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Exploring Structural Environmental Enrichment and Welfare in Captive-Bred Nursehound Sharks (*Scyliorhinus stellaris*)

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Abstract

Sharks play a pivotal role in maintaining the ecological balance of the seas; however, the general loss of biodiversity is particularly affecting this group. Restocking strategies are far less common for elasmobranchs than for teleosts, but in the Balearic Islands, a conservation program aims to breed nursehound sharks (*Scyliorhinus stellaris*) in captivity for subsequent release into the sea, as a conservation measure for these endangered populations. Improving welfare during captive rearing may be important for the success of such programs, yet basic information on the husbandry of this species is lacking. In this exploratory study, we evaluated the effects of two types of structural environmental enrichment (EE), seaweed (SW) and tiles (TL), compared with a non-enriched control, on the growth, body condition, and space use of 45 captive-bred juveniles over six months. Growth did not differ significantly among treatments, although SW individuals showed consistently higher mean length and weight, and enriched animals maintained a better body condition than controls over time. The SW group expanded its core area of use earlier than the other groups, whereas TL individuals progressively aggregated around the tiles. When both structures were offered, naïve control animals mainly selected the seaweed, whereas tile use was only observed in animals previously exposed to them. To our knowledge, these are the first published data on growth, space use, structure selection, and sedation with buffered MS-222 for captive-bred nursehounds. Although these results should be interpreted with caution owing to the use of a single tank per treatment, they provide a baseline for future research on the welfare and rearing of this species in captivity.

Keywords: environmental enrichment; welfare; restocking; space use; sharks; habituation

Key Contribution: Our study provides essential scientific insights into the effects of EE on elasmobranchs, contributing to the development of strategies that support the recovery and conservation of threatened and vulnerable species.

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1. Introduction

The loss of biodiversity in the oceans is widely recognized as one of the most severe global challenges. It is not only significant in its own right, but also plays a fundamental

role in maintaining ecosystems and providing ecosystem services [1]. In this context, the Mediterranean Sea is no exception. Industrial activities, maritime transport, industrial fishing, and other factors negatively affect biodiversity indicators [2]. Despite these pressures, the Mediterranean basin remains a biodiversity hotspot. Although the Mediterranean Sea covers approximately 1% of the ocean's surface, it supports a disproportionately high diversity of fish species, hosting approximately 3–4% of the world's total. The richness of elasmobranch species is particularly notable, with around 7% of all cartilaginous fish species represented in this basin [3–5]. Marine predators have been strongly impacted by human activity, and several species and taxa are currently of urgent conservation concern [6]. Global population declines have been observed in sharks and rays, as their life-history traits (e.g., low fecundity, slow growth and late reproductive maturity) make them particularly vulnerable to anthropogenic impacts [7]. In the Mediterranean Sea, industrial fishing is well documented as the greatest threat to chondrichthyan species [8].

Marine Protected Areas (MPAs) and no-take zones have been established in the Mediterranean Sea under multiple protection frameworks, as part of various conservation strategies aimed at enhancing biodiversity. Another conservation strategy is restocking; however, this is less common in elasmobranchs, although it is most commonly applied for commercially or recreationally valuable species [9,10]. Restocking releases of captive-bred juveniles into wild populations mostly aim to restore spawning biomass [11]. Key elements include developing cost-effective juvenile production, enhancing survival, and minimizing ecological impacts [12]. Despite its success for some species, restocking faces criticism as ineffective and costly, as cultured specimens may show lower survival, growth, reproduction, and genetic diversity [13]. Increasing success in rearing captive-bred sharks and rays in aquaria may support future initiatives for threatened species (e.g., *Orectolobus maculatus*) [14].

In this context, the "Petits Taurons–Acció Stellaris" project was launched in 2021 as a pilot program focused on the breeding, rearing, and release of native shark species in the Balearic Islands (Northwestern Mediterranean Sea). The selected species was the nursehound shark (*Scyliorhinus stellaris*), an elasmobranch in the family Scyliorhinidae and order Carcharhiniformes, inhabiting the continental shelf over rocky and algal-covered substrates. It typically occurs at depths between 20 and 60 m, with records reaching 500 m. It is considered "Vulnerable" by the IUCN [15]. In Spain, there is no specific protection for this species, and both eggs and adult individuals are occasionally caught as bycatch in trawl nets; they are then recovered by specialized technicians from local agencies and taken to species recovery centers. The availability of these specimens facilitates project management and experimentation. However, knowledge of this species remains limited compared to its close relative, *Scyliorhinus canicula* (1335 results on Web of Science vs. 124 in 2023), highlighting the need for more research to close this gap. In the Balearic Islands, the nursehound shark is listed as an "Endangered" species under criteria I and IV of the Balearic Red List of Fishes due to an inferred 50% reduction in biomass over the last or next decade, based on direct observations and current exploitation levels [16].

Captive breeding and post-release survival may be influenced by several factors as early-life experiences shape behavioral phenotypes. In natural contexts, such experiences affect behaviors like predator avoidance and navigation. In contrast, captive environments reduce behavioral flexibility [17]. Hatchery-reared fish must have key behavioral skills to find food and shelter, and avoid predators, increasing survival chances [18]. In aquaculture, several studies support the use of environmental enrichment (EE) as a conditioning tool to improve fish health, cognitive development, and animal welfare [19]. One of the most common strategies is the application of physical or structural EE in captive fish, which adds structural complexity through objects or habitat modifications in the captive environ-

ments [20]. Although no specific studies on nursehounds exist, and elasmobranch research is limited compared to commercial fish, evidence suggests increased spatial complexity improves space use [21], stimulates foraging [22], and reduces stereotypic behaviors such as surface breaking in *Mustelus canis* [23]. Space use has long been used as an indicator of animal welfare [24]; however, it should not be considered a standalone proxy, particularly in sharks and rays, for which validated welfare indicators remain scarce [25]. Shifts in spatial distribution may reflect either positive exploratory behavior or stress-induced searching and escape responses, and these cannot be reliably distinguished without complementary physiological measures. Accordingly, in the present study, space use is interpreted as one component of welfare assessment, together with growth performance, rather than as a definitive welfare metric.

However, simply adding environmental complexity is not enough. Trials with *Raja clavata* show that elasmobranchs change behavior depending on EE type and show diverse preferences [26]. Additionally, elasmobranchs are not a homogeneous group, and species may respond differently to the same stimuli [27]. Therefore, the main objective of the present study is to investigate the effects of two different structural EEs on the growth and space use of captive-reared nursehounds. Specifically, this study aims to assess whether structural EE influences growth performance and spatial distribution within the tank, as potential indicators of welfare in captivity. It also seeks to determine which types of structural EE are preferred by the animals, and whether these preferences change over time. Although these juveniles are intended for restocking purposes, post-release performance was beyond the scope of this study. Rather, the findings are intended to provide a baseline for the development of rearing protocols that incorporate structural EE, whose potential benefits for post-release survival should be evaluated in future field studies.

All the procedures regarding maintenance, handling and sampling of fish were approved by the Animal Experimentation Ethics Committee (CEEA-UIB, Spain; Ref. 217/05/23 approved on 14 July 2024) and were carried out in accordance with the European Directive 2010/63/UE and Spanish Royal Decree (RD53/2013).

2. Materials and Methods

2.1. Study Animals and Settings

This study was carried out at the facilities of the Marine Research and Aquaculture Laboratory (LIMIA IRFAP) located in Port d'Andratx (Spain) (39°32′39″ N 02°22′42″ E). Forty-five juvenile nursehounds (*S. stellaris*) were included in the study. At the start of the experiment, animals had a mean total length of 31.4 ± 2.7 cm (range: 25.5–37.0) and a mean body weight of 126.1 ± 38.3 g (range: 61.9–210.2), with no significant differences among tanks (one-way ANOVA; length: $p = 0.561$; weight: $p = 0.514$). The animals were born in captivity in the aquariums of Cabrera National Park (PNMTAC) during March 2023 and were brought to the LIMIA facilities for this study, which lasted from 1 November 2023 to 10 May 2024. The study had a total duration of 6 months. The animals were tagged with mini passive integrated transponders (PIT-tags) model ID-100^a/1.4 (Trovan Ltd., North Ferriby, United Kingdom) for individual identification. After a 15-day acclimation period, they were randomly distributed into three circular tanks with a capacity of 1000 L (∅ 147 cm), resulting in 15 fish in SW (6 males/9 females), 16 in TL (6 males/9 females and one of undetermined sex), and 14 in C (8 males/6 females). The tanks are part of a semi-open system connected to the sea and filter the water through UV and sand filters, maintaining a salinity of 37 ppt. Oxygenation of the tanks was achieved through a pressurized air system with porous stone diffusers, maintaining a dissolved oxygen concentration (DO) between 7.1 and 9.4 mg/L. The temperature was controlled by recirculating the tanks' seawater (filtered and UV disinfected) through a coil connected to an external fan coil,

ranging from 14.66 to 18.97 °C (mean ± SD = 16.68 ± 2.04 °C). The tanks were covered with opaque curtains and topped with shade meshes to attenuate incoming light. Tanks were illuminated from above with Luxina T8 LED 21 W lamps (Luxna Lighting, Normanton, United Kingdom), providing an intensity of 400 lx at the water surface, under a 12L:12D photoperiod controlled by a timer. The fish were fed three times per week, between 9 a.m. and 12 p.m. This feeding frequency was selected based on established husbandry guidelines for elasmobranchs, which indicate that feeding frequency may vary according to species and life stage. In addition, feeding frequencies of 2–3 times per week have been estimated for captive sandbar and lemon sharks based on extrapolated gastric retention times. A ration equivalent to 7% of body weight (BW) was offered at each feeding event. The feeding ration was established based on the husbandry experience of the facility with juvenile sharks and previous observations of feeding response, growth, and body condition. Species-specific differences in nutritional requirements and feeding rates were also considered, as reported for captive elasmobranchs [28]. The fish were fed a homemade mix containing 10% shrimp, 25% squid, 25% anchovy, and 40% hake. Tanks were siphoned daily to monitor uneaten food and maintain tank hygiene.

2.2. Biometrics and Biological Sampling

A total of six samplings were conducted throughout the study by qualified personnel. The first sampling took place on 15/11/23 (T0), before the first experimental round, while the second occurred on 18/12/23 (T1), following the space-use test and prior to the preference test. This process was repeated for the second and third rounds, with a one-month interval between them. T2 = 17/01/24; T3 = 20/02/24; T4 = 18/04/24 and Tf = 21/05/24. At T0, each fish was individually identified by subcutaneous implantation of a microchip with a unique identification code. During each subsequent sampling, fish were identified by scanning their microchip using a Trovan LID-575 portable microchip reader (Trovan Ltd., North Ferriby, United Kingdom). Once identified, fish were weighed and measured for length.

Prior to handling, fish were sedated with MS-222 (tricaine methanesulfonate), buffered with sodium bicarbonate. This compound is readily soluble in water and is also lipid soluble and readily moves across the gills. It was applied via immersion in a static bath at a concentration of 100 mg/L of water, renewing the dose every 20 fish. Since there is no bibliography on this species and the literature is scarce on similar species [29], we assess this sedation protocol to serve as a basis for future research. The times it took for the fish to reach the different sedation phases, as described by Bowser [30], were recorded. The procedure was performed when the fish reached stage V (total loss of muscle tone and equilibrium; slow but regular ventilation rate; loss of spinal reflexes).

Blood samples (0.5 mL at each time point) were collected in heparin tubes at T0 and Tf. The blood samples were subsequently processed, initially stored at −20 °C, and later transferred to −80 °C until laboratory analysis of general stress indicators, the results of which are part of a separate manuscript currently in preparation.

The objective was to monitor the biometric changes throughout the experiment and assess the potential effects of EE on these parameters. The biometric variables analyzed included total length (cm), weight (g), Fulton's condition factor (K), calculated as [31]

$$K = (W \times 100)/L^3,$$

and specific growth rate (SGR), calculated as

$$SGR = 100 \times (\ln(\text{final weight}) - \ln(\text{initial weight}))/\text{days}.$$

2.3. Experimental Setting

2.3.1. Space-Use Test

To test the influence that different types of structural EE might have on the behavior and welfare of the fish, the experiment was designed as follows: first, the fish were randomly distributed into three tanks, following the specifications mentioned above, and elements of structural EE were placed in these tanks. Following the recommendations of Jones [32], who highlights that the lack of detailed information regarding the different types of physical EE complicates the comparison of studies and the establishment of guidelines for future research, the physical enrichment was defined in detail as follows. Tank 1/Seaweed Treatment (SW) contained algae: plastic elements shaped to mimic various types of algae. These elements were kept no taller than 35 cm and were mounted on two green mesh bases (50 × 60 cm) distributed homogeneously, with a density of 43 bundles per m^2. Tank 2/Tiles Treatment (TL) contained tiles: six conical ceramic tiles measuring 50 × 21 × 16 cm, placed concave-side down to form shelters with a 6.5 cm entrance on each side. Tank 3/Control (C) served as a control, with no EE.

To determine the space use over time, photographs were taken with cameras GOPRO Hero10(GoPro Inc., San Mateo, CA, USA) mounted over the tanks for two hours (9–11 a.m.) at 2 min intervals, twice a week, for four consecutive weeks. After this period, the animals were sampled. The images taken with the cameras in both tests were processed using the ImageJ2™ software (v 1.54) and the FIJI image processing package (v 2.15). To establish a baseline for subsequent statistical analysis, the coordinates of each nursehound, as well as the enrichment and the tank boundary, were determined using the multi-point tool. The following criteria were applied: The tank was represented by 8 equidistant points that follow the contour of the heating tube. The enrichment consisted of 8 points at the vertices of the structures. The fish were represented by a single point located at the center of the head, along the line posterior to the eyes.

From 72 image groups, 61,452 coordinates were obtained. Kernel home range estimates are based on a density estimate calculated from the coordinate points that can be interpreted as a utilization distribution (UD). The UD is an estimate of the amount of time a fish spent in any given area of the home range. Kernel utilization distribution (KUD) analyses were used to assess the space used by nursehounds in each tank based on the photographs taken. The metrics used for the KUD include density (the number of individuals during the recording period) and the area size measured in cm^2. Density was calculated by dividing the number of individuals observed by the total observation area, providing a measure of how concentrated individuals were within the habitat during the recorded period. Area size was determined through home range estimation methods like Kernel Density Estimation (KDE), which identified the areas with the highest usage within the studied habitat. For comparisons between KUDs, models were developed that considered the number of animals, the treatment they were subjected to, and the time of exposure to enrichment. The areas analyzed were those used 95% of the time and those used 50% of the time [33].

2.3.2. Preference Test

After the space-use test and sampling, the distribution of physical enrichment was modified to conduct a preference test. Because each group had been continuously exposed to a single type of structural EE prior to this test, the preference test was designed to assess the selection of structures by animals with different previous experience, rather than intrinsic habitat preference. In this new arrangement, each tank contained one algae mesh (50 × 60 cm) and two tiles (50 × 21 × 16 cm), covering 30% of the total area with EE. As in the space-use test, space-use density was quantified using a heatmap, and the

utilized areas (cm^2) were compared. Video recordings were conducted following the same method described previously for three consecutive days. In this case, 23,817 coordinates were obtained from 18 groups. Furthermore, in the preference test, the number of times the animals entered each of the three defined zones was quantified: one representing the algae EE, another for the tiles, and a third representing the open area. The tanks were then returned to their previous configuration, and recordings were resumed after a one-month interval. This entire process was repeated a third time when the sharks had been in the tanks for four months. During the experiment, three repetitions of each test were conducted to observe how the measured variables changed over time. No issues were encountered during the space-use test. However, in the third phase of the preference test, problems with the recording system prevented us from obtaining reliable data, limiting our analysis to the first and second phases.

2.4. Statistical Analysis

Statistical analysis was performed using R software (v 4.3.0 (21 April 2023 ucrt))with the RStudio (2024.04.2+764) integrated development environment (R version 4.3.0 (21 April 2023 ucrt)). In addition to the default packages, the following packages were used: the graphics were created with the "ggplot2" (3.5.1) and "plotrix" (v 3.8-4) packages. Linear mixed-effects models were fitted with the "nlme"(v 3.1-165) package, and post hoc comparisons were performed with the "emmeans" (v 1.10.3) package. To test the differences that might exist between the different treatments among the variables—length, weight, K, and SGR—the packages "lme4" (v 1.1-35.5) and "car" (v 3.1-2) [34] were used as tools to adjust the linear models and analyze the variance, the "nortest"(v 1.0-4) package was applied in cases where non-normality was confirmed by the Shapiro–Wilk test, and homoscedasticity was assessed using Levene's test. Subsequently, non-parametric Kruskal–Wallis analyses were performed to determine whether the differences between variables were significant. For all of them, the existence of significant differences based on sex and the number of sharks in each tank was tested separately. All analyses were performed at a 95% significance level. Dunn's test was used as a post hoc test to compare between groups when differences were found.

Additionally, to evaluate the consistency of the anesthesia protocol across repeated sampling events, the times required to reach stages III and V of anesthesia were compared among sampling events T1, T2, and T3 using linear mixed models (LMMs). Sampling event was included as a fixed factor and individual shark (PIT-tag ID) as a random factor to account for repeated measurements on the same animals. Sampling event T0 was excluded because a different criterion was used to record stage III.

The "adehabitatHR" (v 0.4.21) package was used for the analysis of space use and spatial distribution [35], which allows Kernel Utilization Distribution (KUD) calculations that indicate the probability that an animal is present in a given position by coordinates. Because each treatment was applied to a single tank, tank and treatment effects are confounded and cannot be statistically separated. Therefore, the observed differences among groups cannot be attributed exclusively to the EE treatments, and the present study should be considered exploratory. KUD areas were analyzed with linear mixed-effects models for repeated measures. Areas were square-root transformed to meet model assumptions. The model proposed considered the factors: number of fish, treatment, and time (days since the start of the experiment). Therefore, sampling date was included as a random intercept to account for the conditions shared by all tanks recorded on the same day. Temporal autocorrelation between consecutive recordings of the same tank was tested by adding a first-order autoregressive (AR1) structure, which did not improve the models (likelihood

ratio test, $p > 0.4$) and was therefore not retained. The most complex of these included the interaction of treatment and date of recording and the number of individuals.

Among the models generated, the best model was selected based on information criteria (AIC), penalizing the more complex models to avoid overfitting, and checking the normality of the residuals to ensure that the model assumptions were met. Regarding the variables analyzed in the space-use test (area and density), the final model included the interaction of treatment and date of recording. Pairwise comparisons between treatments, overall, at each recording date, and of their temporal trends were performed on the estimated marginal means with Bonferroni adjustment.

In the case of the preference test, in addition to calculating the area and density, the proportion of individuals using each EE was also calculated. As this was an analysis of proportions, the quasibinomial family was used within the GLM. The quasibinomial family is more flexible than binomial models in handling heterogeneity, and it allows for dealing with overdispersion. This is particularly useful when the variance exceeds the mean, as is often the case in ecological data with varying individual behaviors [36].

The sample size in the preference test was much smaller (6 vs. 23 in space use), which means each observation had a greater impact on the results, making the model more sensitive to the variability present in the data. It is easier to build a model that generalizes well without being affected by outliers or extreme values with the larger sample size of the space-use test.

3. Results

3.1. Biometrics

Regarding biometry recorded during the study period (Table 1), a significant increase in overall length and weight was observed after 6 months (L: $Z = -11.35$, $p < 0.001$; W: $Z = -10.98$, $p < 0.001$), representing good growth performance of the animals during the experiment. Length, weight, and condition factor (K) were analyzed at three time points: T0 (15/11/23), T3 (20/02/24), three months after starting the experiment, and Tf (21/05/24), six months after the beginning. Length and weight did not show significant differences between treatments at any sampling time ($p > 0.05$; L: $p = 0.579$; W $p = 0.585$) (Table 1), but all of them showed significant differences between the first time (T0) and the final time (Tf). The K condition factor showed no differences at the beginning of the experiment between tanks (T0: $\chi^2 = 0.07$, $p = 0.96$), but significant differences were detected at T3 ($\chi^2 = 7.81$, $p = 0.02$) and Tf ($\chi^2 = 16.25$, $p < 0.001$) between treatments. Indeed, after 3 months (T3), significant differences were only observed between the TL and C groups ($Z = -2.6$, $p = 0.01$), whereas at the end (Tf), the C group differed significantly from the other two groups, showing a K condition factor smaller than the other groups'. The difference with SW ($Z = 2.67$, $p = 0.01$) was smaller than with TL ($Z = 3.96$, $p = 0.001$). The specific growth rate (SGR) showed no significant differences between treatments after 3 months (T3: $\chi^2 = 4.80$, $p = 0.09$), with values ranging from 0.73 ± 0.03 to $0.83 \pm 0.04\%$ day^{-1} (Table 1). However, significant differences were detected at the end of the experiment (Tf: $\chi^2 = 9.02$, $p = 0.01$) between treatments. Indeed, the C group showed a significantly lower SGR ($0.53 \pm 0.02\%$ day^{-1}) than the SW ($0.62 \pm 0.02\%$ day^{-1}; $Z = 2.38$, $p = 0.03$) and TL groups ($0.63 \pm 0.02\%$ day^{-1}; $Z = 2.79$, $p = 0.02$), whereas no differences were found between SW and TL ($Z = -0.37$, $p = 0.71$).

Table 1. Mean values ± SE of initial measurements (T0) after 3 months of rearing (T3) and final measurements (Tf), for length (cm), weight (g), condition factor (K, g/cm^3), and specific growth rate (SGR, %/day) of juvenile nursehounds under different enriched rearing conditions: seaweed (SW) and tiles (TL), and the control group (C). *p* (T0 vs. Tf): paired *t*-test (^{t}) or Wilcoxon signed-rank test (^{w}), depending on the normality of the paired differences (Shapiro–Wilk). Different letters indicate statistically significant differences ($p < 0.05$) between the treatments.

	Time	SW	TL	C	*p*
Length (cm)	T0	32.0 ± 2.62	30.89 ± 2.82	31.35 ± 2.81	0.579
	T3	39.23 ± 2.73	37.12 ± 2.55	38.53 ± 2.63	0.102
	Tf	45.9 ± 2.37	43.5 ± 2.64	44.5 ± 2.31	0.078
p (T0 vs. Tf)		<0.001 ^{w}	<0.001 ^{t}	<0.001 ^{w}	
Weight (g)	T0	134.93 ± 42.74	118.24 ± 37.49	125.82 ± 35.4	0.585
	T3	275.36 ± 67.88	231.31 ± 62.98	276.56 ± 55.59	0.077
	Tf	427.44 ± 97.55	379.37 ± 69.88	334.45 ± 65.13	0.061
p (T0 vs. Tf)		<0.001 ^{t}	<0.001 ^{t}	<0.001 ^{t}	
K (g/cm^3)	T0	0.39 ± 0.04	0.38 ± 0.03	0.39 ± 0.02	0.965
	T3	0.44 ± 0.02 ^{ab}	0.44 ± 0.03 ^{a}	0.47 ± 0.03 ^{b}	0.020 *
	Tf	0.43 ± 0.03 ^{a}	0.45 ± 0.02 ^{a}	0.37 ± 0.04 ^{b}	0.001 **
SGR	T3	0.73 ± 0.04	0.73 ± 0.03	0.83 ± 0.04	0.09
	Tf	0.62 ± 0.02 ^{a}	0.63 ± 0.02 ^{a}	0.53 ± 0.02 ^{b}	0.01 *

Note: Asterisks indicate statistically significant differences: * $p < 0.05$; ** $p < 0.01$.

Induction times were analyzed for sampling events T1, T2, and T3 ($n = 45$ per sampling event). Across these samplings, sharks reached anesthesia stage III after 1.63 ± 0.85 min (mean ± SD) and stage V after 4.95 ± 1.08 min (range: 2.35–7.68 min). Time to stage III differed significantly among sampling events (LMM, likelihood-ratio test: $\chi^2 = 27.54$, df = 2, $p < 0.001$). It was shorter at T2 (1.23 ± 0.76 min) than at T1 (1.70 ± 0.83 min; $p < 0.001$) and T3 (1.96 ± 0.82 min; $p < 0.001$), whereas T1 and T3 did not differ ($p = 0.278$). In contrast, time to stage V did not differ significantly among sampling events ($\chi^2 = 5.71$, df = 2, $p = 0.057$; T1: 5.24 ± 1.08 min; T2: 4.76 ± 1.05 min; T3: 4.85 ± 1.08 min), and no pairwise comparison was significant (Tukey, $p > 0.05$). All animals recovered from anesthesia and returned to their tanks.

3.2. Space-Use Test

The analysis of the spatial distribution of the nursehound revealed different behavioral patterns across treatments as well as over time. While the TL group displayed a small and concentrated distribution at specific points where the structural EE was located, fish in the C group were more dispersed, occupying mainly the periphery of the tank. Although the area of occupation appeared similar for both SW and C groups, the concentration zones of the animals differed. In the heat map (Figure 1), the high-density points (in red) observed in TL and SW groups were associated with the EE and not at the periphery of the tank, as could be seen in the C group.

The KUD 95 represents the area in which there is a 95% chance of finding the fish, while the KUD 50 is the 50% chance and represents the core area, represented by a black line in the heatmap (Figure 1). KUD 95 analyses show significant differences (Table 2) between the SW and TL groups ($p < 0.001$) and non-significant differences between C and TL ($p = 0.18$) and between C and SW groups ($p = 0.13$). However, the temporal trend of TL differed significantly from that of C ($p = 0.003$).

Table 2. Space-use area (cm^2) for KUD95 and KUD50 estimated from recordings during the space-use test. Different letters indicate statistically significant differences ($p < 0.05$) between the treatments.

Treatment 95	Mean (cm^2)	SE	*n*
Control [ab]	4×10^3	1.9×10^3	23
Seaweed [a]	5.2×10^3	2.27×10^3	23
Tiles [b]	3×10^3	2.26×10^3	23
Treatment 50	Mean (cm^2)	SE	*n*
Control [b]	424	212	23
Seaweed [a]	631	286	23
Tiles [b]	317	216	23

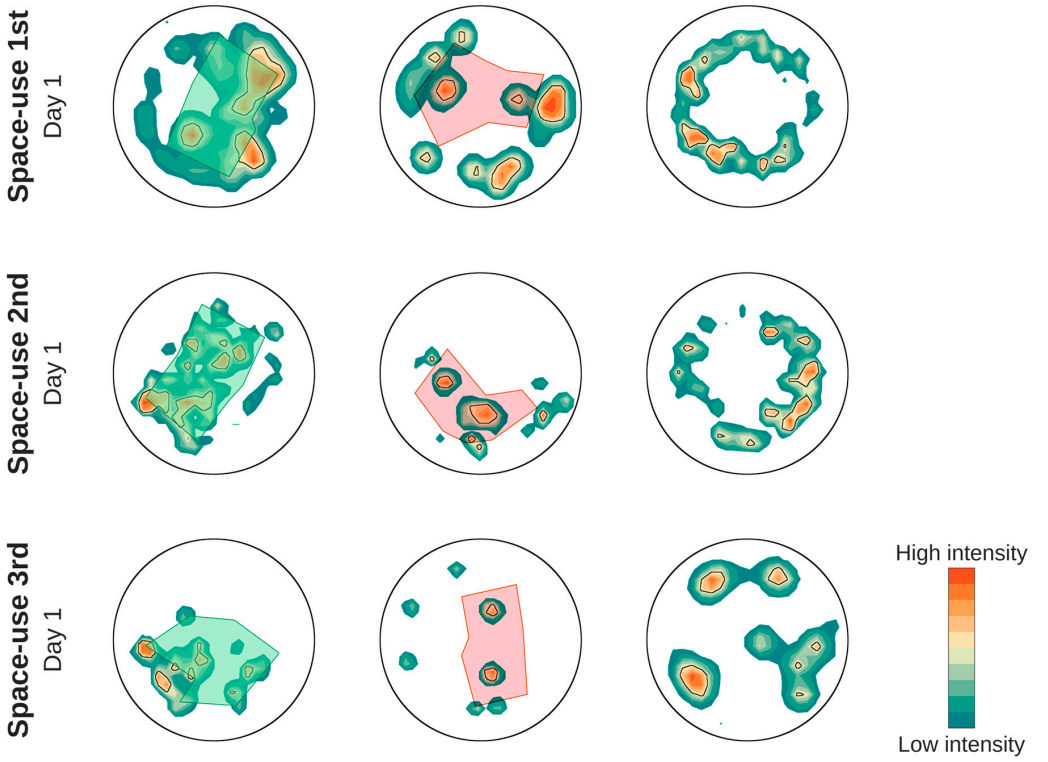


Figure 1. Images of the different treatments and heat maps showing the cumulative spatial distribution of nursehounds within the tank during the first day of each of the three space-use tests, for each treatment (SW, TL, and C). Colors represent kernel utilization distribution (KUD) isopleths, i.e., the percentage of the utilization distribution volume enclosed by each contour, ranging from the 10% isopleth (red; areas of highest use intensity) to the 95% isopleth (blue; outer boundary of space use). Black lines delimits the tank, green polygons are the algae EE, and red polygons are the tiles EE.

Significant differences were detected in the area sizes over time ($p = 0.004$), and the temporal trend differed among treatments (treatment × time interaction, $p = 0.003$). In the temporal analysis of KUD95 by date (Figure 2), the results indicated significant differences ($p = 0.02$) between the area occupied by the nursehounds in the SW (5.5×10^3 cm^2) and TL (2.8×10^3 cm^2) groups after two months of the experiment (beginning 18/01/24). Additionally, significant differences ($p < 0.001$) were observed between the C (5.2×10^3 cm^2) and TL (3.7×10^3 cm^2) groups starting from 18/04/24. No significant differences were found between the SW and C areas.

In the case of KUD50, the analysis shows significant differences (Table 2) between the C and SW groups ($p = 0.004$) and between the SW and TL groups ($p < 0.001$), but no significant differences between the C and TL groups ($p = 0.12$). There are also significant differences in the area sizes over time ($p = 0.004$) and in the temporal trend among treatments ($p < 0.001$)

(Figure 3). The results of the pairwise comparisons with Bonferroni adjustment show the following: From the start of the experiment (23/11/23) until 15/02/24, significant differences were found between the C and SW groups ($p \leq 0.03$), e.g., on 05/12/23, C (458 cm^2) and SW (680 cm^2) ($p = 0.02$). It was not until 18/01/24 that the areas of SW (539 cm^2) and TL (492 cm^2) began to diverge significantly ($p = 0.002$). By 18/04/24, the differences between SW (1058 cm^2) and C (591 cm^2) were no longer significant ($p = 0.32$), while significant differences between C (591 cm^2) and TL (273 cm^2), which began to emerge on 15/02/24 ($p = 0.048$), became clear ($p < 0.001$).

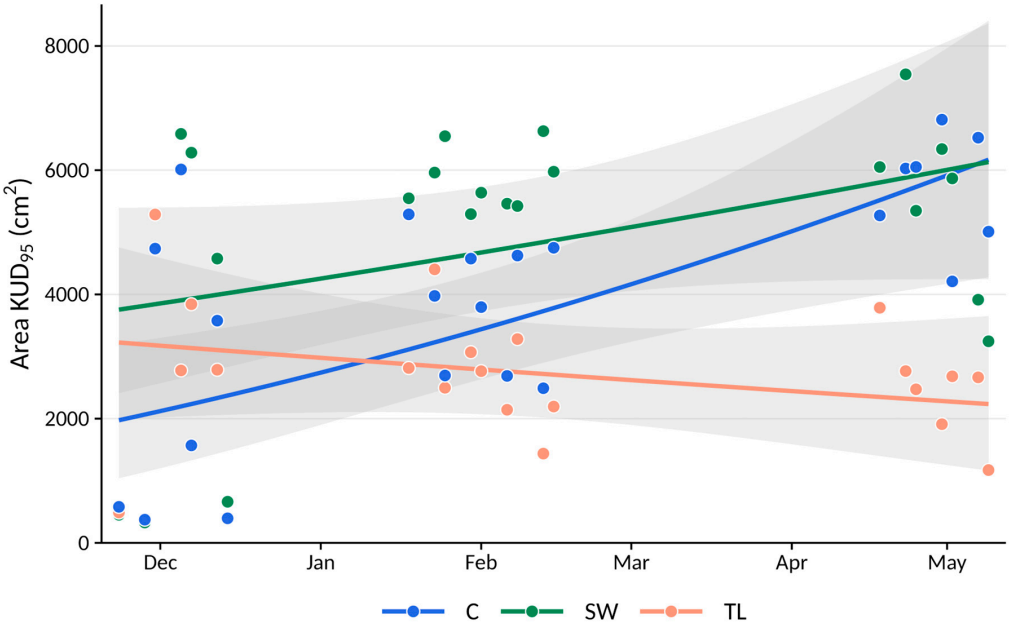


Figure 2. Area of use (cm^2) by treatments calculated using KUD95 in the space-use test. The shaded area is the area of the 95% confidence interval. Lines show the fitted values of a linear mixed-effects model (treatment × time, with sampling date as a random intercept).

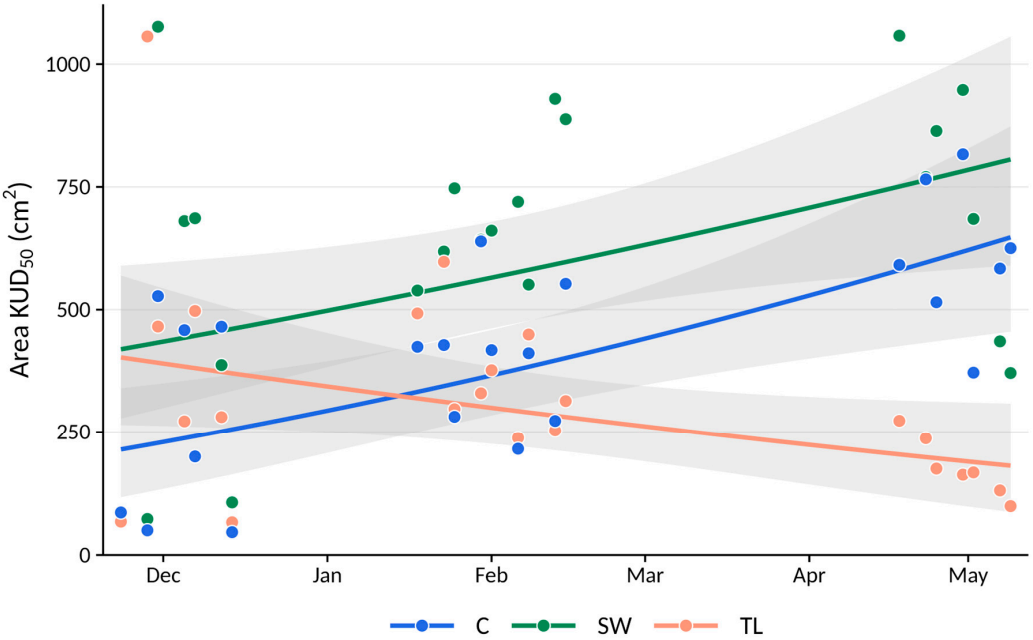


Figure 3. Area of use (cm^2) by treatments calculated using KUD50 in the space-use test. The shaded area is the area of the 95% confidence interval. Lines show the fitted values of a linear mixed-effects model (treatment × time, with sampling date as a random intercept).

3.3. Preference Test

Regarding the area utilized by the sharks, the KUD95 represents the area in which there is a 95% probability of finding the individuals, while the KUD50 represents a 50% probability and defines the core area, depicted by a black line in the heatmap (Figure 4). The KUD95 analysis revealed no significant differences between the SW (0.37 ± 0.13 m^2) and TL (0.40 ± 0.38 m^2) groups (p = 0.82), nor between the SW and C (0.34 ± 0.14 m^2) groups (p = 0.78). Similarly, for KUD50 values, no significant differences were detected between the SW (452 ± 170 cm^2) and TL (310 ± 150 cm^2) groups (p = 0.21), or between the SW and C (324 ± 153 cm^2) groups (p = 0.25).

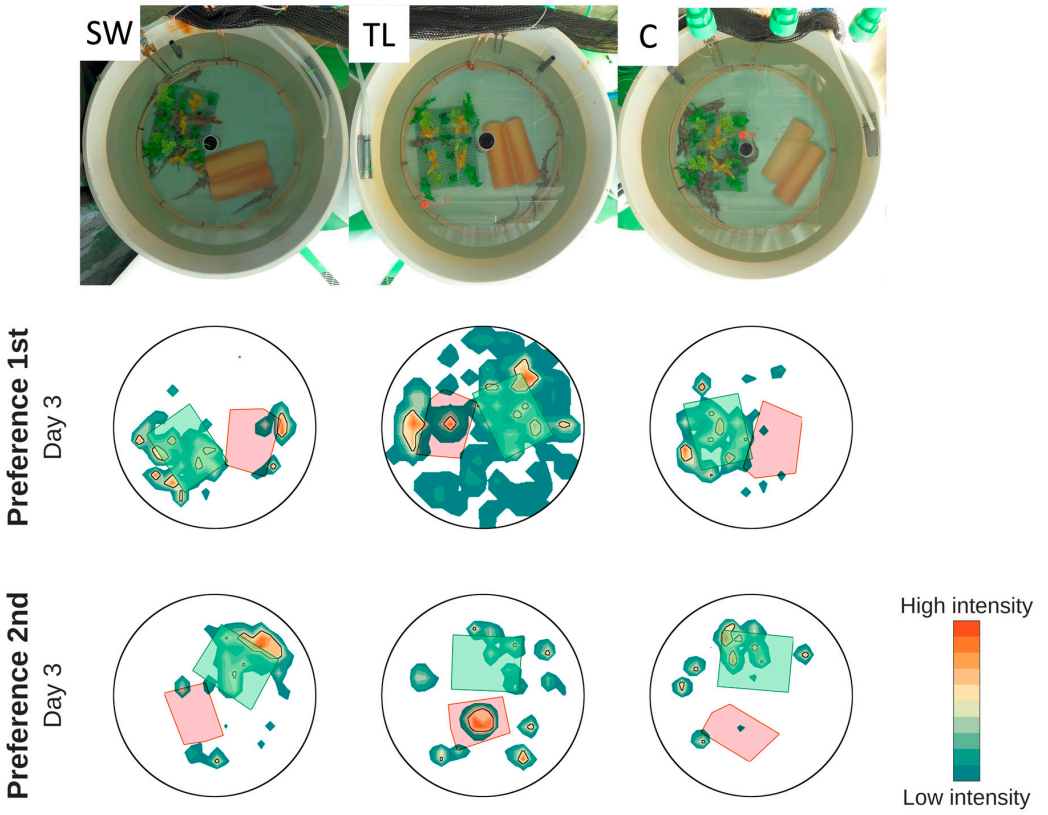


Figure 4. Heat map showing the cumulative spatial distribution of nursehounds within the tank during the two preference tests during the third day of each test. The maps are divided into the three previous treatments (SW, TL and C). Colors represent kernel utilization distribution (KUD) isopleths, i.e., the percentage of the utilization distribution volume enclosed by each contour, ranging from the 10% isopleth (red; areas of highest use intensity) to the 95% isopleth (blue; outer boundary of space use). Black lines delimit delimits the tank, green polygons are the algae EE, and red polygons are the tiles EE.

The preference of the nursehounds for each of the enrichments was calculated based on the proportion of individuals (Table 3) inside or outside each EE. Quasibinomial GLMs revealed a significant effect of treatment group on the proportion of nursehounds in the Seaweed EE ($F_{2,18}$ = 13.23, $p < 0.001$) and the Tiles EE ($F_{2,18}$ = 14.88, $p < 0.001$), but not outside the EEs ($F_{2,18}$ = 2.50, p = 0.11), and the treatment × day interaction was not significant for any variable (all $p \geq 0.42$). Model selection based on QAICc supported a treatment-only model for the Tiles EE and a treatment + day model for the Seaweed EE, reflecting increased Seaweed EE use after the first day of exposure ($F_{1,18}$ = 4.66, p = 0.045). The proportion of animals that preferred the structural enrichment tiles (Tiles EE) differed significantly ($p < 0.01$) between the TL group and the other two groups. Similarly, we see that there was no difference between SW and C groups in the proportion of animals in the Seaweed EE

($p = 0.57$), but there was a significant difference from the TL group ($p < 0.01$). The treatment × day interaction was not significant for any response variable (all $p \geq 0.42$). The treatment × phase interaction was likewise not significant ($p > 0.55$), except for a marginal effect in the Tiles EE ($F_{2,18} = 3.56$, $p = 0.0498$). Model selection based on QAICc supported the treatment-only model for the Tiles EE and a model including treatment and day for the Seaweed EE (ΔQAICc = 1.43 relative to the treatment-only model). This reflects an increase in Seaweed EE use after the first day of exposure ($F_{1,18} = 4.66$, $p = 0.045$; ≈22% on day 1 vs. 39–45% on days 2–4). For the proportion of nursehounds outside the EEs, the null model was the most parsimonious. When comparing the utilized areas between groups, we observed significant differences between the SW group and the Preference test in both KUD95 ($Z = -2.98$, $p = 0.01$) and KUD50 ($Z = -3.34$, $p = 0.004$).

Table 3. Proportion of nursehounds recorded inside each EE or outside during the preference test (mean ± SD).

	% Seaweed EE	% Tiles EE	% Outside EE
SW-group	43.86 ± 15.59%	1.85 ± 3.01%	54.28 ± 14.29%
TL-group	17.18 ± 9.28%	19.6 ± 13.33%	63.20 ± 8.79%
C-group	48.37 ± 17.86%	1.53 ± 2.07%	50.09 ± 17.94%

4. Discussion

This study provides the first preliminary evidence that structural EE may influence the condition and space use of captive-bred nursehounds. Adding complexity to their environment with simple structures enhances natural behaviors associated with their welfare. Results suggest that nursehounds adapted their habits according to the different structural EE to which they were exposed.

Throughout the six-month experimental period, continuous growth was recorded in juvenile nursehounds across all treatments. Although differences among treatments were not statistically significant, individuals in the SW group showed consistently higher mean length and weight than those in the TL and C groups from T3 onward, and this difference tended to widen over time (Table 1). In addition, the control group showed an average weight loss between the final two sampling points (T4 and Tf). Consistent with this, SGR over the whole experimental period was significantly lower in the control group than in both enriched groups, mainly as a result of its reduced growth during the second half of the experiment (SGR T3–Tf: 0.21 ± 0.04% day^{-1} in C vs. 0.49 ± 0.02 and 0.54 ± 0.04% day^{-1} in SW and TL, respectively). Given the lack of statistical significance and the use of a single tank per treatment, this pattern should be interpreted with caution; however, it suggests that potential effects of structural EE on growth may become more evident over longer rearing periods, which warrants further investigation.

Fulton's condition factor (K) allows comparison of within-species length–weight relationships across seasons or under varying environmental conditions [37,38]. Significant differences in K were first detected between the TL-enriched group and the control group, already observable by sampling point T3 (20/02/24). These findings indicate a deteriorating trend in the three-month condition factor, exceeding the potential influence of the third round of space-use and preference tests. To date, no prior studies have been identified demonstrating the beneficial effect of structural EE on elasmobranch body condition, although such effects are well documented in other fish species. In juvenile Atlantic salmon (*Salmo salar*), fish reared at low density in tanks with rocks and plastic plants grew faster in the hatchery than conventionally reared fish [39]. In rainbow trout (*Oncorhynchus mykiss*), physical enrichment reduced aggressive and stereotypic behaviors, increased shoal cohesion, decreased fear-related responses, and improved growth [40]. Likewise, the

presence of cover increased food consumption at ad libitum rations and growth rates in the European minnow (*Phoxinus phoxinus*), although it had no significant effect on the growth of the threespine stickleback (*Gasterosteus aculeatus*), highlighting that growth responses to structural EE are species-specific [41].

The selection of two distinct types of structural EE enabled assessment of how differing enrichment elements influenced behavior. Tiles served as shelters, permitting animals to hide and aggregate beneath them, but may also have acted as opaque obstacles capable of concealing potential threats. Moreover, tiles were firmly anchored to the bottom and did not extend into the water column, thus functioning as enrichment elements that occupied less vertical space and introduced less environmental complexity. Other studies have corroborated that the use of tiles as shelter can be beneficial, as it reduces the standard metabolic costs in *Salmo salar* [42] or improves the estimated post-release survival of conditioned seabreams [43]. Consistently, the lack of shelter can disrupt behavior, increase stress levels, and consequently alter physiological performance [44]. Algae, on the other hand, provided greater spatial complexity; although they occupied the same bottom area as tiles, their elevation into the water column created a more three-dimensional structure, enhancing environmental heterogeneity. According to other studies like the one with tiger muskellunge (*Esox masquinongy* × *E. lucius*), behavioral responses resulting from exposure to vegetation could increase survival after stocking in lakes [45]. Their flexibility allowed nursehounds to move within them, without being dense enough to obstruct visibility.

Spatial use results revealed distinct behavioral dynamics when nursehounds were introduced to a novel environment. No significant differences between treatments were observed in the core area during the initial weeks; however, from T1 onward, the SW group's core area expanded beyond that of the control group, whereas the TL group did not differ from the control until approximately one month later (T2). These results suggest that when exposed to a novel environment, individuals in a more heterogeneous habitat may initiate exploratory behaviors more rapidly and occupy a larger central area. The use of the tank center or periphery is well-defined in welfare terms [24], with the use of the periphery serving as a defense and aggregation mechanism against the only element that offers them some shelter, the walls of the tank itself [46]. By contrast, venturing into open areas is indicative of exploratory behavior and, consequently, positive welfare status [47]. However, an expansion of the area used may also reflect stress-induced searching behavior, and these alternatives cannot be fully distinguished without complementary physiological indicators.

This initial response may attenuate over time as animals acclimatize to their environment. Habituation, a fundamental learning mechanism [48], is documented across taxa and increasingly recognized in sharks [49]. In our study, differences in core area between the SW and control groups were no longer significant after more than four months (18/04/24), which may reflect a progressive acclimatization of the control group to the tank. Nevertheless, seasonal temperature changes and animal growth over this period may also have influenced activity levels and space use. Concurrently, the TL group significantly reduced its used area; however, this should not be interpreted as a negative behavioral change. Although the reasons for aggregation are not fully understood, aggregation has been widely reported in rays and sharks [50]. Evidence shows that, particularly during early life stages, sharks exhibit strong site fidelity, with shallow coastal waters serving as optimal nursery areas that promote juvenile aggregation [51]. Spatial use density plots indicated that initially, TL individuals occupied an intermediate area more complex than the control group, but less than the SW group. Over time, TL individuals concentrated progressively within the shelter provided by the tiles, whereas control animals remained confined to the periphery, and SW individuals were distributed in patches closely associated with the algae meshes.

When discussing natural behaviors such as foraging and aggregation, it is crucial to reference the limited but valuable existing data. For example, Sims [52], using radio-tracking and direct scuba observation, monitored the release of immature nursehounds (*Scyliorhinus stellaris*) along the Irish coast, showing that immature males primarily used rock refuges during the day and moved into deeper waters to forage at night, with similar behaviors confirmed for immature females. Importantly, tracked individuals displayed no abnormal post-tagging behaviors. Consequently, any enrichment element promoting natural behaviors can be interpreted as improving welfare [21,27,53].

Preference tests are widely used in ethology to assess the degree to which fish "prefer" or "avoid" specific environmental features, evaluating consistent choices and tendencies to access resources or traits [54]. In elasmobranchs, there remain clear knowledge gaps regarding cognition [55]. In the present study, the structures selected by nursehounds when both options were offered differed according to their previous rearing conditions. Individuals from the C group, which had not been previously exposed to any EE, mainly selected the algae (48.37 ± 17.86%), with negligible use of tiles (1.53 ± 2.07%), and a similar pattern was observed in the SW group (43.80 ± 15.59% and 1.85 ± 3.01%, respectively). In contrast, TL individuals used tiles (19.60 ± 13.33%) and algae (17.18 ± 9.28%) to a similar extent, and showed the highest proportion outside the EE (63.20 ± 8.79%). As the C group had no prior experience with either structure, its selection may be considered the closest approximation to intrinsic preference in our design, suggesting that algae were the more attractive structure regardless of previous exposure, although a response to novelty cannot be excluded. Moreover, the similarity between the SW and C groups indicates that prior exposure to algae did not further increase their selection. Conversely, tile use was only observed in animals previously exposed to them, suggesting that familiarity with this structure influenced selection, consistent with studies demonstrating learning through habituation in sharks [49]. Therefore, although the preference test was conducted with animals previously exposed to a single type of EE, the inclusion of a naïve control group allows the effects of prior experience to be at least partially distinguished from those of the structures themselves. Future studies should include replicated naïve groups or cross-over designs to confirm these patterns.

The selection of the tile area by TL individuals was more marked in the second than in the first phase of the preference test. However, the loss of the third preference trial (month 4) limits our ability to assess the long-term stability of structure selection and the retention of behavioral patterns. With only two time points, changes between phases cannot be interpreted as consistent temporal trends, and it remains unknown whether selection patterns would have stabilized, shifted, or reversed with longer exposure. Nevertheless, space-use data from the third phase, which were obtained without issues, showed that TL individuals remained progressively concentrated around the tiles, which is consistent with the persistence of this pattern. Longer-term studies with repeated preference assessments, ideally extending to the pre-release period, are needed to evaluate the persistence of these behavioral patterns.

Finally, the observation that the utilized areas did not differ between preference tests and treatment groups, except for the SW group, is consistent with the conclusion that this treatment exerts the most pronounced effects on behavior. Nevertheless, the possibility that environmental changes and handling during sampling acted as stressors, potentially influencing nursehound condition, cannot be ruled out. In this regard, the sedation data provide some insight into the response of animals to repeated handling. Time to reach stage V (4.95 ± 1.08 min) did not differ among sampling events, and all animals recovered and returned to their tanks, indicating that buffered MS-222 at 100 mg/L provided consistent and safe sedation for juvenile nursehounds throughout the experiment. In contrast, time

to stage III was shorter at T2, which coincided with the lowest water temperatures of the study. Since lower temperatures would generally be expected to prolong induction by reducing metabolic and ventilation rates, this difference is unlikely to be explained by temperature alone and might reflect changes in the animals' responsiveness to handling; however, it should be interpreted with caution, as it was not maintained at stage V. Given that no sedation data were available for this species and information for related species is scarce [29], these results provide a baseline for future research and for the handling of nursehounds in captive breeding programs.

This study has several limitations that should be considered. First, each treatment was applied to a single tank, so treatment and tank effects are confounded; although tanks were identical and shared water supply and environmental conditions, our results should be regarded as exploratory. Second, welfare was inferred from growth, body condition, and space use, without paired physiological indicators; blood samples were collected at T0 and Tf, and their analysis of general stress indicators will be reported in a separate manuscript currently in preparation. Third, the preference test was conducted with animals previously exposed to a single type of EE, so selection may reflect familiarity rather than intrinsic preference, and the loss of the month-4 trial prevents assessing the long-term stability of these patterns. Finally, seasonal temperature changes and animal growth may have contributed to the temporal changes in space use, and no post-release data were collected.

Based on these preliminary findings, structural EE may be considered a potential conditioning tool for captive breeding programs targeting nursehound conservation. Specifically, algae were used from the first weeks of exposure and were the structure mainly selected by naïve individuals, promoting exploratory behavior, whereas tiles showed a progressive increase in use over approximately three months, possibly reflecting spatial acclimatization, and enhanced aggregation and refuge behavior. Accordingly, we hypothesize that a coverage of approximately 30% of the tank area, with a predominance of algae (e.g., 75% algae and 25% tiles), could be a suitable starting point for juvenile rearing environments, and that the inclusion of tiles may provide limited benefits when the rearing period is shorter than three months, given the time required for their use to increase. This predominance of algae is further supported by the concern that strong fidelity to tile shelters could be detrimental upon release into the wild, where such structures may be absent. However, neither the proposed proportion nor the minimum rearing period for tiles was tested in the present study, and both require experimental validation.

Although no studies have directly linked structural EE to improved post-release performance in sharks, captivity is known to negatively affect fitness-related traits in the wild [56,57]. In teleosts, EE has been shown to improve early survival, adaptation to natural prey, and feeding success compared to fish reared in barren environments, as reported in *Salmo salar* [39,58], and recent reviews highlight that EE, combined with life-skills training and post-release strategies, can enhance fitness-related traits in fish [59,60]. Based on this evidence and on the behavioral patterns observed in the present study, we hypothesize that structural EE may contribute to better preparing captive-reared nursehounds for release. However, this hypothesis could not be evaluated in the present study, and the responses of sharks upon release remain uncertain. Future research should therefore combine replicated captive experiments with post-release tracking, as telemetry has already been successfully applied to this species [52], to determine whether the behavioral and condition-related differences observed in captivity translate into improved survival and habitat use in the wild. In this regard, the individual PIT-tagging of the animals in the present study would allow their identification in the event of recapture after release.

5. Conclusions

This exploratory study suggests that structural EE is a simple and low-cost tool that may improve rearing conditions for captive-bred nursehounds. Its implementation had no adverse effects on juvenile growth, and enriched animals maintained a better body condition than those in the control group over time. Structural EE was associated with changes in space use, including a faster expansion of the core area in the more complex SW environment and aggregation around shelters in the TL group, behaviors that may be indicative of improved welfare, although this should be confirmed with physiological indicators. In the preference test, naïve individuals mainly selected algae, whereas tile use was only observed in animals previously exposed to this structure, suggesting that both the type of structure and prior experience influence structure selection. Based on these observations, we propose a hypothetical framework for incorporating structural EE into the captive rearing of nursehounds, which should be validated in future studies. Given the use of a single tank per treatment and the absence of post-release data, further research with replicated designs, physiological indicators, and post-release monitoring is needed to assess the effectiveness of EE strategies in rearing programs aimed at restocking. Nevertheless, this study provides novel information on the effects of structural EE in elasmobranchs, a group for which a significant knowledge gap still exists. By focusing on animal welfare, we contribute to the development of strategies that support the recovery and conservation of threatened and vulnerable species. More broadly, we align with the collective effort to mitigate and reverse the negative impacts of anthropogenic pressure, climate change, and biodiversity loss on our planet.

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Institutional Review Board Statement: All the procedures regarding maintenance, handling and sampling of fish were approved by the Animal Experimentation Ethics Committee (CEEA-UIB, Spain; Ref. 217/05/23 approved on 14 July 2024) and were carried out in accordance with the European Directive 2010/63/UE and Spanish Royal Decree (RD53/2013).

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: During the preparation of this work, the authors used (Claude (Anthropic)) (v.Opus 5.5) solely to improve the language, readability, and clarity of the manuscript. The tool was not used to generate, analyze, or interpret data, nor to draw scientific conclusions. All suggestions were critically reviewed, edited, and approved by the authors, who take full responsibility for the content of the publication.

Conflicts of Interest: Author Carlos Díaz-Gil was employed by Xelect Ltd. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MPAs	Marine Protected Areas
EE	Environmental Enrichment
SW	Seaweed (structural EE treatment)
TL	Tiles (structural EE treatment)
C	Control (group with no EE)
PIT-tag	Passive Integrated Transponder
DO	Dissolved Oxygen
K	Fulton's Condition Factor
SGR	Specific Growth Rate
T0, T1, T2, T3, T4, Tf	Sampling time points (initial to final)
KUD	Kernel Utilization Distribution
KDE	Kernel Density Estimation
UD	Utilization Distribution
GLM	Generalized Linear Model
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance

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