

Beyond the Tank: A Transformative Environmental Enrichment of Iconic Species at SEA LIFE Porto

Maria Carlos da Silva Salta Castelo



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**Beyond the Tank: A Transformative Environmental Enrichment
of Iconic Species at SEA LIFE Porto**

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em Ciências do Mar – Recursos Marinhos,
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Maria

Declaração de Honra

Declaro que o presente trabalho é de minha autoria e não foi utilizado previamente noutro curso ou unidade curricular, desta ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras da atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referência. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.

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Abstract

This dissertation provides a comprehensive study on the development, implementation, and evaluation of an environmental enrichment program for a green sea turtle (*Chelonia mydas*) residing at SEA LIFE Porto, relating the impacts of this enrichment to behavioral and spatial patterns, in conjunction with a description of the author's professional experience as an aquarist during the internship period. The study is framed within the context of the paradigm shift of public aquariums from simple exhibition facilities to institutions for conservation, education, and research, which has fostered increased collaboration among these institutions and universities, particularly regarding the growing demand to ensure the welfare of animals kept under human care.

Based on a critical review of the concept of animal welfare, including its physiological, behavioral, and psychological aspects, it highlights the effectiveness of modern assessment models, particularly the Five Domains model. It also points out the role of environmental enrichment as a well-proven approach to encourage natural behaviors, increase behavioral diversity, and reduce abnormal behaviors, supporting the mental and physical health of animals in captivity.

The study carried out systematic observations of the green turtle in its own habitat, registering the use of space and the behaviors exhibited in different experimental conditions: without and with the introduction of a shelter and rotating enrichment devices. Prior to these systematic observations, *ad libitum* observations were conducted to familiarize with the turtle's behavior and inform the development of a detailed ethogram of species-specific behaviors. The systematic observations were then divided into two main studies: one focusing on the effect of shelter introduction on spatial distribution, and the other assessing the impact of a rotating enrichment plan by comparing behavioral sessions with and without enrichment. These approaches aimed to evaluate how structural and dynamic environmental modifications influenced the turtle's behavior, activity patterns, and use of space, ultimately contributing to improvements in welfare assessment and enrichment strategies.

The results showed that the introduction of the shelter significantly influenced the green sea turtle's spatial distribution, with increased occupancy in specific refuge zones such as behind the shelter, and a decreased focus on the feeding area. This change suggests the shelter provided a valued hiding place that reduced exposure to external stimuli, promoting more diverse and natural habitat use. Behavioral analysis indicated that

the shelter contributed to a reduction in stress-related behaviors, such as excessive anticipatory feeding, by encouraging the turtle to explore other areas of the tank.

Following the shelter introduction, the rotating enrichment plan further enhanced the turtle's welfare by leading to a significant increase in behavioral diversity and exploratory actions. The enrichment devices stimulated a broader range of natural behaviors, including active interaction with the environment, and promoted a more balanced use of tank space, especially in underutilized areas. Notably, the presence of enrichment reduced resting and hiding behaviors, while increasing activity levels without simply increasing swimming activity, indicating improved physical and psychological stimulation. These results together demonstrate that both structural shelter and dynamic enrichment devices play complementary roles in enhancing spatial use, behavioral diversity, and overall welfare of the captive green sea turtle.

In parallel, practical experience as an aquarist allowed for the acquirement of technical skills applied to the management and maintenance of aquatic environments, monitoring of physical-chemical water parameters, animal feeding, and support for the biology team, demonstrating the importance of professional training for the effective application of enrichment and welfare practices. This work experience also reinforced the importance of interdisciplinary support through constant collaboration with the biology and veterinary team at SEA LIFE Porto, integrating clinical, behavioral, and environmental data to optimize environmental enrichment and animal management protocols. As such, specific technical training and operational experience proved to be essential for the effective implementation of enrichment practices and the promotion of animal welfare, highlighting the strategic role of the aquarist as an active agent in the ethical and sustainable management of animals under human care.

The study concludes by reaffirming the value of environmental enrichment as an essential tool for promoting welfare in contemporary aquariums, highlighting the need for continuous and multidisciplinary assessments that integrate behavioral and environmental data. Moreover, it emphasizes the relevance of technical training for professionals involved in management, as well as the potential of applied research to guide evidence-based practices, contributing to the ethical and sustainable management of species in human care.

Keywords: *Chelonia mydas*; Environmental Enrichment; Animal Welfare; Animal Behavior; Under Human Care Management; Aquarist; Public Aquariums

Resumo

Esta dissertação apresenta um estudo abrangente sobre o desenvolvimento, implementação e avaliação de um programa de enriquecimento ambiental para uma tartaruga verde (*Chelonia mydas*) residente no SEA LIFE Porto, relacionando os impactos deste enriquecimento com os padrões comportamentais e espaciais, em conjunto com uma descrição da experiência profissional da autora como aquarista durante o período de estágio. O estudo situa-se no contexto da mudança de paradigma dos aquários públicos, que deixaram de ser simples espaços de exposição para se transformarem em instituições de conservação, educação e investigação, fomentando uma maior colaboração entre estas instituições e as universidades, especialmente face à crescente exigência para garantir o bem-estar dos animais mantidos sob cuidado humano.

Baseado numa revisão crítica do conceito de bem-estar animal, incluindo os seus aspetos fisiológicos, comportamentais e psicológicos, destaca a eficácia dos modelos modernos de avaliação, nomeadamente o modelo dos Cinco Domínios. Aponta também o papel do enriquecimento ambiental enquanto abordagem comprovada para incentivar comportamentos naturais, aumentar a diversidade comportamental e reduzir comportamentos anormais, apoiando a saúde mental e física dos animais em cativeiro.

O estudo realizou observações sistemáticas da tartaruga verde no seu próprio habitat, registando a utilização do espaço e os comportamentos exibidos em diferentes condições experimentais: sem e com a introdução de um abrigo e dispositivos de enriquecimento rotativos. Antes destas observações sistemáticas, foram conduzidas observações *ad libitum* para familiarização com o comportamento da tartaruga e para informar o desenvolvimento de um etograma detalhado dos comportamentos específicos da espécie. As observações sistemáticas foram depois divididas em dois estudos principais: um focado no efeito da introdução do abrigo na distribuição espacial, e outro a avaliar o impacto de um plano rotativo de enriquecimento, comparando sessões comportamentais com e sem enriquecimento. Estas abordagens visaram avaliar como modificações estruturais e dinâmicas no ambiente influenciaram o comportamento da tartaruga, os seus padrões de atividade e o uso do espaço, contribuindo assim para a melhoria das estratégias de avaliação de bem-estar e enriquecimento.

Os resultados mostraram que a introdução do abrigo influenciou significativamente a distribuição espacial da tartaruga verde, com aumento da ocupação em zonas específicas de refúgio, como atrás do abrigo, e uma diminuição do foco na área de alimentação. Esta mudança sugere que o abrigo proporcionou um local de esconderijo valorizado que reduziu a exposição a estímulos externos, promovendo um uso do habitat

mais diversificado e natural. A análise comportamental indicou que o abrigo contribuiu para a redução de comportamentos relacionados com o stress, como o comportamento antecipatório de alimentação excessivo, encorajando a tartaruga a explorar outras áreas do tanque.

Posteriormente à introdução do abrigo, o plano rotativo de enriquecimento potenciou ainda mais o bem-estar da tartaruga, levando a um aumento significativo da diversidade comportamental e das ações exploratórias. Os dispositivos de enriquecimento estimularam uma gama mais ampla de comportamentos naturais, incluindo a interação ativa com o ambiente, promovendo uma utilização mais equilibrada do espaço do tanque, especialmente em áreas subutilizadas. Notou-se que a presença do enriquecimento reduziu os comportamentos de repouso e esconderijo, enquanto aumentou os níveis de atividade sem simplesmente incrementar a atividade de natação, indicando uma estimulação física e psicológica melhorada. Estes resultados demonstram que tanto o abrigo estrutural como os dispositivos de enriquecimento dinâmico desempenham papéis complementares no aumento do uso espacial, da diversidade comportamental e do bem-estar geral da tartaruga verde em cativeiro.

Paralelamente, a experiência prática como aquarista permitiu a aquisição de competências técnicas aplicadas à gestão e manutenção de ambientes aquáticos, ao monitoramento dos parâmetros físico-químicos da água, à alimentação dos animais e ao apoio à equipa de biologia, demonstrando a importância da formação profissional para a aplicação eficaz das práticas de enriquecimento e de bem-estar. Esta experiência de trabalho reforçou ainda a importância do apoio interdisciplinar através da colaboração constante com as equipas de biologia e veterinária do SEA LIFE Porto, integrando dados clínicos, comportamentais e ambientais para otimizar os protocolos de enriquecimento ambiental e gestão animal. Assim, a formação técnica específica e a experiência operacional revelaram-se essenciais para a implementação eficaz das práticas de enriquecimento e promoção do bem-estar animal, destacando o papel estratégico do aquarista como agente ativo na gestão ética e sustentável dos animais sob cuidado humano.

O estudo conclui reafirmando o valor do enriquecimento ambiental como ferramenta essencial para promover o bem-estar em aquários contemporâneos, sublinhando a necessidade de avaliações contínuas e multidisciplinares que integrem dados comportamentais e ambientais. Além disso, enfatiza a relevância da formação técnica para os profissionais envolvidos na gestão, assim como o potencial da investigação aplicada para orientar práticas baseadas em evidências, contribuindo para a gestão ética e sustentável das espécies sob cuidado humano.

Palavras-chave: *Chelonia mydas*; Enriquecimento Ambiental; Bem-Estar Animal; Comportamento Animal; Gestão Sob Cuidado Humano; Aquarista; Aquários Públicos

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List of Abbreviations, Acronyms and Symbols

a.m. - ante meridiem

AZA - Association of Zoos and Aquariums

BHAG - Behavior and Husbandry Advisory Group of AZA

CBSG - Captive Breeding Specialist Group of IUCN

CITES - Convention on International Trade in Endangered Species of Wild Fauna and Flora

CMS – Convention on Migratory Species

EAZA – European Association of Zoos and Aquariums

EE – Environmental enrichment

FAWC - Farm Animal Welfare Council

IUCN - International Union for Conservation of Nature

IUDZG - International Union of Directors of Zoological Gardens

m² - Square Meters

p.m. - post meridiem

PRT – Positive Reinforcement Training

QBA – Qualitative Behavioral Assessment

SPAW - Specially Protected Areas and Wildlife

TEDs - Turtle Excluder Devices

WAZA - World Association of Zoos and Aquariums

WZACS - World Zoo and Aquarium Conservation Strategy

WZCS - World Zoo Conservation Strategy

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Conference Presentations

I had the opportunity to present part of the results from my scientific work at 2 conferences in poster format:

1. Castelo, M., Ferreira, A., & Magalhães, A. (2025). *A Green Sea Turtle's Domain: Habitat Use and Preference in a Public Aquarium*. Investigação Jovem da Universidade do Porto 18ª Edição, Porto, Portugal.
2. Castelo, M., Ferreira, A., & Magalhães, A. (2025). *Exploring Space: Habitat Use by a Green Sea Turtle in a Public Aquarium*. Universities Federation for Animal Welfare – International Animal Welfare Conference 2025, Online.

The respective abstracts and posters submitted for the conferences are included in Appendix A.

Chapter 1: General Introduction

1. Evolution of Public Aquariums

Since their origins in the mid-19th century, public aquariums - like zoos - have undergone a deep transformation (Smith, 2023). Once essentially focused on entertaining and fascinating the public with exotic species (Miller et al., 2024), they have progressively been redefined as institutions devoted to education, conservation, animal welfare and scientific research (Rabb & Saunders, 2005). This change reflects the evolving role of society's stance on animal ethics and greater public expectations for the responsible treatment and presentation of captive animals (Powell & Watters, 2017).

The first modern public aquarium exhibit was introduced at the London Zoo in 1853, positioning aquatic species as a central feature of zoological and educational displays (Brunner, 2005). For many years, these facilities worked mostly as animal collections, presenting exotic animals mainly for public entertainment, often without serious regard for their ecological roles or natural behaviors (Gruen, 2014). Early exhibitions prioritized novelty over understanding or empathy and generally offered little insight into species-specific behavior or natural habitats (Miller et al., 2024).

Around the middle of the 20th century, professionals increasingly recognized that ensuring animal welfare was an indispensable part of their institutions' broader objectives (Powell & Watters, 2017). From early on, aquariums have had missions that included maintaining healthy animal populations for conservation, education, recreation, and research (Barongi et al., 2015). An important milestone occurred in 1950 with the release of a key publication by Hediger et al. (1950) emphasizing that the well-being of animals depends more on the quality of their living conditions than the amount of space provided. This publication played a crucial role in shaping the term "Zoological Biology" to depict the scientific management of zoo animals, focusing not only on their survival, but also on the psychological and behavioral fulfillment of the animals.

Over time, institutions increasingly adopted science-based methods in the treatment and showcasing of animals. Priority began to be given to the naturalistic design of exhibits, advancing research on animal behavior and the implementation of management practices that promoted health and successful reproduction (Hancocks, 2001). These changes were largely mission-driven, based on the understanding that animals in high welfare conditions are more likely to prosper, reproduce and contribute effectively to educating the public (Luebke et al., 2016). This transformation was influenced by both internal objectives - such

as ethical responsibility and species sustainability - and external factors, including evolving public concerns and regulatory pressures (Powell & Watters, 2017).

The 1970s also marked a critical moment in the evolution of animal welfare, prompted in part by the ascent of the modern animal rights' movement (Powell & Watters, 2017). Public intellectuals such as Peter Singer played a vital role in this change, particularly with the release of his influential book *Animal liberation: a New Ethics for our Treatment of Animals* in 1975, which raised widespread awareness and led to activism (Singer, 1975). As public concern grew, more attention was paid to the treatment of animals in various sectors, including scientific research, agriculture and institutions such as zoos and aquariums (Powell & Watters, 2017). However, the pressure to improve animal welfare was not just a response to external criticism. Those who work directly with animals - such as zookeepers, aquarists and veterinarians - also began to give greater value to animal welfare. As the profession of zookeeping advanced, welfare was recognized not only as an ethical requirement, but also as a practical necessity to guarantee health, longevity and successful reproduction of animals under human care (Barongi et al., 2015).

As zoos and aquariums redefined their missions, they increasingly incorporated conservation, education, research and recreation as fundamental principles (Barongi et al., 2015). One of the main driving ideas behind this change was the understanding that animals living in stimulating environments with appropriate social dynamics tend to exhibit natural behaviors, adapt better to captivity and enhance educational outcomes for visitors (Luebke et al., 2016; Powell & Bullock, 2014).

By the late 20th century and into the 21st, animal welfare became multidimensional, expanding beyond its traditional veterinary focus to include psychological, behavioral and social well-being (Powell & Watters, 2017). This has led to the development of habitats that mimic natural ecosystems, the formation of social groups based on species-specific needs and enrichment strategies designed to promote mental stimulation and physical activity (Hancocks, 2001).

Over the last three decades, the care of aquatic animals in public aquariums has improved significantly. Aquarists have developed a deeper knowledge of the specific needs of each species, while the rise of professionals trained in aquatic veterinary medicine has boosted health and welfare standards in these institutions (Smith, 2023). This shift towards prioritizing animal welfare reflects more widespread social changes, including greater public awareness of ethical concerns and expectations regarding animals under human care (Powell & Watters, 2017). As Hutchins (2003) observed, zoos and aquariums today face

complex challenges such as the accelerating loss of habitat, the growing attention to animal rights and the lack of connection between urban populations and the natural world.

In response, aquariums have embraced a renewed mission firmly rooted in conservation and ethics (Barongi et al., 2015). Landmark efforts such as the *World Zoo Conservation Strategy* (WZCS) (IUDZG/CBSG, 1993) and the initiatives of the World Association of Zoos and Aquariums (WAZA) have institutionalized this change (Rabb & Saunders, 2005), placing aquariums not only as exhibition spaces but as vital players in species conservation, research, public education and environmental policy (Hutchins & Conway, 1995). This evolution reflects a professional and social commitment to reconceive the aquarium as an institution that fosters understanding, promotes scientific research and actively contributes to the preservation of aquatic life (Rabb & Saunders, 2005).

2. SEA LIFE Porto

This research was conducted at SEA LIFE Porto (1ª Rua Particular, Via do Castelo do Queijo, 4100-379 Porto, Portugal) (SEA LIFE Porto, 2025a) during the 2024/2025 school year, starting in September 2024 and ending in June 2025 (Figure 1).

The SEA LIFE Porto is the second largest public aquarium in the country and the largest in the north of Portugal (civitas Porto, 2025). Opened on June 15, 2009, the center offers an exhibition of more than 3,000 species (freshwater and saltwater) spread over 31 aquariums (Visit Portugal, 2025). With a total area of 5,500 m² (indoor area and outdoor park) (Visit Portugal, 2025), there are themed environments such as the underwater tunnel with a panoramic view of the largest tank in the facility (civitas Porto, 2025), and the Penguin Harbor, the habitat of the Humboldt penguins, which opened in 2019 to commemorate the center's 10th anniversary (Mesquita, 2019).



Figure 1 – Exterior of the SEALIFE Porto building (Author: Maria Castelo).

SEA LIFE centers have as their central missions are the preservation of marine life, animal welfare, environmental education and the involvement of the community. In terms of conservation, they work to protect aquatic species by collaborating with universities and research centers on scientific projects, contributing to the wellbeing of marine populations in their natural habitats and in captivity (Merlin Entertainments, 2025). In addition, they implement initiatives for the reproduction, rescue, rehabilitation and reintroduction of animals into the wild (SEA LIFE Porto, 2025b).

In the educational sphere, they offer enriching learning experiences, using interactive exhibitions and innovative technological tools, to raise awareness of the need to preserve the oceans and stimulate the public's interest in subaquatic life (SEA LIFE Porto, 2025d).

Finally, they encourage the involvement of society through environmental awareness programs, sustainability campaigns and volunteer activities such as beach clean-ups, thus strengthening their contribution to the protection of marine ecosystems (SEA LIFE Porto, 2025c).

3. Understanding Animal Welfare

Animal welfare is a broad concept that has been discussed and evolved over the years (Fraser, 2008). In fact, animal welfare is a complex issue that includes important scientific, ethical, economic and political aspects (Lund et al., 2006). The concept of animal welfare began to be formally considered as a science with the publication of the Brambell report on farm animals, issued by the British government (Brambell, 1965). Historically, three main approaches have been followed to define and, consequently, develop methodologies to assess the level of welfare (Carenzi & Verga, 2016) (Figure 2).

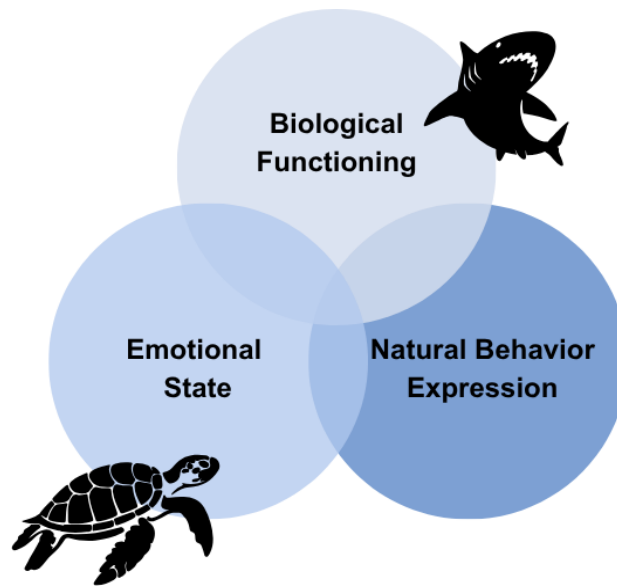


Figure 2 - Main approaches to animal welfare (adapted from Fraser et al. (1997); World Association of Zoos and Aquariums (WAZA) (2015)).

This initial perspective focuses on the biological functioning of living organisms, including processes such as development and reproductive functions, along with general health and behavioral patterns (Carenzi & Verga, 2016). Behavioral responses serve as primary indicators of how organisms react to environmental challenges and can provide immediate insight into how effectively an organism is managing environmental stressors. Ideal conditions of welfare are characterized by the absence of psychological distress or significant stress reactions (Broom & Johnson, 1993).

This methodology is exemplified by definitions that emphasize coping capacity. According to Broom (1986), “an animal's well-being is its state with respect to its attempts to cope with its environment”. This framework is linked to a structured system of biological requirements and is based on evidence that demonstrates how meeting or neglecting these requirements has an impact on maintaining positive welfare standards (Duncan, 2005).

The second approach states that the psychological dimension of animal welfare goes beyond measuring stress levels (Duncan, 2002). Hughes (1976) described welfare as “a state of complete mental and physical health in which the animal is in harmony with its environment”, rather than simply the absence of disease or injury.

This psychological view prioritizes the emotional and mental aspects of animal welfare, treating feelings and emotions as fundamental factors in determining quality of life. It covers not only physical health, but also the animal's subjective experiences. This perspective emerged partly as a critique of purely functional approaches to welfare assessment (Duncan, 2002).

The main difficulty with this feelings-based approach lies in the impossibility of directly measuring subjective experiences, since it is not possible to scientifically access internal emotional states. However, researchers can maintain scientific rigor by studying how the satisfaction or neglect of animals' biological needs affects their well-being. This requires careful consideration of what constitutes genuine biological needs, while avoiding anthropomorphic projections (Morton et al., 1990).

The third approach prioritizes natural life, stating that animals should be free to express their innate behaviors and rely on the adaptations that evolution has given them (Carenzi & Verga, 2016).

To summarize, assessing animal well-being in zoos requires recognizing that animal welfare comprises more than just physical health - which is typically defined as being free of illness and injury - because true animal welfare must consider both the physical and psychological conditions of the animals. Therefore, any assessment methodology must include both aspects (Mendl, 2001).

The concept of the Five Freedoms was first introduced in the Brambell (1965) and later refined by the Farm Animal Welfare Council (Farm Animal Welfare Committee (FAWC), 1993) and has been a useful tool in identifying welfare problems in animals under human care. The Five Freedoms were initially developed for farm animals but have also been used for zoo animals (Tallo-Parra et al., 2023). The Five Freedoms (Brambell, 1965; Farm Animal Welfare Committee (FAWC), 1993) are as follows:

- **Freedom from hunger and thirst** (by ready access to fresh water and a diet to maintain full health and vigor);
- **Freedom from discomfort** (by providing an appropriate environment including shelter and a comfortable resting area);
- **Freedom from pain, injury or disease** (by prevention or rapid diagnosis and treatment);
- **Freedom to express normal behavior** (by providing sufficient space, proper facilities and company of the animal's own kind);
- **Freedom from fear and distress** (by ensuring conditions and treatment which avoid mental suffering).

The Five Freedoms have been increasingly challenged for their narrow focus on preventing negative states, as they neither reflect advances in our understanding of animals' underlying biological processes nor acknowledge the vital role of positive experiences in true welfare (Mellor, 2016). Critics argue that simply ensuring the absence of suffering is

insufficient, as animals must also be provided with opportunities to experience “positive welfare”, or pleasure (Lawrence et al., 2019).

To address these shortcomings, the Five Domains Model was developed (Figure 3). This framework assesses an animal’s welfare by considering the balance of positive and negative emotions over time, integrating both physiological and psychological measures (Mellor & Beausoleil, 2015). By recognizing that an animal can be outwardly healthy yet still suffer (e.g., exhibiting stereotypies) or that stress responses are not always indicative of poor welfare (Duncan, 2005), the Five Domains Model offers a more nuanced, evidence-based approach that captures both sides of the emotional spectrum (Mellor & Beausoleil, 2015).

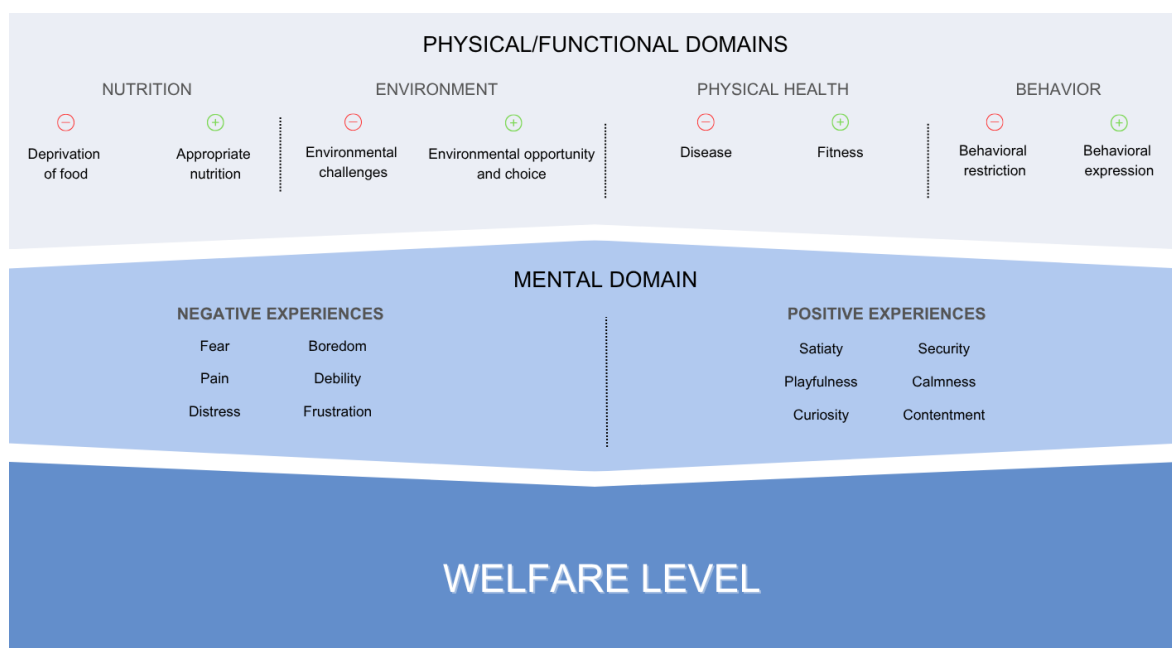


Figure 3 - The Five Domain's Model (adapted from World Association of Zoos and Aquariums (WAZA) (2015)).

In synthesis, promoting animal welfare relies on the development and use of science-based assessment tools (Tallo-Parra et al., 2023), which are essential for identifying welfare problems and tracking improvements over time (Carenzi & Verga, 2016). The most comprehensive understanding of animal welfare must include animals' physical and mental states. Ongoing research in this field not only deepens our understanding of animals' needs and experiences but also provides practical information to improve animal care. By applying the findings of applied ethology, one can improve housing, equipment and management practices (Grandin, 1993), ultimately creating environments that allow animals to express natural behaviors and cope better with their surroundings. Through this integrated approach, we are closer to guaranteeing the true welfare of the animals under human care (Tallo-Parra et al., 2023).

4. Evaluating Animal Welfare

Animal welfare assessment has progressed significantly, especially in recent years, moving from simple health monitoring to comprehensive, multi-faceted assessments that address both the physical condition and mental well-being of animals (Jones et al., 2022). It is important to develop welfare assessment frameworks that can be applied to different types of species and institutions (Racciatti et al., 2022).

Current advances in animal welfare assessment emphasize the need to use scientifically validated methodologies that take into account the various aspects of an animal's experience (van der Staay et al., 2025). These frameworks recognize that a comprehensive welfare assessment goes beyond the absence of negative experiences, such as illness or distress, and must also consider positive welfare indicators (Boissy et al., 2007). These indicators include the expression of species-typical behaviors, the presence of social interactions and the provision of opportunities for choice and environmental control (Jones et al., 2022; Kagan et al., 2015). By integrating both the absence of adverse conditions and the presence of positive outcomes, contemporary welfare assessment aims to provide a more detailed and holistic comprehension of animal welfare (Martínez-Yáñez et al., 2025).

Animal welfare assessment relies on the systematic measurement and analysis of various indicators that provide information on the physical and mental state of animals (Martínez-Yáñez et al., 2025; Tallo-Parra et al., 2023). These indicators are typically divided into two main categories: resource-based indicators and animal-based indicators, each providing a distinct perspective on welfare status (Manteca et al., 2016).

Resource-based indicators, also referred to as input-based indicators or environment-based indicators, assess the features of the environment provided to animals, rather than measuring the direct responses of the animals (Jones et al., 2022). They cover factors such as enclosure size and design, temperature, lighting conditions, and feeding protocols (Jones et al., 2022; Salas et al., 2024). Though generally simpler to quantify and produce consistent results, these indicators do not always accurately reflect an animal's welfare status, as the presence of appropriate resources alone does not guarantee good welfare without corresponding positive responses from the animals (Benn et al., 2019; Sherwen et al., 2018).

Animal-based indicators, also known as output-based indicators, are direct measurements taken from the animal and are considered the most accurate reflection of its true state of welfare (Manteca et al., 2016). These indicators evaluate how the animal

responds to its environment and management, providing information on its true ability to cope with the conditions it encounters (Martínez-Yáñez et al., 2025). They also have particular value for individual validity, as animals can experience the same environment differently (Tallo-Parra et al., 2023).

Given that behavior is a direct expression of an animal's experiential state, **behavioral indicators** are the most common animal-based measures used in welfare assessment (Binding et al., 2020), mainly due to their non-invasive nature and low cost (Hill & Broom, 2009; Hosey et al., 2023). These include:

1. **Abnormal behaviors** - stereotypies, which are repetitive and unchanging behaviors with no apparent immediate function, often indicating frustration or neurological dysfunction (Mason, 1991). Other forms of abnormal behavior include self-injurious actions and apathy (Manteca et al., 2016; van Zeeland et al., 2009).
2. **Changes in normal behaviors** - focuses on changes in the frequency, duration, or intensity of behaviors typical of the species (Manteca et al., 2016). This can include social, maintenance, anticipatory, and other types of behaviors. Monitoring these behaviors helps identify subtle changes in animal welfare (Tallo-Parra et al., 2023).

Physiological indicators reflect internal biological processes affected by welfare (Manteca et al., 2016). They can include heart and respiratory rates, heterophil/lymphocyte ratios, immunoglobulin levels, neuroendocrine, immunological, cardiovascular, and biochemical biomarkers (Tallo-Parra et al., 2023). One of the most well-known indicators includes cortisol/corticosterone measurements, which provide information about stress responses (Binding et al., 2020).

Finally, **physical indicators** serve as directly observable measures of body condition, injuries, and overall physical appearance, and are important for detecting acutely emerging welfare concerns (Martínez-Yáñez et al., 2025).

Recent years have seen several frameworks proposed and validated applying these principles in practice (Tallo-Parra et al., 2023). The most significant progress has been the development of species-specific protocols, which allow for a more accurate and detailed assessment of animal welfare (Salas et al., 2024). Among the most widely used models is the Five Domains Model (Mellor, 2016), in which the four physical domains - nutrition, environment, health, and behavior - influence the fifth domain, which reflects the mental state and affective experience of the animal. These physical domains correspond to the principles of the Welfare Quality® protocol, which focuses on standardized criteria assessed through indicators, the former being adapted to the biological characteristics of each species (Botreau et al., 2007). Although the protocol was initially designed for farm

animals, it has been successfully adapted for different species kept in zoos and aquariums, as in the case of bottlenose dolphins (*Tursiops truncatus*) (Clegg et al., 2015).

Most recently, the EPI-DOM approach has emerged as an innovative framework, integrating basic epidemiological concepts (EPI) with a new classification of animal welfare domains (DOM). EPI-DOM makes a clear distinction between indicators measured directly in animals and external factors that impact their welfare, thus offering a comprehensive analysis that considers physical, mental, and behavioral dimensions collectively. In addition, this approach classifies risk factors into three broad domains: management, environment, and interaction, making it highly versatile for application across different species and animal production systems (Martínez-Yáñez et al., 2025).

Some of the main advantages of these protocols include the use of measures that can be validated and verified for reliability, as well as their adaptation to the needs and biological characteristics of each species (Jones et al., 2022). On the other hand, the main limitation lies in the small number of specific protocols that have been developed. For most species found in zoos, there are still no specific, validated protocols for assessing welfare, which highlights the need for further progress and continued investment in this area (Salas et al., 2024; Tallo-Parra et al., 2023).

5. Environmental Enrichment: A Definition

Enrichment is widely recognized as one of the most effective strategies animal keepers can employ to promote individual welfare (Mellen & Sevenich MacPhee, 2001; Young, 2003). According to Reinhardt and Reinhardt (1998), environmental enrichment consists of “providing stimuli that encourage the manifestation of behavioral and mental activities typical of the species in environments with few stimuli”. This definition emphasizes the importance of enabling animals to express their natural behaviors, which are often restricted in captive situations.

Alternatively, Young (2003) advances the idea that the definition of environmental enrichment should be based on the goals to be achieved, prioritizing the intended results rather than the concept itself. According to the author, the main intentions of enrichment include: (1) expanding the variety of behaviors exhibited; (2) reducing the frequency of abnormal behaviors; (3) increasing the repertoire of behaviors considered natural or typical of the species; (4) encouraging positive use of the environment; and (5) strengthening the animal's ability to overcome challenges in a manner compatible with its natural responses.

Environmental enrichment is recognized as the introduction of sensory stimuli or the provision of different options in the environment, with the aim of broadening the behavioral possibilities of the animals that live there (de Azevedo et al., 2007). This approach highlights the dynamic and multifaceted nature of enrichment, covering both physical and psychological aspects (Young, 2003).

In a more general sense, the Behavior and Husbandry Advisory Group of the Association of Zoos and Aquariums (AZA) (BHAG, 1999) defines environmental enrichment as a process designed to enhance both the living conditions and the care provided to animals in zoos, with consideration for their behavioral biology and natural history. This dynamic approach involves making adjustments to enclosures and management practices to broaden the range of behaviors animals can express and encourage the development of species-specific abilities, ultimately supporting their well-being.

The relevance of environmental enrichment is further accentuated by WAZA, which considers enrichment to be a fundamental part of animal welfare and administration in zoos and aquariums. According to the World Zoo and Aquarium Conservation Strategy (WZACS) (World Association of Zoos and Aquariums (WAZA), 2005), these institutions are uniquely placed to carry out research into animal welfare, behavior and management, including the creation and evaluation of enrichment methods. This type of research is essential for improving insight into the best ways to promote the physical and psychological health of animals kept under human care.

Enrichment generally consists of introducing stimuli or options in the environment, as mentioned by de Azevedo et al. (2007). These factors aim to stimulate one or more of the animal's senses and can come from different sources, such as caretakers, zoo visitors or the environment itself (Hoy et al., 2010).

Animal enclosures tend to be less complex than the natural habitats to which animals have adapted throughout evolution (Bachetti et al., 2024). For this reason, the introduction of enrichment devices - be they structural, dietary, sensory, cognitive or social - plays a fundamental role in expanding the behavioral repertoire of species kept in captivity (Hoy et al., 2010) (Figure 4). These strategies encourage the emergence of exploratory behaviors, strengthen social interactions and increase both the variety and frequency of natural behaviors. It is worth noting that a well-implemented enrichment program can also contribute to reducing undesirable behaviors, such as stereotypies and other abnormal

repetitive behaviors, which are often associated with a poor state of welfare (Bachetti et al., 2024; Mason et al., 2007).



Figure 4 - Types of enrichment under the 5 main categories (Adapted from (Sea World)).

In essence, although selecting the most appropriate enrichment strategies for different species or groups is a complex task - requiring attention to the specific needs and preferences of each animal - the central purpose of any enrichment program remains clear: to provide a variety of opportunities that favor both the psychological and physical well-being of the animals (Young, 2003). As highlighted by Bloomsmit et al. (1991), it is essential to adopt a comprehensive approach, involving multiple categories of enrichment, to promote the welfare of animals kept in captivity. By applying these strategies in a careful and structured way, institutions, such as zoos and aquariums can encourage the manifestation of natural behaviors and improve the health of the animals in their care, thus ensuring high standards of animal welfare (Maple & Perdue, 2013).

6. Main Objectives

This dissertation, developed in collaboration with the SEA LIFE Porto team, is structured into two complementary chapters: one focused on scientific research and the other on professional experience and development during the internship. Its development is guided by two central objectives, which combine the practical application of animal welfare research with the acquisition of professional skills essential for the effective practice and management of zoological institutions.

Based on the needs expressed by the company, two main objectives were established:

1. Development and implementation of an environmental enrichment plan for the green sea turtle (*Chelonia mydas*) resident in the aquarium's central tank;
2. Building technical skills related to the work of an aquarist, particularly in the operation of aquatic life support systems and the application of animal welfare procedures, in a professional context and as part of a specialized team.

Chapter 2: The Influence of Environmental Enrichment on a Resident Green Sea Turtle (*Chelonia mydas*): a Study Case

1. Framework for Developing Environmental Enrichment Programs

Environmental enrichment has become an essential element in the routine management of animals kept under human care, and it's been used to promote animal welfare in various zoos and aquariums (Mellen & Ellis, 1996; The Shape of Enrichment Inc., 2025). Public sensitivity and concern for animal welfare has been increasing in recent years (Grunert et al., 2018), highlighting the need for systematic and implemented environmental enrichment programs given the perception that these programs improve animal welfare (Schütz et al., 2020).

As a fundamental principle of animal caretaking, enrichment aims to enhance both physiological and psychological health (Shepherdson et al., 1998), and it has become an important focus in applied ethology (Carenzi & Verga, 2016). In this regard, environmental enrichment most often refers to intentional changes made to an animal's environment that lead to improved biological functioning. These positive changes can be observed through outcomes such as improved health, greater reproductive success over an animal's lifetime, enhanced behavioral flexibility, positive mental states and improved emotional regulation (Littlewood et al., 2023; Newberry, 1995). However, despite the benefits that environmental enrichment provides, some of these plans have no effect on animal welfare and, in some cases, may even have a negative impact. This highlights the need for rigorous planning, implementation, and ongoing evaluation (Taylor et al., 2023). Mellen and Sevenich MacPhee (2001) assert that environmental enrichment should follow a holistic approach, considering the animal's environment, under human care, as a whole. That means using knowledge about each animal's natural behavior and individual history to adjust its environment in terms of housing, socialization, and caretaking (like training, feeding, cleaning, etc.). Therefore, to develop an environmental enrichment plan for a specific animal, there are a few steps that should be followed (Figure 5).

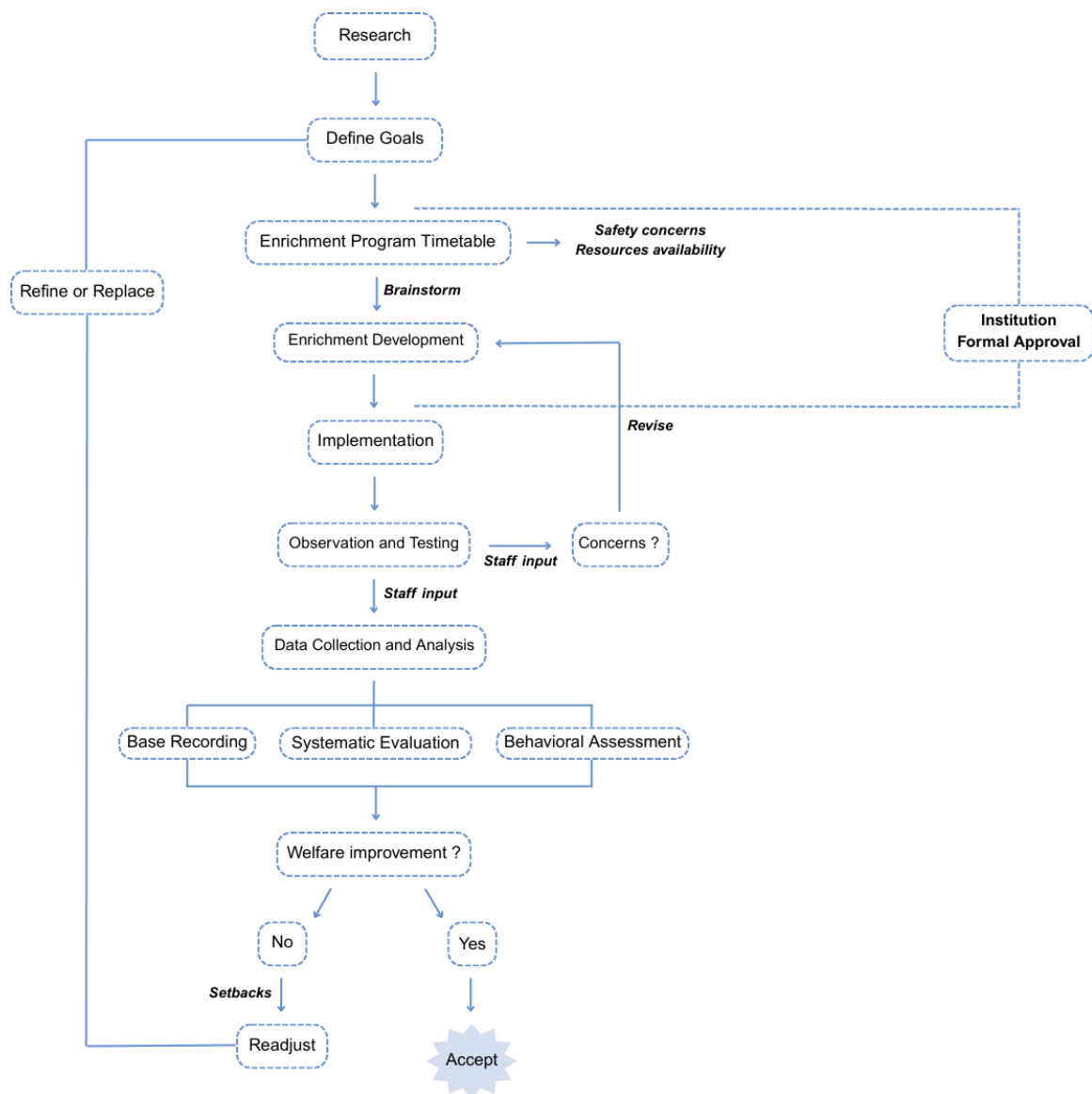


Figure 5 – Step by step on how to build an environmental enrichment program (Adapted from (Cipreste et al., 2010; The Shape of Enrichment Inc., 2025).

To build an effective enrichment plan, a continuous approach is required, starting with the creation of **clear objectives** and supported by in-depth **research**. This research should collect detailed information on individual animal histories, develop databases of species behavior in the wild and under human care, and identify species-specific safety requirements (Cipreste et al., 2010). It is essential to take a proactive stance, ensuring that any changes to the animals' environment are based on this knowledge with the inclusion of their natural characteristics, needs, individual experiences, and developmental stage (infant, juvenile, or adult) (Mellen & Sevenich MacPhee, 2001).

A second step for developing an enrichment program focuses on **planning** the interventions and obtaining **formal approval** from the institution. This step requires setting up formal and rigorous institutional processes for submitting, reviewing, and approving new

enrichment efforts. Roles and responsibilities must be clearly assigned and scheduled, with detailed specifications of each team member's duties and timelines. Equally important is to ensure that all proposals are evaluated for safety concerns and that the necessary resources - including materials, financing, and staff time - are secured prior to implementation to ensure full and effective execution (Cipreste et al., 2010; Mellen & Sevenich MacPhee, 2001).

Once the enrichment plan has been formally approved, the **implementation** phase begins, putting the plan into action in the animals' environment. Enrichments are implemented in accordance with the previously approved plan, whilst progress is monitored to ensure conformity and enable any problems to be quickly resolved. The benefits of a routine are that it creates consistency, facilitates reporting, and helps assess effectiveness. This step links planning to measuring welfare outcomes and must be rigorously executed to enable an accurate assessment (Cipreste et al., 2010).

A fourth step involving comprehensive **documentation** and **data collection** is necessary to accurately determine the success of an enrichment program. To do this, a multi-level approach to recording is needed, so there should be three levels of data collection. First, there's basic recording, where keepers note any noteworthy events related to enrichment initiatives in their daily reports. Second, systematic evaluation uses standardized scales to assess direct evidence, such as behaviors observed in animals in response to enrichment, and indirect evidence, such as signs that animals have interacted with enrichment items, particularly in the case of new or significant enrichment items. The third is scientific evaluation, in which the team performs structured behavioral studies in three phases: before the introduction of enrichment, during its implementation, and after its removal (Cipreste et al., 2010; Mellen & Sevenich MacPhee, 2001). This method allows for a thorough and reliable assessment of the effects of enrichment. Ensuring that results are valid, reliable, and repeatable, research on enrichment should follow established methodologies and experimental protocols used in behavioral science (Martin & Bateson, 2007). Such rigorous documentation supports accurate evaluation, informs necessary adjustments, and ultimately promotes evidence-based improvements in animal welfare.

Step five, **evaluation** and **analysis**, systematically reviews the data collected during the documentation phase to assess the effectiveness of the enrichment activities. That process analyzes keepers' evaluations and behavioral observations to check for trends in the animals' responses over time, giving valuable input on how the animals interact with different enrichment items or activities. Careful analysis in this step makes it possible to tell how well specific enrichment efforts are working and suggest the best times and ways to

use them for maximum effect. The assessment methods should be validated using standard techniques for collecting animal behavior data that provide reliable, repeatable, and quantifiable results. This evidence-based approach allows caretakers to make well-informed decisions regarding which enrichment strategies are most beneficial to the animals, and which should be continued, modified, or replaced in future planning cycles (Mellen & Sevenich MacPhee, 2001).

Finally, a final stage of **readjustment** and continuous **improvement** ensures that enrichment programs remain dynamic, evidence-based, and consistently meet the needs of the animals. This phase involves careful analysis of evaluation data to identify which strategies have been most successful and which require modification or replacement. It then begins a new round of the framework, setting updated goals that reflect both the successes achieved and the challenges identified during the evaluation (Mellen & Sevenich MacPhee, 2001). This cyclical approach is essential to maintaining the plan's long-term effectiveness, as it permits enrichment strategies to continually evolve in response to the animals' needs, to new research findings, and to a better understanding of species-specific requirements. Rather than treating enrichment as a static intervention, the readjustment stage ensures that enrichment programs remain adaptive and progressively more effective in promoting animal welfare (Taylor et al., 2023).

2. Behavioral Assessment as a Tool for Animal Welfare Improvements

Behavioral tools play a fundamental role in assessing animal welfare, by providing effective methods for evaluating the quality of life of animals and offering credible evidence to support decision-making in zoos and aquariums (Miller et al., 2023; Wark et al., 2019). Continuously monitoring behavior and space use enables keepers to identify significant trends and patterns that are specific to each individual, which helps in recognizing and adapting individual care to improve welfare (Wark et al., 2019). This type of long-term monitoring can also identify behavioral patterns of each individual, which allows managers to discern whether any deviation means that welfare has been compromised (Hill & Broom, 2009).

Several methods for behavior assessment have been developed and adapted to different species under human care. Reliable, consistent, and reproducible results are crucial in this type of welfare assessment, as they can contribute significantly to improvements in animal management (van der Staay et al., 2025).

Time Budgets and Behavioral Diversity

Behavioral assessments based on time budgets presuppose that the ratio of time an animal spends in positive or negative behavioral states is reflective of its overall welfare (Tallo-Parra et al., 2023; Watters et al., 2019). Rather than focusing on the presence or absence of specific behaviors, the aim is to capture whether animals are able to engage in their full range of natural behaviors, which is usually taken as a positive indicator of welfare (Watters et al., 2019). Time budget data can be summarized into diversity indexes, most notably the Shannon-Wiener Diversity Index (H index), used to quantify behavioral diversity (Miller et al., 2023). High behavioral diversity generally signals that an animal's environment allows for engagement in a variety of species-specific behaviors, while low diversity may indicate compromised welfare, such as inadequate enclosure design, social isolation, or lack of enrichment (Tallo-Parra et al., 2023).

The link between time spent on a diverse repertoire of natural behaviors and good welfare is supported by evidence from many species: management practices known to improve welfare, such as enrichment or animal training, tend to increase behavioral diversity. Nonetheless, these analyses must also take context into account: increases in negative behaviors (such as stereotypies) can increase diversity scores without truly representing positive welfare. Thus, the inclusion and careful interpretation of “negative” behaviors are crucial for accurate assessment (Miller et al., 2023).

Qualitative Behavioral Assessment (QBA)

Qualitative Behavior Assessment is a holistic assessment method developed by Wemelsfelder (2007), during which the observer captures and evaluates the behaviors and emotional state of animals using descriptive terms such as “calm,” “anxious,” “tense,” etc. In contrast to traditional ethograms—a collection of species-specific behaviors—, in this case, the observer can select the terms that best depict the animal's behavior, thus potentially detecting subtle changes that are not detected by quantitative methods. It can correlate rather well with other welfare indicators and is best used in tandem with other assessment tools, since it requires consistent validation of the descriptive terms and the reliability between observers across species and contexts (Andreasen et al., 2013).

Keepers' Ratings

Similar to QBA, this kind of assessment is based on direct observation by keepers and their interpretation of animal behaviors and expressions (Tallo-Parra et al., 2023). However, in contrast to the formal observations of QBA, keepers' ratings are based on everyday interactions, which allows caretakers to benefit from their ability to notice subtle

changes in animals' behavior and attitudes. Initially developed for farm animals, qualitative assessments of animals' individual welfare by zookeepers can also be integrated into the daily routines of zoos and aquariums. This approach is particularly advantageous because it requires less time than other methods, providing a practical way to monitor welfare, especially where resources for systematic direct observation are limited (Whitham & Wielebnowski, 2009).

Research shows that keeper assessments, when collected systematically, are highly reliable and correlate with more formal welfare indicators. However, some caution is needed due to potential biases, such as misconceptions about animal needs or difficulties in interpreting the behavior of non-mammalian species (Tallo-Parra et al., 2023).

Cognitive Bias Testing

More recently, a promising new method has arisen focused on cognitive bias—changes in perceptual ability caused by the emotional state of animals—in which animals are trained to recognize positive and negative cues and are then presented with an ambiguous stimulus. The assumption is that animals experiencing a negative affective state are more likely to interpret ambiguous signals as predicting a negative event, compared to animals in a more positive state (Mendl et al., 2009).

Although cognitive bias tests have great potential to uncover internal states that traditional behavioral assessments may overlook, their application in zoos and aquariums is limited by the training and resources required for its implementation. Nonetheless, as a validation tool or for specific welfare investigations, it is proving increasingly valuable (Clegg, 2018).

Space Use and Preference Testing

In environments under human care, it is important to assess how animals use their space and seek to understand their preferences, which allows gathering information about the positive and negative aspects of their space (Ross et al., 2009). One of the most common ways to do this is through preference tests (Fraser et al., 1993). Although space use is not exclusively influenced by preferences of the environment, but also by social and biological factors, allowing animals to choose between different characteristics of their environment can reveal valuable information that enables keepers to make more informed choices about habitat design, thereby enhancing welfare (Estevez & Christman, 2006; Wark et al., 2019).

To summarize, contemporary zoos and aquariums rely on a wide range of behavioral tools—both quantitative and qualitative—to assess and improve animal welfare. These

reflect a collective effort to develop credible, scientifically-based methods that are practical for practitioners and accurately represent the true welfare of animals in human care (Jones et al., 2022). Through integrating and combining these multiple approaches, institutions can meet increasing welfare standards and guarantee that management decisions are evidence-based, ultimately promoting the welfare of animals in their care (Miller et al., 2023).

3. Green Sea Turtle (*Chelonia mydas*)

3.1 Biological Overview

The green sea turtle (*Chelonia mydas*) (Linnaeus, 1758) who belongs to the family Cheloniidae, is a specimen with a complex life history that is difficult to track and study because of the large spatial and temporal scales involved (Bowen et al., 1992). This species is considered one of the most widely distributed marine reptiles, inhabiting subtropical and tropical waters worldwide, and to a lesser extent, temperate waters (Figure 6) (Hirth, 1997; Seminoff et al., 2015).

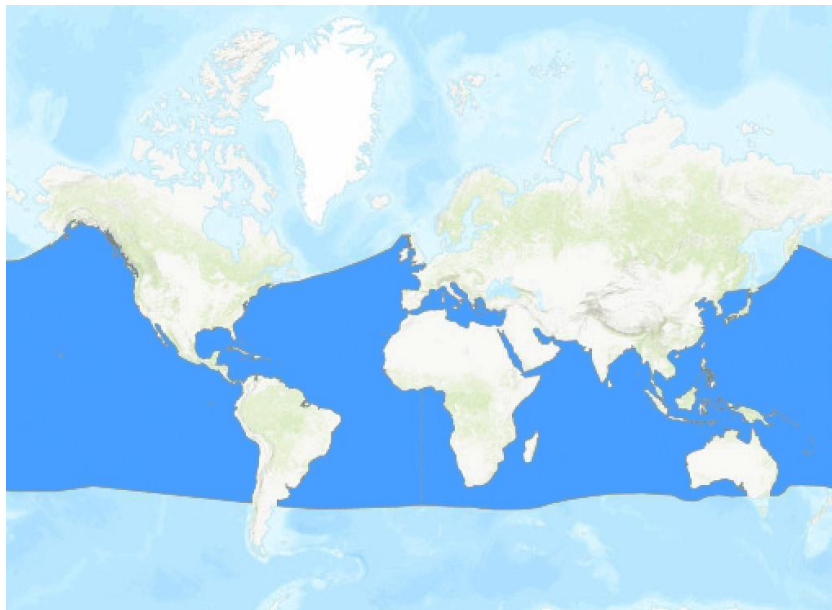


Figure 6 – Worldwide distribution of the green sea turtle (*Chelonia mydas*) (Intense blue) (Adapted from (Seminoff, 2004)).

There's record of nesting grounds in over 80 countries (Hirth, 1997) and although their movements are less understood, it is believed that green sea turtles inhabit coastal waters of over 140 countries (Groombridge & Luxmoore, 1989), which makes green sea turtles a cosmopolitan species (Alvarez-Varas et al., 2022).

In terms of morphology, the green sea turtle is considered the largest hard-shelled sea turtle and second in size against the leatherback (*Dermochelys coriacea*) (Lagueux, 2001). Adults usually weight between 150 and 200 kg, with a curved carapace length of 95 to 120 cm (occasionally up to 140 cm). The carapace is broadly oval, with five vertebral scutes, four pairs of costal scutes, and 12 pairs of marginal scutes, all non-overlapping. Distinctive traits of this species include a single pair of elongate prefrontal scales, a somewhat small head with a blunt beak, and four postorbital scales behind each eye (Carr, 1952; Hirth, 1997). Adult's plastron ranges in color from white cream to yellow, while the carapace varies from brown to olive with radiating or mottled markings. Oddly, the term "green" in their name actually refers to the greenish hue of its subdermal fat, as a result of their herbivorous diet (Carr, 1952).

As the majority of sea turtles, this is a highly migratory species which uses a wide range of broadly separated localities and habitats during their lifetimes (Hirth, 1997). Upon leaving their nests, hatchlings orient towards the ocean and begin an oceanic phase, where they take advantage of gyres (major current systems) to swim passively, which serves as open-ocean developmental grounds (Salmon et al., 1992). After a period of 2-10 years, juveniles go to neritic developmental areas where they forage and grow until maturity (Bolten, 2003). In these neritic habitats, juveniles undergo a dietary shift, from an omnivorous or carnivorous oceanic diet to a singular herbivorous dominated by seagrass and macroalgae (Bjorndal, 1997).

Growth rates are generally slow and are highly variable across populations, so it takes a prolonged time to reach sexual maturity – the longest of any marine turtles' species (Hirth, 1997). Estimate ages range widely from: 20-40 years in Atlantic and Australasian populations (Frazer & Ladner, 1986; Limpus & Walter, 1980), 9-58 in Hawaii (Balazs, 1982) and can be as low as 12-20 years in Caribbean Mexico (Zurita et al., 2012).

Reproduction is characterized by a long-distance migration between the foraging ground and the natal nesting beach (Meylan, 1982). Every 2 to 5 years, these migrations are carried out by both males and females and may cross oceanic zones. Females exhibit strong site fidelity to their natal rookeries, contributing to genetic structuring at surprisingly fine spatial scales. On average, females produce approximately 100 eggs per clutch (Hirth, 1997). Temperature during the middle third of the incubation period is directly related with sex determination of the hatchlings. In green turtles, the pivotal temperature—the constant incubation temperature at which an equal proportion of males and females is produced—differs between populations, typically ranging from about 28.0 to 30.3 °C (Godfrey &

Mrosovsky, 2006). During non-breeding periods, adults reside in coastal neritic feeding zones (Seminoff, 2023).

Molecular studies have also revealed that green sea turtles can be distributed in 11 distinct phylogeographic lineages. Most evidence points to an evolutionary origin in the Indo-Pacific, followed by colonization of the Central/Eastern Pacific and the Atlantic basin (Figure 7) (Jensen et al., 2013).

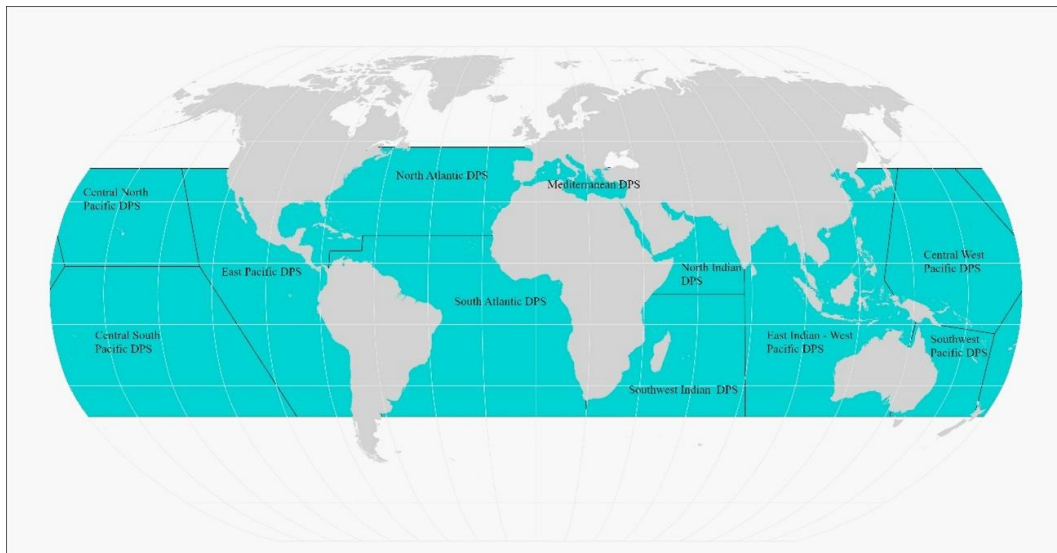


Figure 7 - 11 distinct population segments of green sea turtles worldwide (NOAA Fisheries, 2025).

3.2 Behavior in the Wild

In the wild, green sea turtle perform a wide variety of behavior, most of which are linked to their foraging, reproduction, resting routines and long-distance migrations. However, much about their behavioral patterns, particularly those occurring underwater, remains unclear (Wood, 2023).

This species is mostly active during the day, rotating between feeding and resting (de Boer et al., 2021). These tend to be most active in the mid-morning and mid-afternoon, although it also feeds on moonlit nights and in the hours before sunrise (Ogden et al., 1983). They spend most of their time submerged, holding their breath for minutes when active and extending to hours during resting or nighttime (Bjorndal, 1980). Resting sites are diverse and include coral ledges, caves, boulder fields, algal pastures or even open waters, usually far from feeding grounds (Bjorndal, 1980; Seminoff et al., 2006). Some of these sites, especially the ones with vertical features, appear to assist sea turtles in avoiding predation and remain motionless while conserving energy (Seminoff et al., 2006).

As described in section 3.1, green sea turtles undergo a dietary shift throughout their life stages. These turtles exhibit strong site fidelity, frequently returning to the same foraging grounds (Ogden et al., 1983). As they transition to nearshore habitats, their diet becomes primarily herbivorous, characterized by grazing on seagrasses and algae, although there's been evidence of adults occasionally consuming animal matter such as sponges and mollusk eggs (Bjorndal, 1997). Furthermore, green turtles have the unique habit of basking in the sun on the beach for hours, possibly to synthesize vitamin D due to their herbivorous diet (de Boer et al., 2021).

Green turtles frequently engage in grooming or self-maintenance behaviors. In reef habitats, they establish symbiotic relationships with cleaner fish that feed on epibiota attached to their carapace (Booth & Peters, 1972). In areas lacking these fish, sea turtles actively rub against sponges, rocks, or other hard surfaces to remove algae and/or crusting organisms (Heithaus et al., 2002). This rubbing behavior often involves rotating both the carapace and the plastron, providing tactile stimulation as well (Wood, 2023).

Mating behavior among green sea turtles happens close to nesting beaches following long migrations (Limpus et al., 1992). During breeding season, males exhibit intense sexual aggression, roaming around lagoons and attempting to mount any large object (Booth & Peters, 1972). Courtship involves head and flipper movements, biting, circling, and nibbling. Females have control over acceptance of mating and resist unwanted advances through avoidance. Successful males cling to the female's carapace using their claws during copulation. The females can mate with several males, leading to multi-parentality clutches, and have the ability to store sperm for several years (de Boer et al., 2021).

They are predominantly solitary. Aggregations are generally coincidental, taking place at feeding or breeding sites, lacking social organization (Ogden et al., 1983). Though males are highly aggressive during the breeding season, aggression out of breeding season is typically subdued. At feeding or resting locations, disputes over space are often resolved with circular displays, eye contact, and open-mouth postures, avoiding physical confrontations (Schofield et al., 2006).

3.3 Major Threats and Conservation Concerns

Green sea turtles (*Chelonia mydas*) have been connected to coastal civilizations for many years, nonetheless, over the last four centuries, unsustainable harvesting has led several populations to extinction and drastically has diminished others. Despite their long

cultural significance, this intense exploitation remains a critical historical and contemporary threat to their survival (Escobedo-Bonilla et al., 2022).

This species is classified as Endangered by the International Union for Conservation of Nature (IUCN) (Seminoff, 2004). Green sea turtles are protected internationally through multiple agreements like Annex II of the Specially Protected Areas and Wildlife (SPA) Protocol to the Cartagena Convention (United Nations Environment Programme, 1990), Appendix I of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (CITES, 1973) and Appendices I and II of the Convention on Migratory Species (CMS) (CMS, 1979). Unfortunately, despite all the efforts made in an international and regional level, enforcement gaps and continued legal and illegal harvesting of eggs and adults still persist, undermining effective protection of nesting and foraging populations (NOAA Fisheries, 2025).

Accidental mortality due to fisheries bycatch is a noteworthy ongoing threat. Often, turtles get caught in gillnets, trawls and longlines which results in high mortality through drowning and injury (Seminoff, 2023). The development of Turtle Excluder Devices (TEDs) has reduced some of these incidents, especially in the Americas (National Research Council, 1990).

The loss of habitat also poses a serious threat to green turtles. Nearshore urban development results in the degradation and erosion of nesting beaches, while pollution and habitat disturbance degrade essential foraging habitats near the coast, such as seagrass beds (Shaver et al., 2020). Furthermore, climatic change is currently emerging as a major threat, by raising nest temperatures, which causes distortions in sex ratios due to temperature-dependent sex determination in turtles. These threaten population viability, with the potential for site extinctions (Blechs Schmidt et al., 2020).

Despite the positive impact of protective laws and international agreements on green turtle conservation, significant challenges persist. To secure the species' survival across its migratory routes, it is essential to improve fisheries monitoring, safeguard critical habitats, reduce both legal and illegal harvesting, and implement forward-looking strategies to address climate change effects.

[3.4 Behavior Under Human Care](#)

Green sea turtles under human care display a range of behaviors that both mirror those seen in the wild and reveal their adaptive responses—whether positive or negative—to the captive environments in which they are maintained.

In overcrowded captive settings, like turtle farms, green turtles are often faced with behavioral challenges such as failed attempts to bask, preventing turtles from regulating their physiology, and frequently exhibit increased aggression, territoriality, and hierarchical disputes (Arena et al., 2013). Such competition is rare in the wild, since green turtles are typical solitary grazers (Heithaus et al., 2002). However, in these environments, weaker individuals often lose out to stronger ones, resulting in significant health and welfare concerns. Additionally, controlled feeding schedules disrupt their natural feeding patterns, causing elevated stress and social tension (Arena et al., 2013).

Reproductive stress is another major concern in overcrowded environments. Such conditions often lead to excessive male harassment of females, causing both psychological stress and physical injuries (Arena et al., 2013). Additionally, aggression and even cannibalism have been reported, driven by stress, hunger, deficiencies in the environment, and nutritional imbalances. Furthermore, poorly sized enclosures that fail to meet the turtles' minimum space requirements result in frequent boundary exploration, which is indicative of escape attempts (Arena et al., 2013; Kanghae et al., 2014).

Human handling can as well provoke strong stress responses that can be translated into escape behaviors, cloacal evacuations, regurgitation and aggression directed at handlers. Other stress-related abnormal behaviors/stereotypies consist of jaw rasping, pseudo-vocalisations, poor resting postures, and repetitive swimming patterns which are indicative of compromised welfare (Arena et al., 2013).

Similarly, when housed in appropriate environments and provided with appropriate enrichment, sea turtles under human care exhibit behavior indicative of positive welfare. There's been reported that turtles engage in exploratory behaviors (attentive and non-repetitive swimming), focused behaviors (purposeful interaction with objects by biting, pushing or rubbing) (Therrien et al., 2007; Wood, 2023). Self-grooming behavior, involving the use of cleaning tools, has been observed and is a reflection of a natural maintenance routine, although the ingestion of inappropriate materials, such as broom bristles, poses risks (Brill et al., 1995; Therrien et al., 2007). Resting behavior is natural and signals satisfaction when it occurs in natural proportions, but it becomes problematic if it is excessive and accompanied by lethargy (>75% of daily activity budget) (Warwick et al., 2013).

Behavioral patterns of green turtles under human care highlight the need for well-designed enclosures, appropriate densities, and meaningful enrichment to encourage natural behaviors and reduce stress-related disturbances. Implementing husbandry practices suited to the species' needs, that are adequately spacious and enriched, can

promote positive behavioral outcomes and significantly improve the welfare of green turtles under these conditions (Shepherdson et al., 1998; Wood, 2023).

3.5 Environmental Enrichment in Sea Turtles

Environmental enrichment (EE) for sea turtles is a valuable tool used to encourage natural behaviors, reduce stress and promote overall welfare under human care settings. By altering the environment to provide stimuli that mimic natural conditions, EE increases activity and mental stimulation (Wood, 2023). There's been evidence of use of various types of EE devices to provide stimulation and encourage natural behaviors in sea turtles.

New food items can be provided, sometimes hidden in devices such as buckets, tubes, water jugs with holes, stimulating turtles to engage in foraging behaviors for longer periods of time. Lettuce feeders enable grazing behavior while also serving as hiding places. These approaches increase engagement, promote natural feeding behaviors, and can encourage physical exercise (Diggins et al., 2022; Therrien et al., 2007; van de Merwe et al., 2009).

Physical complexity within the enclosure mimics natural habitats as well as supports natural behaviors. These include shallow areas for resting, deeper areas with caves or tubes for hiding, and environmental elements such as water currents, waves, rain, or waterfalls. It encourages exploration, reduces boredom, and allows for individual enclosure, respecting the solitary nature of turtles (Brill et al., 1995; Burghardt, 2005; Therrien et al., 2007).

Cognitive enrichment sets up turtles to solve problems or persist in tasks to get rewards. Activities can include association training, puzzles that require manipulation, and persistence tasks, such as obtaining frozen food from ice blocks or hard-to-reach places (Diggins et al., 2022). These exercises stimulate mental abilities, promote decision-making, and have long-term health benefits. This enrichment needs to be customized based on the turtles' preferences and physical abilities (Wood, 2023).

Even though sea turtles are usually solitary animals (Heithaus et al., 2002), cohabitation with compatible fish species can provide both social and visual enrichment and may help green turtles engage in their natural cleaning behaviors (Balazs et al., 1994). In addition, human interaction in controlled aquarium conditions increases the turtles' familiarity with their caretakers, reducing stress and promoting positive social experiences (Claxton, 2011).

4. Study Objectives

In collaboration with SEA LIFE Porto, this case study aims to assess the impact of environmental enrichment interventions (introduction of a shelter and a rotating enrichment plan) on the behavior, spatial distribution, and overall welfare of the green sea turtle (*Chelonia mydas*) residing in this aquarium.

To achieve this, the following objectives were established:

1. To evaluate the effect of introducing a shelter on the spatial distribution of the turtle within the tank:
 - Assess changes in space occupancy patterns before and after the introduction of the shelter;
 - Determine whether the use of refuge areas increases with the availability of the shelter;
 - To compare activity levels, use of the shelter and spatial distribution between morning and afternoon periods;
 - To determine whether the effects of the shelter on behavior and space use are modulated by the time of day.
2. To assess the effect of a rotating enrichment plan on behavioral diversity and space use:
 - Analyze the behavioral profile of the turtle in the presence and absence of enrichment devices, with a focus on natural behaviors, unwanted behaviors and responses to new stimuli;
 - Evaluate behavioral diversity using the Shannon Diversity Index;
 - Compare activity levels, use of enrichment and spatial distribution between morning and afternoon periods;
 - Determine whether enrichment effects are modulated by the time of day;
 - Identify preferences for enrichment items and determine whether they promote a more balanced use of the aquarium.

This approach is intended to help improve animal welfare practices and fulfill SEALIFE Porto's mission of conservation and environmental education.

5. Materials and Methods

5.1 Study Site

The ocean tank, called “Kingdom of Salacia”, is SEA LIFE Porto's largest aquarium. With an approximately square shape, it holds around 550,000 liters of water, with depths varying between 5 meters in the shallowest area and 7 meters in the deepest. This aquarium is home to 108 marine organisms of 24 different species, including a green sea turtle (*Chelonia mydas*), two zebra sharks (*Stegostoma tigrinum*), four blacktip reef sharks (*Carcharhinus melanopterus*), a southern stingray (*Hypanus americanus*) and schools of fish such as the golden trevally (*Gnathanodon speciosus*), among others. To guarantee the well-being of all these species, the water is carefully regulated to maintain a temperature range of 22-25°C, with a pH between 8.2 and 8.4 and salinity of 26-31 ppt.

The tank can be viewed from six different angles, thanks to the acrylic panels that provide panoramic views of its interior. On the ground floor, viewers can overlook the aquarium through two small windows called “Rockpool windows” (Figure 8A), a large panoramic “balcony” (Figure 8B) and the underwater tunnel (Figure 8C), which crosses the tank and offers an immersive experience of the ecosystem created inside. On the first floor, there are three additional windows (Figure 8D, E, F), allowing the observation of the aquarium from different perspectives.

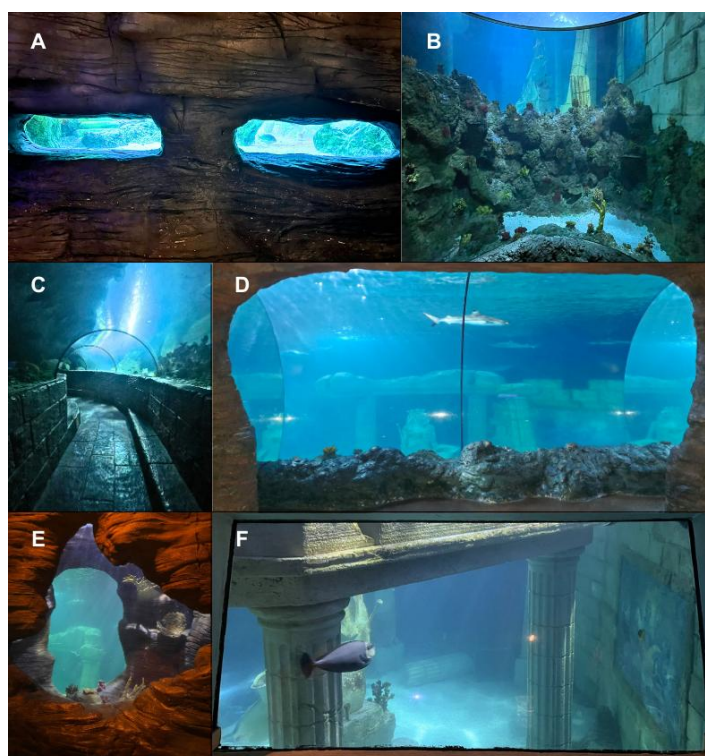


Figure 8 - Perspectives of the Aquarium; A- “Rockpool windows”; B- “Balcony”; C - Underwater Tunnel; D, E and F – 1st floor views (Author: Maria Castelo).

To ensure the proper maintenance and feeding of the animals, the ocean tank has three dedicated technical rooms. The first is located at the beginning of the first floor (Figure 9 - green circle), next to the panoramic window, and is used for feeding one of the zebra sharks, the southern stingray, the four blacktip reef sharks and the other fish. The second room is in the “Ray Bay” (Figure 9 – red circle), shared with two other tanks, and is mainly used for storing materials used in cleaning and maintenance of the tank, such as equipment for siphoning. The third and largest technical room, “Ocean Platform” (Figure 9 – white arrow), the only one outside the exhibition area, is the access point for divers from the biology team and is used for maintenance operations, handling or transference of animals or other specialized interventions. This room also has a platform dedicated to feeding the green sea turtle and, on one of its sides, the second zebra shark is fed.

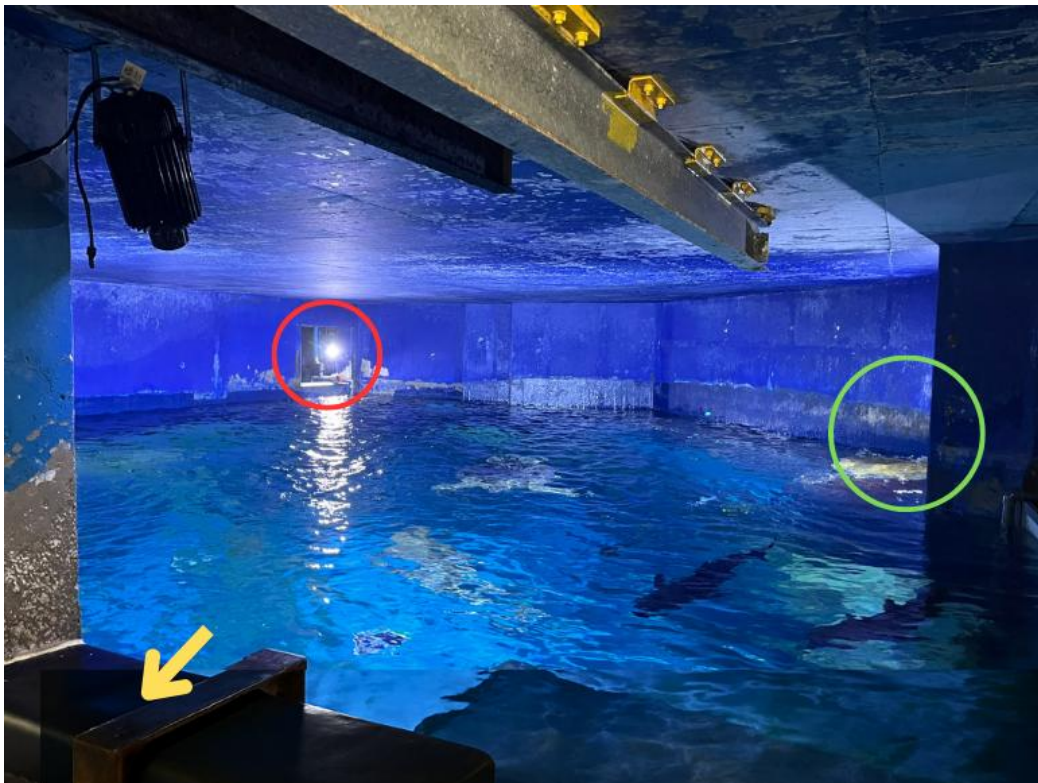


Figure 9 – View of the central tank surface from the perspective of the “Ocean Platform” technical room (yellow arrow) and the windows of the two other technical rooms – “Ocean view” technical room (green circle) and “Ray Bay” (red circle) (Author: Maria Castelo).

For the purposes of the study, the aquarium was divided into zones according to ecological value and function, based on prior observation of the turtle and its behavior within the space. This division was essential for subsequent analysis of the sea turtle's use of space and implementation of environmental enrichment devices.

A division into 13 distinct zones was therefore established (Figure 10), in which, for the purposes of further analysis, zone 7 was subdivided into two additional zones (7E -

partially inside the shelter and 7A – behind the shelter) due to the introduction of a specific environmental enrichment device (shelter).

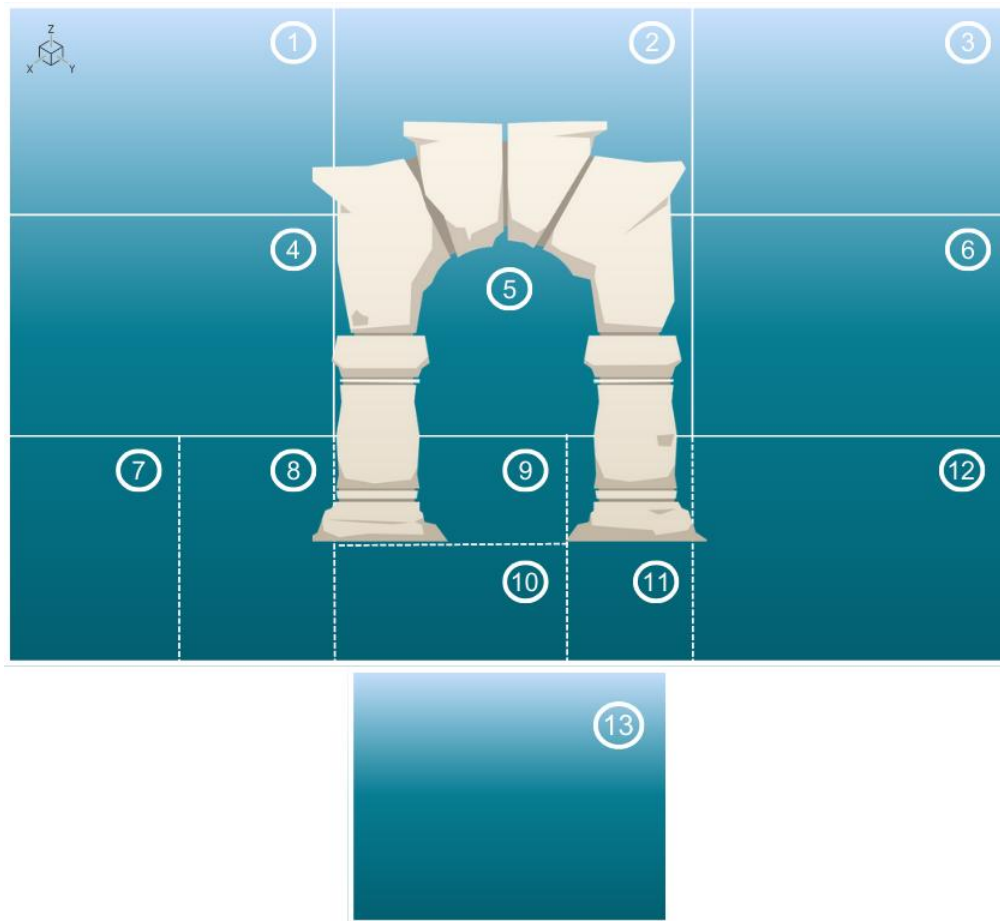


Figure 10 - Graphical representation of the central tank with the division of zones by depth: **surface** (A1-A3); **middle** (A4-A6) and **bottom** (A7-A13) (Author: Maria Castelo).

The zones can be grouped according to depth: surface (A1-A3), middle (A4-A6), and bottom (A7-A13). In addition, some areas were classified according to their functional value: the feeding area (A1), as this is where the platform where the turtles' feeding routine takes place is located, free swimming areas (A2-A6) and resting areas (A9 and A13). For a more comprehensive overview of the area divisions, please consult Appendix B.

5.2 Study Subject

For this study, the focus was on the green sea turtle (*Chelonia mydas*) residing in the ocean tank at SEA LIFE Porto. It's a male named "Mariza," born in a turtle farm in the Cayman Islands between 2001 and 2003.

The turtle was transferred to SEA LIFE Brighton, where it remained between December 2005 and February 2006. In the same month, it was transferred to SEA LIFE

Benalmadena, where it remained until May 2011. Finally, still in May 2011, while still juvenile, the turtle was transferred to SEA LIFE Porto, where it is currently an adult, with an estimated age of 24 years (Figure 11).

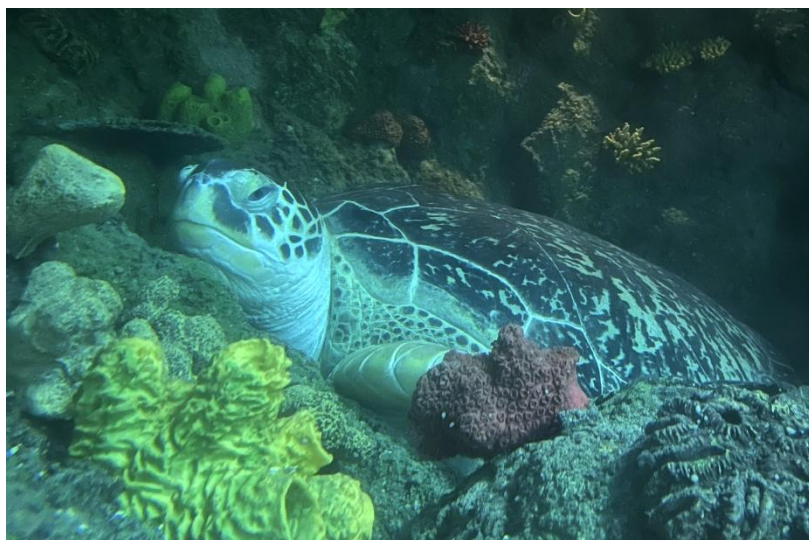


Figure 11 – The green sea turtle “Mariza” (24 years old) (Author: Maria Castelo).

Mariza is one of the most iconic resident animals at SEALIFE Porto, serving as one of the biggest draws for visitors but also as a symbol for the promotion of conservation and care for nature.

5.3 Preliminary Observations

Between October and December 2024, *ad libitum* observations were conducted, exclusively on working days and without a pre-established schedule. Any new or relevant behaviors exhibited by the animal during passages through the aquarium were immediately recorded. These observations were intended to familiarize myself with the animal's behavioral patterns and to develop a specific ethogram for the specimen under study.

A total of 28 behaviors, detailed in Table 1, were recorded and serve as the basis for the systematic observations. To further ensure the completeness of the ethogram, information from the research literature was added, incorporating behaviors observed in other members of the species that, while not exhibited by the individual studied, are relevant for a broad and accurate characterization of the species' behavioral repertoire. A more detailed version of the ethogram is provided in Appendix C.

Table 1 - Categories and descriptions of behaviors displayed by green sea turtles.

Category	Behaviors	Description	References
Rest	Sleep/Rest.	Behaviors associated with periods of inactivity, during which the turtle reduces its movements and energy consumption.	(Therrien et al., 2007); <i>Ad libitum</i> observation.
Locomotion	Crawl; Dive; Surfacing; Horizontal swimming; Surface swimming; Turn.	Refers to all movements that allow a change in position or orientation within your environment.	(Lowry, 2019); <i>Ad libitum</i> observation.
Aggression	Biting animal; Flipper slap.	It encompasses harmful or threatening behaviors directed at another individual.	(Therrien et al., 2007); <i>Ad libitum</i> observation.
Breathing	Respiration; Between breaths.	Behaviors related to the breathing process.	(Lowry, 2019; Therrien et al., 2007); <i>Ad libitum</i> observation.
Grooming	Scratch with the flipper.	Self-directed behaviors performed to maintain the cleanliness, health, and good condition of an animal's body surfaces.	(Lowry, 2019; Therrien et al., 2007); <i>Ad libitum</i> observation.
Feeding	Grazing; Eating.	It consists of a series of activities focused on locating, collecting, and eating food.	(Diggins et al., 2022); <i>Ad libitum</i> observation.
Interaction with Environment	Push; Pull; Touch; Rubbing; Interaction with diver; Biting object.	Behaviors wherein the animal physically interacts with items or elements of its environment, either for exploration, manipulation, maintenance, or communication.	(Lowry, 2019; Therrien et al., 2007); <i>Ad libitum</i> observation.

Table 1 - (Continued).

Category	Behaviors	Description	References
Hiding	Hiding.	The turtle positions itself in such a way as to reduce its visibility, detectability, or accessibility to potential disturbances.	(Therrien et al., 2007); <i>Ad libitum</i> observation.
Defecation	Defecation	Refers to the expulsion of solid waste from the digestive tract through the cloaca.	<i>Ad libitum</i> observation
Abnormal Behavior	Gafting of jaw; Pseudo-vocalisation; Pattern swimming; Rest in poor posture; Boundary exploration.	References to atypical or unusual behaviors that deviate from the species' standard behavioral repertoire, often indicating stress, illness, injury, or environmental disturbances.	(Arena et al., 2013; Diggins et al., 2022)
Out of Sight	Out of sight	Times when the animal is out of sight due to leaving the visual field, being behind obstacles, or in areas not directly observable, including when its behavior cannot be determined.	(Lowry, 2019); <i>Ad libitum</i> observation.

5.4 Systematic Observations

Systematic observations were carried out over a period of five months, between the months of January and June 2025. During this period, observations were conducted for Objective 1 of this study, the Shelter Place Experiment, and for Objective 2, the Assessment of the Rotating Enrichment Plan. Because of time constraints, last two phases to take place had to be carried out partially simultaneously. Direct observations took place at the underwater tunnel, on the ground floor, to provide the most thorough view of the entire tank area. The two studies were organized as follows:

5.4.1 Shelter Placement Experiment

Shelter

The main goal of the shelter was to provide a hiding place that would allow the turtle to reduce its exposure to external stimuli, such as visitors, thus providing a safer and less stimulating environment. This shelter simulates the natural conditions found in the wild, where turtles use underwater cavities or other refuges to rest, conserve energy, and avoid predators (Seminoff et al., 2006).

For this experiment, a commercially used water storage drum was used as the EE hiding device, which was placed in area 7 of the tank. With the introduction of the shelter, two new subzones were formed within this zone 7: 7E - inside the shelter and 7A - a cave that formed between the shelter and a decorative column.

Spatial Use Observations

Observations were conducted in two distinct phases to evaluate the effect of a permanent hiding place on the turtle's spatial use. Baseline observations (total of 88 observations – phase 1) were carried out from January 15th to February 14th, prior to the introduction of the hiding place, serving as control data. In this phase 1, four observations were made daily (9 a.m., 12 p.m., 3:30 p.m., and 4:40 p.m.), Monday through Friday for 22 days.

Following the installation of the permanent hiding place (phase 2), 88 observations were conducted from March 6th to June 12th to assess changes in spatial distribution. This design allowed comparison between the turtle's space use in the absence and presence of the shelter. In the second phase, the main change was that weekly observations were reduced to Tuesdays and Thursdays to allow objective 2 to be carried out concurrently.

In both phases, a focal animal sampling sessions were conducted to assess the time (in seconds) that the turtle spent in each of the predefined areas of the tank with the aid of a digital chronometer. Each session had a duration of 10 minutes, during which, whenever the animal moved to a new zone (a change of zone was only considered when the head and front flippers were in the new zone), a "lap" was recorded on the chronometer. Simultaneously, an audio record was made with a smartphone recorder, in which each "lap" was verbally matched to the respective zone to aid in recording and minimize possible errors in the records.

5.4.2 Assessment of the Rotating Enrichment Plan

To evaluate the effects of environmental enrichment (EE) on turtle behavior and space use, observations were conducted under two conditions: with EE and without EE (control). EE were introduced three times a week—Mondays, Wednesdays, and Fridays—at random, ensuring, whenever possible, that each EE was present at least once every two weeks (this was not always possible due to logistical constraints), either in the morning or in the afternoon. The aim of this approach was to maintain the novelty of the EE and prevent habituation or learning. An example of the timetable used to record the application of the different enrichments is presented in Appendix D.

Selecting Environmental Enrichment Devices (EE)

Each enrichment device used in this study was selected based on existing scientific as well as grey literature from specialists and professional aquarium associations regarding the behavior of green sea turtles both under human care and in the wild, and adapted to the individual characteristics of the turtle in study. Considering these factors, as well as handling requirements, a detailed enrichment plan was proposed and discussed with the SEA LIFE's biology team. EE devices were selected in a way that would provide opportunities for the green sea turtle to engage in foraging behavior, to increase exploratory swimming and use of the available space, and to stimulate tactile sensitivity. EE devices were presented three times per week for a duration of 30 minutes, ensuring consistency of exposure and allowing for structured behavioral observations.

The placement of environmental enrichment (EE) devices was primarily guided by the logistics and available space within the tank. Devices that required larger areas, such as the grazing mat and the brush used by the diver, were positioned in the most spacious zones—specifically areas 4 and 8—to allow for ample use. Additionally, to encourage the turtle to explore the entire tank, the remaining enrichment items were strategically distributed with this objective in mind.

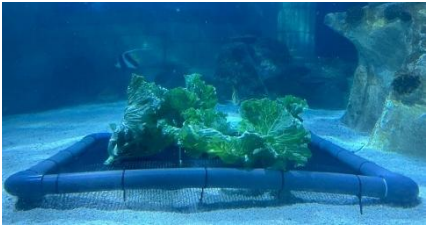
Regarding the shelter, zone 7 was selected as the most suitable location due to its more secluded nature, providing the turtle with a less visible, sheltered space for resting. However, it was essential that all enrichments, including the shelter, be placed in locations that allowed for continuous observation to ensure monitoring of the turtle's behavior at all times.

Table 2 shows the enrichment devices selected for this study. The table identifies each EE, and provides a picture of each, along with its type, objective, expected response to EE, and designated placement area within the enclosure.

Table 2 - Overview of environmental enrichment devices utilized, including their picture, type, purpose, stimulated response, and placement area.

Enrichment Device	Device Photo	Type of Enrichment	Objective	Promoted Response	Area Placement
Diver with Scrub		Sensorial Social	Promote social interaction between the animal and the diver. Encourage animal welfare and the expression of maintenance behaviors.	Encourage interaction with the diver as well as rubbing behavior.	Areas 4, 5, 8, 10
Fixed Scrub		Sensorial	Encourage animal welfare and the expression of maintenance behaviors.	Encourage rubbing behavior.	Area 3
Shelter		Structural	Provide the animal with the option to hide and remain out of sight during rest periods.	Promote the opportunity to hide, staying completely out of sight.	Area 7 (7E – partially inside the shelter; 7A – Behind the shelter)

Table 2 – (Continued).

Enrichment Device	Device Photo	Type of Enrichment	Objective	Promoted Response	Zone Placement
Yoga Ball with Spikes		Sensorial Structural	Encourage superficial exploration of the aquatic environment and engagement in playful activities.	Encourage swimming and interaction with the device, as well as exploration of the various areas.	Areas 1, 2, 3
Cantaloupe		Sensorial Dietary Structural			
Grazing Mat		Dietary	Encourage occupation of deeper areas. Promotion of time use, encouraging natural behavior of the species.	Stimulate grazing behavior and increase occupancy of the deeper areas.	Area 8
Tube with Vegetables		Dietary Cognitive	Encourage deeper area use and provide a cognitive challenge that promotes time use and problem-solving.	Engage in foraging behavior by investigating, biting, and pulling at the vegetables inside the tube.	Area 11

Behavioral and Spatial Use Observations - Enrichment

Observation sessions took place twice a day, in the morning between 12 p.m. and 1:30 p.m., and in the afternoon between 3 p.m. and 4 p.m.. Each session lasted 30 minutes and was divided into: 20 minutes dedicated to behavioral recordings and 10 minutes to assessing space use for the second phase of the study one.

For the 20 minutes of behavioral recordings, instantaneous observations with annotations every 30 seconds of the behavior exhibited and area occupied was used (Appendix E). To mark the 30-second intervals, a mobile app called "Intervals" was used, which emitted a sound signal at each interval.

For these observations, the behavioral categories Rest, Locomotion, Aggression, Breathing, Grooming, Hiding, and Abnormal Behavior were used. However, when analyzing specific behavioral changes in response to environmental enrichment, individual behaviors rather than broad categories were examined. These specific behaviors included Grazing, Eating, Push, Touch, Rubbing, Interaction with diver, and Biting Object (Table 2), allowing for more precise detection of enrichment-induced behavioral modifications. The areas used were the same, except for areas 7E and 7A, which were included in area 7.

5.5 Statistical Analysis

A series of complementary statistical analyses were conducted to investigate how space use changed with the introduction of a shelter. All analyses were performed in GraphPad Prism (v10.0).

Effect of Shelter on Spatial Distribution

To assess whether the distribution of time spent in the zones shifted with the introduction of the shelter, the temporal points within each condition were grouped together. A two-way ANOVA was performed with *Areas* (15 areas) and *Shelter Condition* (before vs. after) as factors.

Due to the sensitivity of this parametric approach to infringements of normality and independence assumptions, we adopted a conservative threshold for statistical significance ($p < 0.01$) for this analysis, in order to reduce the risk of type I errors.

To explore which particular zones had contributed to the overall effect, the percentage of time spent on each zone prior to and after the shelter was compared using the Wilcoxon test, treating each zone as a paired observation. This non-parametric

approach was opted for due to the non-normal distribution of the data at the zone level (Shapiro-Wilk test, $p < 0.05$).

Effect of the Time of Day on Space Utilization

To determine whether spatial use varied depending on the time of day, two separate two-way ANOVAs were performed—one for the *Before* condition and one for the *After* condition—with *Zone* and *Time of day* as factors. This allowed one to determine whether the time of day modulated spatial occupancy in each phase independently. Again, due to deviations from normality, significance was considered only for results with $p < 0.05$.

Interaction Between the Shelter and the Time of Day

In order to assess whether the effect of the shelter varied throughout the day, a two-way ANOVA was performed with *Shelter Condition* and *Time of Day* as factors. Here, the response variable was either the total time spent in each area (grouped by zones) or the average per zone. The analysis assessed if the impact of shelter was more pronounced at certain times of the day through interaction effects. As above, results were interpreted conservatively ($p < 0.01$).

Effect of Environmental Enrichment on Behavior and Spatial Distribution

To evaluate the effect of enrichment on behavior, Wilcoxon signed-rank tests were applied for each behavior to compare behavioral frequencies between conditions (with EE vs. without EE).

For behaviors with a high proportion of zero values, Fisher's exact test was applied to compare the presence or absence of behavior between conditions. A one-tailed test was chosen a priori because the environmental enrichment devices were expected to increase these behaviors.

Behavioral diversity was assessed using the Shannon Diversity Index, which considers both the richness and uniformity of the different behaviors exhibited by the individual:

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

H – Shannon Diversity Index

S – Number of behaviors observed

p_i – Proportion of the total observations represented by behavior (i.e. frequency of behavior i divided by total behaviors)

Behavioral diversity was assessed using Hill numbers, a unified framework that quantifies diversity by incorporating both richness (number of behaviors) and evenness (uniformity in behavior distribution). Specifically, the Shannon Diversity Index (Hill number of order $q=1$) was calculated to reflect the effective number of distinct behaviors exhibited by the individual:

$$\begin{aligned} \text{Hill numbers} \\ \text{when } q > 0 \text{ and } q \neq 1 \quad {}^qD &= \left(\sum_{i=1}^S p_i^q \right)^{1/(1-q)} \\ \text{when } q = 1 \quad {}^1D &= \exp \left(- \sum_{i=1}^S p_i \ln p_i \right) \end{aligned}$$

To determine whether environmental enrichment increased activity and exploratory behavior, the total frequency of *Total Activity* behaviors (includes all behaviors with the exception of rest, hiding and breath) and *Total Exploratory* behaviors (includes all the behaviors related to the interaction with the environment – grazing, eating, push, pull, touch, biting object, rubbing and diver interaction) per session were compared between enriched and non-enriched conditions using a Wilcoxon signed-rank test, as the data were not normally distributed and a directional hypothesis was established a priori.

As to assess whether the time of day affected behavior and whether the effect of enrichment depended on the time of day, a two-way ANOVA was conducted on the categories of *Total Activity* and *Total Exploration*. These aggregate measures were used instead of individual behaviors, as the latter contained a high proportion of null values.

A two-way ANOVA was performed on area occupancy to assess whether the time of day affected space use and whether the effect of enrichment depended on the time of day. Preference for enrichment devices was assessed by comparing the time spent interacting with each device using a Kruskal-Wallis test, since the assumptions of normality were not met, followed by multiple post hoc comparisons to identify specific differences between EE devices.

6. Results

6.1 Effect of Shelter on Spatial Distribution

The two-way ANOVA analysis revealed a significant main effect of *Area* ($F(4.827, 839.9)=123.0, p<0.0001$), meaning that the proportion of time the individual spent varied significantly between different areas, regardless of the presence or absence of shelter.

Meanwhile, the main effect of *Shelter* was not significant ($F(1, 174)=0.6934$, $p=0.4062$), which suggests that when all areas were viewed together, adding a shelter did not independently change the overall time distribution. More importantly, there was a significant interaction between *Area* and *Shelter* ($F(14, 2436)=2.945$, $p=0.0002$), indicating that changes in spatial distribution after shelter introduction depended on the specific area being considered. That is, the effect of the shelter was not uniform across all areas - some areas were preferentially used or avoided after the introduction of the shelter (Figure 12).

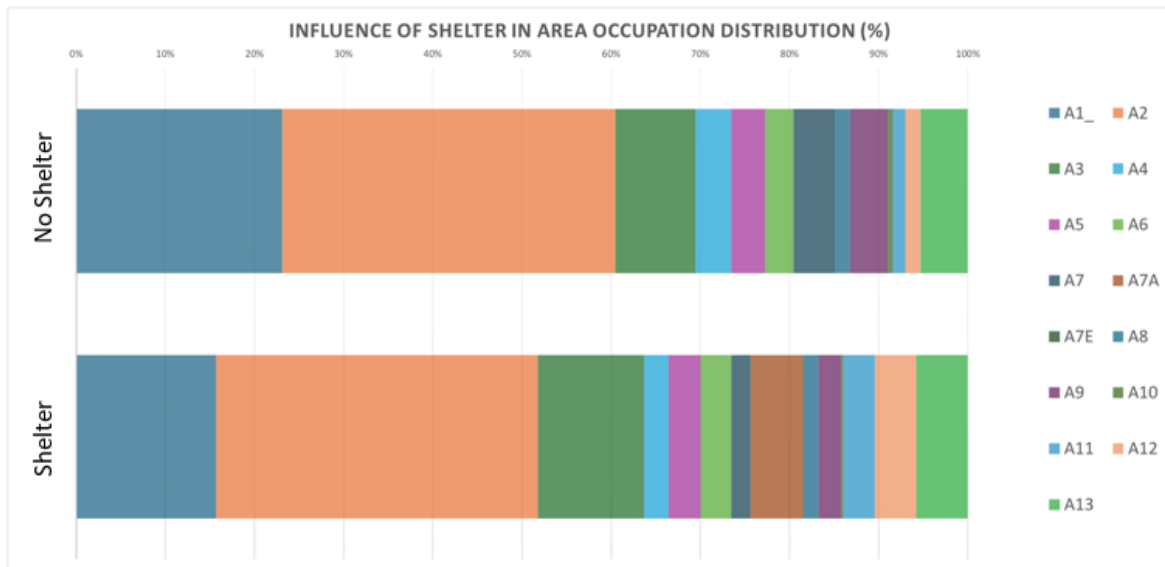


Figure 12 - Proportion of time the animal spent in each area of the aquarium under the conditions *No Shelter* and *Shelter*, calculated as the number of observations in each area divided by the total number of observations. These values represent space utilization or relative occupancy.

The non-parametric analysis (Wilcoxon signed-rank test) comparing space use in each area before and after the introduction showed significant change in space use in three areas (Table 3).

Table 3 - Mean percentage of occupation for each aquarium area before and after shelter introduction, with results from Wilcoxon signed-rank tests.

Area	Mean Occupation (No Shelter)	Mean Occupation (Shelter)	W	P-value
1	23.24	15.82	-1721	<0.0001
2	37.44	36.19	-353.0	0.4575
3	9.10	11.95	930.0	0.0449
4	4.06	2.76	-646.0	0.0526
5	3.86	3.68	-95.00	0.7830
6	3.19	3.39	-13.00	0.9660
7	4.68	2.21	-311.0	0.0694

Table 3 – (Continued).

Area	Mean Occupation (No Shelter)	Mean Occupation (Shelter)	W	P-value
7E	0	0.125	3.000	0.5000
7A	0	5.89	36.00	0.0078
8	1.68	1.64	-36.00	0.8317
9	4.26	2.55	-72.00	0.2186
10	4.26	2.56	0.0997	-143.0
11	1.4	3.52	146.0	0.1960
12	1.68	4.66	93.00	0.1621
13	5.37	5.79	-24.00	0.8492

Legend - For each area, the table shows the average proportion of time spent before ("No Shelter") and after ("Shelter") shelter placement, the Wilcoxon test statistic (W), and the corresponding p-value. Statistically significant differences ($p < 0.01$) are indicated in bold.

Noticeably, after the introduction of the shelter, there was a considerable decrease in the time spent in Area 1, with occupancy falling from 23.24% to 15.82% ($p < 0.0001$). By contrast, Areas 3 and 7A showed significant increases in occupancy: Area 3 increased from 9.10% to 11.95% ($p = 0.0449$) and Area 7A went from no occupancy (0%) to 5.89% ($p = 0.0078$) after the shelter was set up. All other areas showed no significant change in occupancy ($p > 0.01$), indicating that the impact of the shelter was concentrated in specific areas rather than causing widespread changes in spatial distribution.

6.2 Effect of the Time of Day on Space Utilization

To explore temporal patterns of spatial occupancy, two separate two-way ANOVAs were performed—one for the condition without shelter and one for the condition with shelter—with *Area* and *Time of Day* as factors. This analysis assessed whether space use differed across times of day and whether certain areas were preferentially occupied at specific periods.

In both the *No Shelter* and *Shelter* phases, a highly significant main effect of *Area* was found ($p < 0.0001$), meaning the amount of time spent in each zone varied considerably from one area to another, regardless of when observations were taken. However, the main effect of *Time of day* was not significant in either condition ($p > 0.9999$), indicating that, when looking across all zones, overall occupancy did not change as a function of the time period.

There was a significant interaction between *Area* and *Time of day* in both conditions ($p < 0.0001$), corroborating that the influence of time on space use depended on the specific

area. That shows that temporal changes in occupancy were limited to certain zones, while others maintained consistent usage patterns throughout the day.

Post hoc analyses revealed pronounced temporal changes in occupation and can be corroborated by Figure 13. Area 1 showed the most pronounced temporal effects under both conditions: in the *No Shelter* condition, occupation was low in the morning (9 a.m.) but increased significantly across all afternoon periods (12 p.m., 3:30 p.m., and 4:40 p.m.) (all $p < 0.01$). This trend is visually clear in the heatmap, with deeper purples in the morning turning to shades of yellow and orange throughout the day. Under the *Shelter* condition, there was a reduction in the occupation of Area 1 across all periods, compared with *No Shelter* condition.

Area 2 exhibited similar patterns in both conditions, with significant temporal shifts occurring primarily between morning and afternoon periods, where occupancy increased. Under *No Shelter*, occupation differed significantly across multiple comparisons (9 a.m. vs. 12 p.m., 3:30 p.m., and 4:40 p.m.; 12 p.m. vs. 3:30 p.m.; all $p < 0.01$), while the *Shelter* condition also showed significant differences between morning and afternoon periods (9 a.m. vs. 12 p.m., 3:30 p.m. and 4:40 p.m.; all $p < 0.05$).

Although differences are not statistically significant, the heatmap visually suggests altered occupancy patterns in zones 7A, 9, and 13 between conditions. For instance, area 7A shows an increase in occupancy at 4:40 p.m., while areas 9 and 13 experienced a decrease and increase in occupancy at 9 a.m., respectively.

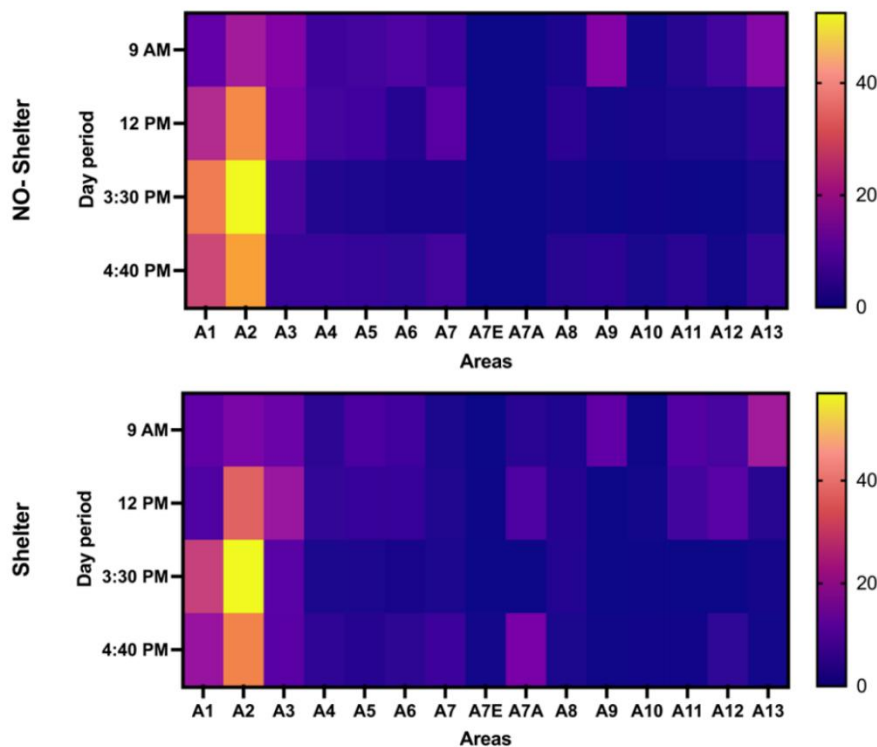


Figure 13 – Temporal and spatial distribution of area occupancy under *No Shelter* and *Shelter* conditions.

6.3 Interaction Between the Shelter and the Time of Day

To determine whether the effect of the shelter varied throughout the day, a two-way ANOVA was performed with Shelter Condition and Time of Day as factors. This analysis revealed whether the impact of the shelter on space occupancy was consistent across all periods or more pronounced at specific times of day. The testing showed that neither the main effect of *Time of Day* nor the main effect of *Shelter Condition* were significant ($p > 0.9999$ and $p = 0.9608$, respectively), meaning that when all areas were considered together, no factor independently (*Shelter Condition* or *Time of Day*) influenced overall space use. Moreover, there was no significant interaction between *Shelter Condition* and *Time of Day* ($p > 0.9999$), demonstrating that the presence or absence of the shelter had no differential effect on spatial occupancy at different times of the day. These results indicate that the impact of the shelter on space use remained consistent throughout the day and was not either stronger or weaker at any specific time. No time-modulation of the effect of the shelter was detected when calculating the average occupancy across all areas, as all p-values were well above the conservative significance threshold of $p < 0.01$.

6.4 Effect of Environmental Enrichment Devices on Behavior and Spatial Distribution

6.4.1 Impact of Enrichment on Behavior

Figure 14 descriptively illustrates the percentage of frequencies of the different behaviors performed by the turtle under standard conditions (without the presence of enrichments) and with the presence of environmental enrichment. The bar chart visually demonstrates that the presence of EE devices results in greater diversity of behaviors.

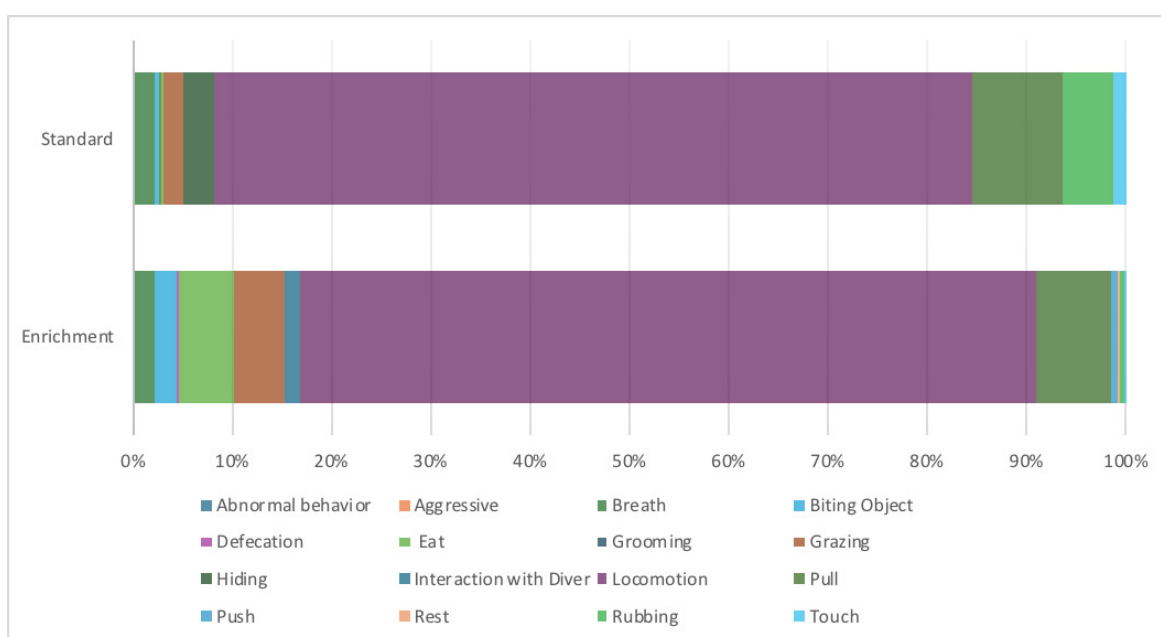


Figure 14 – Behavioral profile of the resident turtle in both *Standard* and *Enriched* conditions. Percentage of frequency of observed behavioral categories.

The calculation of the Shannon Diversity Index (Figure 15) showed that behavioral diversity increased with the introduction of the enrichments (1.018 - *Standard* and 1.266 - *Enriched*). The Hill numbers calculated from the Shannon Diversity Index values were approximately 2.77 for the Standard condition and 3.55 for the Enriched condition. These values represent the effective number of distinct behaviors exhibited by the individual under each condition, corresponding to an increase of approximately 28.15% in behavioral diversity with enrichment.

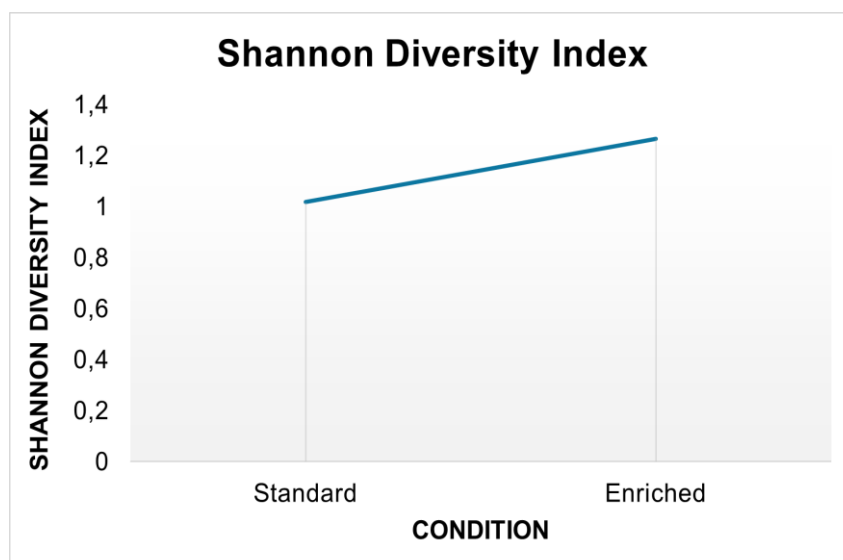


Figure 15 – Impact of enrichment on the behavioral diversity of the green sea turtle, calculated using the Shannon Diversity Index.

Further analysis verifies this shift between conditions. Figure 16 details the impact of introducing EE devices on each behavior.

Resting behavior (A) significantly decreased in the presence of enrichment, with a marked decrease in frequency compared to standard conditions ($W = -101.0$; $p = 0.00025$, one-tailed Wilcoxon test). On the contrary, no significant differences were observed in *locomotion* (B) ($W = -87.00$; $p = 0.2222$), *breath behavior* (C) ($W = -7.000$; $p = 0.4846$), or *grazing behavior* (D) ($W = -4.000$; $p = 0.4748$). Meanwhile, the frequency (%) of *eating behavior* (E) was significantly higher with the presence of the enrichment devices, occurring in 31.4% of the enriched sessions compared to only 5.7% in the standard sessions ($n = 35$; $p = 0.0059$, Fisher's exact test, one-tailed).

Behavioral interactions with the environment, such as *push* (P), *pull* (G), *touch* (H), *biting object* (I), and *diver interaction* (K) were all more frequent or exclusively present during enrichment sessions, but did not reach statistical significance with one-tailed tests (all $p > 0.05$).

Conversely, *rubbing* (J) and *hiding* (L) behaviors were more common under standard conditions. *Rubbing behavior* showed a non-significant decrease with the addition of enrichment ($p=0.099$, Fisher's exact test, one-tailed). The frequency (%) of *hiding behavior* was significantly reduced by enrichment and entirely absent, from about 14% to 0%, when enrichment devices were present ($p=0.0268$, Fisher's exact test, one-tailed), signaling a clear difference between the two conditions.

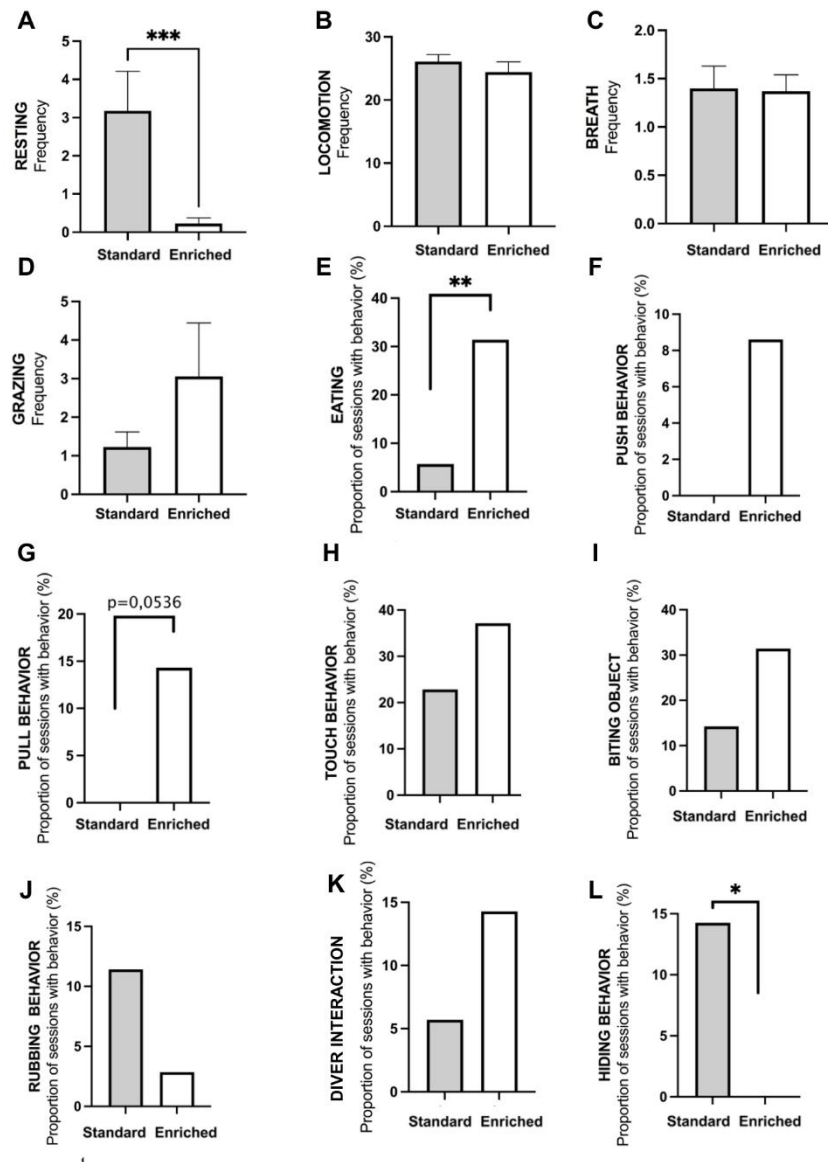


Figure 16 – Effect of environmental enrichment on behavioral categories exhibited by the captive green sea turtles under *Standard* and *Enriched* conditions. Graphs A–D depict behavioral frequencies, while graphs E–L present proportions, given the zero-inflated distribution of the dataset. Significant differences are indicated by asterisks (** $p < 0.01$, *** $p < 0.001$).

Results also reveal that the introduction of enrichment had a significant impact on both the overall activity and exploratory behavior of the turtle. *Total Activity* (percentage of frequency on all active behaviors except resting, hiding, and breathing) was significantly higher within enriched conditions compared to the standard setup ($W= 205.0$; $p=0.0336$)

(Figure 17A). The activity levels increased from approximately 30% in standard conditions to about 35% with enrichment. Likewise, *Exploratory Behavior* (including all interactions with the environment, such as grazing, eating, pushing, pulling, touching, biting objects, rubbing, and diver interaction) showed a significant increase with enrichment ($W= 254.0$; $p=0.0057$). Exploratory behavior increasing from around 3% under standard conditions to over 10% with the introduction of enrichment (Figure 17B).

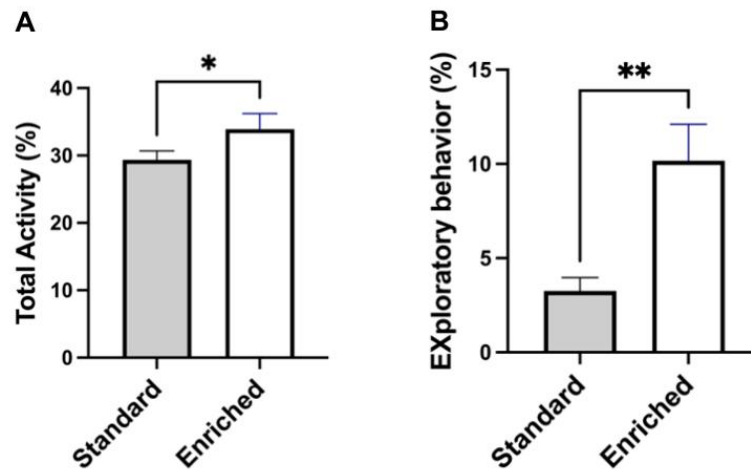


Figure 17 - Total activity (A) and exploratory behavior (B) of the resident green sea turtle under *Standard* and *Enriched* environmental conditions. Columns represent the percentage frequency of observed behaviors under each experimental condition. Significant differences are indicated by asterisks (* $p<0.05$, ** $p<0.01$).

A two-way ANOVA was used to analyze which periods of the day the turtle preferred the environmental enrichment. The analysis showed that environmental enrichment had a significant effect on exploratory behavior, while time of the day (12 p.m. and 3 p.m.) and the interaction between both conditions did not have significant effects.

Exploration behavior was significantly higher in the enrichment condition compared to the standard environment ($F(1,66)=11.01$, $p=0.0015$), regardless of the period of the day. Neither the main effect of *Day Period* ($F(1,66)=0.004542$, $p=0.9465$) nor the interaction between *Day Period* and *Environment* ($F(1,66)=0.4974$, $p=0.4831$) reached significance, indicating that enrichment promoted increased exploration consistently at both 12 p.m. and 3 p.m., with no evidence for time-specific or interactive effects (Figure 18).

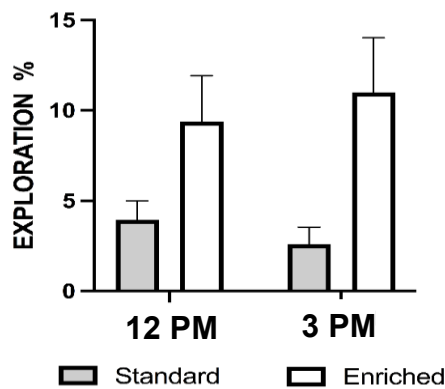


Figure 18 – Exploratory behavior by the resident green sea turtle under *Standard* and *Enriched* conditions at 12 p.m. and 3 p.m.. Columns represent the percentage frequency of observed behaviors under each experimental condition.

6.4.2 Spatial Use and Distribution Under Enrichment

The results testing the effects of *Enrichment* introduction on the spatial distribution of the turtle showed a highly significant main effect of *Area* ($F(4.957, 337.1)=32.78$, $p<0.0001$), meaning that the proportion of frequency on the different zones varied greatly, regardless of the enrichment condition. Although there was no significance of the main effect of *Enrichment* ($F(1.68)=0.04837$, $p=0.8266$), there was a significant interaction between both conditions ($F(12, 816)=2.289$, $p=0.0073$), which means the effect of the enrichment introduction on space occupancy changed depending on the specific area.

Figure 19 visually shows these findings, showing that the relative occupancy of different areas (A1-A13) differs between standard and enriched conditions.

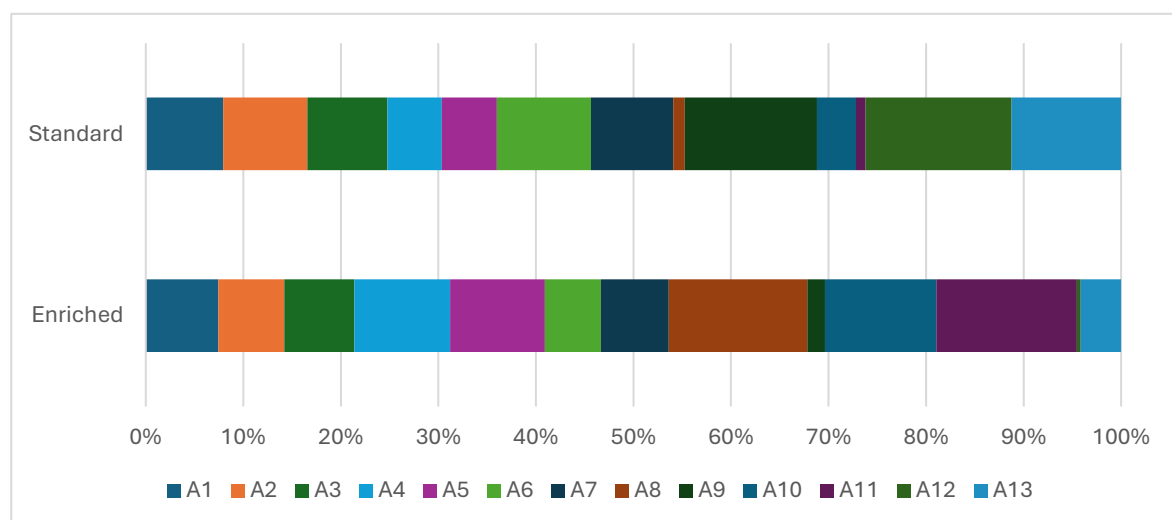


Figure 19 - Distribution of spatial area use by the resident green sea turtle under *Standard* and *Enriched* conditions.

Post hoc analysis identified the specific areas where enrichment significantly changed space distribution. The proportion of frequency in area 2 changed significantly between conditions ($p=0.0065$), showing a decrease in occupancy in enriched conditions. Areas 8 and 11 also showed significant differences between standard and enriched conditions ($p=0.0033$ and $p=0.0069$, respectively), with an increase of occupancy when the enrichment was present. All other areas didn't reach statistical significance ($p>0.05$).

Spatial distribution analysis revealed that the turtle's use of space was extremely selective in both conditions, revealing a tendency toward specific zones. In both standard and enriched conditions, the effect of *Area* was highly significant ($F(12,208)=2.487$ – *Standard*; $F(12, 429)=14.69$ – *Enriched*, $p<0.0001$), demonstrating a persistent preference for specific areas.

The effect of *Time of Day* was analyzed separately for each condition to determine whether the turtle preferred specific zones at particular times, regardless of the area. Under the standard condition, there was a significant interaction between *Time of Day* and *Area* ($F(12, 208)=2.487$, $p=0.0046$). Post hoc analysis also revealed that a notable change in occupancy occurred between 12:00 p.m. and 3:00 p.m. in areas 1, 2, and 12. Instead, under enriched conditions, space distribution remained consistent across time periods, with neither *Time of Day* ($F(1, 429)=2.952e^{-5}$, $p=0.9957$) nor the interaction between *Time of Day* and *Area* ($F(12, 429)=0.8394$, $p=0.6097$) being statistically significant.

The heatmaps (Figure 20) visually support these results, showing pronounced use of areas 1 and 2 in both conditions and, despite not reaching significance, in the enriched condition, it is possible to perceive a more ample distribution of space use.

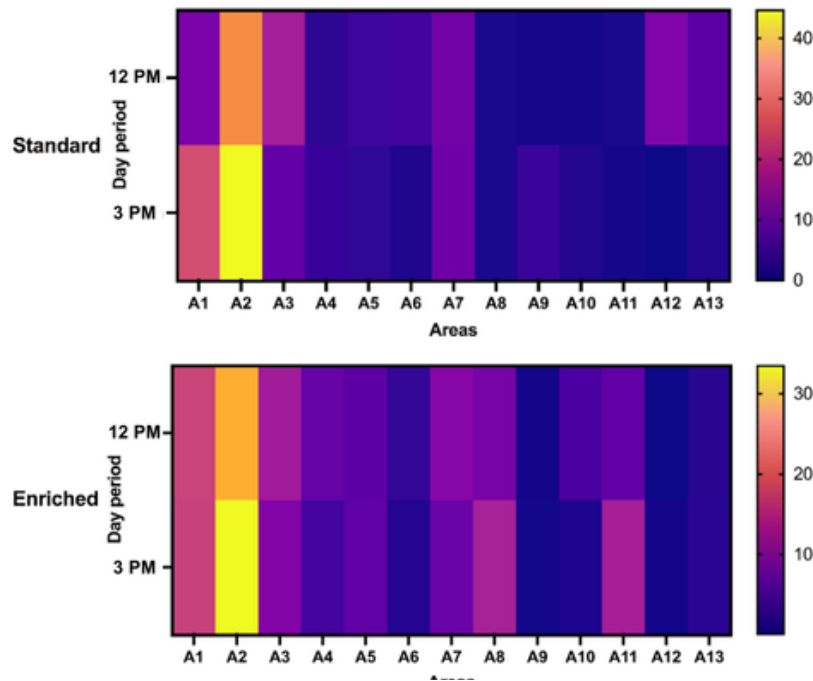


Figure 20 - Spatial area use by a resident green sea turtle under Standard and Enriched conditions at two-time periods (12 p.m. and 3 p.m.).

To evaluate whether the turtle showed preferences for one or more enrichment items, a Kruskal–Wallis test was performed. The test results noticed a significant difference in the frequency of *Exploration* behaviors stimulated by the different EE devices ($H(5)=17.84$, $p=0.0031$), which demonstrates that there was a clear preference between objects by the turtle (Figure 21). Results indicate that the highest levels of exploration activity are with the tube and the lowest activity with the fixed scrub.

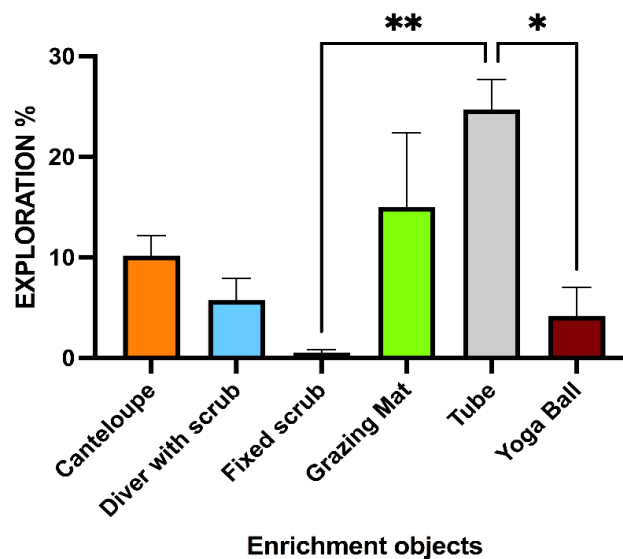


Figure 21 - Exploration behavior percentages of a resident green sea turtle in response to different enrichment objects. Columns represent the percentage frequency of observed behaviors under each experimental condition.

Multiple comparisons revealed that the tube significantly stimulated more explorative behavior when compared to both the fixed scrub ($p=0.0014$) and the yoga ball ($p=0.0427$). No other significances were found between the remaining device pairs ($p=0.05$).

7. Discussion

The findings of this case study provide valuable insights into a deeper understanding of spatial utilization and behavioral patterns of a resident green sea turtle (*Chelonia mydas*) at SEA LIFE Porto Aquarium, in response to environmental enrichment interventions. It's important to underline that this research was conducted with the intention of promoting the welfare of this individual, by evaluating the impact of environmental enrichment interventions, and therefore the inferences made should be interpreted with caution and are not meant to be generalized to other individuals of the same species or others.

This study first addressed the impact of a shelter in the form of an environmental enrichment. The shelter was distinct from the rotating enrichment plan implemented in the subsequent phase, as it functioned as a permanent enrichment feature designed to provide consistent hiding opportunities and potentially modify spatial distribution patterns and behavioral expression in the long term. Both methodological approaches were developed with distinct objectives: the shelter intended to assess how stationary structural enrichment influences habitat use and behavior, while the rotating enrichment program aimed to promote exploratory behavior, increased activity levels, and behavioral diversity through various stimuli. By implementing these studies independently, we were able to distinguish the specific effects of each enrichment approach and gain a clearer understanding of how each one individually contributes to the welfare and behavioral diversity of the resident green sea turtle.

The results regarding the effect of the shelter as a stable refuge show that the shelter had a significant impact on the spatial distribution of the resident green sea turtle, particularly among specific areas. Occupancy in the feeding zone (area 1) decreased from 23.24% to 15.82%, while there was a relevant increase in occupancy in free swimming surface zone (area 3), from 9.10% to 11.95%. The reduced occupancy in the feeding area might suggest a decline in anticipatory feeding behavior, indicating that the shelter encourages the turtle to be more involved with its environment, rather than waiting for food. This behavioral modification represents a potential increase in welfare, as the animal shows less fixation on the expectation of feeding and a greater ability to express species-specific

behaviors. Area 7A's occupancy increased significantly from 0% to 5.89%, which was particularly interesting because area 7A provided a cavity behind the shelter that allowed the turtle to hide completely, unlike area 7E, where only it was possible for its head to be protected inside the shelter itself. The turtle demonstrated a surprising application of the shelter: instead of sheltering inside it as was expected, it positioned itself behind it to form a partially hidden refuge. This behavior highlights the value of providing enrichment objects that enable animals to display their natural behaviors in a variety of ways and proposes a flexible use of environmental elements to satisfy their desire for a calm place. The preference for hiding places is in accordance with the proposals of Brill et al. (1995) and the EAZA guidelines (de Boer et al., 2021), which emphasize the necessity and importance of introducing refuge areas that allow turtles to rest without the pressure of external stimuli (e.g., visitors) (Therrien et al., 2007). Furthermore, in the wild, as observed by Houghton et al. (2003) in juvenile hawksbill turtles (*Eretmochelys imbricata*), they take advantage of underwater cavities that create three-dimensional microhabitats with low light, which provide protection from predators and currents and help them conserve energy during rest, neutralizing mild positive buoyancy at depth.

While the introduction of the shelter led to changes in specific areas, no significant differences were observed in the remaining areas. This suggests that the shelter influenced space use in a targeted manner, primarily affecting zones of high relevance to the turtle, while overall patterns in other parts of the tank remained stable. These results indicate that permanent structural enrichment can modify behavior selectively, highlighting the importance of considering both affected and unaffected areas when assessing the impact of enrichment on spatial distribution and welfare.

The influence of time of day on spatial distribution appear to be consistent with the natural routines of turtles in the wild, however, it is important to note that the time budgets of turtles under human care may differ from their wild counterparts (Wood, 2023). SEA LIFE's turtle shows an increase occupancy of areas 1 (feeding zone) and 2 (free swimming surface zone) at 12 p.m. and 3:30 p.m.. These results are expected, as the main feeding of this animal routinely occurs in area 1 at 11:30 a.m. and again at 2:30 p.m. A similar temporal pattern of space use at the same time periods was also reported by Bjorndal (1980) on captive green sea turtles in the Bahamas, where the feeding time peaks occurred in the mid-morning and mid-afternoon.

The significant decrease in occupation of area 1 with the presence of the shelter, alongside an increase in occupation in area 3, particularly at 12 p.m., (and also in Areas 12 and 13, although not significantly) indicating that the turtle spent less time in the feeding

area and more time in other zones. This result suggests that the turtle redistributed its activity across the surface which may reflect a reduction in food-anticipatory stress. By spending less time near the point of food provision, the turtle appears less focused on waiting for feeding, potentially indicating increased comfort provided by the shelter. Whilde et al. (2021) observed that food-based enrichment significantly reduced the time juvenile green sea turtles spent resting, while increasing species-typical active behaviors such as focused investigation and feeding during rehabilitation. Several turtles showed a notable reduction in stress-related behaviors and shifted their activity toward more determined exploration and feeding. These findings could suggest that environmental enrichment and comfort-promoting features may encourage turtles to change their focus away from the food supply site and instead distribute their activity more naturally and with reduced stress. This behavioral adjustment parallels the redistribution of activity and reduction in anticipatory stress for food observed in this study.

The periods during which the turtle spent the most time at the surface (Areas 1, 2, and 3) were around 12 p.m. and 3:30 p.m., while the least time at the surface was observed at 9:30 a.m. These patterns align with findings previously reported in the literature as reported by Ogden et al. (1983) which observed juvenile green sea turtles on the coast of St. Croix, U.S. Virgin Islands, and noticed that surfacing behavior of the turtles was most evident on mid- to late mornings (10 a.m.-11 a.m.) and again on the mid-afternoon (3:00 p.m.-4p.m.). They also reported low periods of surfacing during the early morning, midday and late afternoon. These literature data are in agreement with our results, which indicate that minimal time at the surface was recorded during the early morning and late afternoon periods. In fact, the results indicate a low surface occupation at 9 a.m. and *ad libitum* observations indicate that Mariza rested between 8:30 a.m. and 9a.m., in zones 9 and 13, typical resting sites of the turtle in its environment. Other research also corroborates that these low periods have been happening in turtle resting areas ((Booth & Peters, 1972; Witzell, 1982). The introduction of the shelter increased its occupancy in area 7A, particularly at 4:40 p.m., which corroborates with the low surfacing periods in the late afternoon. The majority of hard-shelled sea turtle are believed to be really inactive at night (Ogden et al., 1983) and periods of rest during the day are common (Schofield et al., 2006).

Interestingly, aside from the surfacing periods on area 2 (free swimming surface zone), the high occupancy in this area by the turtle during the day, perhaps could be explained by the presence of an active water inlet pipe producing a current. This may indicate a preference for the tactile or hydrodynamic stimulation provided by flowing water, reminiscent of documented behaviors in related species. It was described that pig-nose

turtles or fly river turtles (*Carettochelys insculpta*) would orient themselves towards the stream of water to a satisfactory position and remain motionless. This behavior could be related to comfort or even provide a sensation of pleasure. These sightings make sense considering the study of Burghardt (2005) on turtles in riverine environments with strong currents. EAZA guidelines also recommend the use of flow pumps in captive ambients of turtles from the Cheloniidae family, that create laminar flows since it stimulates normal swimming patterns and maintain physical condition (de Boer et al., 2021). Also, experiments with juvenile green sea turtles showed response to increasing current velocities by swimming upstream, altering swimming modes between 'dog-paddle' and synchronous forelimb flapping depending on current speed (Davenport et al., 1997). Thus, the turtle's preference for area 2 may reflect an intrinsic drive to interact with water currents.

The evaluation of how time of day and shelter influenced the turtle's overall spatial distribution proved to be non-significant, when considering the habitat collectively. Moreover, there was no interaction between these factors, which means that the influence of the shelter on occupancy was uniform throughout the day. This suggests that while the turtle adjusted its preferences for particular zones in response to the shelter (e.g., increased use of the hiding spot in area 7A and decreased use of the area 1 - feeding zone), the habitat use patterns on a broader scale remain stable over the daily cycle. In practical terms, the shelter affected the selection of specific microhabitats rather than fundamentally altering the turtle's daily rhythm or general space utilization within the tank. Moreover, the absence of a time-dependent modulation indicates that the advantages provided by the shelter are perpetually available and beneficial, highlighting its value as a stable, enriching feature. Therefore, the shelter was maintained during the following enrichment phase since the turtle made active use of it, particularly as a refuge, which is crucial from an animal welfare perspective. However, this design choice means that all comparisons between enrichment and non-enrichment conditions were made with the shelter already present, potentially influencing behavioral and spatial outcomes. Therefore, the results from the enrichment phase should be interpreted with this consideration in mind as the effects of the enrichment plan are discussed in the following data.

An enrichment phase in this study provided a really important chance to assess not only behavioral changes in a long-term under human care green sea turtle but also contextualize these changes within the scope of established and emerging knowledge about the well-being of sea turtles under human care. In this individual's case, *ad libitum* observations indicated an absence of abnormal behaviors (such as stereotypies or patterned swimming) both before and after the addition of the enrichments. Therefore, the

primary purpose of implementing environmental strategies was to improve the turtle's welfare and increase its behavioral diversity.

The introduction of enrichment plan for the resident green sea turtle led to a clear and significant increase in behavioral diversity, which was verified by an increase in the Shannon Diversity Index from 1.018 (standard condition) to 1.266 (enriched condition). Hill number's calculations showed a significant increase in the diversity of the green turtles' behaviors after the introduction of the enrichment plan, from approximately 2.77 in the standard condition to 3.55 in the enriched condition, representing an increase of about 28%. This improvement in behavioral diversity is biologically significant, as it reflects greater mental engagement, motivation, and physical activity, all essential components of animal welfare. Enriched environments stimulate natural behaviors, reduce the incidence of behaviors related to inactivity and stress, and ultimately promote the overall psychological and physiological health of captive sea turtles. These findings are consistent with previous studies that show that environmental enrichment enhances the welfare of sea turtles by encouraging active exploration and the expression of natural behavior repertoires, which are essential for their well-being and adaptability both under human care and in potential rehabilitation scenarios (Therrien et al., 2007; Whilde et al., 2021; Wood, 2023).

Further findings indicate that, although there was a significant increase in total activity and exploratory behaviors under enriched conditions, locomotion did not increase significantly between conditions. This suggests that the turtle did not simply swim more but rather directed its activity toward a wider range of species-specific behaviors—such as investigating, foraging, or interacting with the enrichments. Therrien et al. (2007) also found similar results when analyzing behavioral differences in both these conditions in three loggerhead sea turtles (*Caretta caretta*) and one blind green sea turtle, noting a significant increase in exploratory and focused behaviors. He also noted a significant reduction in resting periods under enriched conditions, which follows along with the findings of this study that revealed significant decreases in resting and hiding behaviors. Although it's expected a certain level of quiescence in sea turtles, excessive lethargy can signal chronic stress and/or indicate poor welfare (Warwick et al., 2013). On contrary, increased activity levels reflect a positive physical and mental stimulation (Ethier & Balsamo, 2005).

Data on the spatial distribution of the turtle showed that, in both conditions, feeding area (area 1) and free swimming surface zone (area 2) remained the most occupied, suggesting that the presence of enrichment did not significantly alter the turtle's spatial habits. Even so, it was possible to observe greater occupation of areas 8 and 11 during the enrichment sessions, since these were the areas where the grazing mat and tube were

introduced, respectively. Although not significant, these findings reveal that well-designed enrichment devices can lead turtles to forage or explore more underutilized areas (Therrien et al., 2007).

Both under standard and enriched conditions, the turtle displayed clear and consistent selectivity in their use of space, with certain areas—particularly areas 1 and 2—being greatly preferred. This suggests that the animal's occupation of space was not random but rather motivated by long-term preferences. Under standard housing conditions, these spatial choices were moreover influenced by the time of day, as evidenced by significant interactions between area and temporal factors, with marked changes in occupancy observed between midday and early afternoon. Such findings suggest that temporal dynamics could have a role in determining space use when environmental resources are limited.

Conversely, the addition of enrichment seemed to stabilize spatial patterns, eliminating diurnal fluctuations in area preference. This stability could indicate that enrichment reduces temporal variability in activity patterns, possibly by providing consistent opportunities for engagement throughout the enclosure. Although statistical analyses did not indicate a significant broadening of space use under enriched conditions, there is a trend toward a more evenly distributed use of the available environment. Taken together, these outcomes highlight the influence of environmental enrichment in modulating the relationship between spatial selectivity and temporal variation, while underlining the animal's enduring preference for specific zones within the enclosure (Puehringer-Sturmayr et al., 2023).

The assessed the turtle's preference among the enrichment devices, as indicated by the relative frequency of use of each device, revealed that the enrichments were not equally effective in stimulating exploratory behaviors. The data revealed that the tube with vegetables was the EE device that most stimulated this range of behaviors, which may suggest a specific preference from the turtle. Wilde et al. (2021) also noted this difference in the effect of enrichments among several green sea turtles, where behavioral responses varied depending on the individual. Such variability in individual responses to EE devices further proves that EE programs need to be adapted and personalized to meet the specific needs of individuals (Cipreste et al., 2010; Wood, 2023). Information on the types of enrichment preferred and the frequency of their use is important, as should be considered when designing management and husbandry strategies.

8. Conclusion

The present study demonstrated that the inclusion of a shelter clearly influenced spatial distribution, increasing occupancy in hiding areas and allowing the turtle the option to hide in a less stimulating location. Temporal patterns of space use were consistent with natural daily routines, and the effects of shelter stayed consistent throughout different times of the day. Furthermore, EE effectively improved the welfare of a long-term resident green sea turtle (*Chelonia mydas*) under human care. The introduction of the EE devices led to an increase in behavioral diversity, particularly of exploratory behaviors, indicating an improvement in physical and psychological stimulation.

This research highlights the importance of creating individualized EE programs that address the needs and preferences of the animal, as supported by the response to various enrichment objects. Enrichment interventions can positively influence the behavior and welfare of marine turtles by promoting natural behaviors, increasing environmental exploration, and providing preferred refuges. In addition, the use of less frequented areas in the aquarium during enrichment periods illustrates the potential of enrichment to encourage a more balanced use of space.

These findings underscore the importance of incorporating carefully planned, science-based enrichment programs into captive care to enhance both psychological well-being and overall health. This study contributes valuable information for the assessment of welfare and enrichment of sea turtles under human care, providing more practical insights for improving captive management and conservation efforts.

Chapter 3: Experience as an Aquarist at SEA LIFE Porto

1. Introduction

Animal keepers play a fundamental and indispensable role in the current management of animal welfare in zoos and aquariums. As prime caregivers and key observers of animals under human care, keepers occupy a unique position that allows them to combine daily management with careful monitoring of welfare (Whitham & Wielebnowski, 2009). Through routine and often long-term interactions with each animal, keepers gain in-depth knowledge of each animal's normal behavior patterns and reactions to various environmental and social changes (Meagher, 2009). This daily contact gives them the ability to notice slight behavioral or physical changes that may be early signs of welfare problems, which may go unnoticed during occasional veterinary examinations or inspections (Whitham & Wielebnowski, 2009).

Rather than simply observing the animals, animal keepers directly and significantly affect animal welfare as a result of their daily management practices, including daily care routines, environmental enrichment practices, and training that meet the physical and psychological needs of the animals (Cole & Fraser, 2018). Decisions regarding feeding, enclosure design, and social grouping are based on the practical knowledge accumulated by keepers, allowing them to be flexible in their approach to meet the specific needs of each species and individuals (Rose & Lewton, 2025). The development of positive, long-term relationships between handlers and animals can further improve welfare by reducing animal stress and increasing cooperation during veterinary procedures (Fernandez & Martin, 2021; Pasteur et al., 2024).

Animal keepers are the keystone of modern welfare management in zoos and aquariums, acting as skilled observers and actively contributing to animal welfare through their close and constant contact with the animals in their care (Carlstead et al., 2019; Meagher, 2009; Whitham & Wielebnowski, 2009). This direct, everyday contact puts them in a unique position to provide comprehensive, contextualized assessments of welfare, recognizing subtle behavioral and physiological changes that often escape more formal assessment protocols, and thereby serving as an irreplaceable connection between scientific welfare standards and practical animal care (Whitham & Wielebnowski, 2009). In order to maximize these benefits and align themselves with current ethical, scientific, and social expectations, zoos and aquariums should consistently invest in formal caretaker training programs, provide adequate resources and institutional support, and formally integrate keepers' insights into standardized welfare assessment protocols (Bacon et al.,

2023; Wielebnowski et al., 2002). Supporting the vital role of animal keepers through professional development and organizational support is not only beneficial, but fundamental to achieving and maintaining the highest standards of animal welfare (Bacon et al., 2023; Ward et al., 2020).

2. Key Objectives

This chapter aims to cover and describe the practical activities I took part in during my curricular internship at SEA LIFE Porto, as part of my experience as an aquarist. Alongside the scientific project, I had the opportunity to assist the biology team across a range of tasks, mostly related to animal welfare and husbandry practices.

These were the main goals:

- 1. Application of Existing Environmental Enrichment Strategies** - In this section, detailed information is provided on the implementation of the environmental enrichment plan specifically designed for the Humboldt penguins (*Spheniscus humboldti*), with the aim of stimulating natural behavior and promoting the animals' welfare.
- 2. Continuing the Training of the Humboldt Penguins** - Description of the feeding training activity previously implemented as an alternative to the standard technique used by other keepers. Such conditioning was intended to facilitate the voluntary handling of the penguins.
- 3. Assisting Across Aquarist Roles and Tasks** - Taking part of the biology team's daily and occasional routine tasks, especially those directly linked to animal welfare, explaining the responsibilities taken on to make sure the aquarium runs efficiently and the species are properly cared for.

3. Application of Existing Environmental Enrichment Strategies

As part of my internship, one of my responsibilities was to implement the environmental enrichment program developed the previous year for the Humboldt penguins (*Spheniscus humboldti*) in collaboration between a master's student and the biology team at SEA LIFE Porto.

The implementation of environmental enrichment plans is a fundamental component of the management and maintenance of public zoos and aquariums, given the direct effect

it has on the physical and psychological welfare of the animals (Mellen & Sevenich MacPhee, 2001). The colony at SEA LIFE Porto is composed of 8 resident penguins, with 4 males and 4 females paired into 4 couples. In April 2025, one of these pairs successfully bred, raising 2 chicks and increasing the colony to 10 individuals. This program was therefore designed to promote the welfare of the resident penguins by encouraging and stimulating the natural behaviors typical of the species. In particular, the aim was to increase swimming time and exploratory behavior. To achieve this, various types of devices - sensory, structural, dietary, social, and cognitive - were used, all of which had been approved and tested the year before, in order to stimulate both the physical and mental aspects of the species (Figure 22).



Figure 22 - Environmental enrichment devices used in the program (Author: Maria Castelo).

For part of my internship, I was given the responsibility of preparing and implementing this EE program on a daily basis. Because of time constraints and the necessity of fulfilling other internship objectives, the program was only implemented between October and December 2024. These sessions lasted between 30 minutes to 1 hour and took place at specific times of the day: in the morning between 10:30 a.m. and 11:30 a.m. or in the afternoon between 3 p.m. and 4 p.m..

Apart from directly monitoring how the penguins interacted with the devices during the enrichment sessions, filming was also done to get a more detailed understanding of the impact and reaction of the animals to the enrichment. However, due to time limitations, it wasn't possible to analyze these videos during the internship. Nevertheless, the records acquired during the sessions were entered by the biology team into the Animal Welfare App by OERCA (OERCA, 2025) database, a specialized software for animal care designed for zoos, animal sanctuaries, and wildlife rehabilitation centers. This platform allows for integrated management of all aspects related to animal welfare and facility operations. It

allowed the creation of a historical record of the program's implementation, which will facilitate further monitoring and management of the plan, demonstrating the caretakers' ongoing care and dedication to promoting animal welfare.

4. Continuing the Training of the Humboldt Penguins

This topic followed the same foundation as described on topic 3. This work took place throughout the entire internship, with training sessions (Figure 23) being held daily, sometime between 8 a.m. and 10 a.m., in the technical room with the door always open to the outdoor area, allowing the penguins to freely choose to come and go. To supplement the training, another team member recorded the number of fish eaten by each individual and monitored the penguins' entry, in order to individualize the training. The applied method complemented the training already established by the team, with sessions conducted two to three times per week, alternating between different approaches.

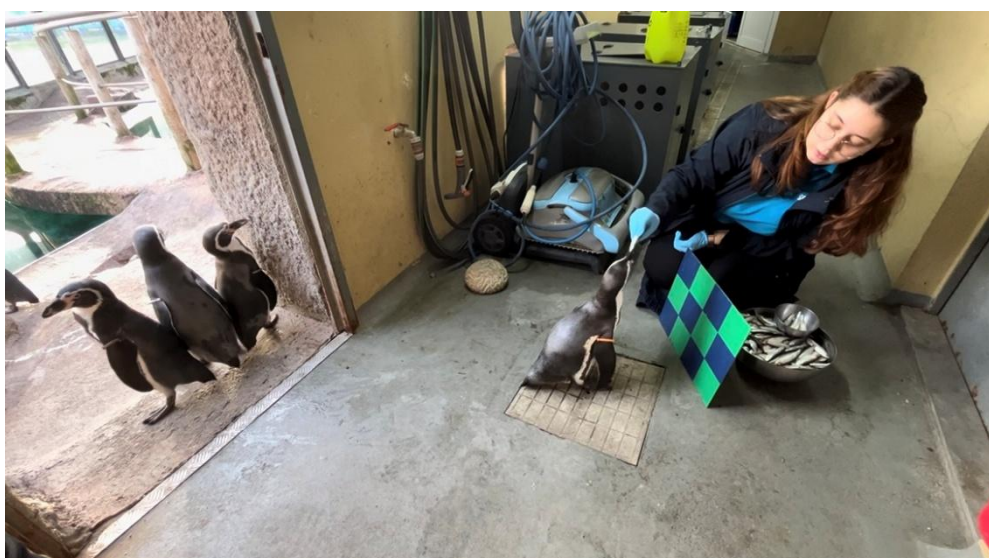


Figure 23 - Demonstration of the alternative training method (Author: Maria Castelo).

The training was conducted in several gradual stages to ensure that the penguins felt comfortable and confident around the keeper. First, the penguins were encouraged to enter the indoor area and stay close to the keeper and a visual cue, using small rewards to motivate them. Over time, they became more comfortable around the keeper, showing no fear, which was evident from the calmness with which they remained still nearby. The training then progressed to getting the penguins used to physical contact, starting with light touches and slowly increasing the pressure, simulating the way they would be held. In this way, they became used to having the keeper's hands on both sides of their bodies and to the positioning required to be held safely.

In the following steps, the penguins were introduced to the sensation of being lifted, initially causing some unease, but gradually accepting this contact calmly. An important milestone was reached when they managed to remain calm and still during the holding phase, minimizing stress and avoiding regressions in training. However, it was not possible to advance beyond the phase of the methodology, in which the penguin tolerated being held but could not yet be lifted. The next planned steps would include teaching the penguins to lie down with their legs and feet extended, an unnatural position that may require assistance, and getting them used to sitting on the keeper's lap at a height to facilitate medical procedures. Due to the change in height, this step has to be implemented gradually because of the animals' likely uneasiness.

Such conditional training, especially through positive reinforcement (PRT), is essential to safeguard the welfare of penguins, as it significantly reduces stress during veterinary and handling procedures. By transforming potentially stressful experiences into cooperative and voluntary interactions, the psychological well-being of the animals and the effectiveness of the care they receive are promoted (Laule et al., 2003). Providing penguins with choice and control over their environment plays an essential role in their welfare, reducing the fear and anxiety commonly experienced in these settings. In addition, conditional training strengthens trust between keepers and animals, reinforcing the human-animal bond and improving long-term outcomes in care management (Ward & Melfi, 2013).

So as to optimize the monitoring and recording of training sessions, I took the initiative to create a record table (Appendix F), where keepers could write their names, the time of the training session, and the type of training performed. This allows for more effective control of the sessions, facilitates continuity of the method, and ensures individualization and consistency in the penguins' training and feeding.

5. Assisting Across Aquarists Roles and Tasks

5.1 Daily Tasks

Quarantine

SEA LIFE Porto's quarantine (Figure 24) facility provides a specialized area for the detention and treatment of newly acquired specimens, and exhibition animals that require medical intervention or health monitoring. While in quarantine, animals are isolated, where body condition, behavior, feeding patterns and water quality can be closely monitored under controlled conditions. This allows aquarists to detect and treat parasite-related issues,

infections, or stress-related problems before the animals join the public exhibition, protecting the resident animals (Hadfield & Clayton, 2011).



Figure 24 - Overview of SEA LIFE Porto's quarantine facility (Author: Maria Castelo).

The quarantine facility comprises 14 tanks of varying sizes and depths, organized by type of aquatic system: fresh (cold freshwater), ambient (cold saltwater), and tropical (warm saltwater). If required, it is also possible to isolate any of the tanks and adapt the water parameters as necessary. This configuration allows for the accommodation of a wide variety of aquatic species with different environmental requirements. It also serves as a storage area for equipment needed for daily maintenance tasks, including siphoning equipment, strategic items to recreate hiding places, tubing, and other specific materials. It also has a disinfection station consisting of two basins: one with water and specialized disinfectant and the other with water to ensure that all equipment is thoroughly cleaned.

Daily quarantine tasks include preparation of two daily feedings (morning and afternoon) for the animals, with diets tailored to the specific needs of each species present. Regular cleaning of the tanks is then done through siphoning to remove feces, detritus, or food debris in order to prevent changes in water quality and maintain optimal conditions for the animals.

Water Tests and Parameters

Maintaining water quality is essential to ensure the welfare of animals in aquariums. Small changes in the chemical parameters of the water can trigger stress responses in animals or, in more extreme cases, lead to mortality. Therefore, it is crucial to establish a rigorous routine of water quality analysis.

Given that it is impossible to test all the aquariums in the exhibition in a single day, the biology team at SEA LIFE Porto organizes a weekly schedule that distributes the

different aquariums over the course of the week. Every week, all isolated aquariums are analyzed, as well as one aquarium representing each of the main aquatic systems (the largest tank in each system). In addition, a rotating test of the remaining tanks is conducted each day, ensuring that all aquariums in the exhibition and quarantine are tested at least once a month.

Daily samples are collected in the morning, prior to any other tasks, to obtain an accurate assessment of the water condition. Water quality tests (Figure 25) are then conducted, preferably in the morning, to allow for immediate intervention if any parameters are outside the ideal range. All tanks are tested for ammonia (NH_3), nitrites (NO_2^-), and nitrates (NO_3^-). For aquariums with corals, samples are also analyzed for phosphates (PO_4^{3-}), magnesium (Mg^{2+}), alkalinity (KH), and calcium (Ca^{2+}).



Figure 25 - Water quality testing (Author: Maria Castelo).

As a complement to water quality tests, daily measurements are taken with a multiparameter probe (Figure 26) on all weekly tested tanks and on the rotating tank of the day. The probe records pH, dissolved oxygen (%DO), salinity (ppt), and temperature ($^{\circ}\text{C}$)



Figure 26 - Multiparameter probe for measuring water parameters (Author: Maria Castelo).

values, providing a comprehensive overview of the physical and chemical conditions of the water.

Monitoring is performed based on guidelines established by the company, whereby the values must fall within the ranges stipulated for each tank. All data is recorded daily in the Animal Welfare App by OERCA (OERCA, 2025).

Feeding Routines

At SEA LIFE Porto, feeding routines are carefully structured to ensure the nutritional welfare of all animals under human care. This process begins in the food preparation area, where all daily food preparation is based on the specific needs of each animal (Figure 27).

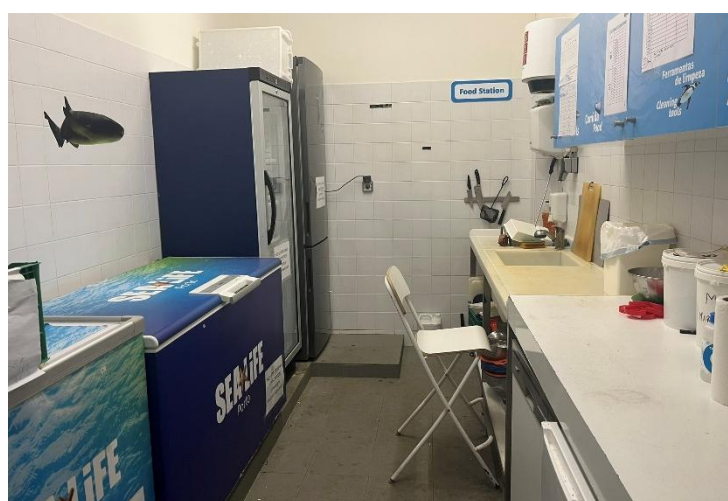


Figure 27 - SEA LIFE Porto's kitchen (Author: Maria Castelo).

The kitchen is fully equipped with all the equipment needed to prepare the animals' food, including three freezers – one for frozen fish and two for storing frozen preparations, mussel meat, shrimp, and clams – and two refrigerators: one for fish meals and another exclusively for vegetables for the green sea turtle (Figure 28B). All fish feeds are prepared on the same day, using fish that has been left to defrost in the refrigerator since the previous day (Figure 28A). The number of pieces and total weight of each meal are adapted according to previously established individual diets. In the case of the sea turtle, its feed is prepared the day before, following the specific quantities defined in its feeding plan. The meals are supplemented with vitamins specific to each species (Figure 28C), with the number of tablets per individual adjusted according to their body weight. For the turtle, a cube of sugar-free gelatin is added to its diet to help maintain hydration, with the sugar-free formulation being crucial to avoid introducing sugars that are not part of the turtle's natural diet. This extra supplementation is important to ensure that the animals receive all the essential nutrients, especially since the fish used is deep-frozen to prevent the transmission of possible pathogens, a process that can reduce the nutritional value of the fish.



Figure 28 - Feeding preparation; A - Fish feeding; B – Turtle’s feeding; C - Vitamin supplementation (Author: Maria Castelo).

The feedings are distributed as follows: a first round is held in the morning, before the exhibition opens to the public, during which the specific diets for the species present in each tank are distributed throughout the displays. In the afternoon, a second round is held, intended only to reinforce the feeding in selected tanks, complementing the morning routine. The feedings of the sharks (blacktip reef shark - *Carcharhinus melanopterus* and zebra shark - *Stegostoma tigrinum*), the rays (southern stingray - *Hypanus americanus* and cow nose rays - *Rhinoptera bonasus*), and the green sea turtle take place simultaneously with the lectures, offering visitors the opportunity to observe the feeding behavior of these animals. Species labeled as “special,” such as common octopus (*Octopus vulgaris*), Mediterranean slipper lobster (*Scyllarides latus*), red scorpionfish (*Scorpaena porcus*), moray eels (*Enchelycore pardalis* and *Gymnothorax favagineus*), and others, are only fed on Mondays, Wednesdays, and Fridays.

Another responsibility related to feeding was preparing the live brine shrimp culture columns (Figure 29). Each culture must undergo a 24-hour maturation period until the eggs hatch. This food is used to feed the common jellyfish and corals, which, in addition to frozen food, receive a complementary diet consisting of live organisms.



Figure 29 - Brine shrimp culture columns (Author: Maria Castelo).

To guarantee food quality control, the minimum and maximum temperatures of all freezers and refrigerators are recorded daily, including the medicine refrigerator located in the office. Records are also kept of the number of pieces of food prepared and actually consumed by the elasmobranchs at each meal. In addition, the amount of each vegetable used in the turtle's diet is documented until the total weight of the meal is reached. These records allow us to monitor the animals' feeding behavior and identify fluctuations over time, providing essential data for the continuous optimization of nutritional protocols and for the early detection of possible changes in the animals' health condition.

Exhibition Siphoning and Water Changes of Isolated Systems

In order to ensure optimal water quality, daily maintenance is required through two tasks: siphoning and partial water changes. So as to guarantee regularity and a high degree of cleanliness, siphoning (Figure 30) is scheduled weekly and distributed among the biology team staff. Nevertheless, in tanks with particularly sensitive species (seahorses, razorfish, and common jellyfish), siphoning is carried out every morning, preferably before the center opens to the public. This process helps remove excess food, faeces, and debris deposited in the sediment, preventing the accumulation of toxic components.



Figure 30 - Exemplification of the siphoning routine (Author: Maria Castelo).

In terms of logistics, in the case of smaller or easily accessible tanks, siphoning can be carried out from the outside. However, in larger or hard-to-reach systems, specialized equipment—waders or diving gear—is required to clean from the inside of the tank directly.

Although the siphoning process ultimately removes some of the “unclean” water, partial water changes are still essential to help maintain the stability of the physical and chemical parameters of the water. Thus, during this task, a volume of water is estimated and discarded, and replaced with “new” water which is introduced directly into the tank. In some cases, an intermediate step is necessary to adjust the water parameters, namely salinity, so the water from the system is placed in a tub – “salt mix” – where salt is added until the ideal salinity for that tank is reached.

By carrying out such tasks, it's possible to rigorously monitor water quality, keeping it at an optimal level while promoting the animals' welfare and health.

Penguins' Feeding and Habitat Cleaning

Every morning, before opening to the public, the Humboldt penguins at SEA LIFE Porto are given their primary feeding, and their habitat is thoroughly cleaned. The facility has its own technical room, equipped with a kitchen dedicated exclusively to preparing the penguins' food, and an exclusive quarantine area for the penguins. It is also the place where the penguins' nest boxes are accessible, as well as the external part of the habitat (Figure 31).



Figure 31 - Penguins' technical room (Author: Maria Castelo).

The day begins with the preparation of food in the kitchen (Figure 32A). The fish that is stored and is part of the penguins' diet – capelin, sprat, or herring – is kept in a freezer and as needed, is transferred to the refrigerator where it is defrosted and kept ready for daily consumption (Figure 32B). Since the fish is deep-frozen for health reasons, and to ensure that the animals meet their nutritional requirements, each penguin is supplemented with a vitamin capsule per day, which suppresses these demands. In addition, every Friday, instead of vitamins, each penguin is given an anti-malarial drug to prevent avian malaria (Figure 32C).



Figure 32 - Penguin feeding preparation; A – Kitchen; B - Prepared food; C - Vitamin supplements (Author: Maria Castelo).

The moment of the first feeding takes place inside the technical room: the penguins are trained to enter and individually climb onto a scale—conditioning training that facilitates veterinary procedures—and are fed by hand by a keeper (Figure 33). Simultaneously, another staff member records the number of fish consumed by each penguin.



Figure 33 - Feeding time (Author: Maria Castelo).

Following feeding, the technical room, nests, and outdoor habitat are then cleaned (Figure 34). Cleaning is carried out using a hose to wash the floor and surfaces, ensuring thorough cleaning both indoors and outdoors. Due to the proliferation of algae, the pool is cleaned with the aid of a special underwater robot, and the glass is brushed and washed to maintain visibility. A disinfection product is also applied to all areas for thorough sanitation.



Figure 34 - Cleaning routine (Author: Maria Castelo).

In addition to the morning feeding, there is a second afternoon feeding session that takes place at the same time as the lecture about the penguins, allowing visitors to observe the animals' feeding behavior up close. In this case, the feeding takes place outside, where the penguins approach the keeper to receive their food. Again, the fish eaten by each individual is documented. At the end of the day, all daily food consumption data is transferred to an Excel document, which allows the feeding behavior of each individual to be monitored over time.

5.2 Occasional Tasks

Handling of Large Animals

During my internship, I had the opportunity to assist in the handling of the sea turtle, a large animal that requires care and procedures adapted to its size and morphology.

To guarantee a quick intervention in emergency situations or during veterinary procedures, it is necessary to remove the turtle from the water in a safe and controlled manner that minimizes the stress caused to the animal. For this, a special vest is used, designed specifically for sea turtles to ensure maximum comfort and prevent injury. After adjusting the vest to the turtle, it is hoisted by a hoist, moving the turtle from the tank to the outside. This procedure must be performed as quickly and carefully as possible to ensure the animal's welfare at all stages (Figure 35).



Figure 35 - Green sea turtle handling procedure (Author: Maria Castelo).

One of the procedures I had the opportunity to assist with was weight checks. This procedure is regularly carried out to monitor the animals' body mass index and facilitate the early identification of any nutritional imbalances. I also assisted in more complex veterinary procedures, such as X-rays and blood sampling (Figure 36). My role was to assist the biology team in calming the turtle as much as possible in order to reduce stress and restrict movements that could pose a risk to the animal and the team. All work was carried out in close coordination with the veterinary team to ensure that procedures were conducted efficiently and with the utmost care.



Figure 36 - Blood sampling (Author: Maria Castelo).

This experience deepened my understanding of handling large animals in an aquatic environment, highlighting the importance of team coordination and cooperation in order to respond as efficiently as possible to the specific needs of each animal.

Reception and Acclimatization of New Specimens

Upon arrival of new animals, certain precautions must be taken to ensure their successful integration into the exhibition.

Prior to arrival, the team confirms that the quarantine tanks are properly prepared and adjusted to the requirements of the species being received. Water parameters are checked—in particular temperature, salinity, pH, and dissolved oxygen—and water quality analyses are performed (ammonia, nitrites, and nitrates). The remaining equipment is inspected to ensure that the tanks are in proper condition.

Upon arrival, the animals are packaged in individual bags containing conditioned water to ensure they can withstand the long transport periods. After an initial assessment of each animal's condition, the bags are transferred to quarantine for acclimatization. The bags are transferred and placed on the surface of the quarantine tank without being opened

for about 30 minutes, a process that is only feasible due to the close similarity in environmental parameters between the arrival conditions and those inside the quarantine tank. This thermal acclimatization process gradually equalizes the temperature of the transport water with that of the tank, reducing the risk of thermic shock to the animals. Once this phase of thermal acclimatization is complete, the water in the bags is partially replaced with water from the tank, in progressive increments, until it is completely changed. This process of progressive dilution prevents osmotic shock and allows the animals to safely adjust to the parameters of their new environment.

After rechecking the parameters and confirming that the water in the bags is uniform with that in the tank, the animals are carefully relocated inside the tank, where they remain under observation and receive the treatments specified in the protocols until they are ready to be placed on the exhibit.

Deep Cleaning of Exhibition Tanks

The exhibition tanks are cleaned regularly, which involves daily tasks such as siphoning and cleaning the acrylics. But sometimes more thorough cleaning is needed.

During these interventions, all theming elements are removed, washed, brushed, and disinfected before being reinstalled. The tank surfaces are also brushed, and any debris is discharged directly into the sewer using a hose. For the control of *Aiptasia* – fast-growing invasive anemones that damage marine ecosystems – an appropriate product is injected to ensure effective elimination.

At the same time, a large water change is carried out, as during cleaning there is an accumulation of suspended impurities, which are eventually removed. After thoroughly sanitizing the entire tank, substrate is replaced to smooth the bottom and theming is placed back in order to maintain the original design. This preservation of the initial layout is considered essential, as it is presumed to represent the most suitable environmental configuration for the resident species. Finally, renewed water is added until the ideal level is reached.

All of this process demands time and precision, along with special attention to the marine organisms present, ensuring that all processes are carried out safely and carefully (Figure 37).



Figure 37 - Tank that I was responsible for deep cleaning (Author: Maria Castelo).

6. Conclusion

During my internship at SEA LIFE Porto, I was actively involved in a range of essential responsibilities to promote and support animal welfare. I contributed to the continuation of the positive reinforcement training program for Humboldt penguins, with the aim of facilitating voluntary compliance during handling and veterinary procedures. In addition to training, I assisted with various aquarist tasks, including the implementation of an environmental enrichment program designed to stimulate natural behaviors and improve the resident penguins' well-being. My duties also included critical husbandry practices such as preparing species-specific diets, performing regular cleaning of quarantine and exhibition tanks, and adhering to strict water quality monitoring protocols to maintain optimal living conditions.

Importantly, I had the opportunity to share my ideas with the biology team, some of which were integrated into their daily routines, contributing to the continuous improvement of animal care and welfare management practices.

Such comprehensive involvement allowed me to develop practical skills and deepen my understanding of the sensitive balance required between animal care, welfare assessment, and facility management. Overall, this experience highlighted the indispensable role of animal keepers as caregivers and observant assessors of welfare, demonstrating that effective animal management requires the integration of scientific knowledge with practical experience to ensure high standards of health and welfare in zoological institutions.

Chapter 4: Ultimate Considerations

1. Final Discussion

The conjugation of scientific research and professional aquarist's experience constitutes an essential progress in modern animal welfare management in zoological institutions. This thesis evidenced how the systematic application of environmental enrichment programs, combined with specialized behavioral monitoring and daily husbandry practices, establish an integral framework for assessing and enhancing the welfare of animals under human care. These findings add to the increasing amount of research that supports evidence-based welfare assessment methods, while also highlighting the key role of animal care professionals in turning research into real welfare improvements.

Using quantitative methods to assess behavior, especially the Shannon Diversity Index, provided objective measurement criteria that complemented the subjective observations of the keepers. This dual approach meets the methodological concerns raised by Diggins et al. (2022), which stressed the need for both quantitative and qualitative assessment tools when evaluating the welfare of sea turtles. Behavioral changes documented in this study - increased exploratory behaviors, higher environmental interaction, increased behavior diversity and more balanced space use - represent measurable improvements in psychological welfare.

Everyday observations and husbandry routines demonstrate the critical role of animal care personnel in assessing and improving welfare. This finding is strongly supported by Jones et al. (2022), who emphasized that keeper experience represents an indispensable element of comprehensive welfare monitoring systems. Documenting daily routines, environmental parameters, and behavioral observations provides the basis for evidence-based management decisions that directly affect animal welfare outcomes.

The interdisciplinary approach demonstrated in this study - combining systematic research-based methodologies with professional knowledge in animal husbandry - ultimately guarantees that scientific discoveries can influence practical applications, while professional observations guide research priorities and methodological refinements. By detecting subtle behavioral changes, recording individual preferences, and adjusting management protocols based on daily observations, animal keepers practice applied ethology that perfectly complements formal research protocols.

The efficacy of the environmental enrichment program implemented in this study demonstrates the value of combining structured research protocols with expert care practices. Wood (2023) emphasized that long-term welfare management in captive sea turtles requires integrated approaches that address both immediate behavioral needs and long-term psychological welfare. The systematic rotation of enrichment devices, coupled with ongoing behavioral monitoring, created opportunities for choice and environmental control that are recognized as fundamental components of positive welfare states. Complementing these efforts, the introduction of shelter as a long-term enrichment provided the turtle with a stable and secure refuge, promoting natural resting behaviors while offering continuous opportunities for environmental choice and control. Together, these strategies support the comprehensive welfare essential for long-term positive outcomes under human care conditions.

This study's findings contribute to the limited but growing literature on environmental enrichment applications in reptile species, particularly marine turtles. The successful implementation of species-specific enrichment protocols, validated through behavioral assessment methods, provides a framework that can be further customized for other individuals or other chelonian species under human care. This contribution is particularly valuable given the relevance of sea turtle conservation and the role of zoological institutions in supporting conservation efforts through research, education, and rehabilitation programs.

This study's unique partnership between systematic behavioral assessments and daily animal care practices provides a useful model for raising welfare standards in zoos and aquariums. In rigorously validating keepers' insights with scientific methodology and ensuring that research findings lead to concrete improvements, it provides a sustainable system for continuous welfare enhancement that benefits both individual animals and conservation efforts. Ultimately, the advancement of animal welfare in zoological settings does not depend on choosing between research or practice, but on the integration of both approaches to benefit from their complementary strengths and ensure the best outcomes for animals under human care.

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Appendices

Appendix A

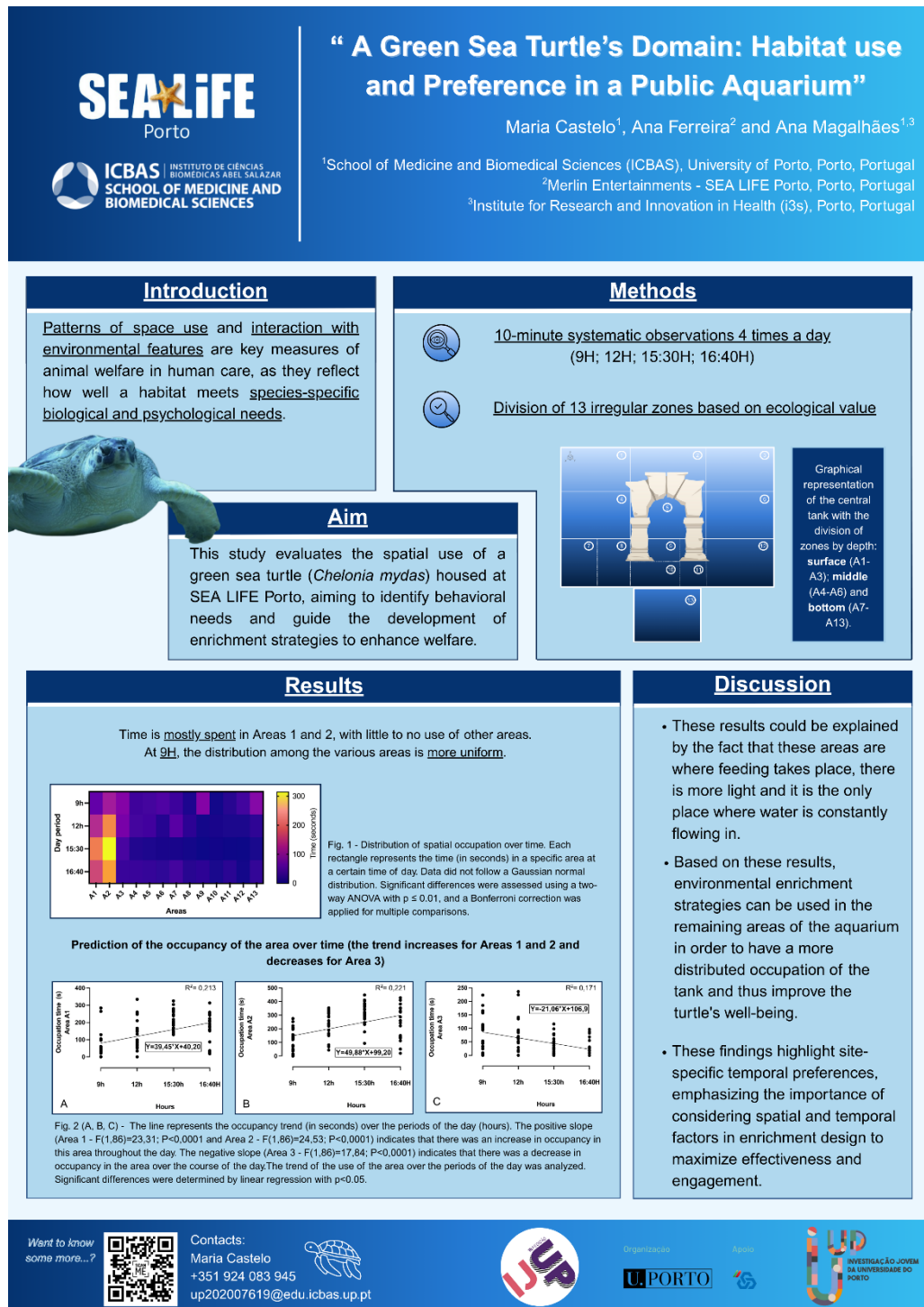
1. Investigação Jovem da Universidade do Porto, 18ª Edição, ICBAS, Porto, Portugal.



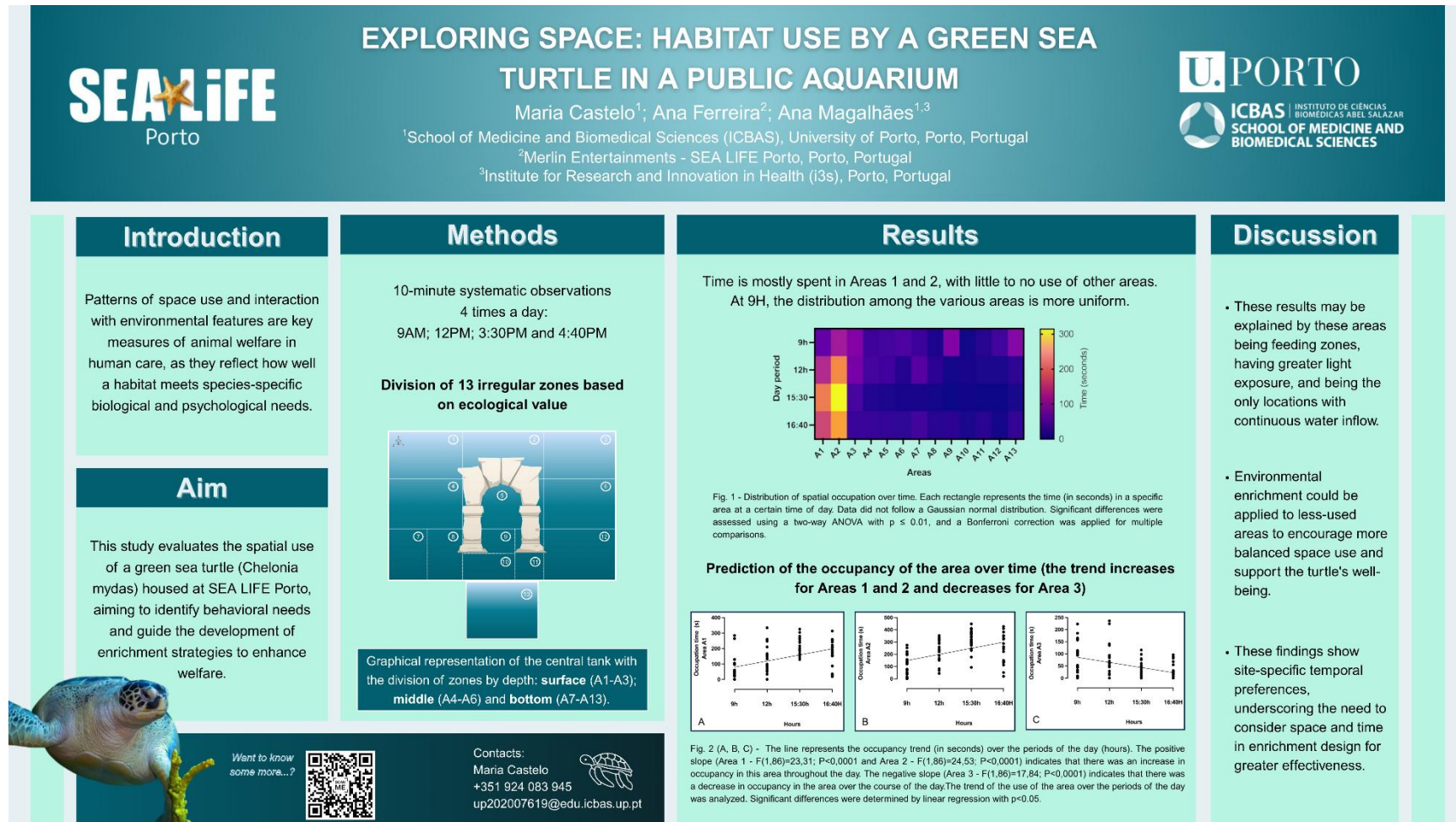
2. Universities Federation for Animal Welfare – International Animal Welfare Conference 2025. Online.



3. Poster presented at conference nº1.



4. Poster presented at conference nº2.



Appendix B

Detailed division of the tank into zones.



Appendix C

Ethogram of green sea turtle's behavior at SEA LIFE Porto.

Category	Behavior	Code	Description
Rest	Sleep/Rest	SR	Periods of inactivity in which the animal remains on a surface, eyes open or closed. Can either be completely lying down or with the front of the body elevated by the anterior flippers with slight head movements.
Locomotion	Crawl	LC	Move along the bottom with fore and hind flippers.
	Dive	LD	Vertical swimming in the direction to the bottom. (including diagonal directions)
	Surfacing	LS	Vertical swimming to the surface. (including diagonal directions)
	Horizontal swimming	LHS	Swim horizontally on the same level as the water column. (could be at different levels of the water column)
	Surface swimming	LSS	Swimming on the surface (first 5 cm of water column)
	Turn	LT	Twist at the same point to change direction.
Aggression	Biting Animal	ABA	The animal bites other species in the tank (e.g. the tail of the stingray).
	Flipper Slap	AFS	Slapping the flipper on the surface of the water to project water or hit another individual with the flipper.
Breathing	Respiration	BR	The animal rises to the surface and puts its head out of the water.
	Between breaths	BB	Swim close to the surface in between breaths.

Category	Behavior	Code	Description
Grooming	Scratch with the Flipper	GS	Scratching the face with the anterior flipper.
Feeding	Grazing	FG	Staying at the bottom digging up the substrate with mouth in search of food.
	Eating	FE(E)	Locate the food, extend the neck, bite or grasp and swallow. It can include the food on the device (FEE) or scattered around the tank (FE).
Interaction with environment	Push	P	Pushing an object or food with their snout or anterior flippers.
	Pull	IP	Pulling an object or food with their mouth.
	Touch	IT	Touch an object with a part of the body.
	Rubbing	IR	Leaning on and rubbing against walls, floor or objects.
	Interaction with diver	ID(R)	Approach, follow or touch the diver. Can approach to be scrubbed (IDR).
	Biting Object	IBO	The animal bites elements present in the tank.
Hiding	Hiding	H	Head or first third of the body inside a hiding place.
Defecation	Defecation	D	Expelling feces.
Abnormal Behavior	Grafting of jaw	GJ	Rasping of rhamphotheca (jaw).
	Pseudo-vocalisation	PV	Squeaks or whines
	Pattern swimming	PS	Continuous, patterned repetition of a random, non-targeted swimming.
	Rest in poor posture	RP	Flopped and lifeless rather than propped up on front slippers on a surface.

Category	Behavior	Code	Description
Abnormal Behavior	Boundary exploration	BE	Swimming close to the edges of the tank while hitting their head on the walls.
Out of sight	Out of sight	OS	The animal is out of the observer's range of vision.

Appendix D

Example of the environmental enrichment plan timetable (two-week schedule, alternating enrichment devices randomly).

	1 st Week				2 nd Week			
	M	No.	A	No.	M	No.	A	No.
Monday	G1-1	1	WE	2	WE	7	G2-1	8
Tuesday	No Observation				No Observation			
Wednesday	WE	3	G1-2	4	G2-2	9	WE	10
Thursday	No Observation				No Observation			
Friday	G1-3	5	WE	6	WE	11	G2-3	12
Weekend	No Observation				No Observation			

Legend:

G1 → G1-1: Yoga Ball with Spikes; G1-2: Tube with Vegetables; G1-3: Fixed Scrub

G2 → G2-1: Cantaloupe; G2-2: Grazing Mat; G2-3: Diver with Scrub

M → Morning

A → Afternoon

[Appendix E](#)

Behavior and area observation record table.

Date:

Time:

Enrichment: YES ☐ NO ☐

Time	Area	Rest	Loco	Aggre	Breath	Groom	Graz	Push	Pull	Touch	Rub	Int.Diver	B. Obj	Hiding	Defec	Abnorm	OS
00:00																	
00:30																	
01:00																	
01:30																	
02:00																	
02:30																	
03:00																	
03:30																	
04:00																	
04:30																	
05:00																	
05:30																	
06:00																	
06:30																	
07:00																	
07:30																	
08:00																	
08:30																	
09:00																	

Time	Area	Rest	Loco	Aggre	Breath	Groom	Graz	Push	Pull	Touch	Rub	Int.Diver	B. Obj	Hiding	Defec	Abnorm	OS
09:30																	
10:00																	
10:30																	
11:00																	
11:30																	
12:00																	
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18:30																	
19:00																	
19:30																	
20:00																	

[Appendix F](#)

Record table for penguin training.

Humboldt Penguin Training Record

Month:

Day	Staff	Time	Type of training	
			Scale	Target
1				
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