreas costeiras urbanizadas, como a Marina de Lagos. Embora as áreas naturais continuem a ser insubstituíveis, as Biohuts oferecem uma solução viável para mitigar os impactos negativos da urbanização costeira. Além disso, a eficácia das Biohuts no recrutamento de espécies com potencial interesse económico sugere que estas estruturas podem contribuir para a sustentabilidade das pescas em regiões costeiras densamente urbanizadas. A longo prazo, a implementação contínua e a expansão do uso dos Biohuts, juntamente com estratégias de conservação de habitats naturais, poderão desempenhar um papel fundamental na gestão dos recursos marinhos e na conservação da biodiversidade.

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1. Introduction

1.1. Habitat degradation

Marine ecosystems, covering over 70% of the Earth's surface, are vital hotspots of biodiversity under increasing threat due to human activities (Pekel et al., 2016; Trathan et al., 2015; Pikitch, 2012; Hanski, 2011). Coastal areas stand as highly important habitats that are crucial for fish recruitment and the sustainability of biodiversity (Hutchings et al., 2002; Biagi et al., 1998; Menge., 1991). The significance of these zones in nurturing the early life stages of fish has become increasingly apparent as we recognize their multifaceted roles and ecological contributions in terms of species richness and for being essential marine habitats that act as recruitment areas, nurseries, and refuges for fish larvae/juveniles (and other organisms) (Dias et al., 2016; Solari et al., 2015).

Amongst the most important coastal areas in terms of recruitment and being a very productive ecosystem, estuaries globally play a critical role in fish recruitment, serving as nursery areas for many marine species (Babler et al., 2011; Amorim et al., 2017; Arevalo et al., 2023). However, these ecosystems have been strongly affected by habitat loss due to urbanization (Amorim et al., 2017; Stamp et al., 2022).

Estuaries are characterized by being highly dynamic systems; environmental stressors such as changes in salinity, particularly in the mid-to upper estuary regions, restrict biodiversity, with species richness typically decreasing from the estuary mouth to upstream regions (Wildish, 1977; McLusky, 1971; Attrill et al., 1996). Despite their relatively low biodiversity compared to adjacent marine or freshwater systems, estuaries remain highly productive ecosystems (Attrill et al., 1996). Due to the large amount of detrital material and for being very dynamic systems, the estuaries are known for being a more suitable habitat for detritivorous, generalist and or opportunistic species which often leads to the dominance of these fish in the estuarine areas (Constanza et al., 2013; Franco et al., 2008; Babler et al., 2011; Carassou et al., 2017).

The degradation of estuarine habitats diminishes their role in fish recruitment, potentially affecting the survival of juvenile fish populations and the nursery carrying capacity (Amorim et al., 2017; Stamp et al., 2022). With rapid urbanization becoming a growing problem, the degradation and loss of habitat increases (Chase et al., 2020; Rahman, 2023). With natural shallow coastal waters being one of the most affected areas and the urban infrastructure falling

short as a replacement for the ecosystem services that natural habitats provide (Zhang et al., 2020; Dias et al., 2016).

1.2. Possible solution - Biohut

The growth of communication channels has increased public awareness of the importance of fish recruitment, biodiversity loss, habitat restoration, and sustainable fisheries (Cooke et al., 2014; Arlinghaus et al., 2002). This has resulted in increasing the pressure for conservation measures to be taken on the shorelines, with a possible solution being the restoration of these essential habitats and the implementation of artificial nurseries (Mercader et al., 2019; Jacob et al., 2018).

Habitat restoration can indeed restore important nurseries that offer refuge and nourishment to recruiting fish, thereby altering positively the overall expected number of fish recruited and, consequently, the number available to fishermen (Camp et al., 2019). Amidst the challenges posed by rapid urbanization, particularly along coastal areas, innovative ecological engineering solutions have been developed as is the case of the artificial structures named Biohuts (Boissery et al., 2023; Nystrup Lund, 2021).

The Biohuts were developed by Ecocean, a French company specialized in aquatic ecological restoration (Boissery et al., 2023). They are artificial habitats acting as a nursery made with recyclable materials designed to promote the ecological health of coastal areas while enhancing fish recruitment and marine biodiversity (Boissery et al., 2023; Nystrup Lund, 2021). Overall, there are more than 5200 Biohut nurseries worldwide, with these structures being found in 54 harbors just in Europe (Ecocean, 2023).

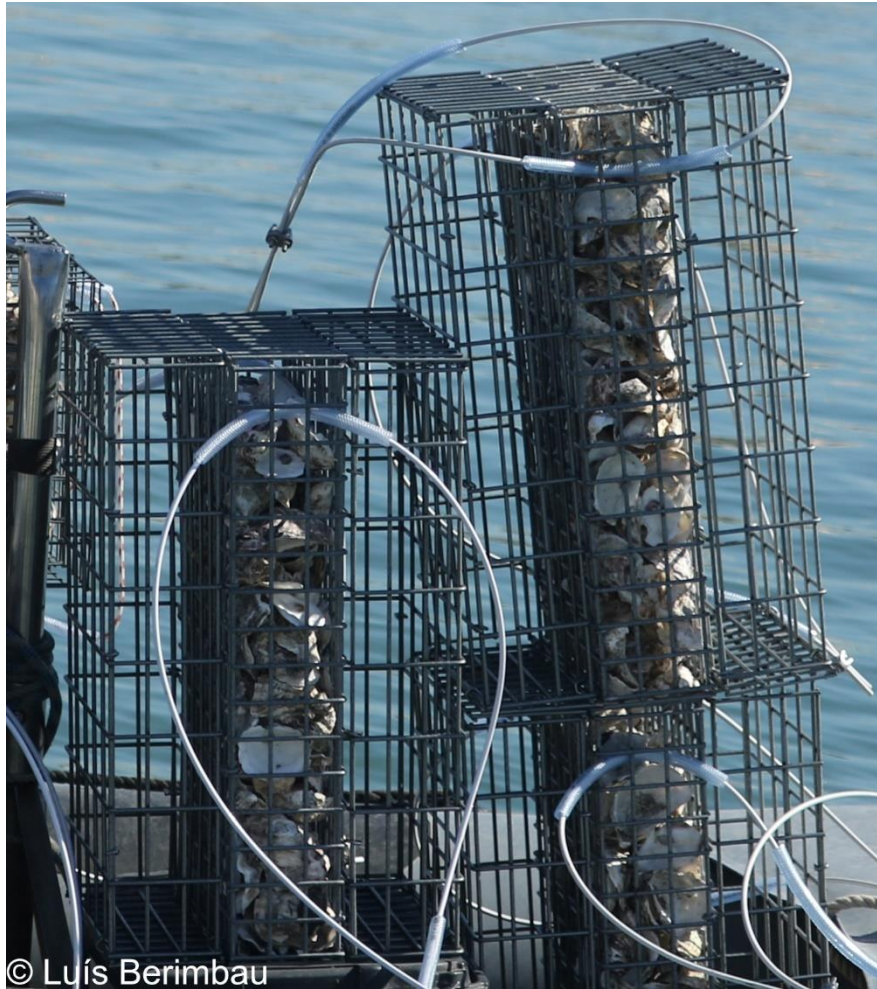


Figure 1.1. Biohuts before the installation on the pontoons.

Every Biohut recreates the nursery role through the two-cage system (fig. 1.1). The inner compartment is filled with natural substrate, usually empty oyster shells (Boissery et al., 2023; Ecocean, 2019). These shells become a habitat for settlement of various species of algae, crustaceans, worms, etc., and provides forage and protection for small fish (Boissery et al., 2023). This module is rounded by one or more empty ones, whose mesh size allows only small fish to go inside the structure, keeping their predators on the outside (Boissery et al., 2023).

Different specific designs of the Biohuts were created depending on the purpose, their location and the goals of local conservation and restoration efforts (Boissery et al., 2023). For example, Ecocean also created smaller Biohuts (mini-Biohuts), with these structures being quite useful for analyzing the vagile fauna and for educational purposes as they can be easily lifted out of the water (Ecocean, 2019).

Biohuts also proved to be valuable tools for research and monitoring (Varenne et al, 2023; Mercader et al., 2019; Lossent et al., 2018; Bouchoucha et al., 2016). In a broader context, the introduction of these structures enables scientists to confirm the species inhabiting these environments, monitor shifts in biodiversity over time, and assess the efficacy of biohuts in enriching local biodiversity (Varenne et al., 2023; Mercader et al., 2019; Mercader et al., 2017).

In addition, the Biohuts can be used to evaluate factors such as recruitment periods and the stay duration for a diverse array of fish species, including those of commercial significance for the fisheries. Furthermore, the artificial nurseries can furnish valuable insights into the dynamics of marine life in urbanized coastal regions, and consequently contribute to studies on fish recruitment (Varenne et al., 2023; Mercader et al., 2019; Mercader et al., 2017).

1.3. The importance of studying abundance and biodiversity

Knowledge of fish abundance is crucial for studying recruitment, as recruitment is directly linked to the number of spawners in a population (Barrowman & Myers, 1996). By analyzing fish abundance, researchers can estimate the reproductive output of the adult population (Barrowman & Myers, 1996). Additionally, abundance data helps in understanding the factors that limit recruitment and provides insights into population dynamics, habitat quality and the overall resilience of species within an ecosystem (Hilborn, & Walters, 2013; Caley, 1993).

The portfolio effect is an ecological concept that suggests that biodiversity contributes highly to enhancing the stability, adaptability, and resilience of ecosystems (Moore et al., 2021; Maselko et al., 2020; Link, 2002). This concept is known for its importance in terms of ecosystem-based fisheries management (Moore et al., 2021; Maselko et al., 2020; Schinder et al., 2015; Link, 2002). Essentially, it posits that in habitats with a high species richness, a level of redundancy is established, meaning that if one species is lost, it can be more readily replaced by another organism (Moore et al., 2021; Schinder et al., 2015).

Within this framework, it is important to study biodiversity to understand the efficiency of the artificial nurseries' functions and for assessing their suitability as favorable habitats for fish settlement during recruitment (Moore et al., 2021; Maselko et al., 2020; Schinder et al., 2015).

1.4. Recruitment

Among all the complexities within marine ecosystems, the phenomenon of fish recruitment stands as one of the most important factors influencing the abundance and diversity of fish populations (Chambers, 2012; Allen & Hightower, 2010; Sissenwine, 1984).

The ecological process considered as fish recruitment comprises all the life stages from eggs to larvae and to juvenile fish (recruits) that will eventually play a crucial role in shaping adult fish populations (Houde et al., 2022; Fuiman & Higgs, 1997; Houde, 1987). One factor that normally differentiates the recruits from the adult population is their inability to breed (Camp et al., 2020). For each species, this recruitment process occurs during a certain time considered as the recruitment period (Duffy-Anderson et al., 2005). At an early stage of the recruitment (settlement), the larval fish start to associate with a certain habitat, with the period of time that each species stays in one habitat during the recruitment considered as the stay duration (White et al., 2019).

The recruitment is affected by environmental factors, including changes in temperature, salinity, currents, habitat availability, and biological factors such as predation, competition and the availability of suitable food sources (Arevalo et al., 2023). In a general way recruitment is affected by two major parameters, density-dependent mortality and density-independent mortality. Density-independent mortality is not related to density or population size, as is the case of pollution and habitat destruction (Lorenzen & Camp, 2019).

On the contrary, density-dependent mortality is related to the density or size of the population; as the population size increases, the intensity of this factor also increases (Lorenzen & Camp, 2019). Some of the most common density-dependent-mortality mechanisms are predation, diseases and resource competition (Lorenzen & Camp, 2019).

Some studies have recognized the settlement phase as the start of density-dependent mortality, marking the beginning of recruitment (Walters and Juanes, 1993). As fish grow during the recruitment, they become less vulnerable to predators as fewer predators are large enough to prey on them (Burbank et al., 2023; Nunn et al., 2012; Sissenwine, 1984). Eventually, when the fish reach a significant size, density-dependent mortality decreases, indicating their transition to the recruited or recruit size category (Nunn et al., 2012; Holm, 1990).

Another crucial aspect related to fish recruitment is natural mortality (Lorenzen & Camp, 2019; Vetter, 1998). It represents the rate of death caused by natural factors within a population,

including environmental influences, diseases, predation, and competition (Allen & Hightower, 2010; Sissenwine, 1984). This concept excludes impacts from fishing and other anthropogenic activities (Vetter, 1998).

Survival also plays a vital role in the context of recruitment, influencing the number of individuals who persist (Schnute et al., 1989; Sissenwine, 1984). High natural mortality rates can limit survival, affecting the overall success of recruitment in fish populations (Fogarty & O'Brien, 2016; Vetter, 1998; Sissenwine, 1984).

Fish recruitment studies started gaining more attention around the mid-20th century (Houde, 2008; Nakken, 2008). During this period, researchers were more focused on quantitative methods in fish recruitment studies and had limited technological tools for studying fish recruitment associated with basic sampling techniques (Hilborn & Walters., 2013; Ricker, 1954). Recent studies have developed, expanded the quantitative tools, and created improvements in data collection, enabling the analysis of larger, long-term datasets in less time through computer modeling and simulation (Haddon, 2020; Miller, 2007; Johnson, 1995).

Although earlier literature related to fish recruitment recognized how important some ecological concepts were, the latest studies have a more multidisciplinary approach (genetics, oceanography, ecology and biology) to explain the complex interactions related to fish recruitment (Ottoni et al., 2023; Haddon, 2020; Wallace et al., 2015).

Earlier studies were also more focused on a certain species or a certain fishery while some of the more recent studies have a more global perspective, enabling to perceive and establish connections between marine ecosystems and changes in environmental conditions (Bueno-Pardo et al., 2019; Katara, 2014; Laarman & Schneider, 1938).

The sampling techniques have expanded and adapted greatly through the years to better understand the fish recruitment of the species studied, and according to the environment they are found in (Harrisson et al., 2014; Allen & Hightower, 2010). Although the techniques used in fish recruitment studies have been improved to minimize harm, some of the techniques are more invasive, requiring the fish to be caught, subjected to stress or tagged, as is the case of trawl surveys (Jurvelius et al., 2010; Allen & Hightower, 2010).

In contrast, some techniques cause minimum or no harm to the fish during these studies as is the case of the underwater visual census using transects, with the only disturbance being caused by the presence of the divers (Bennett et al., 2009; Samoilys & Carlos, 2000; Halford &

Thompson, 1994). The data is recorded in real-time and provides the abundance, size and species composition of juvenile fish (Bennett et al., 2009; Samoily & Carlos, 2000; Halford & Thompson, 1994). This method also can provide some information in terms of habitat preferences and fish behavior, while being a cost-effective technique, especially in shallow areas (Pais & Cabral, 2017; Bennett et al., 2009; Samoily & Carlos, 2000).

Some of the biggest challenges related to the underwater visual census are the species and size identification as the fish that are actively moving (Edgar et al., 2004). One possible solution to counter these challenges is the combination of underwater visual census with photography for later identification and analysis of reference points with software to estimate the size (Pais & Cabral, 2017, Davis et al., 2014).

1.5. Objectives

It is generally recognized that the marinas often result in significant habitat disruption within sheltered coastal zones previously utilized as vital nursery grounds by various fish species (Selfati et al., 2018). This study aims, to assess the potential of the artificial habitats (Biohuts) to enhance fish abundance and biodiversity compared with artificial areas inside and outside the Marina of Lagos and with the natural area. The study also aims to assess the recruitment of the greatest number of species and compare how the recruitment varies between the different zones.

2. Methodology

2.1. Area of study

The Biohuts made their debut in Portugal within the southern mainland region (Algarve), specifically in the Marina de Lagos which is located at 37°06'27"N 8°40'26"W. The city of Lagos where the marina is present is characterized by having one of the largest bays in Europe with around four kilometers, and the marina itself is connected with the Atlantic Ocean through the Bensafrim estuary (Agapito, 2010; Ramos-Pereira et al., 2010).

In total, three different areas within Marina de Lagos were selected for the implementation of Biohuts, as shown in Figure 2.1. In each of these areas, five Biohuts and one mini-biohut were

suspended from the marina platforms, resulting in a total of fifteen Biohuts and three mini-biohuts. These particular Biohuts had an outer mesh size of 5 cm while the mini Biohuts had a mesh size of 2.5 cm. Three control areas were also selected, one inside the marina, and the other two outside, with one more upstream while the other one is downstream in “Ribeira de Bensafrim” (Figure 2.1).



Figure 2.1. Aerial view of Marina the Lagos with the control and Biohut areas. Retrieved from Google Maps, 12 March, 2023.

Both the Biohut and control areas were carefully selected taking into account the different areas present in the marina that are subjected to different environmental variables. Zone C is an area that is more protected, with less boat traffic and slightly more upstream than the other areas, while zone A is the opposite. Zone B is an intermediate area with not much boat traffic and slightly more downstream than zone C.

The same principle was applied in terms of choosing the control areas outside the marina, with one area upstream (control area B) and the other one downstream (control area A) of “Ribeira de Bensafrim” (Figure 2.1). Control zone A is near the gas station and has both support pontoons and rocks alongside the coast, while Control zone B has less disturbances related to boat activity but is near a spillway of the wastewater treatment plant.



Figure 2.2. Natural area and the 6 different transect areas. Retrieved from Google Earth, 15 August, 2024.

The control area inside the marina was implemented in the center of the marina in one area that is not much affected by boat traffic and is not too much downstream or upstream of “Ribeira de Bensafrim”, to represent well the habitat functions that the structures of Marina de Lagos has. Apart from this, a natural area was also selected on the bay outside of the marina (6 transects), more concretely in the rock formations between the “Praia da Batata” and “Praia dos Estudantes”, with the main objective of comparing with the control and Biohut areas, and to also get information about fish recruitment in the natural area (Figure 2.2).

2.2. Data collection

Sampling started on the 28th of September 2023 and ended on the 21th of June 2024. The periodicity of the underwater visual census was once per month and when recruitment was observed, the effort was increased by two visual censuses per month.

To collect the data regarding fish recruitment, the form in Annex A associated with a waterproof notebook was used during each underwater visual census while snorkeling. Accordingly, two transects of 10 meters are done for each Biohut area; one of the transects involves passing by two Biohuts, while the other would go past the other three Biohuts. For every transect when reaching a Biohut, a stop of at least 1 minute was made around the structure to better collect the data, observe the species, quantify the number of individuals, estimate the total length of the fish and film the fauna presents inside and around the artificial nursery.

Additionally, inside the marina, three transects of 10 meters were defined in the control area, and three transects of the same length were also performed in each control area outside the marina, making a total of 6 transects outside of the marina. Regarding the natural area, a total of 6 transects of 10 meters each were done in the shoreline.

Each transect typically lasted between 5 to 10 minutes, these underwater visual censuses were made with the diver moving slowly and at the surface in the transects to reduce possible disturbances on fish during the survey. In the case of the transects done inside the marina, the diver surveyed under the floating pontoons which enable an observation beam of 180 degrees with 2 meters for each side and 2 meters of depth, while outside the marina (natural area and control area outside the marina) the observer is limited to the shoreline having only an observation beam of 90 degrees with 2 meters to only one side and 2 meters of depth.

Some of the transects were filmed using a GoPro underwater camera and a flashlight when the visibility and the shadow of the Marina structures would not give a clear observation of the fish present. After each transect in an area, another transect was done if necessary, where the observer would collect some pictures for species, size and abundance identification using a TG-6 Olympus camera. Almost all the data regarding the underwater visual census was preferably collected during high tide.

In terms of fish identification two main guides were used; respectively the “Atlas of post-larval fish of the Northwestern Mediterranean Sea” (Crec'hriou & Lenfant, 2015) and “Manual de identificação de peixes ósseos da costa continental portuguesa: Principais características diagnosticantes” (Martins & Carneiro, 2018). The checklist “Ichthyofauna of Portugal: taxonomic diversity, common and scientific names of marine fishes” by Carneiro et al. (2019), was also used.

Regarding the evaluation of the size of the fish, the total length was estimated, with eleven size classes created (table 2.1). Taking into account that the fish were not caught and to minimize the measurement error, at an initial phase, the observer was submitted to training by estimating the sizes of different small objects underwater with the aid of the laminated sheet with the form present in Annex A.

Table 2.1. Sizes classes of fish observed during the survey.

Size class	1	2	3	4	5	6	7	8	9	10	11
Range in Centimeters	<1	1 to 1.5	1.5 to 2	2 to 2.5	2.5 to 3	3 to 3.5	3.5 to 4	4 to 5	5 to 6	6 to 7	>7

In cases of uncertainty and to enhance the mitigation of measurement errors, photographs of the fish were taken, and the software ImageJ was employed. This software converted the dimensions of a known reference object into pixels, enabling the measurement of the total length of the fish based on the number of pixels displayed, as can be seen in Figure 2.3, where the outer mesh size is the reference length near the fish.

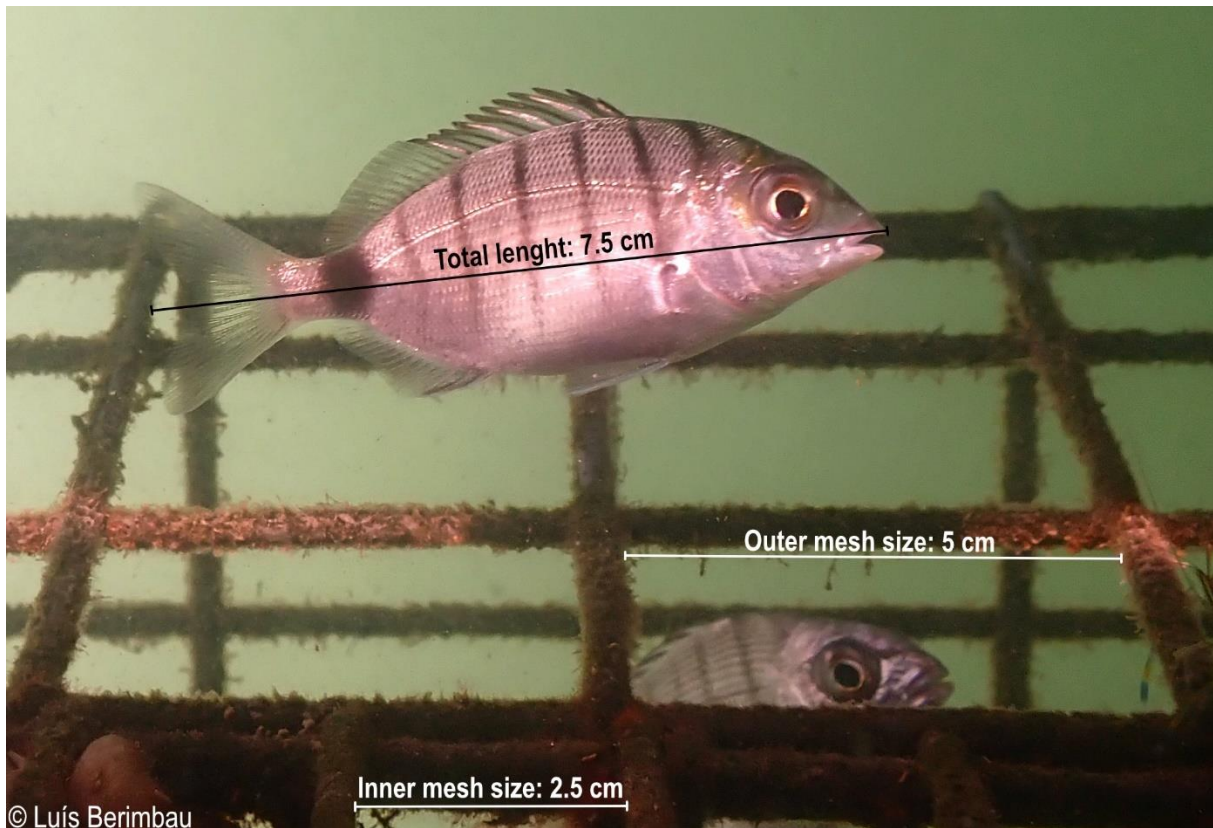


Figure 2.3. Post-processed image showing the measurement of the total length (7.5 cm) of a *Diplodus sargus*, just above a Biohut. The outer mesh size is 5 cm (reference length), and the inner mesh size is 2.5 cm.

For accurate measurement, the fish must present one of its sides at a perpendicular 90-degree angle to the camera (Figure 2.3). The size estimations were mostly done on days when the visibility underwater was more than 1 meter, and when the fish would get near enough to have clear high-definition pictures. To increase the sharpness in the measurements the pictures were processed (changes in contrast, shadows and vibrances parameters) using Affinity Photo, the images were all converted to 300 DPI, and only then were measurements done using ImageJ.

Apart from the previously mentioned data, information on some environmental variables was recorded during the surveys, namely cloud cover (%), wind velocity (knots), water temperature (°C), air temperature (°C), swell outside the marina (meters), visibility underwater (meters), pollution, if there was rain in last 24 hours and the condition of the Biohut (verify if the structures were well fixed, damaged or what organisms covered the structure). Additionally, it was also recorded if fish were around or inside the biohuts (less than 1 meter) or not.

To gather this data different methods were used; the cloud cover, pollution and the condition of the biohut were evaluated visually. The cloud cover was evaluated on a scale of 0 (no cloud

cover) to 8 (complete cloud cover), while the pollution was analyzed in terms of detecting fouling, plastic debris and oil slicks presence in each area. To assess the conditions of the biohuts, each structure is analyzed in terms of integrity.

Regarding visibility, a Secchi disk was used, and the water temperature was obtained using a mercury-in-glass thermometer. The rest of the variables information was acquired through the network of meteorological stations of the Portuguese Institute for the Sea and the Atmosphere (IPMA , 2023). To improve the species identification, abundance and size estimation, the data was collected taking into account some of the environmental variables. The underwater visual census was preferably done whenever possible on days with no rain in the last 24 hours, less pollution, visibility above or equal to 1.5 meters and the swell outside the Marina was not too strong (high).

2.3. Data analysis

For the evaluation of biodiversity, the Shannon diversity index (H) and Simpson diversity index (D) were used (Equations 1 and 2). To calculate both indexes, all of the data was compiled in an Excel file where the calculations were made excluding the unidentified fish. The indexes were calculated for the 4 different zones, but then were also calculated for each different transect area inside the zones with the objective of comparing them.

$$H = -\sum(pi * \ln(pi))$$

Equation 1

$$D = 1 - \frac{\sum ni(ni - 1)}{N(N - 1)}$$

Equation 2

The Shannon-Weaver (H), given in Equation 1, is a way to measure the species diversity of a certain community (Magurran, 2004; Spellerberg & Fedor, 2003; Shannon & Weaver, 1949). Values of H range from 0 to infinite (this value is limited by the number of species in the sample), with p_i being the proportion of each species in the sample (division between the individuals of a specific species by the total number of individuals in a community). The higher the value of H, the higher the diversity and the evenness of species in a particular community (Magurran, 2004; Spellerberg & Fedor, 2003; Shannon & Weaver, 1949). In contrast when H has a low value it indicates that there is less species diversity in the community (Magurran, 2004; Spellerberg & Fedor, 2003; Shannon & Weaver, 1949).

The Simpson index (D), quantifies the diversity of species within ecological communities (Okpiliya, 2012; Simpson, 1949), where n_i is the number of organisms that belong to species i and N is the total number of organisms (Okpiliya, 2012; Simpson, 1949). The values for Simpson's Diversity Index ranges between 0 and 1, with higher values indicating lower diversity and a bigger dominance (Okpiliya, 2012; Simpson, 1949). Sometimes the interpretation of the results is counterintuitive when using the Simpson diversity index, for this reason the the Simpson Index of Diversity was used ($1 - D$) has it can be seen in Equation 2 (Shitikov & Rozenberg, 2005). For Simpson Index of Diversity the values have the same range but the higher values (close to 1) indicates a greater species diversity (Shitikov & Rozenberg, 2005).

In order to estimate the recruitment, the sizes classes, the number of fish and the species were considered (Table 2.1). To distinguish what was recruitment the length at first maturity for each species was compared with the size classes, and the fishes that were below the length at first maturity were considered as recruits (Table 2.2). In the literature, sometimes the length at first maturity was given by a range, and in those cases the lowest value of that range was the one considered.

Table 2.2. Length at First Maturity and the respective sources, for fish species observed during the underwater visual census

Species	Length at first maturity (cm)	Reference
<i>Atherina</i> spp.	4.25 cm	Küçük et al. (2012)
<i>Balistes capriscus</i>	20.26 cm	Kacem & Neifar (2014)
<i>Coryphoblennius galerita</i>	4 cm	Milton (1983)
<i>Ctenolabrus exoletus</i>	12 cm	Skiftesvik & Halvorsen (2019)
<i>Ctenolabrus rupestris</i>	6 cm	Skiftesvik & Halvorsen (2019)
<i>Dicentrarchus labrax</i>	31.7 cm	Kennedy & Fitzmaurice (1972)
<i>Dicentrarchus puntactus</i>	17.24 cm	Mehanna (2006)
<i>Diplodus annularis</i>	13.4 cm	Mouine et al. (2012)
<i>Diplodus cervinus</i>	22 cm	Pajuelo et al. (2003)
<i>Diplodus puntazzo</i>	19 cm	Mouine et al. (2012)
<i>Diplodus sargus</i>	21.9 cm	Mouine et al. (2012)
<i>Diplodus vulgaris</i>	17.0 cm	Mouine et al. (2012)
<i>Gobius cobitis</i>	12.0 cm	Potts & Swaby (1992)
<i>Gobius paganellus</i>	6.0 cm	Azevedo & Simas (2000)
<i>Liphophrys pholis</i>	8 cm	Monteiro et al. (2005)
<i>Lithognathus mormyrus</i>	13.4 cm	Türkmen & Akyurt (2003)
Mugilidae	35.0 cm	Bem-Tuvia (1986)
<i>Mullus surmuletus</i>	13.2 cm	Arslan & İşmen (2013)
<i>Oblada melanura</i>	18.83 cm	Daban et al. (2020)
<i>Parablennius icognitus</i>	3.5 cm	Kotrschal & Goldschmid (1981)
<i>Pomadasyus incisus</i>	15.6 cm	Pajuelo et al. (2003)
<i>Sardina pilchardus</i>	10.2 cm	Basilone et al. (2021)
<i>Sarpa salpa</i>	16.5 cm	Aissat-Ziamni et al. (2020)
<i>Scomber colias</i>	20.82 cm	Bouzzammit et al. (2022)
<i>Scomber scombrus</i>	23.2 cm	AO El-Aiatt & A Sh Shalloof (2020)
<i>Serranus atricauda</i>	16 cm	García-Díaz et al. (2006)
<i>Sparus aurata</i>	32.6 cm	Chaoui et al. (2006)
<i>Spondylisoma cantharus</i>	20.1 cm	Gonçalves & Erzini (2000)
<i>Symphodus melops</i>	9 cm	Skiftesvik & Halvorsen (2019)
<i>Syngnathus acus</i>	6.1 cm	Gurkan et al. (2009)
<i>Trachurus trachurus</i>	14.9 cm	Rahmani et al. (2020)

Some species were seen during the underwater visual census but are not presented in Table 2.2. This happened when no information was displayed in terms of length at first maturity and therefore the recruitment for this species was not calculated and included in the study.

With the objective of comparing the fish recruitment abundance across the different sites, the total recruit density was calculated, as the transects in the Biohut zones had twice the volume

of those in the Control and Natural areas, and therefore comparing the number of recruits would not be possible.

The Biohut transect area measured 2 meters deep by 4 meters wide by 10 meters long, while in other zones, the transect area values were the same except only 2 meters wide, resulting in 80 square meters for the Biohut zones and 40 square meters for the other areas. These areas were then multiplied by the number of transects in each zone per underwater visual census (6 transects per zone), and further multiplied by the total number of UVCs (16). This calculation resulted in a total volume of 7,680 cubic meters for the Biohut zones and 3,840 cubic meters for the other zones.

To calculate the recruit density for a certain taxa, the number of recruits was divided by the corresponding volume of the transect area, with the result being the number of recruits per cubic meter. With this measure, comparisons were then made between the Biohut, Control Inside the Marina, Control Outside the Marina and the Natural areas.

Regarding the statistical analysis, the Software R was used. Although the sample size was considerably large, the data was not normally distributed according to the Shapiro-Wilk normality tests, with the same being verified in the Q-Q plots and with some extreme outliers also being verified on the boxplots.

The non-parametric chi-squared Kruskal Wallis test was then employed for most of the analysis, when evaluating one quantitative and one qualitative variable. If significant differences were obtained when using the Kruskal Wallis test, pairwise comparisons were done if needed using the pairwise Wilcoxon test. This comparison was made mainly between the different zones and different transect areas in terms of abundance, biodiversity and recruitment. Linear regressions were also used to evaluate the relationships between some environmental variables and recruitment.

3. Results

3.1. Sample description

From the visual censuses conducted from the 28th of September to 21th of June 2024, 42 different fish taxa have been identified as can be seen in Table 3.1; some of these fish species can be observed in Figure 3.1. In total, 47,427 individuals were observed, with the highest number found in the Biohut areas (21,918 individuals), followed by the natural zone with 16,094 individuals (Table 3.1). The areas with fewer individuals observed were the control zone outside the marina (8,314 fish) and the control zone inside the marina with 1,101 fish seen (Table 3.1).



Figure 3.1. Some of the fish species that were photographed in the biohut areas of the Marina de Lagos; A- *Parablennius gattorugine* B- *Diplodus sargus* C- *Gobius paganellus* and D - *Atherina* spp.

The most abundant taxa identified was *Atherina* spp., with a total of 19,635 observed individuals (Table 1). This was followed by Mugilidae, which had 9,280 individuals, and *Diplodus sargus*, which had 6,860 individuals (Table 3.1). Other species that stood out due to their high numbers were *Sardina pilchardus* (3,799 individuals), *Sarpa salpa* (1,933

individuals), and *Diplodus puntazzo* (720 individuals) (Table 3.1). On the other hand, *Syngnathus acus* was the least common species, with only two individuals counted (Table 3.1).

Examples of species that were found in comparatively smaller quantities than the most abundant species were, *Boops boops* (665 individuals), *Coris melanura* (350 individuals), *Oblada melanura* (335 individuals), and *Diplodus vulgaris* (308 individuals) (Table 3.1). Other taxa, with number of individuals ranging from 202 to 248, were *Pomadasys incisus* (248 individuals), *Dicentrarchus labrax* (243 individuals), *Parablennius gattorugine* (189 individuals), and *Diplodus cervinus* (157 individuals) (Table 3.1). Some other sightings were *Symphodus melops* (154 individuals), *Lithognathus mormyrus* (118 individuals), *Sardinella maderensis* (109 individuals), *Dicentrarchus punctatus* (96 individuals), and *Liphophrys trigloides* (91 individuals) (Table 3.1).

Some of the taxa that were less frequently seen were *Trachurus trachurus* (78 individuals), *Gobius cobitis* (77 individuals), *Coryphoblennius galerita* (71 individuals), and *Symphodus bailloni* (66 individuals) (Table 3.1).

Alternatively, species that were overall less abundant and fewer than 60 individuals included *Sparus aurata* (55 individuals), *Gobius paganellus* (45 individuals), *Mullus surmuletus* (35 individuals), and *Scomber colias* (33 individuals) (Table 3.1). The most rarely seen species were *Parablennius pilicornis* (25 individuals), *Diplodus annularis* (22 individuals), *Labrus merula* (19 individuals), *Microlipophrys canevae* (14 individuals), *Balistes capriscus* (14 individuals), and *SpondylIOSoma cantharus* (12 individuals) (Table 3.1). The species that were rarely encountered were *Ctenolabrus exoletus* (11 individuals), *Salaria pavo* (10 individuals), *Liphophrys pholis* (10 individuals), *Parablennius incognitus* (7 individuals), *Labrus bergylta* (5 individuals), *Scomber scombrus* (5 individuals), and *Serranus atricauda* (2 individuals) (Table 3.1).

Table 3.1. Abundance and distribution of fish species in Biohuts, Control and Natural zones, ranked from most to least abundant (Total)

Species	N° of individuals observed				Total
	Biohuts	Control Inside the Marina	Control Outside the Marina	Natural Zone	
<i>Atherina</i> spp.	9895	546	4861	4333	19635
Mugilidae	7393	485	1248	154	9280
<i>Diplodus sargus</i>	1761	63	1030	4006	6860
<i>Sardina pilchardus</i>	1217	0	0	2582	3799
<i>Sarpa salpa</i>	153	0	212	1568	1933
<i>Diplodus puntazzo</i>	197	0	100	423	720
<i>Boops boops</i>	0	0	74	591	665
<i>Coris Melanura</i>	0	0	1	349	350
<i>Oblada melanura</i>	85	0	94	156	335
<i>Diplodus vulgaris</i>	15	0	54	239	308
<i>Pomadasy incisus</i>	121	0	127	0	248
<i>Dicentrarchus labrax</i>	125	6	40	72	243
<i>Ctenolabrus rupestris</i>	0	0	0	220	220
<i>Parablennius gattorugine</i>	183	0	2	4	189
<i>Diplodus cervinus</i>	29	0	27	101	157
<i>Symphodus melops</i>	4	0	0	150	154
<i>Lithognathus mormyrus</i>	0	0	0	118	118
<i>Sardinella maderensis</i>	0	0	0	109	109
<i>Dicentrarchus punctatus</i>	23	1	26	46	96
<i>Liphophrys trygloides</i>	0	0	0	91	91
<i>Trachurus trachurus</i>	60	0	18	0	78
<i>Gobius cobitis</i>	9	0	68	0	77
<i>Coryphoblennius galerita</i>	0	0	0	71	71
<i>Symphodus bailloni</i>	2	0	1	63	66
<i>Sparus aurata</i>	18	0	4	33	55
<i>Gobius paganellus</i>	21	0	15	9	45
<i>Mullus surmuletus</i>	0	0	0	35	35
<i>Scomber colias</i>	11	0	0	22	33
<i>Parablennius pilicornis</i>	12	0	0	13	25
<i>Diplodus annularis</i>	0	0	0	22	22
<i>Labrus merula</i>	0	0	0	19	19
<i>Microlipophrys canevae</i>	0	0	0	14	14
<i>Balistes capriscus</i>	0	0	0	14	14
<i>Spondyliosoma cantharus</i>	9	0	3	0	12
<i>Ctenolabrus exoletus</i>	0	0	0	11	11
<i>Salaria pavo</i>	0	0	10	0	10
<i>Liphophrys pholis</i>	0	0	0	10	10
<i>Parablennius icognitus</i>	0	0	0	7	7

<i>Labrus bergylta</i>	0	0	0	5	5
<i>Scomber scombrus</i>	0	0	0	5	5
<i>Syngnathus acus</i>	2	0	0	0	2
<i>Serranus atricauda</i>	0	0	0	2	2
Unidentified individuals	573	0	299	427	1299
Total number of individuals	21918	1101	8314	16094	47427
Total number of Individuals per Volume (m^3)	2.854	0.287	2.165	4.191	2.470
Total number of species	23	5	21	36	42

While some fish taxa were observed across all sampling areas, such as *Atherina* spp., Mugilidae, *Diplodus sargus*, *Dicentrarchus labrax*, and *Dicentrarchus punctatus* (Table 3.1), other species were restricted to specific locations. Notably, *Serranus atricauda*, *Scomber scombrus*, *Labrus bergylta*, *Liphophrys pholis*, *Parablennius incognitus*, *Ctenolabrus exoletus*, *Balistes capriscus*, *Microlipophrys canevae*, *Labrus merula*, *Diplodus annularis*, *Mullus surmuletus*, *Coryphoblennius galerita*, *Liphophrys trygloides*, *Sardinella maderensis*, *Lithognathus mormyrus*, and *Ctenolabrus rupestris* were exclusively observed in the Natural Zone (Table 3.1). Additionally, *Salaria pavo* was only detected in the Control Outside the Marina, whereas *Syngnathus acus* was uniquely recorded in the Biohut zones (Table 3.1).

In addition to the taxa previously mentioned, other species displayed varying levels of habitat presence across the sampling areas (Table 3.1). *Sardina pilchardus* was observed exclusively in the Natural Zone and the Biohuts while *Sarpa salpa* was primarily seen in the Natural Zone, with some presence in the Control Outside the Marina (Table 3.1). *Diplodus puntazzo* was detected in the Biohuts, Control Outside the Marina, and the Natural Zone but was absent from the Control Inside the Marina (Table 3.1).

Regarding *Boops boops* and *Coris melanura* both were found in the Natural Zone and Control Outside the Marina (Table 3.1). *Oblada melanura* and *Diplodus vulgaris* were observed in the Biohuts, Control Outside the Marina, and the Natural Zone but not inside the Marina (Table 3.1). *Pomadasys incisus* was found only in the Biohuts and Control Outside the Marina (Table 3.1). *Gobius cobitis* and *Trachurus trachurus* were observed exclusively in the Control Outside the Marina (Table 3.1).

Additionally, *Parablennius gattorugine* and *Diplodus cervinus* were mainly seen in the Biohuts and the Natural Zone, while *Symphodus melops* was observed mostly in the Natural Zone (Table 1). In contrast, *Sparus aurata* and *Gobius paganellus* were found in multiple habitats but were less abundant in the Marina areas (Table 3.1).

There were a total of 1,299 unidentified individuals, with the Biohuts and the Natural zone having the biggest numbers of individuals, respectively, 573 and 427 individuals (Table 3.1). The Control Zone Outside the Marina displayed 299 unidentified fish while on the Control Zone Inside the Marina no unidentified fish were recorded (Table 3.1).

3.1.1 Size Classes

Concerning size classes, the overall observed fish were predominantly bigger than 7 cm (size class 11) with a total number of 27,151 fishes, this value was followed by the size class 10 (3,935 individuals) which corresponds to individuals that have a total length between 6 and 7 cm. (Figure 3.2).



Figure 3.2. Number of individuals according to the size classes.

According to the Kruskal-Wallis test it was seen that there were significant statistical differences in the distribution of the number of individuals by the different 11 size classes ($\chi^2=231.68$, $df = 10$, $p\text{-value} < 0.001$).

3.1.2 Fish proximity with the biohuts

A Kruskal-Wallis test revealed a significant difference in the abundance of fish individuals around and within biohut transect areas ($\chi^2 = 21.738$, $df = 1$, $p < 0.001$). The pie chart further demonstrates this, with 64.28% of individuals observed inside the biohuts and 35.72% outside, which corresponds respectively to 14,088 and 7,830 individuals (Figure 3.3).

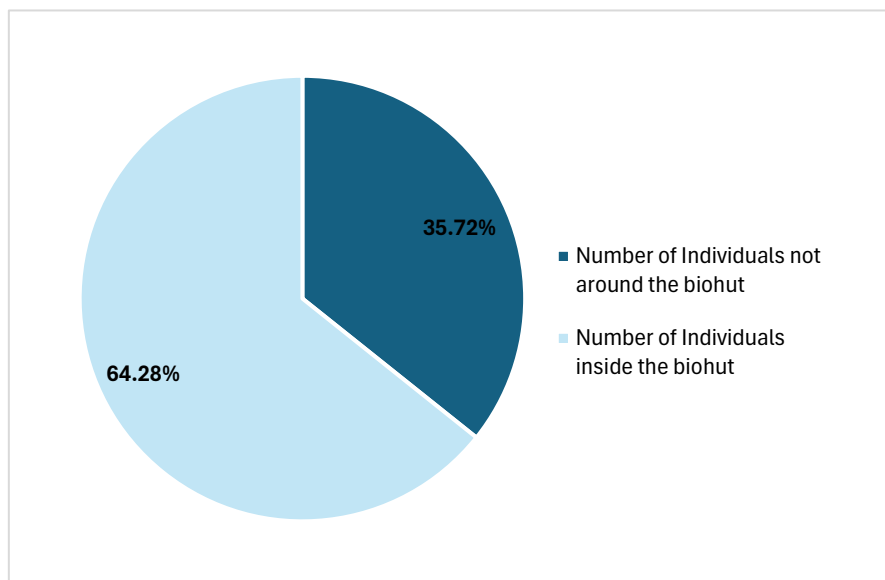


Figure 3.3. Percentage of Fish Within and Outside Biohut Transect Areas

3.2. Differences in abundance according to the location

Regarding the density in number of individuals per cubic meter, the Natural Zone exhibited the highest density at 4.191 individuals per cubic meter (Figure 3.4). Biohuts recorded a density of 2.854 individuals per cubic meter, followed by the Control Outside the Marina with 2.165 individuals per cubic meter (Figure 3.4). The Control Inside the Marina showed the lowest density, with 0.287 individuals per cubic meter. Overall, the total density across all sampling locations combined was 2.470 individuals per cubic meter (Table 3.1).

The density of individuals per cubic meter varied across the sampling sites with significant statistical differences being verified across 4 zones according to the Kruskal Wallis test, $\chi^2 = 109.57$, $df = 3$, $p < 0.001$.

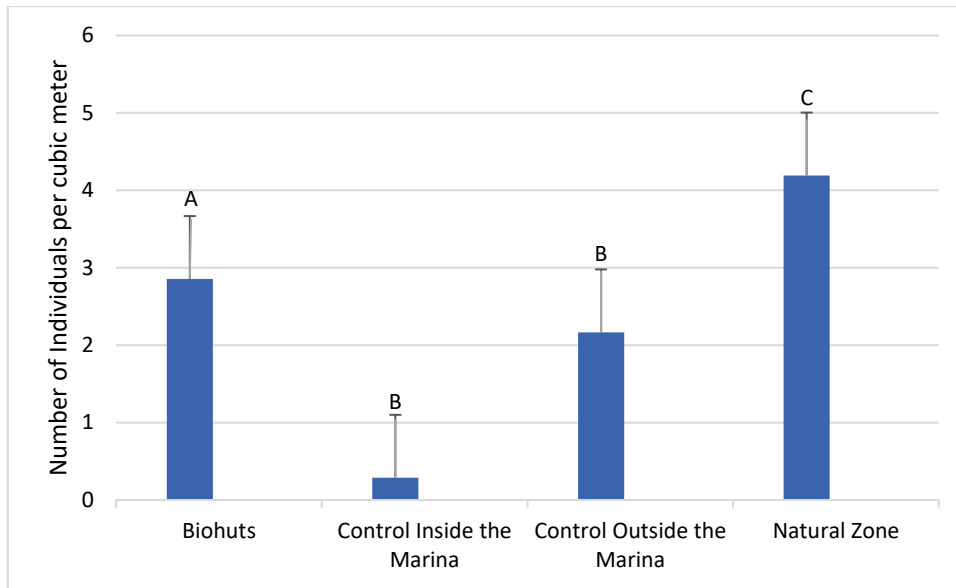


Figure 3.4. Comparison of Fish Density in Biohuts and Control Zones

Pairwise comparisons using the Wilcoxon rank sum test indicated that there were significant differences between the Biohuts and the Control Inside the Marina ($p < 0.001$) and the Biohuts and the Control outside the Marina ($p < 0.001$). Also, significant differences in terms of the density of individuals were seen between Biohuts and the Natural Zone ($p < 0.001$) as well as the Control outside the Marina and the Natural Zone ($p = 0.003$).

On the contrary, no significant differences were found between the zones Control Inside the Marina and Control Outside the Marina ($p = 0.055$), while significant differences were also found between the Control Inside the Marina and the Natural Zone ($p < 0.001$).

3.2.1. Variation of fish abundance regarding the different transect zones

Considering the density of fish, significant statistical differences were verified through a Kruskal Wallis test between the 7 different transect zones $\chi^2 = 141.89$, $df = 6$, $p < 0.001$. Through the pairwise Wilcoxon Rank Sum Tests it was possible to verify several significant differences between different transect zones (Table 3.2).

Comparisons within the Biohut transect zones revealed no significant difference between Biohut Zone A and Biohut Zone B ($p = 0.897$), and no significant differences were obtained

between Biohut Zone B and Biohut Zone C ($p = 0.897$; Table 3.2). However, Biohut Zone A differed significantly from Biohut Zone C ($p < 0.001$; Table 3.2).

Taking into account the Biohut transect zones and control transect zones, clear significant differences were revealed, particularly involving between Biohut Zone A and Control A outside the marina ($p < 0.001$). A similar pattern was observed between Biohut Zone A and Control B outside the marina ($p < 0.001$). Significant differences were also found between Biohut Zone A and the control zone inside the marina ($p < 0.001$), indicating distinct conditions between these areas (Table 3.2).

When considering the comparison between Biohut Zone B and Control A outside the marina no significant differences were seen with a p-value equal to 0.072 (Table 3.2). However, significant differences were found between Biohut Zone B and Control B outside the marina ($p = 0.001$) and between Biohut Zone B and the control inside the marina ($p = 0.001$; Table 3.2).

For Biohut Zone C, there was no significant difference between this zone and Control A outside the marina ($p = 0.072$), but significant differences were observed when comparing Biohut Zone C to Control B outside the marina ($p < 0.001$) and the control inside the marina ($p = 0.001$; Table 3.2).

Table 3.2. Pairwise Comparisons of fish density Across different transect zones using the Wilcoxon Test

Zone A	Zone B	p-value	Significance
Biohut zone A	Biohut zone B	< 0.001	Significant
Biohut zone A	Biohut zone C	< 0.001	Significant
Biohut zone A	Control A outside the marina	< 0.001	Significant
Biohut zone A	Control B outside the marina	< 0.001	Significant
Biohut zone A	Control Inside the Marina	< 0.001	Significant
Biohut zone A	Natural zone	< 0.001	Significant
Biohut zone B	Biohut zone C	0.897	Not Significant
Biohut zone B	Control A outside the marina	0.072	Not Significant
Biohut zone B	Control B outside the marina	< 0.001	Significant
Biohut zone B	Control Inside the Marina	< 0.001	Significant
Biohut zone B	Natural zone	< 0.001	Significant
Biohut zone C	Control A outside the marina	0.072	Not Significant

Biohut zone C	Control B outside the marina	< 0.001	Significant
Biohut zone C	Control Inside the Marina	< 0.001	Significant
Biohut zone C	Natural zone	< 0.001	Significant
Control A outside the marina	Control B outside the marina	0.149	Not Significant
Control A outside the marina	Control Inside the Marina	0.013	Significant
Control A outside the marina	Natural zone	0.001	Significant
Control B outside the marina	Control Inside the Marina	0.153	Not Significant
Control B outside the marina	Natural zone	0.077	Not Significant
Control Inside the Marina	Natural zone	0.631	Not Significant

Comparisons regarding the fish density between Biohut zones and the Natural Zone consistently showed significant statistical differences (Table 3.2). A highly significant difference was observed between Biohut Zone A and the natural zone ($p < 0.001$). Significant differences were also found between Biohut Zone B and the Natural Zone ($p < 0.001$) and between Biohut Zone C and the Natural Zone ($p < 0.001$; Table 3.2).

Analysis of the control transect zones outside the marina showed that there were no significant differences between Control A and Control B in regard to fish density ($p = 0.149$; Table 3.2). Yet, significant differences were found between Control A outside the marina and the control inside the marina ($p = 0.013$), significant differences were also observed between Control A outside the marina and the natural zone ($p = 0.001$), indicating (Table 3.2).

No significant differences were found between Control B outside the marina and the control inside the marina ($p = 0.154$) or between Control B and the natural zone ($p = 0.077$; Table 3.2). Finally, no significant difference was detected between the control inside the marina and the natural zone ($p = 0.631$; Table 3.2).

3.3. Biodiversity

The number of fish taxa varied according to the different areas as can be seen in Figure 3.5, where the Natural Zone was the zone with the highest number of fish taxa (36), followed by the Biohuts zone with 23 fish taxa. With just two less fish taxa, the Control Zone Outside Marina had a total of 21 fish taxa (Table 3.5). The Control Zone Inside the Marina obtained a

substantially lower number of fish taxa than the other zones with only 5 fish taxa recorded (Figure 3.5).

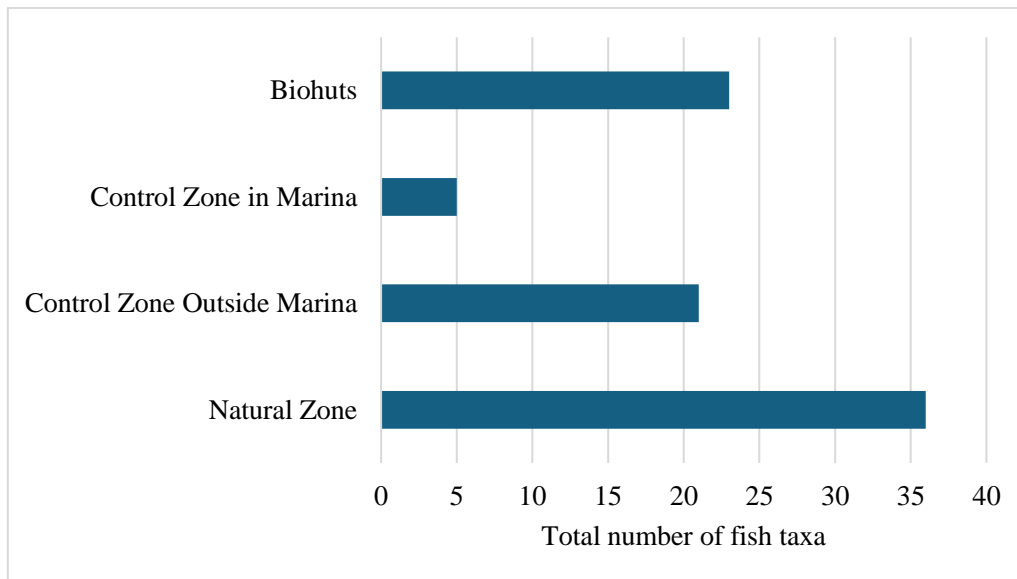


Figure 3.5. Comparison of Fish Taxa Diversity in the Biohuts and the control zones.

3.3.1. Simpson and Shannon Diversity Indices

Considering the Simpson Diversity Index and the Shannon Diversity Index and the different zones, the Natural Zone has the highest Simpson Diversity Index (0.817), followed by the Biohuts (0.655) and Control Zone (0.590; Table 3.3). The Control Zone in Marina has the lowest Simpson Diversity Index (0.557; Table 3.3).

Table 3.3. Simpson and Shannon Indices in the Biohuts and the Control Zones.

Zones	Simpson Diversity Index	Shannon Diversity Index
Biohuts	0.655	1.362
Control Marina	0.557	0.907
Control Zone	0.590	1.352
Natural Zone	0.817	2.117

Similar to the Simpson Diversity Index, the Natural Zone has the highest Shannon Diversity Index (2.117), followed by the Biohuts (1.362) and Control Zone (1.352; Table 3.3). The Control Marina has the lowest Shannon Diversity Index (0.907; Table 3.3).

Relatively different values for Simpson Diversity Index and Shannon Diversity Index were also obtained between the multiple transect zones in the Biohuts. For the Simpson Diversity Index the Biohut zone A had the highest value with 0.672 followed by the zone C (0.634) and the zone B (0.571; Table 3.4).

Table 3.4. Simpson and Shannon Indices in the 3 different Biohut zones

Biohuts	Simpson Diversity Index	Shanon Diversity Index
A	0.672148203	1.404699849
B	0.570547236	1.002322166
C	0.633747931	1.398051849

When considering the Shannon Diversity Index, it was observed that the Biohut transect zone A was the one with the highest index (1.405; Table 3.4). The second highest Shannon diversity index value was the Biohut transect zone C (1.398), while the zone that got the smallest value was zone A with 1.002 (Table 3.4).

Taking into account the Control Zone Inside the Marina, the differences between the different transect areas concerning The Simpson Diversity Index and the Shanon Diversity Index were rather small (Table 3.5).

Table 3.5. Simpson and Shannon Indices in the 3 different Control Zone Inside the Marina transect zones.

Control Zone Inside the Marina	Simpson Diversity Index	Shannon Diversity Index
CM1	0.545	0.885
CM2	0.531	0.905
CM3	0.536	0.845

The transect zone CM1 was the zone that displayed the highest Simpson Diversity Index value (0.545), followed by CM3 (0.536) and CM2 (0.531; Table 3.5). As for the Shannon Diversity Index in these three different transect Control Zones Inside the Marina different results were obtained. The highest Shannon Diversity Index value was 0.905 which corresponds to the CM2 transect zone, the second highest was CM1 (0.885; Table 3.5). The zone that got the smallest value in terms of the Shannon Diversity Index was CM3 with 0.845 (Table 3.5).

Regarding the Control Zone Outside the Marina and the Simpson Diversity Index, it was seen that the Control Zone Outside the Marina B had the smaller values both for the Simpson Diversity Index (0.519) and for the Shannon Diversity Index (1.189), while the zone A obtained 0.723 for the Simpson Diversity Index and 1.598 for the Shannon Diversity Index (Table 3.6).

Table 3.6. Simpson and Shannon Indices in the Control Zone Outside the Marina transect zones.

Control Zone Outside The Marina	Simpson Diversity Index	Shannon Diversity Index
A	0.723	1.598
B	0.519	1.189

Among the six Natural Zone zones, 6, 5, and 3 exhibited the highest Simpson and Shannon Diversity Indices. Specifically, zone 6 recorded the highest values for both indices (Simpson: 0.883, Shannon: 2.187), followed by zone 5 (Simpson: 0.809, Shannon: 2.063) and zone 3 (Simpson: 0.770, Shannon: 1.864; Table 3.7).

Table 3.7. Simpson and Shannon Diversity Indices in the different Natural transect zones.

Natural Zone	Simpson Diversity Index	Shannon Diversity Index
1	0.423	1.028
2	0.719	1.754
3	0.770	1.864
4	0.649	1.353
5	0.809	2.063
6	0.831	2.187

On Natural Zone 1 the lowest values were obtained (Simpson: 0.423, Shannon: 1.028), Natural Zone 4 had higher values when compared to Zone 1 (Simpson: 0.649, Shannon: 1.353). Natural

Zone 2 obtained 0.719 for the Simpson Diversity Index and 1.754 for the Shannon Diversity Index (Table 3.7).

3.4. Recruitment

The recruitment data reveals considerable variation among species, with some taxa showing high recruitment numbers while others are represented by very few individuals (table 3.8). According to the Kruskal-Wallis test significant statistical differences were obtained between the distribution of the number of recruits by the different species where recruitment was observed ($\chi^2 = 408.15$, $df = 13$, $p < 0.001$).

There was a total of 18,868 recruits distributed by 14 fish taxa, with Mugilidae having the highest number of recruits with 6,801 individuals. *Diplodus sargus* also showed a substantial number of recruits, with 5,263 individuals, making it the second most abundant fish taxa in terms of recruitment (Table 3.8). *Atherina* spp. exhibited a significant recruitment with 5,016 individuals, further highlighting its strong presence (Table 3.8).

Table 3.8. Number of Recruits per Fish Species.

Species	Number of Recruits
Mugilidae	6801
<i>Diplodus sargus</i>	5263
<i>Atherina</i> spp.	5016
<i>Sarpa salpa</i>	862
<i>Diplodus puntazzo</i>	410
<i>Pomadasys incisus</i>	184
<i>Sardina pilchardus</i>	113
<i>Oblada melanura</i>	82
<i>Diplodus cervinus</i>	54
<i>Trachurus trachurus</i>	36
<i>Diplodus vulgaris</i>	22
<i>Lithognathus mormyrus</i>	21
<i>Symphodus melops</i>	3
<i>Ctenolabrus exoletus</i>	1

In contrast, species such as *Sarpa salpa* (862 individuals) and *Diplodus puntazzo* (410 individuals) showed moderate recruitment when compared to the top fish taxa recruiters (Table

3.8). Several species exhibited relatively low recruitment, *Diplodus vulgaris* had only 22 recruits, *Lithognathus mormyrus* had 21 recruits, and *Trachurus trachurus* had 36 recruits (Table 3.8). *Symphodus melops* (3 individuals) was also sparsely represented (Table 3.8).

The species *Ctenolabrus exoletus* (1 individual), had the lowest number of recruits (Table 3.8). *Oblada melanura* and *Sardina pilchardus* had 82 and 113 individuals, respectively, showing slightly better recruitment but remaining on the lower end of the spectrum (Table 3.8). Another species that had similar results was *Pomadasys incisus* which recorded 184 recruits.

3.4.1. Recruitment by months

The Figure 3.6. presents the fish recruitment throughout the specified period. Commencing in September 2023, the recruitment was initiated with a relatively modest influx of new individuals. However, as the months progressed, a steady upward trajectory became apparent (Figure 3.6). October and November witnessed increases in recruitment numbers, albeit at a gradual pace (Figure 3.6). December experienced a minor downturn, marked a more pronounced surge, signaling a transition toward a more robust recruitment phase (Figure 3.6). While January marked a more pronounced surge, with the overarching upward trend persisted (Figure 3.6).

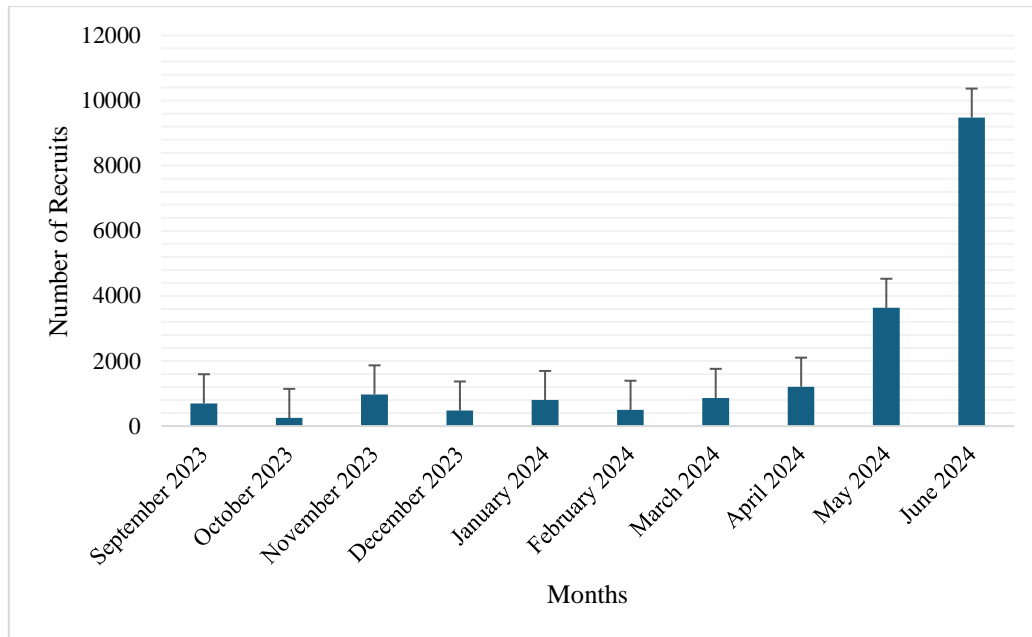


Figure 3.6. Seasonal Variation in Fish Recruitment

February and March observed further increments in recruitment, at a more subdued rate (Figure 3.6). April displayed a renewed rise in terms of number of recruits, setting for a considerable large increase in May and June (Figure 3.6). The month of June recorded the highest value with a total of 9489 recruits recorded during that month (Figure 3.6).

According to the Kruskal-Wallis chi-squared test, these differences between the distribution of the number of individuals by the different months were statistically significant ($\chi^2 = 221.15$, $df = 9$, $p < 0.001$; Figure 3.6).

The distribution of fish recruitment across different species from September 2023 to June 2024 was analyzed using a Kruskal-Wallis test (Figure 3.7). The results indicated statistically significant differences in recruitment patterns among the species across the months, as demonstrated by the Kruskal-Wallis chi-squared test ($\chi^2 = 115.6$, $df = 9$, $p < 0.001$).

The results presented in Figure 3.7, demonstrate the recruitment dynamics of the various fish species across different months, highlighting distinct seasonal and species-specific patterns. *Atherina* spp. exhibited recruitment during all the months with fluctuations in terms of number of recruits throughout the observation period (Figure 3.7). Recruitment peaked in November 2023 with 673 recruits, followed by a reduction and another marked increase in March 2024, reaching 490 recruits (Figure 3.7). This species displayed a sharp rise in May 2024 with 536 recruits before slightly declining to 302 in June 2024 (Figure 3.7).

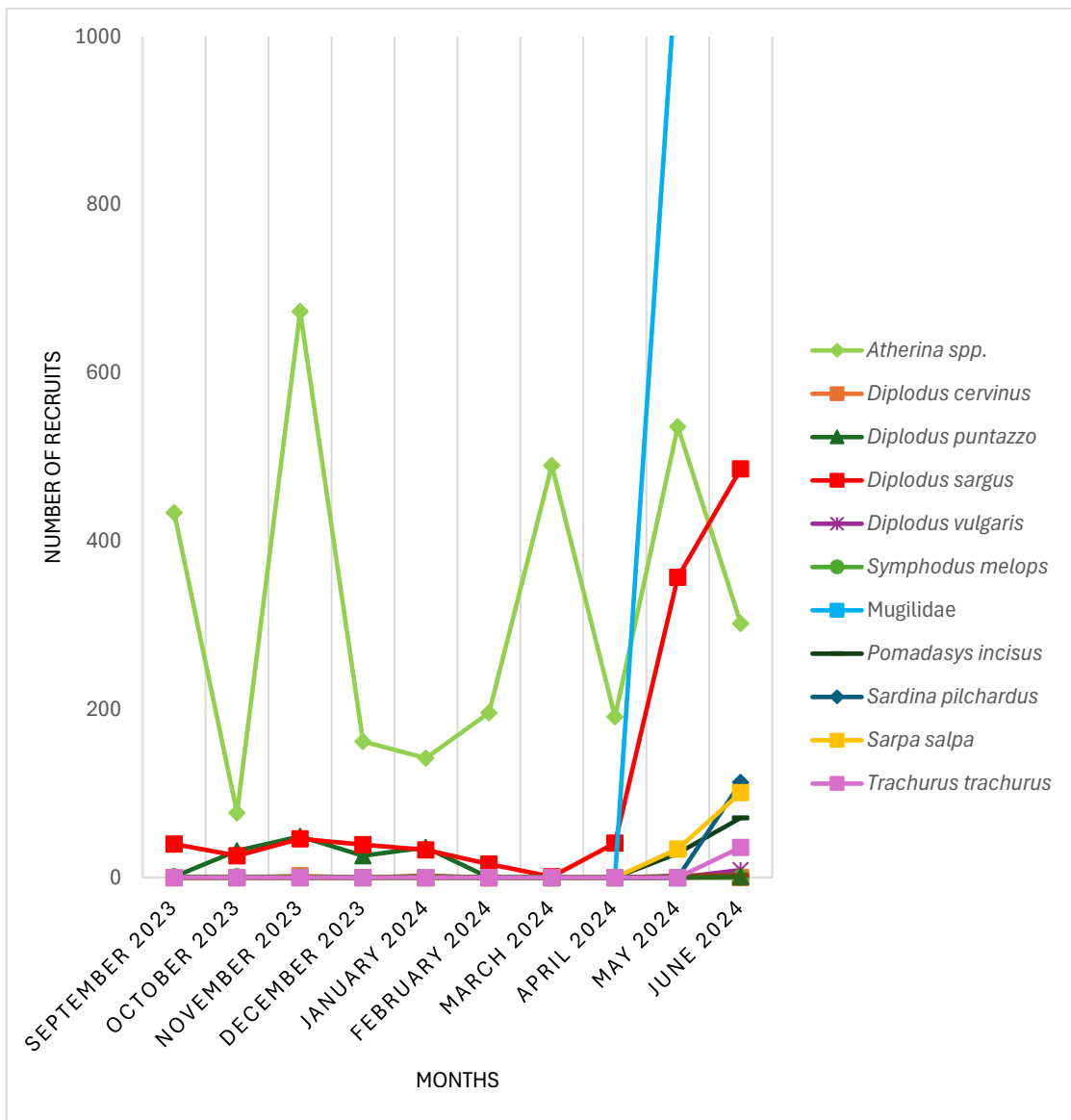


Figure 3.7. Monthly fish recruitment by species from September 2023 to June 2024

Diplodus cervinus recruitment was absent in all the months except for November 2023 where it exhibited only two recruits (Figure 3.7). *Diplodus puntazzo* showed intermittent recruitment, with some peaks early in the observation period, this species recruitment was seen in September 2023 (1 recruit), October 2023 (32 recruits), November 2023 (49 recruits), December 2023 (26 recruits), January 2024 (36 recruits) and on June 2024 (2 recruits). *Diplodus sargus* demonstrated a more consistent presence throughout all the months, with a notable increase in recruitment in May 2024 and June 2024, where 357 recruits and 486 recruits were respectively

recorded (Figure 3.7). The months where *Diplodus sargus* had less recruitment were February 2024 with 16 recruits and March 2024 with only 1 recruit (Figure 3.7).

The species *Diplodus vulgaris* was absent from the recruitment data until June 2024, when 9 recruits were recorded (Figure 3.7). *Symphodus melops* exhibited a comparatively very low recruitment, with only one recruit detected in September, October, and November 2023, and no further presence for the remainder of the study period (Figure 3.7).

According to the figure 3.7. Mugilidae displayed a dramatic spike in recruitment in May 2024, with 1,107 recruits, followed by an even larger increase in June 2024, reaching 4,776 recruits (Figure 3.7). In the rest of the months, no recruitment was verified for Mugilidae (Figure 3.7).

Pomadasys incisus exhibited no recruitment until May and June 2024, when 29 and 71 recruits were recorded, respectively (Figure 3.7). *Sardina pilchardus* was similarly absent for most of the study period with recruitment only being observed in June 2024 with 113 recruits (Figure 3.7). *Trachurus trachurus* was also absent until June 2024, when 36 recruits were recorded (Figure 3.7). The species *Sarpa salpa* only displayed recruitment more towards the end of the study period, first appearing in May 2024 with 34 recruits and increasing to 101 recruits in June 2024 (Figure 3.7).

3.4.2. Recruitment by zones

The recruit density data for the different zones indicates notable differences among the various zones studied (Table 3.9). The Biohuts recorded the highest recruit density at 1.395 individuals per cubic meter, the Natural Zone showed a slightly lower but still relatively high recruit density of 1.296 individuals per cubic meter (Table 3.9).

In contrast, the Control Zone Inside the Marina had the lowest recruit density at 0.073 individuals per cubic meter (Table 3.9). The Control Zone Outside the Marina exhibited a moderate recruit density of 0.754 individuals per cubic meter, which is higher than the Inside Marina control but lower than the Biohuts and Natural Zone (Table 3.9).

Table 3.9. Recruitment Density in Different Zones

Recruitment	Recruits Density
Biohuts	1.395
Control Zone Inside Marina	0.073
Control Zone Outside Marina	0.754
Natural Zone	1.296

A Kruskal-Wallis test was conducted and statistically significant differences in the distribution of recruit density was seen among the four zones: Biohuts, Control Zone Inside Marina, Control Zone Outside Marina, and Natural Zone ($\chi^2 = 68.792$, $df = 3$, $p < 0.001$; Table 3.9). Indicating significant differences in recruit density across these zones (Table 3.9).

When comparing Biohuts to other zones, a highly significant difference was observed between Biohuts and the Control Inside the Marina ($p = 0.010$), indicating notable differences in recruitment patterns between these zones. Furthermore, there is an even more pronounced significant difference between Biohuts and Control Outside the Marina ($p < 0.001$), with the same being seen between Biohuts and the Natural zone ($p < 0.001$).

The comparison between the Control Inside the Marina and the Control Outside the Marina did not show a significant difference ($p = 0.1635$). Additionally, no significant difference was found between the Control Inside the Marina and the Natural zone ($p = 0.529$). Comparing the Control Outside the Marina to the Natural zone also resulted in a significant difference ($p = 0.036$).

3.4.3. Species Recruitment in the different transect areas

The table 3.10 presents data on the recruitment of different fish taxa within Biohuts, expressed as the number of recruits per cubic meter. A total of 11 species are represented, highlighting the diversity of fish in the Biohut zones (Table 3.10).

Among the species recorded, the Mugilidae family shows the highest recruitment density, with 0.7660 recruits per cubic meter, accounting for more than half of the total observed recruitment (Table 3.10). *Atherina* spp., represents the second most abundant fish taxa within the Biohuts zones, with a recruitment density of 0.4171 per cubic meter (Table 3.10). *Diplodus sargus*, has

a recruitment density of 0.1413 per cubic meter, making it the third most abundant species in the Biohuts (Table 3.10). In contrast, other *Diplodus* species, such as *Diplodus vulgaris*, *Diplodus puntazzo* and *Diplodus cervinus* displayed lower recruitment densities of 0.0012, 0.0190 and 0.0003 recruits per cubic meter, respectively (Table 3.10).

Table 3.10. Recruit Density of Fish Species in Biohuts

Biohuts	
Species	Number of recruits per cubic meter
Mugilidae	0.7660
<i>Diplodus sargus</i>	0.1413
<i>Diplodus vulgaris</i>	0.0012
<i>Sarpa salpa</i>	0.0176
<i>Atherina</i> spp.	0.4171
<i>Diplodus puntazzo</i>	0.0190
<i>Diplodus cervinus</i>	0.0003
<i>Sardina pilchardus</i>	0.0147
<i>Pomadasys incisus</i>	0.0130
<i>Trachurus trachurus</i>	0.0047
<i>Symphodus melops</i>	0.0004
Total	1.3952

According to table 3.10, there was also recruitment on the species *Sarpa salpa* with recruitment densities of 0.0176 per cubic meter, respectively. In the Biohuts areas recruitment was also seen in the species *Sardina pilchardus*, *Pomadasys incisus*, and *Trachurus trachurus*, with recruitment densities of 0.0147, 0.0130, and 0.0047 recruits per cubic meter (Table 3.10). *Symphodus melops* was the species with the lowest recruitment densities in the Biohuts, 0.0004 per cubic meter (Table 3.10).

Table 3.11 presents data on the recruitment of fish species within the control zone located inside the marina, expressed as the number of recruits per m³. Only three fish taxa were recorded: Mugilidae, *Diplodus sargus*, and *Atherina* spp., with the total recruitment density being 0.0734 recruits per unit volume (Table 3.11).

Table 3.11. Recruit Density of Fish Species in the Control Zone Inside Marina.

Control Zone Inside Marina	
Species	Number of recruits per cubic meter
Mugilidae	0.0464
<i>Diplodus sargus</i>	0.0078
<i>Atherina</i> spp.	0.0193
Total	0.0734

In the control zone inside the marina and according to table 3.11 it was seen that Mugilidae is the most abundant taxa, with a recruitment density of 0.0464 per cubic meter. *Atherina* spp. follows as the second most abundant species, exhibiting a recruitment density of 0.0193 per cubic meter (Table 3.11). *Diplodus sargus*, with a recruitment density of 0.0078 per cubic meter, is also present in the control zone, though in smaller numbers (Table 3.11).

Regarding the control zones located outside the marina, a relatively diverse community of juvenile fish species was seen, with a total of nine species recorded and a combined recruitment density of 0.7542 recruits per cubic meter (Table 3.12). Among the species observed, *Atherina* spp. has the highest recruitment density, with 0.3232 recruits per cubic meter, followed by the Mugilidae, with a recruitment density of 0.1927 per unit cubic meter (Table 3.12).

Table 3.12. Recruit Density of Fish Species in the Control Zone Outside Marina

Control Zone Outside Marina	
Species	Number of recruits per cubic meter
Mugilidae	0.1927
<i>Diplodus sargus</i>	0.1750
<i>Diplodus vulgaris</i>	0.0031
<i>Sarpa salpa</i>	0.0112
<i>Atherina</i> spp.	0.3232
<i>Diplodus puntazzo</i>	0.0094
<i>Diplodus cervinus</i>	0.0010
<i>Oblada melanura</i>	0.0167
<i>Pomadasys incisus</i>	0.0218
Total	0.7542

Diplodus sargus also displayed a relatively high recruitment density of 0.1750 per cubic meter, underscoring the suitability of the control zone outside the marina for this species (Table 3.12). Other *Diplodus* species displayed recruitment in the control zone outside the marina, such as *Diplodus vulgaris*, *Diplodus puntazzo*, and *Diplodus cervinus*, are also present, though in much lower densities respectively, 0.0031, 0.0094, and 0.0010 recruits per cubic meter (Table 3.12).

Sarpa salpa and *Oblada melanura* were present in moderate numbers, with recruitment densities of 0.0112 and 0.0167 recruits per cubic meter, respectively. *Pomadasys incisus* showed a slightly higher recruitment density, with 0.0218 recruits per cubic meter (Table 3.12).

Considering the recruitment densities of fish taxa in the natural zone, a diverse fish community was observed, with a total recruitment density of 1.296 recruits per unit cubic meter distributed among 9 different fish taxa (Table 3.13). The most abundant species in this zone was *Diplodus sargus*, which exhibited a notably high recruitment density of 0.9052 recruits per cubic meter, indicating its significant presence in the ecosystem (Table 3.13). *Sarpa salpa*, another fish taxa in the natural zone, was the second most abundant, with a recruitment density of 0.1781 recruits per cubic meter (Table 3.13).

Table 3.13. Recruit Density of Fish Species in the Natural Zone

Natural Zone	
Species	Number of recruits per cubic meter
<i>Diplodus sargus</i>	0.9052
<i>Diplodus vulgaris</i>	0.0003
<i>Sarpa salpa</i>	0.1781
<i>Atherina</i> spp.	0.1297
<i>Diplodus puntazzo</i>	0.0594
<i>Diplodus cervinus</i>	0.0125
<i>Ctenolabrus exoletus</i>	0.0003
<i>Oblada melanura</i>	0.0047
<i>Lithognathus mormyrus</i>	0.0055
Total	1.2956

The species *Atherina* spp. was also relatively well-represented, with a recruitment density of 0.1297 recruits per cubic meter, highlighting their role within this marine environment (Table

3.13). *Diplodus puntazzo* was present at a density of 0.0594 recruits per cubic meter, further demonstrating the dominance of the *Diplodus* genus in this zone (Table 3.13). *Lithognathus mormyrus*, had a recruitment density of 0.0055 recruits per cubic meter (Table 3.13).

Several species were present at low densities, including *Diplodus vulgaris* (0.0003 recruits per cubic meter), and *Ctenolabrus exoletus* (0.0003 recruits per cubic meter), which suggests their limited but persistent presence in the natural zone (Table 3.13). *Oblada melanura* had also a considerably low value with a recruitment density of 0.0055 recruits per cubic meter (Table 3.13).

3.4.4. Recruitment in the different transect areas

The analysis of recruit percentages in different Biohut transect zones (Figure 3.8) shows that Biohut zone C accounted for the highest proportion, with 49.44% of the total recruits, representing 11 species and 5,298 individuals. Biohut zone B follows with 41.95% of the recruits, consisting of 4 species and 4,495 individuals. Biohut zone A, was the least abundant zone in terms of recruitment, with 8.60% of the recruits, comprising 4 species and 922 individuals (Figure 3.8).

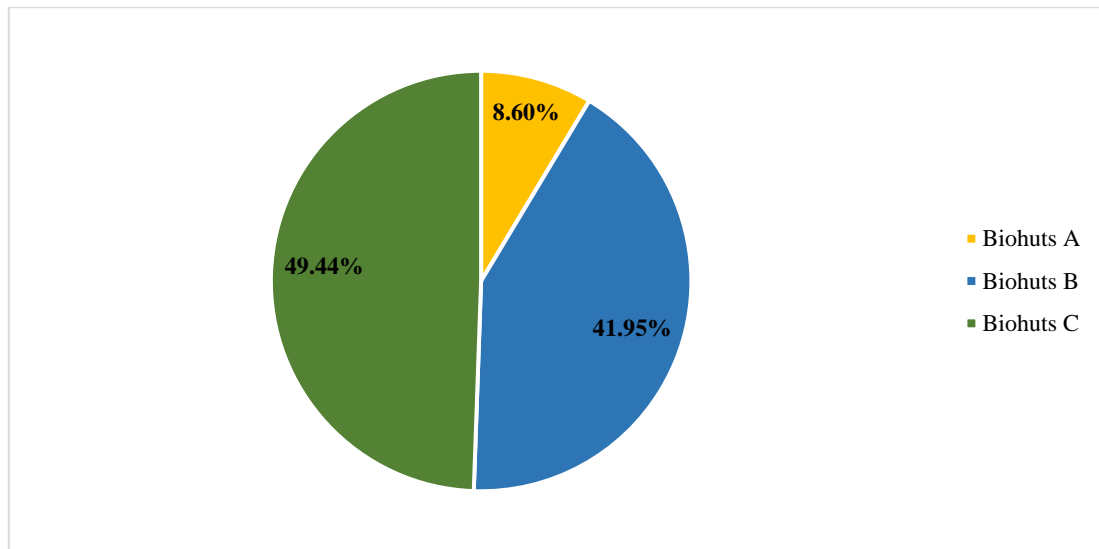


Figure 3.8. Comparison of Recruits Percentage in the Different Biohut transect zones.

A Kruskal-Wallis chi-squared test reveals a significant difference among the three Biohut zones in terms of number of recruits ($\chi^2 = 10.89$, $df = 2$, $p = 0.004$). Pairwise comparisons using the Wilcoxon rank sum test further highlight significant differences between zones A and B ($p = 0.002$) and between zones A and C ($p = 0.005$), while no significant difference is observed between zones B and C ($p = 0.242$).

Figure 3.9 compares the recruit percentages in the control transect zones outside the marina. Control B represents the majority, with 80.97% of the recruits, consisting of 9 species and 2,345 individuals. In contrast, Control A accounts for 19.03% of the recruits, with 6 species and 551 individuals (Figure 3.9).

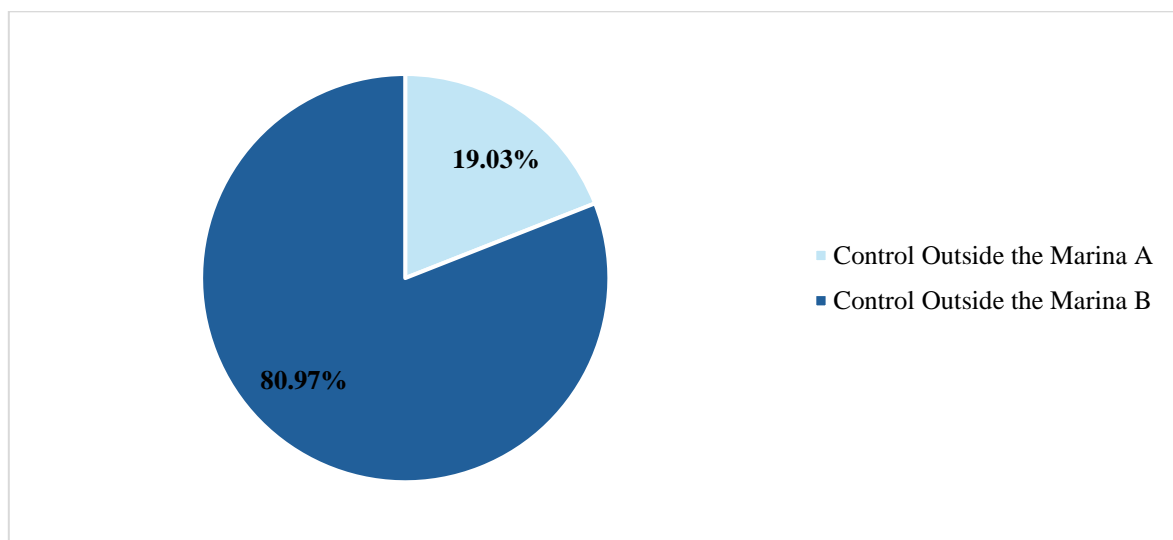


Figure 3.9. Comparison of percentage of recruits in the Control Outside the Marina transect zones.

A Kruskal-Wallis test was conducted to test the differences between the two control zones outside the marina, resulting in a chi-squared value of 0.674 with 1 degree of freedom and a p-value of 0.412. This result indicates no significant difference in recruit densities between the zones Control Outside the Marina A and Control Outside the Marina B (Figure 3.9).

3.4.5. Environmental variables and the recruitment

The scatter plot presented in the figure 3.10., demonstrates the relationship between visibility (in meters) and the number of recruits. The data points exhibit a wide dispersion, with a majority clustering at lower visibility values between 0 and 3 meters (Figure 3.10).

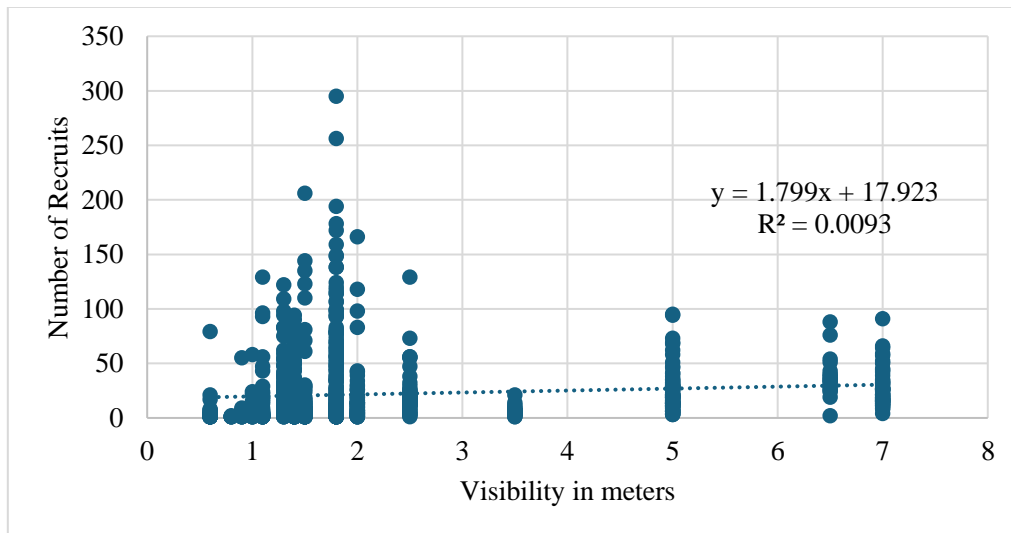


Figure 3.10. Correlation Between Visibility and Number of Recruits

The distribution of points shows that recruit numbers are highest when visibility is around 1 to 2 meters, with several sightings exceeding 100 recruits (Figure 3.10). Beyond 2 meters of visibility, recruit numbers generally decrease, with fewer instances of high recruit numbers. The trend line fitted to the data has a positive slope with the equation $y = 1.799x + 17.923$ and an R^2 value of 0.0093, indicating a very weak correlation between visibility and the number of recruits (Figure 3.10).

The relationship between the swell height outside the marina (in meters) and the number of recruits can be seen in the scatter plot present in figure 3.11. The y-axis represents the number of recruits, ranging from 0 to over 300, while the x-axis shows swell heights ranging from 0 to 2.5 meters (Figure 3.11).

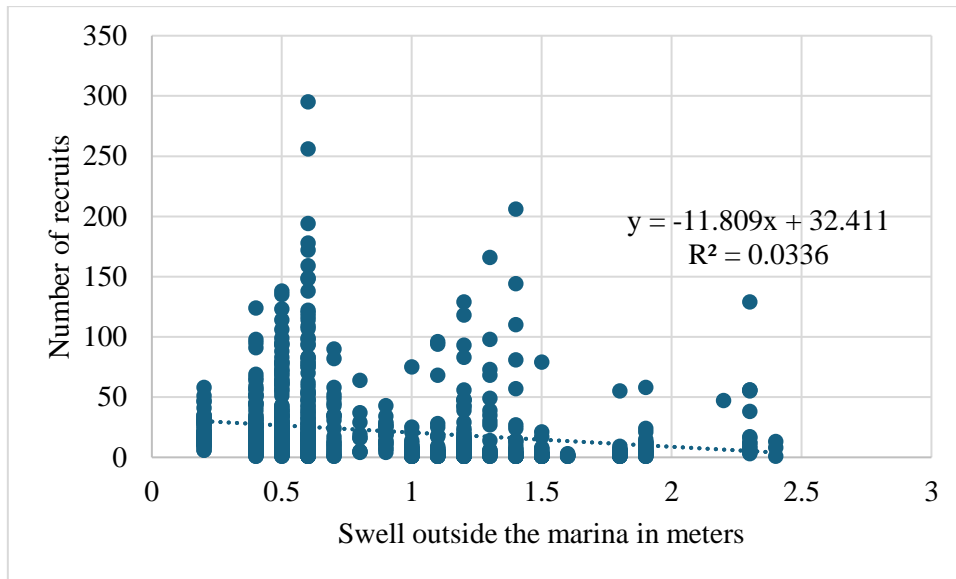


Figure 3.11. Correlation Between Swell Outside the Marina and the Number of Recruits

The data points show clustering at lower swell heights, particularly between 0 and 1 meter, where recruit numbers frequently exceed 50 and occasionally surpass 100 recruits (Figure 3.11). There are several outliers, including one point where the number of recruits exceeds 300 at a swell height of approximately 0.5 meters (Figure 3.11).

The fitted trend line has a negative slope, represented by the equation $y = -11.809x + 32.411$, indicating a general decline in the number of recruits as swell height increases (Figure 3.11). The R^2 value of 0.0336 suggests a weak negative correlation (Figure 3.11).

The scatter plot illustrates the relationship between water temperature (in degrees Celsius) and the number of recruits (Figure 3.12). The y-axis represents the number of recruits, which ranges from 0 to over 300, while the x-axis shows water temperature, spanning from 15 to 21 degrees Celsius (Figure 3.12).

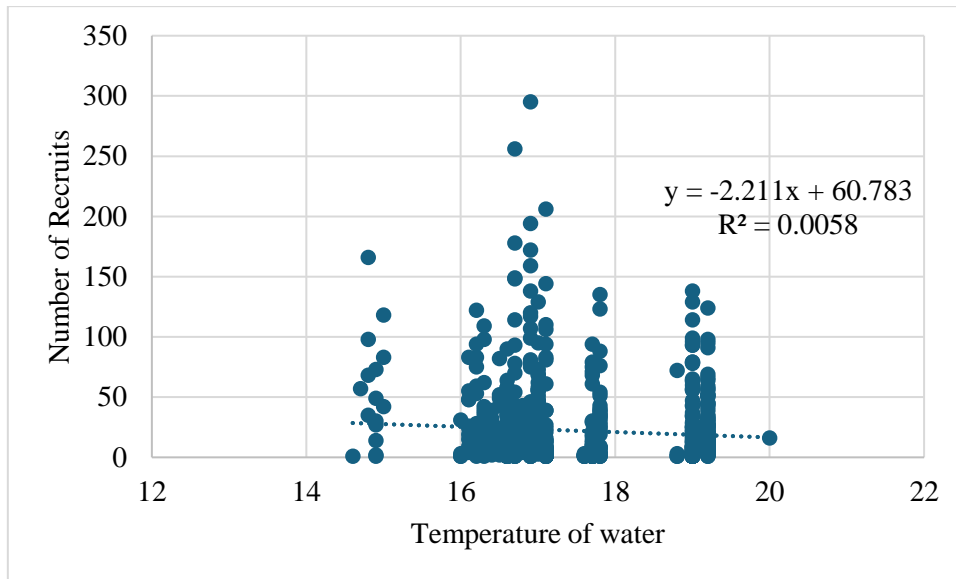


Figure 3.12. Correlation Between Temperature of the Water and the Number of Recruits

The data points are predominantly clustered between temperatures of 15 and 18 degrees Celsius, where the number of recruits frequently varies from 0 to around 50, with some notable outliers reaching or exceeding 100 recruits (Figure 3.12). A few extreme points even show recruit numbers almost surpassing 300 recruits at temperatures slightly above 15 degrees Celsius, highlighting considerable variability in recruitment at lower temperature ranges within this spectrum (Figure 3.12).

The fitted trend line has a negative slope, described by the equation $y = -2.211x + 60.783$, indicating a slight decrease in the number of recruits as water temperature increases (Figure 3.12). However, the correlation is very weak, with an R^2 value of 0.0058 (Figure 3.12).

3.5. Other observations

Apart from the fishes many other organisms including species of Crustaceans, Ascidacea, Bivalves, Cnidaria, Echinoidea and Polychaeta were observed on the Biohuts. Some of the Biohuts were partially covered by algae, mostly Chlorophyta. The pontoons and pillars inside the marina mainly had some crustaceans and bivalves, but not as many as the Biohuts. Green algae were the most abundant and partially covered the pontoons. On this subject, the control outside the marina was more similar to the biohut areas, however, no Echinoidea were seen in

this area. Crustaceans, Polychaeta and Ascidacea were considerably less common in this area when compared with the biohut area.

The natural area was by far the one with the most diversity of benthic organisms, with the rocks being almost completely covered with crustaceans, bivalves and algae. However, in some parts of the transect there was a considerable quantity of the invasive algae *Regulopterix okumurae* covering the rocks. This algae was also presented in the water column in big aggregations which reduced the visibility.

Rocks in the natural area had a lot of rugosity and were full of barnacles, while the rocks in the control zone outside and inside the marina did not have many porosities and presented rather flat surfaces. In the control zone outside the marina on most of the days fouling was observed on top of the rocks and when in suspension would often reduce the overall visibility. This was also the case inside the marina but mostly due to particles and suspension. In two of the days there was a considerable amount of fouling on top of the biohuts and in the Marina structures overall.

In terms of ecosystem functions, various fish species were observed seeking refuge on the Biohuts or feeding on benthic organisms present on the Biohut structures. *Parablennius gattorugine* was seen feeding upon tube worms and small *Mytilus* sp. with *Diplodus sargus* first opening the shell of *Mytilus edulis*. Also, *Palaeomon elegans* were very abundant in the Biohut areas and was seen being preyed upon by many fish species.



Figure 3.13. *Parablennius pilicornis* guarding the nesting area inside the inner cage of the Biohut.

Fish from the Mugilidae family congregated around the Biohut areas, while juvenile *Diplodus sargus* were observed removing parasites from the Mugilidae scales, showcasing a cleaning station ecosystem service. Although recruitment was not detected in the last two underwater visual censuses, two *Parablennius pilicornis* nesting areas were seen on top of the Biohuts (Figure 3.13).

Of the many predator-prey interactions observed, one most frequently seen inside and outside the marina while doing the underwater visual census, was *Dicentrarchus labrax* and *Dicentrarchus puntactus* preying upon *Atherina sp.* In the natural area, probably the most frequent predator-prey interactions was between *Dicentrarchus labrax* and *Sardina pilchardus*.

Some birds (*Ardea cinerea*, *Egretta garzetta* and *Phalacrocorax carbo*) were seen preying inside and around the marina area, but only *Phalacrocorax carbo* was seen hunting near the biohut areas. No interactions of this type were seen in the natural area.

4. Discussion

4.1. Abundance

The present study results suggest that there are statistically significant differences in the abundance between the 4 different areas, and consequently between some of the transect zones (Table 3.2; Figure 3.4). The Biohuts affected positively fish density, being almost 10 times greater than the control zone inside the marina (Table 3.1; Figure 3.4). Multiple factors might explain the difference between the control zone inside the Marina and the Biohuts.

Introducing artificial structures increases the complexity of the habitat, which might boost fish abundance (Gratwicke & Speight, 2005). Since multiple organisms were present in the Biohuts, it can support more ecosystem functions for this habitat and offer more resistance to these communities against invasive species (Lyons & Schwartz, 2001; Stachowicz et al., 2002; Gamfeldt et al., 2015), therefore contributing to a more resilient ecosystem (Stachowicz et al., 2002).

Habitat complexity plays an important role in variations of abundance, with more complex habitats having more abundance of fish than less complex habitats (Gratwicke & Speight, 2005; Ferrari et al., 2018). The same principle was seen in this study; installing the Biohuts increased the fish density in those areas, with this area having a statistically significant greater fish abundance per volume than the control zones inside and outside the marina.

Changes that occurred to the marina and the surrounding area are mainly due to urbanization and some other anthropogenic actions, as is the case of spillways, particularly the spillway of the wastewater treatment plant. These factors might partially explain why lower values were obtained in terms of fish density on both types of control zones in the marina area, when compared with the natural and the Biohut area (Jackson, 2001; Emslie et al., 2014). However, the control zone outside the marina had a higher fish density when compared to the control inside the marina (Table 3.1). This was expected, as the structures inside the marina (less habitat complexity) do not provide as much refuge or surface area as the rocks in the control zones outside the marina, which normally affects fish abundance and biodiversity (Johnson et al., 2003; Gratwicke & Speight, 2005; Ferrari et al., 2018).

Habitat complexity and communities take time to develop, the Biohuts were installed rather recently only in February of 2023 (Kovalenko et al., 2012). If the Biohuts were immersed for a longer period (years) there was a possibility that the results in terms of abundance, diversity

and recruitment would be even better. According to literature, this was already seen, the immersion period of the Biohuts affected the abundance and the diversity of species, with Biohuts that are subjected to longer immersion periods being the ones with higher biodiversity and abundance (Varenne et al, 2023)

The natural area is being affected by the presence of *Rugulopteryx okamurae* which is an invasive brown algae species in the study area, that is known for having negative consequences for the abundance of fish (García-Gómez et al., 2020; Liulea et al., 2023). However, as would be expected the natural area had a considerably larger fish density than the other zones, as this habitat is more pristine with fewer disturbances. (Gratwicke & Speight, 2005; Ferrari et al., 2018).

4.2. Species diversity

When comparing the biodiversity with the abundance similar results were obtained; the area with fewer disturbances (natural zone) had the largest number of taxa (Figure 3.5). This therefore suggests that it is probably the more stable habitat in terms of ecosystem functions (Loreau et al., 2001).

The impact of the urbanization of the coastal areas and other human-related disturbances might be one of the reasons for the difference in the number of taxa present in the natural and the Control zone in the marina. This difference might be explained however by the fact that estuarine ecosystems normally are considered as having low biodiversity (Attrill et al., 1996). The Biohuts and the control zone outside the marina displayed a considerable number of species in common and a slight number of species with the natural area, which may indicate the biohut area has somewhat more similar habitat functions with the control area than the natural area (Gamfeldt et al., 2015).

The Natural Zone shows the highest levels of biodiversity, with a Simpson index of diversity Index of 0.817 and a Shannon Diversity Index of 2.117, indicating a well-balanced and diverse fish community with a low level of dominance, since the Simpson index of diversity is far from 0 (Table 3.3). This high diversity suggests that the Natural Zone is likely the most suitable habitat for more fish recruitment, providing a stable and resource-rich environment where juvenile fish can thrive (Tilman, 1999).

While the Biohuts do not replicate the natural zone's species diversity, they still provide a relatively supportive environment for fish recruitment (Table 3.3). However, the species diversity might have been greater if the Biohuts had already been installed for a longer period before the study (Varenne et al, 2023). The presence of moderate species diversity according to both indexes present in Table 3.3 suggests that the Biohuts may serve as a functional, though artificial, refuge for recruiting fish, potentially enhancing fish abundance, biodiversity and recruitment in areas where the natural habitat is lacking (Kovalenko et al., 2012).

The Control Zone outside the marina according to Table 3.3 showed a biodiversity level lower but similar to the Biohut zone, with a Simpson Index of 0.590 and a Shannon Index of 1.352, with the rock substrate probably providing some availability of food and shelter. However, there was a bigger difference in terms of both indexes between the Control zone Inside the marina and the Biohut zone, despite the proximity of the Control inside the marina to the Biohut zone (Table 3.3). Highlighting the difference that the artificial nurseries make in habitat complexity and therefore in boosting fish diversity. In a general overview of this study, and only considering fish diversity, the natural zone emerges as the most suitable zone for having more fish recruitment, followed by the Biohuts (Table 3.3). While the control zone inside the marina appears to be the least favorable for recruiting fish.

Upon comparing the biodiversity of the three biohuts zones (A, B and C) and according to the biodiversity indexes it was seen that zone A and C had very similar results, with zone A having slightly larger values than C (Table 3.4). Indicating that they support a fish community with greater biodiversity and equitability compared to zone A.

The fact that Biohut zone B had lower index values might be partially attributed to marine traffic in this area. When boats pass the artificial nurseries, particularly at higher speed, the waves agitate the Biohuts, which exposes them to greater disturbances, ultimately affecting their biodiversity, some species have a preference for less disturbed areas (Abdulla & Linden, 2008; Celi et al., 2016; Weilgart, 2018).

However, from what was seen during the underwater visual census zone A also suffers from marine traffic disturbance, and for that reason another factor that might explain this difference is that zones A and C are somewhat near the rocky shoreline of the marina, while zone B is more distant and more exposed, which might result in this area not being so attractive for some species (Johnson et al., 2003). Another important factor that might explain why Biohut zone A,

had greater values regarding the diversity indexes may also be related to this area being the one nearest to the lower reaches of the river and species diversity is known for decreasing from the mouth to the upper reaches of the estuaries (McLusky, 1971; Attrill et al., 1996).

Regarding the control inside the Marina, no major differences were seen between the biodiversity indexes in the different transect areas as it would be expected, as the composition and complexity of the habitat in the area are the same (Table 3.5). However, when considering the two different control zones outside the Marina (A and B), bigger differences were seen both in the Simpson and Shannon indexes, with zone A having greater values (Table 3.6). This difference might be explained with the fact that when the tide rises fishes including recruits enter brackish water systems as it is the case of the estuarine area of “Ribeira of Bensafrim”. Afterwards, they start to settle at the first suitable habitats they encounter (Gratwicke & Speight, 2005). This might result in more biodiversity in A which is the most downstream area.

The fact that control zone A is closer to the mouth of the river also indicates that more species diversity should be expected as there are normally fewer environmental stresses provoked by the estuarine environment (McLusky, 1971; Attrill et al., 1996).

Since the control zone outside the Marina of Lagos is more upstream, it is implied that the water there has less salinity and therefore this might not be a suitable habitat for every species, especially the ones that are not so tolerant of brackish waters (Chilton et al., 2021; Eick & Thiel, 2014). This pattern of decreasing biodiversity of saltwater fish taxa with the progress further upstream has been seen in other studies (Chilton et al., 2021; Eick & Thiel, 2014). This might result in why there is a smaller diversity index when compared with the other zone, as can be seen in Table 3.4.

In the Natural Zone transects, there appears to be a certain tendency for species diversity to increase with distance offshore (Table 3.7). This is probably mostly related to two factors; the first is that although the surf zone provides habitat for a diversity of fishes the wave action and currents might not be ideal for every fish taxa seen during the study and particularly for the recruitment stage when the swell is strong (Watt-Pringle & Strydom, 2003; Olds et al., 2018).

The other factor is that the invasive algae *Rugulopterix okamurae* has washed ashore and sometimes large piles of this algae are seen in the intertidal area, and at the shallowest area of the subtidal area, which might represent a more inhospitable habitat for fish, particularly in the areas that are more near the shore (García-Gómez et al., 2020; Liulea et al., 2023).

Since the marina is located adjacent to a freshwater stream area, having therefore more brackish water and being a different habitat than the natural area the species composition and ecosystem functions may differ, which highlights the necessity of restoring and preserving this habitat (Thrush et al., 2013).

The findings of this study align with other literature suggesting that more complex habitats tend to have higher fish species richness and abundance than less complex ones (Gratwicke & Speight, 2005). Overall the considerable number of species observed in the Biohuts compared to the control zones within the marina evidences a positive impact in the restoration of natural habitat and might also explain the increase in fish abundance in these particular areas of the Marina. As it is described in the literature, species richness is known to play a positive role in the restoration process (Loreau et al., 2001; Zhang & Zhang, 2006).

4.3. Recruitment

Considering the recruitment there was a highly significant statistical difference between the number of recruits of the different taxa ($p < 0.001$), the most abundant taxa were the ones that had the highest number of recruits, more concretely Mugilidae, *Diplodus sargus* and *Atherina* spp. (Table 3.8). According to the literature these species are known for being abundant coastal species in Portugal, particularly tolerant to brackish water and use estuarine areas to reproduce (Marais, 1988; Arruda et al., 1991 Ribeiro et al., 2006 Abecasis et al., 2009; Ribeiro et al., 2012)

In the time scale of our study, it was seen that there was recruitment more or less throughout all the months with values never exceeding 2000 recruits, until April 2024 (Figure 3.6). After this month there was a very steep increase in recruitment which coincided with the period that more species recruitment was verified (Figure 3.6). This suggests that most species seen during the underwater visual census are late winter or early spring spawners and that is why the peaks of recruitment are in spring and early summer.

There was another small peak of recruitment in November 2023, this was the period when both *Atherina* spp. and *Diplodus puntazzo* had the biggest number of recruits (Figure 3.7). According to the literature the juveniles of *Diplodus puntazzo* are known for settling during October and November in the Northwest of the Mediterranean Sea, which agrees with the pattern displayed

in this study, further evidencing that the recruitment for this species is seasonal (Vigliola et al., 1998).

Regarding *Atherina* spp. recruitment, and according to the literature the spawning period peak for *Atherina boyeri* and *Atherina hepsetus* occurs during spring, which is relatively similar to what was seen in this study, with May and March being the second and third months with most recruitment for this genus (Fernandez-Delgado et al., 1988; Moreno et al., 2005). Although November was the month with the most recruitment for this genus, this result is not completely unexpected as in previous studies recruitment was also seen during late winter and autumn including in Portugal (Moreno et al., 2005; Pombo et al., 2005).

November 2023 was the only month where recruitment was verified for *Diplodus cervinus*, but with only two recruits almost no relations can be established for this species according to seasonality (Figure 3.7). This is also the case for other species such as *Diplodus vulgaris*, *Symphodus melops*, *Pomadasys incisus*, *Sarpa salpa* and *Sardina pilchardus*. However, since adult individuals of most of these species were not abundant during the underwater visual census, a smaller spawning and recruitment is probably to be expected which might explain these results (Barrowman & Myers, 1996).

Atherina spp. was the only taxa that had recruitment throughout all the months, while *Diplodus sargus* had recruitment except in March 2024 (Figure 3.7). However, there was a strong increase in recruitment for this species in the last three months; this seasonality was also seen in the Northwest Mediterranean sea, with the settlement of juvenile fishes of *Diplodus sargus* being detected in May and June which coincides exactly with the months where most recruitment was seen for this species in this study (Vigliola et al., 1998). The fact that recruitment for *Diplodus sargus* was also observed in the other months may also suggest that there might be other spawning periods, or can partially represent juvenile fish from the previous recruitment period that are growing in the study area (Boehlert & Mundy, 1988).



Figure 4.1. Mugilidae recruits in the Control outside the marina in May.

Mugilidae, similarly to *Diplodus sargus* had high levels of recruitment in the last three months, but no recruitment was verified during the remaining months (Figure 3.7). Although concrete data regarding the spawning period is lacking, there are signs that it occurs in the Spring with the first recruits starting to appear in April and May. This spawning period for Mugilidae was identical to the available literature for this taxa (Arruda et al., 1991; Hickling, 1970).

According to the literature the spawning period for *Sardina pilchardus* occurs mainly from October to March, starting normally in September and ending in April (Nunes et al., 2011; Basilone et al 2021). In Lagos however, recruitment was only verified for this species from May to June, which might indicate that the spawning period in the region of Algarve occurs later, or that the recruits started to settle in the study area at the end of the spawning period (Figure 3.7). Changes in the spawning period in the western and southern Iberian Peninsula are mainly related to environmental factors fluctuations have also been seen in previous studies which might suggest differences related with the spawning period of *Sardina pilchardus* may occur in the Algarve (Stratoudakis et al., 2007; Santos et al., 2018).

The recruitment between the different zones was significantly different ($p < 0.001$), this result was expected as the areas are quite different in terms of anthropogenic pressures, abiotic factors and habitat complexity. The Biohuts area, although not the natural habitat, had the highest

number of recruits per volume while the Control zone inside the marina had the lowest, which indicates the Biohuts can effectively enhance recruitment (Table 3.9).

The control zone outside the marina displayed a larger value of recruits per volume than the control inside the marina, and this suggests that despite the destruction of the natural habitat around the marina of Lagos, those areas are more suitable for fish recruitment, probably due to the rocks along the shore which offer some refuge and foraging areas for some fish species (Cheminée et al., 2016).

The Natural zone had a slightly lower density of recruits than the Biohuts zone, but considerably larger than the other areas (Table 3.9). Although this value was still high compared to the ones obtained in the study, it might have been affected by the poor visibility combined with big quantities of *Rugulopteryx okamurae* in suspension on some of the days that the underwater visual census was done (Liulea et al., 2023).

There is also some probability that this algae in suspension deeply affects the fish abundance, biodiversity and recruitment by covering the sea bottom (crevices and porosities) that recruits might use to forage (Ferrari et al., 2018; García-Gómez et al., 2021; Liulea et al., 2023). There is even information in the literature that the ingestion of raw *Rugulopteryx okamurae* causes adverse effects on fish development, which therefore can have a detrimental effect in the recruitment phase (Vizcaíno et al., 2024). Overall, there was more resemblance in terms of recruitment species between the Biohuts and the control areas.

When considering how the different species recruitment varied according to the different transect areas it was seen that the Biohuts areas attracted a greater variety of species, with 11 different taxa being recorded, compared to only three taxa in the control zone inside the marina and nine species in the control zone outside the marina and the natural area (Table 3.10). This suggests that Biohuts are efficient and may serve as important refuges or nurseries for a range of juvenile fish.

No Mugilidae recruitment was seen in the natural zone, with the biggest recruitment values in the Biohuts and the control inside the marina (Table 3.10; Table 3.12; Table 3.13). This might be related to the adaptability of this taxa to changes in the coastal area and the preference for brackish waters, but also to the fact there is a feeding preference for flat surfaces, as is the case of the structures inside and the rocks outside the marina (Koutrakis, 2015; Whitfield, 2016;

Castellini et al., 2019). In contrast, in the natural areas, the rocks have more porosities and rugosity which is not as preferable a habitat for detritivorous and herbivore fishes (Cardona, 2016; Ferrari et al., 2018). Furthermore, it has been reported that Mugilidae use the estuarine areas to take advantage of the rich resources and for shelter from predators (Blaber & Whitfield, 1977).

Recruitment of *Diplodus sargus* was seen in every zone but had an undoubtedly larger number of recruits per cubic meter in the natural area (0.9052 recruits/m³), which indicates a habitat preference for the natural habitat (Table 3.10; Table 3.11; Table 3.12; Table 3.13). As the natural area rocks are full of porosities and crevices where mollusks and crustaceans were abundant, it perhaps prefers this habitat, as according to another study carried out in the Algarve on artificial reefs, this species was strongly associated with prey availability and normally would use these structures as artificial feeding areas (Leitão et al., 2007). As there is probably more food availability in the natural area and there are not so many environmental stresses, it might be a motive why there was a higher preference for this area.

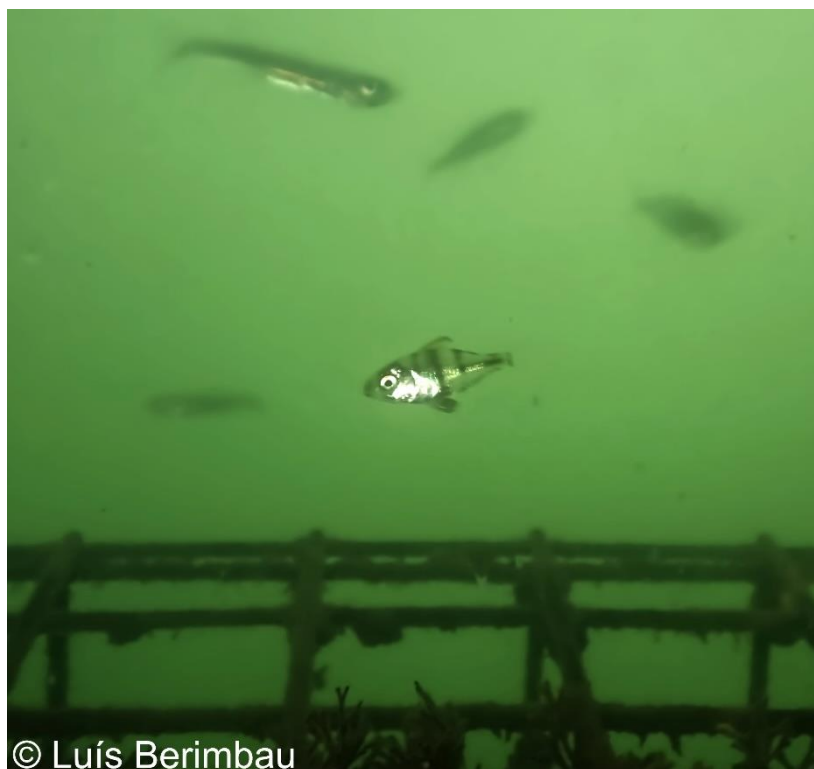


Figure 4.2. *Diplodus puntazzo* recruit above one Biohut with *Atherina* spp. in the background.

Diplodus puntazzo recruitment was seen in most of the areas but with a greater value in the natural zone, although with a low density of approximately 0.06 recruits per cubic meter, which might indicate this species has a similar recruitment habitat preference as *Diplodus sargus* (Figure 4.2). Other taxa were seen at rather small values in terms of recruit density; always less than 0.01 recruits per cubic meter, which might indicate that these fish are not as abundant as the other ones in the study area or have a preference for other habitats in terms of recruitment.

The significant recruitment of species like Mugilidae and *Atherina* spp. in both the Biohuts and control zones suggests that these species are highly adaptable to both artificial and natural environments. However, the lower recruitment of other species in the marina control zone implies that the marina environment may be less suitable for a diverse range of fish species, possibly due to factors such as reduced water quality, habitat complexity, or increased human activity (Gratwicke & Speight, 2005; Fitzgerald et al., 2007 Ferrari et al., 2018)

The species *Sarpa salpa* showed higher recruit density in the control zone outside the marina and even a greater value in the natural zone where it obtained a total of 0.1781 (Table 3.10; Table 3.12; Table 3.13). This preference may be linked to the availability of food in these areas (Houde, 2016). *Sarpa salpa* is a herbivorous species, and the control zone outside the marina is characterized by rocky formations with flat surfaces, which serve as a potential preferential habitat for detritivorous and herbivorous fish (Havelange et al., 1997; Goldenberg & Erzini, 2014; Ferrari et al., 2018).

The natural area has a different shoreline and substrate, it supports a more pristine benthic community compared to urbanized areas, making it potentially more attractive for *Sarpa salpa* recruits. Also, the surface rugosity and porosities typical of the natural area according to the literature have greater algae biomass, with this being observed during the underwater visual census (Bergey & Cooper, 2015).

Both habitats featured rocky formations where schools of juvenile *Sarpa salpa* were observed taking refuge, though more frequently in the natural area. The ideal conditions in the natural area, including abundant food availability (as the rocks were completely covered in algae) and greater habitat complexity, further explain the higher recruitment and overall abundance of this species in that area (Table 3.1; Table 3.13).

According to the literature, estuarine ecosystems, characterized by dynamic environmental conditions, tend to favor generalist, omnivorous, and opportunistic fish species due to their ability to occupy broad ecological niches (Richmond et al., 2005; Elliott et al., 2007; Dolbeth

et al., 2008; Stuart-Smith et al., 2015; Mosman et al., 2023). This is in agreement with our results, as *Diplodus sargus*, Mugilidae, and *Atherina* spp. exhibited the highest recruitment rates in both the Biohut zone and control zones, both inside and outside the marina, and were the most abundant species in these areas (Table 3.10; Table 3.11; Table 3.12; Table 3.13).

Diplodus sargus is documented as a generalist, opportunistic, and omnivorous species (Figueiredo et al., 2005; Leitão et al., 2007). Its wide-ranging diet allows *D. sargus* to thrive in estuarine environments where food resources fluctuate significantly. Similarly, Mugilidae species demonstrate dietary shifts when inside the estuary, transitioning from a more specialized diet in the surf zone to a generalist diet (Garcia et al., 2018). *Atherina* spp. also display opportunistic feeding behaviors, particularly shifting from planktonic prey to benthic organisms during periods of low plankton availability (Bartulovic et al., 2004; Chrisafi et al., 2007). This dietary flexibility and opportunistic feeding evidence why these taxa were so abundant and had greater values of recruits per cubic meter.

Regarding the distribution in the different areas, most of the recruitment in the Biohut zone happened in the transect zones B (41.95 %) and C (49.44 %) as it can be seen in Figure 3.8, it was verified that the distribution of the recruits is significantly different between the different transect zones ($p = 0.004$). The reason why Biohut zone A displayed a significantly different lower value when compared with zone B might be related to the fact that the location of Biohut zone A is in an area where the maritime tourism boats are moored and in general, there are a lot of boat traffic as it is near the entrance of the marina. This traffic, and sometimes lesser gentle approaches to the pontoons agitated the artificial nurseries, which combined with noise sometimes would scare away the recruits during the underwater visual census (Sandström et al., 2005; Jung & Swearer, 2011).

Biohut zone C might have had the highest value since is a more protected zone, with less boat traffic side in the marina and also is nearer to rock formations inside the marina which might attract more fish (Sandström et al., 2005; Jung & Swearer, 2011). Biohut zone B, was the zone where the artificial nursery impact was probably more intriguing, one of the big differences of this zone is that although it isn't near any rock formations and in the center of the marina (more exposed), it had a considerable percentage of recruits (Figure 3.8). This might be explained by the fact that recruits when entering the center of the marina wouldn't probably settle in the pontoons and instead would settle and aggregate around the Biohuts. Which generated a comparatively high percentage of recruits when comparing with the Biohut zone A (Figure 3.8).

The control outside marina zone B accounted for around 80% of the recruitment as can be verified in Figure 3.9, and this might be justified by this area being more upstream in a lesser disturbed area with no boat traffic (Sandström et al., 2005; Jung & Swearer, 2011). However, according to literature there is evidence that when the tide rises, juvenile fishes settle in the first suitable substratum, but this was not seen in this study (Gratwicke & Speight, 2005). Control zone A is more downstream and in proximity to the fueling station, and is the first area to be influenced by the tidal currents that can be important for recruitment and dispersal (Drew & Eggleston, 2006). Apart from the anthropogenic disturbances, the canal of “Ribeira de Bensafrim” straightens in the control zone A area, and both the effect of tidal currents and the currents due to the stream were stronger which might also be difficult for recruits settling around this area (Jenkins et al., 1997; Drew & Eggleston, 2006).

No strong correlations were seen regarding the recruitment and the environmental variables (Figure 3.10; Figure 3.11; Figure 3.12). However, there was a weak and positive correlation between the visibility and the number of recruits, although this might have happened due to easier detection of recruits as visibility improved. Having somewhat good visibility was a problem with the underwater visual census being cancelled on some days or carried out under limited conditions. To get a better understanding if there is any relationship between this variable and the number of recruits, the underwater visual census should be carried out with the same frequency for different visibilities.

A negative weak correlation was obtained between the number of recruits and the swell outside the marina, which might suggest that the number of recruits declines in the study areas when the swell is greater as the small fish may take refuge at a greater distance from the shore to avoid disturbances related with greater turbulence and strong currents (Watt-Pringle & Strydom, 2003; Olds et al., 2018).

The temperature of water showed a very weak positive correlation with the number of recruits, although it is known to be an important environmental variable influencing fish recruitment (Colombano et al., 2022; Lourenço et al., 2023). The fact that this correlation is very weak might be a result of the shifts in water temperature caused by climate change, which therefore affects fish recruitment seasonality including for estuarine species (Morrongiello et al., 2014; Colombano et al., 2022; Lourenço et al., 2023).

4.4. Other observations

Since a considerable number of these individuals had very small sizes, combined with poor visibility (caused by suspended particulate matter) and the avoidance behaviors displayed by some fish, there were instances where identifying the taxa was challenging. In such cases, it was only possible to estimate the number of fish present, which in this sense corresponds to 1,299 individuals that could not be taxonomically identified.

Regarding the size classes, most of the observed fish were 7 cm or larger, which was expected for several reasons. Size class 11 includes all fish larger than 7 cm, making it the group with the largest size range, so it is natural for more fish to fall within this category (Figure 3.2). Another contributing factor is that recruitment was not detected for all species in this study; for those species, only fish of post-recruitment sizes were counted (mostly size class 11). Size class 10 also had a considerable number of individuals, likely due to larger recruits being more resilient to environmental changes and experiencing lower mortality rates compared to the early stages of recruitment (Anderson, 1988; Smith & Botsford, 1998; Johnson, 2007). Additionally, for some species observed during the underwater visual census, this size already corresponds to post-recruitment, which is associated with higher survival rates (Chambers & Trippel, 2012).

Considering the other sizes in Figure 3.2, a slightly higher cluster of values (greater than 2000) from class 2 to 5 was observed, after which the values slightly decreased until near size class 10 (6 to 7 cm), possibly correlating with the density-dependent and independent mortality of recruits as they grow (Walters and Juanes, 1993; Lorenzen & Camp, 2019). However, the smaller size class had the lowest value of individuals, possibly due to the fact fishes of this size are harder to detect particularly when the visibility was not ideal as it was with some of the underwater visual census days.

One possible explanation for the substantial number of individuals in some of the smallest size classes and even more for the largest size classes is that the marina is located within an estuary, a well-known habitat commonly used as a nursery (recruitment) and feeding area by multiple species (Elliott & Hemingway, 2008; Solari et al., 2015).

A statistically significant difference was seen in how fish are distributed in the biohut areas, with almost 65 % of the fishes being inside or at less than 1 meter from the biohut (Figure 3.3). This, combined with the fact that the Biohut zones had the highest number of recruits per volume is another strong indicator that these artificial nurseries indeed recreate essential habitats as nurseries and for refuge (Martinho, F. 2020).

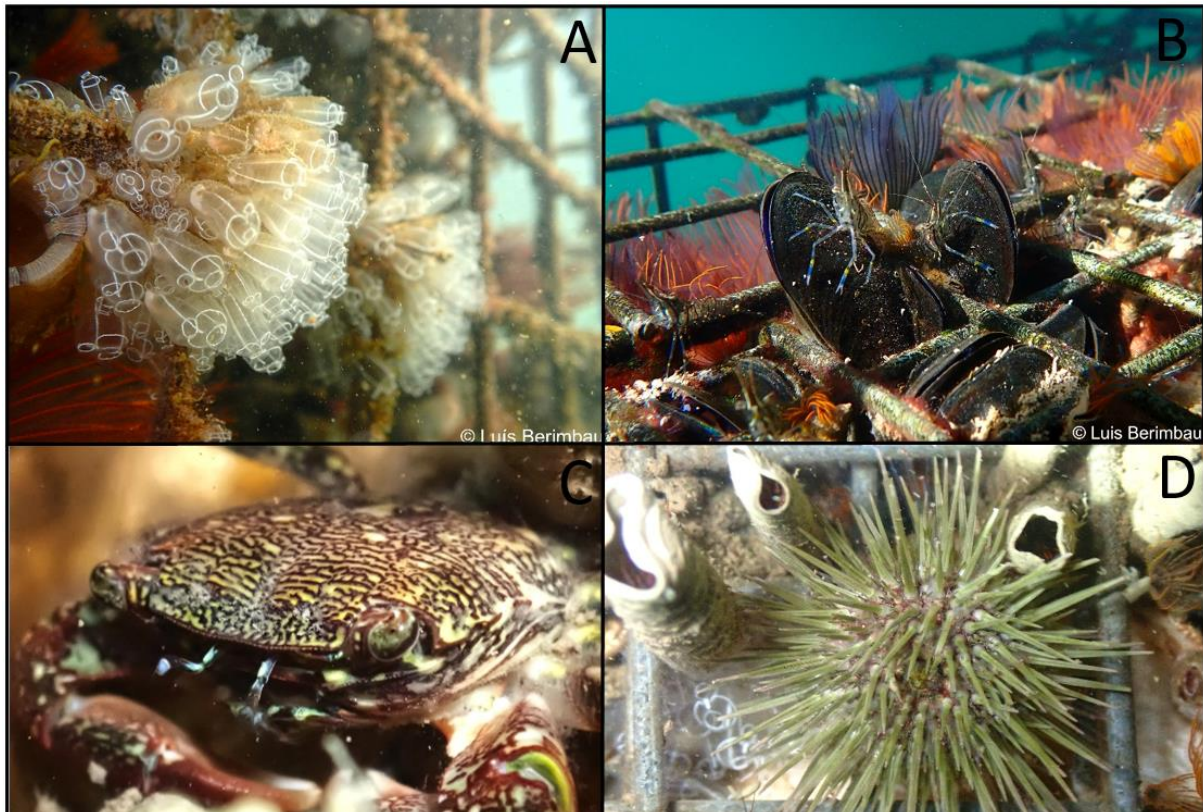


Figure 4.3. Some of the benthic organisms photographed in the Biohuts; *A- Ascideacea*, *B- Palaemon elegans* on top of *Mytilus sp.*, with *polychaetes* in the background, *C- Pachygrapsus marmoratus* and *D – Echinoidea*.

The fact that the Biohuts also had a considerable amount of other benthic organisms also suggests that this artificial habitat enhances the resilience of the habitat and minimizes the negative impacts of the anthropogenic pressures that happen in the marina (Schindler et al., 2012). Some of the organisms that were common in the biohuts, as is the case of *Mytilus sp.*, *Ostrea edulis* and organisms from the *Ascideacea* are effective at filtering large amounts of water, removing suspended particles, nitrogen, and even heavy metals (Draughon, 2010; Carmichael et al., 2012; Broszeit et al., 2016; Colozza et al., 2017; Tzafiriri-Milo et al., 2019; Albentosa 2023).

The presence of higher trophic fishes such as *Dicentrarchus labrax* is evidence that these structures create enough habitat complexity that they might become important foraging areas for these taxa inside the marina (Rilove et al., 2007; Ferrari et al., 2018). This was confirmed with sightings of *Dicentrarchus labrax* preying on unsuspecting fish that were farther from the Biohuts and could not take refuge in time. *D. labrax* was the most common higher trophic fish seen in the areas inside at outside the marina (including the Biohuts), this was expected as this species is known for using estuarine habitats as nursery and feeding grounds (Rogdakis et al., 2010). The fact this species can have an opportunistic feeding behavior, adapting their diet based on prey availability gives them an advantage to proliferate in the estuarine area of the study (Rogdakis et al., 2010; Mosman et al., 2023).

Since the Biohuts are under the pontoons, most of the piscivores birds that were present in the study area except for *Phalacrocorax carbo* would hunt from the top of the structures and rocks of the marina. However, since the biohuts are below the pontoons they could not fish there which makes these artificial nurseries even more effective in offering refuge. Another important factor of the Biohuts as a refuge is that their structure offers shelter but with open space all around each structure, which helps the smaller fish (recruits) to more easily detect the predatory fish from far away, giving them time to enter within the inner cage and escape (Karino & Kuwamura 1997; Rilov et al., 2007)

5. Conclusion

This study aimed to assess the effectiveness of Biohuts in enhancing fish abundance, biodiversity, and recruitment, while also utilizing these structures to monitor the recruitment of various species in the Marina de Lagos. The findings demonstrated that Biohuts significantly increase fish abundance and diversity compared to control zones by enhancing habitat complexity, offering refuge, providing foraging opportunities, and serving as spawning grounds (as was seen with *Parablennius pilicornis*). Evidence shows that the increased biodiversity supported by Biohuts can enhance ecosystem functions, potentially leading to a more resilient environment.

The control area within the marina exhibited the lowest fish abundance and species richness, further highlighting the fact that the marina's existing structures do not provide sufficient habitat complexity for fish. As expected, the natural area showed higher levels of fish abundance and biodiversity, but the differences compared to Biohut zones were not substantial, demonstrating the effectiveness of Biohuts in habitat restoration.

It was also observed that recruitment and spawning periods varied between the different species, with different taxa displaying distinct preferences for recruitment habitats and certain months. While some species preferred the natural area, others favored the estuarine zone (including Biohut and control zones outside the marina) for settlement. Biohuts attracted a broader range of species for recruitment and had the highest recruitment density, underscoring the importance of habitat restoration in urbanized coastal areas. The natural area showed a slightly lower but comparable recruitment density, which may be linked to the presence of the invasive algae *Rugulopterix okumurae*.

Most of the species were identified as late winter to early spring spawners. The most abundant taxa in terms of recruitment in the study area were Mugilidae, *Diplodus sargus* and *Atherina* spp. Highlighting their adaptability as generalist feeders in estuarine ecosystems. The structures contributed to habitat complexity and resilience, as evidenced by the presence of benthic organisms like *Mytilus sp.* and *Ostrea edulis*, which contribute to water filtration and improve overall ecosystem health.

Although no strong correlations were seen between environmental variables and the recruitment there are certain tendencies that suggest that these variables may impact the recruitment. As is the case of the swell, where higher values were associated with decreased recruit density in the

study area. Areas that were less disturbed by boat traffic showed higher values of recruitment density, and the upper reaches of the estuary in the study area had less biodiversity than the lower reaches.

It is important to note that the relatively recent installation of Biohuts and the limited duration of visual censuses may not fully capture their long-term impact. Continued monitoring is essential to accurately assess their effectiveness in enhancing fish abundance, biodiversity, and recruitment over time.

There were some limitations to this study that could have influenced the results, namely the visibility that sometimes was not ideal, mainly due to discharges of the spillway of the wastewater treatment plant and the presence of an invasive algae species in suspension, affecting the abundance, identification and size estimation of the fishes. It was not possible to make underwater visual census during every month of the year (July and August missing), possibly influencing the identification of the seasonality of the recruitment. The knowledge gap in some species length at first maturity, did not allow the verification of recruits for these species. As this was not an extensive fish recruitment study, to make more concrete relations regarding fish recruitment, more environmental data should be collected with greater precision.

In conclusion, our study suggests that Biohuts have significant potential to positively impact coastal areas affected by urbanization, highlighting the need for ongoing research and conservation efforts to address marine habitat loss due to urban development. Furthermore, our findings underscore the importance of investigating fish recruitment, for a better understanding of fish population dynamics, identification of critical habitats and assessment of species resilience in changing environments.

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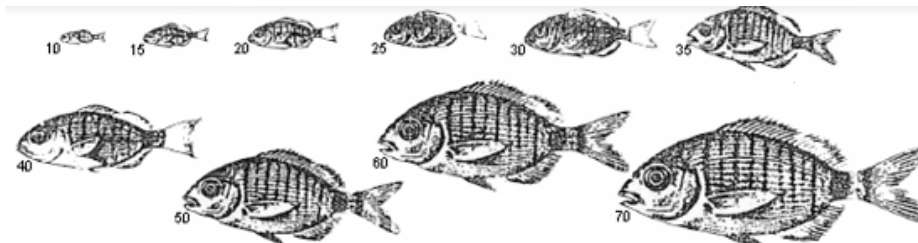
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Annexes

Supplementary material

Annex A: Data collection form for underwater visual census supplied by Ecocean.



PLACE	DATE	HOUR	OBSERVER	
Wind :	Weather conditions :		T° water :	T° air :
Swell :	Visibility :		Rain last 24h?	
Technical and ecological comments + fouling:		Marina map		
Condition of the Biohut® :				
A				
B				
C				
Seawall				