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The Impact of Food Enrichment on the Behavior of Cownose Ray (*Rhinoptera bonasus*) Kept under Human Care

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Abstract: The cownose ray (*Rhinoptera bonasus*) faces vulnerability primarily due to unregulated fishing, resource overexploitation, and habitat degradation. Consequently, individuals maintained under human care play a pivotal role in species conservation, particularly when their welfare is prioritized. Achieving optimal welfare in aquarium settings relies heavily on effective management practices, notably environmental enrichment. However, research on the efficacy of such techniques for cownose rays remains limited. Thus, this study sought to evaluate the impact of various food enrichment items on the behavior of four individuals at the São Paulo Aquarium in Brazil. The project encompassed three phases: baseline, enrichment, and post-enrichment. Enrichment items, designed to mimic the species' natural foraging behavior, included an ice block containing food, food hidden in vegetables fixed to structures at the bottom of the tank, a tray with substrate and food, and a perforated plastic container with food inside. Behavioral observations utilized focal sampling with instantaneous recording every minute. Results showed increased foraging activity in the post-enrichment phase, whereas swimming increased and following behaviors decreased during the enrichment phase. Additionally, foraging behaviors predominantly occurred near the aquarium bottom. Overall, findings suggest that enrichment items effectively stimulated natural behaviors in cownose rays and were very attractive to the fish, advocating for their integration into species management protocols to enhance welfare.

Keywords: aquarium; environmental enrichment; elasmobranchs; fish welfare

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1. Introduction

Elasmobranchs are among the most threatened taxa in the world, and among rays, the cownose ray (*Rhinoptera bonasus*) is classified as vulnerable by the International Union for the Conservation of Nature (IUCN) due to the decline in its global population [1], caused by unregulated fishing and resource overexploitation [2]. The cownose ray is a cartilaginous fish with benthopelagic and gregarious characteristics, often forming huge schools [3,4]. Like many Myliobatiformes, they prey mainly on benthic invertebrates living in the infauna and epifauna, such as mollusks, crustaceans, polychaetes, and echinoderms [5,6]. This species is one of the best adapted in the group for benthopelagic life, with a diet specialized in prey with low mobility and weak electrical stimuli [7].

The cownose ray locates its prey by mechanoreceptive or electroreceptive detection [7], excavating the substrate with movements of its pectoral fins until unearthing the prey and using water jets from its mouth to better separate the prey from the sediments [5,8]. It also has cephalic lobes that assist in excavation and suction of the prey towards the mouth, which is crushed by its teeth [8]. This species has a jaw adapted for durophagous feeding, enabling it to break the hard shell of its prey thanks to its robust jaw and resistant teeth [5,9]. When manipulating food in its mouth, the cownose ray separates the edible from the inedible parts of the prey, discarding the shell pieces and swallowing the soft parts [8].

Many misconceptions exist about how rays are limited to instinctive responses and simple behaviors. Still, it is increasingly evident that they have a complex behavioral repertoire and exhibit high learning capacity [10,11]. In this context, when subjected to human care, the stimuli offered must be part of the environmental complexity, as they can allow animals to express appetitive and consummatory behaviors, allowing them to face positive experiences that influence welfare levels [12]. How food is offered to animals kept under human care (in zoos and aquariums) may be responsible for allowing appetitive behaviors to be consummated [13–15]. Food items are usually offered to animals at the same period of the day (at the same hour each day), in trays, and chopped up, thus offering no challenge in acquiring or handling the food [16–18]. This lack of challenge means that feeding times are very short, leading the animal to display unwanted displacement or behaviors, such as abnormal ones [19]. In addition, anticipatory behaviors can appear before food is offered, which can mean a decrease in animal welfare [20]. The scientific literature is replete with examples demonstrating how variations in the timing of food provision and the manner of offering food (whole items, with acquisition and manipulation challenges) can stimulate animals to exhibit normal foraging and feeding behaviors, thereby significantly enhancing animal welfare [21–24]. The literature still provides little information on effective environmental enrichment techniques for rays and sharks, with physical structures being the most common [25–29]. However, only structural enrichments are insufficient to meet these animals' biological and ethological demands.

Among enrichments for fish, studies demonstrate that food enrichments, when designed to make food acquisition more challenging, encourage foraging behavior [30,31]. However, there is a need to develop more complex enrichments that challenge and encourage the natural behaviors of rays [32]. Another important aspect of maintaining fish under human care is understanding how the animals utilize their space. Fish tanks should be of sufficient size and quality to promote full utilization, providing suitable micro-environments and stimuli for the species maintained within them [33]. Fishes under human care do not typically use all available spaces equally, with the elements present in the environment influencing their usage. For example, fishes often frequent areas where food is provided, as well as hiding and resting areas, since these spaces offer essential stimuli and elements for their survival [34,35]. Depending on the species, more exposed areas and those closer to visitors may be more or less utilized [28]. Evaluating how fish use their tank can help identify the necessary stimuli for their biological needs [36], assist in renewing tank environments [12], and inform the development of optimal management protocols [37]. This can increase fish welfare [38]. In this context, this project aimed to evaluate the effect of different food enrichment items on the behavioral responses and foraging stimulation of cownose rays under human care and how the cownose rays utilized the available space of the tank The hypothesis formulated was that the items offered would stimulate the rays' foraging, feeding, and activity (swimming), and that the tank usage would be directly related to the feeding areas.

2. Materials and Methods

The present study was conducted at São Paulo Aquarium (São Paulo, SP, Brazil; 23°35′36.5″ S 46°36′51.1″ W) from October 2022 to January 2023. Four adult individuals of cownose rays were studied, one female and three males (all individuals are adults, with no estimated age, and having an average weight of 10.38 ± 2.54 kg; the rays were allocated to the aquarium following the decision of Brazilian environmental regulation agencies), already acclimated to the environment. The animals were housed in a multi-species tank (bony fish, elasmobranchs, and turtles: Southern stingray (*Dasyatis americana*), blacktip reef shark (*Carcharhinus melanopterus*); whitetip reef shark (*Triaenodon obesus*); nurse shark (*Ginglymostoma cirratum*); Atlantic goliath grouper (*Epinephelus itajara*); Atlantic tarpon (*Megalops atlanticus*); green moray (*Gymnothorax funebris*); French angelfish (*Pocamanthus paru*); and green turtle (*Chelonia mydas*), with a capacity of 1 million liters of artificial saltwater in a controlled environment. Feeding occurred 3×/week. This project was approved by the research and ethics in animal research committees of the São Paulo Aquarium and was conducted in compliance with international animal welfare standards [39]. The animals were not exposed to stress, there were no invasive procedures or tests that resulted in physical injury to the animals, and none died during the study.

The tank design consisted of two distinct parts in terms of area and depth: one with a depth of 1 m above the visitor area and another of 6 m, facing the visitors (to the right) (Figure 1). Due to the greater visibility and higher presence of the rays in the shallower area, observations were conducted in the upper part of the tank. The shallower section featured a glass surface for visitors below to view the animals. Conversely, the deeper portion had a substrate on the bottom where different structural enrichment items were positioned, such as logs, rocks, and hiding spots.

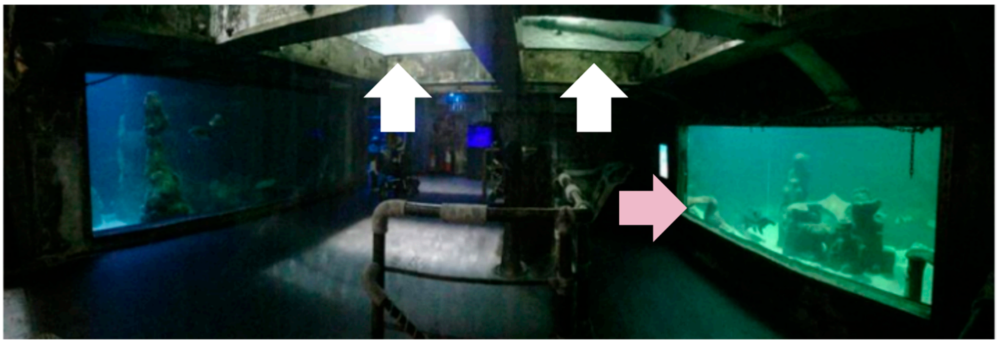

Figure 1. Study tank where the cownose rays were kept with other species. The white arrows represent the upper area of the tank, with a depth of 1 m. The pink arrow represents the area of the tank with a depth of 6 m. Photo: Helen Colbachini.

A period of preliminary observations was necessary to build the ethogram (Table 1) and establish the activity pattern of the individuals. The method employed was ad libitum, with a total of 20 h of observation, one hour per day, during October and November 2022 [40]. Subsequently, the project was divided into 3 phases, following an ABA protocol [41]: (A) baseline: observations conducted before feeding, for a total of 50 min/daily, totaling 20 h; (B) enrichment phase, for 20 min/daily totaling 20 h; (A) post-enrichment phase, for 50 min/daily totaling 20 h. Data collection occurred daily between 1:30 pm and 3:30 pm. All data were collected by a single researcher positioned at the top of the tank, providing a comprehensive view of both quadrants of the aquarium. To observe the animals, the researcher moved calmly along the top of the tank without altering the behavior of the rays (as confirmed during preliminary observation periods). If an individual moved out of the researcher's field of view, it was recorded as "not visible" in the field notes.

In these 3 phases, the behavioral sampling method was focal with instantaneous records at one-minute intervals [40]. Some behaviors, such as biting, were exhibited in rapid bouts and might not coincide with the sampling intervals of the instantaneous records. All records of this behavior included in the analysis coincided with the moment of instantaneous recording, as they occurred quickly but with high frequency. Thus, many of these behaviors were recorded despite using instantaneous recording. The identification of the animals was possible due to the presence of natural marks or anatomical differences among individuals, such as notches, fin spots, or sex. For each record, the executed behavior, the depth of the animal (surface, 1 m, and 6 m), and the tank location (quadrants 1 or 2) were recorded. The determination of the location was made by establishing a virtual division of the tank into two quadrants of the same size, with the first comprising the shallow part of the tank (1 m depth) and the second encompassing the deeper part of the tank (6 m depth) (Figure 2).

Table 1. Ethogram of the cownose rays used to evaluate the effects of food enrichment on their behavior.

Behavior	Description
Swimming	The animal's movement with the oscillatory movement of its pectoral fins (from bottom to top).
Following	When an animal swims closely behind another individual, within 1 m of proximity, matching its pace and route, this pursuit can occur in the following forms: male behind another male, male behind a female, or female behind a male.
Mating	The behavior begins with a high-speed chase of the male behind the female until he catches up, seizing one of her pectoral fins. By holding it this way, he positions himself underneath her, ventral to ventral, allowing the clasper to penetrate the cloaca. This position is maintained for approximately 1 min until the male releases her fin and then withdraws the clasper.
Route change	During swimming, the animal abruptly changes its course, making a sharp turn to the right or left.
Foraging	The animal searches for and explores different areas of the tank for food, positioning itself vertically or horizontally in the chosen spot and swimming slowly along the substrate with its cephalic lobes activated and touching it.
Eating	Food intake with activation of the cephalic lobes, which assist in the suction of food, directing it towards the mouth. It occurs in the water column or close to the substrate.
Enrichment interaction	Behavior in which the animal interacted with the items offered to them, including attempts to interact and any direct contact with it.
Human interaction	This occurs when the animal and the diver make any physical contact, either by simply touching them or by directly feeding from their hand. This behavior was registered only with the divers and not with the visitors through the glass.
Escaping	When the ray makes physical contact with another individual, it swiftly withdraws from the location of the encounter at high speed.
Avoiding	At any moment, the animal almost made contact with an individual of the same or another species but changed its course just before.
Colliding	Sudden encounters between two individuals of the same or another species involving physical contact and subsequent escape by one or both after the interaction.
Underside hitting	Interaction in which one male, swimming from bottom to top, hits the ventral part of another male with its head.
Biting (intraspecific)	The animal approaches another ray, swimming from top to bottom, with its cephalic lobes activated, and touches its rostrum to the dorsal part of the other ray, causing it to flee.
Biting (interspecific)	The ray approaches an individual of another species, swimming from top to bottom, with its cephalic lobes activated, causing the other individual to flee upon contact.
Not visible	When the animal cannot be sighted for behavior recording.

Components of the cownose ray diet previously defined by the São Paulo Aquarium nutrition department were used to prepare the food enrichment items. The rays' diet consisted of 2.5 kg of a mixture of fish (sardines, anchovies, mullets, whiting, etc.), mollusks (squid, clams, and mussels), and crustaceans (shrimp). It was offered once a day, always at 2:20 pm and in quadrant 1. Despite other species sharing the same enclosure, the enrichment items were carefully selected to prevent other animals (sharks, morays, turtles, angelfishes, goliaths, and tarpons) from accessing the food during the interaction of the studied rays [enrichments provided in the shallowest quadrant (Q1)]. Thus, four food enrichment items were used: an ice block containing food, food hidden in vegetables (chicory, *Cichorium intybus*, Asteraceae) secured to heavy structures that kept the enrichment at the bottom of the tank, a tray with substrate and food), and a perforated plastic container with food inside (in all enrichments, the food was the same as in the original diet, divided between the items offered that day). All enrichment items were previously approved by the institution's technical team and offered randomly, in pairs (two of the same item offered simultaneously), alternating types throughout the study. The enrichment items remained in the tank throughout the data collection period. Most of the time, the cownose rays ate all the food in the enrichment item, but if parts of the enrichment food remained until the end of the data collection, they were removed from the tank. The cownose rays did not fear the items since they had been offered sporadically. In this way, the rays interacted with the items immediately after being placed in the tank. The enrichment items and the rays' regular diet were only offered at central points in quadrant 1. This was done to make it easier for the aquarium technicians to monitor the rays' health daily and to make cleaning the area after feeding easier, in addition to avoiding the area where the sharks swam and competed with them. The food was arranged within the enrichment items in a manner to minimize or prevent collection by other species in the enclosure. However, the use of these items, albeit infrequently, by Southern stingrays was observed.

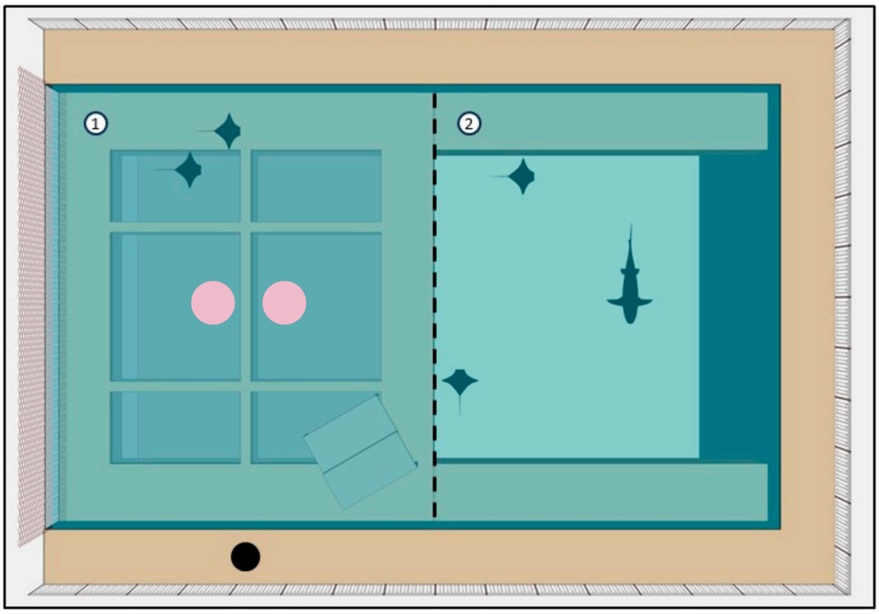

Figure 2. Schematic top view of the cownose ray tank, divided into two quadrants. Quadrant 1 was the shallowest (1 m depth), while quadrant 2 was the deepest (6 m depth). The figure depicts two stingrays in quadrant 1, and two stingrays and a shark in quadrant 2 (depicted as dark silhouettes). Cownose rays' food was always placed in quadrant 1. Pink circles represent the areas where food and environmental enrichment items were offered to the rays. The black circle represents the position of the researcher during data collection.

For the statistical analysis, the collinearity between the variables was assessed by calculating the Variation Inflator Index (VIF). All results were less than 2, indicating some degree of collinearity but not to the extent of being significant in the following analysis [42]. Therefore, the Generalized Linear Mixed Model (GLMM) was used to evaluate the variation in ray behaviors (response variables) according to the type of enrichment, the individual, the study period, and the interaction between the enrichment type and the individuals (explanatory variables). The day of the experiment was used as a random variable.

Additionally, the Spread of Participation Index (SPI) was calculated for each individual in each of the three phases of the study (baseline, enrichment, and post-enrichment) to evaluate space use in the tank [43]. The SPI generates a score ranging from 0 to 1. A score of 1 indicates highly uneven use of the enclosure, whereas a score of 0 suggests perfectly proportional use of all enclosure zones [44]. SPI analysis was conducted in R 4.0.0 [45]. To compare differences in the space used in each study phase, we conducted Wilcoxon signed rank tests after testing data for normality using Anderson–Darling normality tests. Furthermore, the depth data columns were tested for normality using the Anderson–Darling normality test. Since none of these showed data with a normal distribution, the Friedman test with Dunn's post hoc test was conducted. Finally, to verify if there was a difference in the use of the different environmental enrichment items, the Anderson–Darling normality test was conducted to test the data for normality. All data showed a normal distribution. Therefore, the parametric two-way ANOVA test was conducted with Tukey's post hoc test. All statistical analyses were conducted using Minitab 19 software.

3. Results

A total of 9600 behavior records were made on the rays' exhibited behaviors during the study. The most exhibited behaviors were swimming and enrichment interactions, while the least exhibited behaviors were intra- and interspecific biting (Table 2). Mating was not recorded during the study.

Table 2. Descriptive statistics of the recorded behaviors of cownose rays kept in São Paulo Aquarium. N = number of samples; SE = Standard error; SD = standard deviation; Min = minimum value; Q1 = first quartile; Q3 = third quartile; Max = maximum value.

Behavior	N	Mean	SE	SD	Min	Q1	Median	Q3	Max
SW	120	27.94	1.50	16.42	1.00	10.00	34.50	42.00	50.00
FOL	120	2.33	0.30	3.28	0.00	0.00	1.00	4.00	15.00
ROU	120	0.31	0.07	0.79	0.00	0.00	0.00	0.00	4.00
FO	120	5.94	0.63	6.94	0.00	1.00	3.50	8.00	31.00
EAT	120	0.08	0.07	0.74	0.00	0.00	0.00	0.00	8.00
ENR	120	2.70	0.40	4.37	0.00	0.00	0.00	5.75	16.00
ESC	120	0.07	0.02	0.25	0.00	0.00	0.00	0.00	1.00
AVO	120	0.24	0.05	0.57	0.00	0.00	0.00	0.00	3.00
COL	120	0.03	0.02	0.18	0.00	0.00	0.00	0.00	1.00
HIT	120	0.03	0.02	0.18	0.00	0.00	0.00	0.00	1.00
BIT1	120	0.03	0.01	0.16	0.00	0.00	0.00	0.00	1.00
BIT2	120	0.03	0.01	0.16	0.00	0.00	0.00	0.00	1.00
NV	120	0.20	0.06	0.71	0.00	0.00	0.00	0.00	5.00

The behaviors displayed by the rays were influenced, to some extent, by the type of enrichment, the individual, the treatment, and the interaction between the type of enrichment and the individual (Figure 3). Intraspecific biting was also more exhibited by male 1 ($F = 4.38$, $p = 0.006$). Human interactions were more expressed during the enrichment phase ($F = 215.08$, $p < 0.001$). Colliding was more expressed with the ice block item ($F = 3.01$, $p = 0.03$), while intraspecific biting was more expressed when the vegetables were offered ($F = 2.69$, $p = 0.008$). The other behaviors were not influenced by any of the variables.

Male 3 exhibited more swimming behavior than any other evaluated individual (Figure 3a). The following behavior was also influenced by the individual and the study period. In this case, male 1 and female exhibited this behavior the most, especially before the insertion of the enrichment. It decreased substantially during the provision of the enrichment items and increased after the removal of the enrichment (Figure 3b,d).

The route change behavior varied according to the individual and the study period, being exhibited only by male 1 and occurring mainly before the insertion of the enrichment (baseline phase) and shortly after the removal of the items, and did not occur during the enrichment phase (Figure 3c,e). The foraging behavior was influenced by the study phase, occurring shortly before the enrichment use, slightly increasing during the enrichment phase, and significantly increasing after removing the items (Figure 3f).

Regarding the use of the aquarium, it was observed that the individuals utilized the quadrants differently during the three phases of the study, with the highest frequency in quadrant 1 (Table 3). Female, male 1, and male 2 showed a preference for quadrant 1, while male 3 showed a preference for quadrant 2 (Table 3). Quadrant 1 was statistically more used than quadrant 2 in all three phases of the study despite the preference for quadrant 2 of male 3 (baseline: Z = 3.86, $p < 0.001$; enrichment: Z = 5.44, $p < 0.001$; post-enrichment: Z = 4.25, $p < 0.001$; N = 40, DF = 1 for all comparisons).

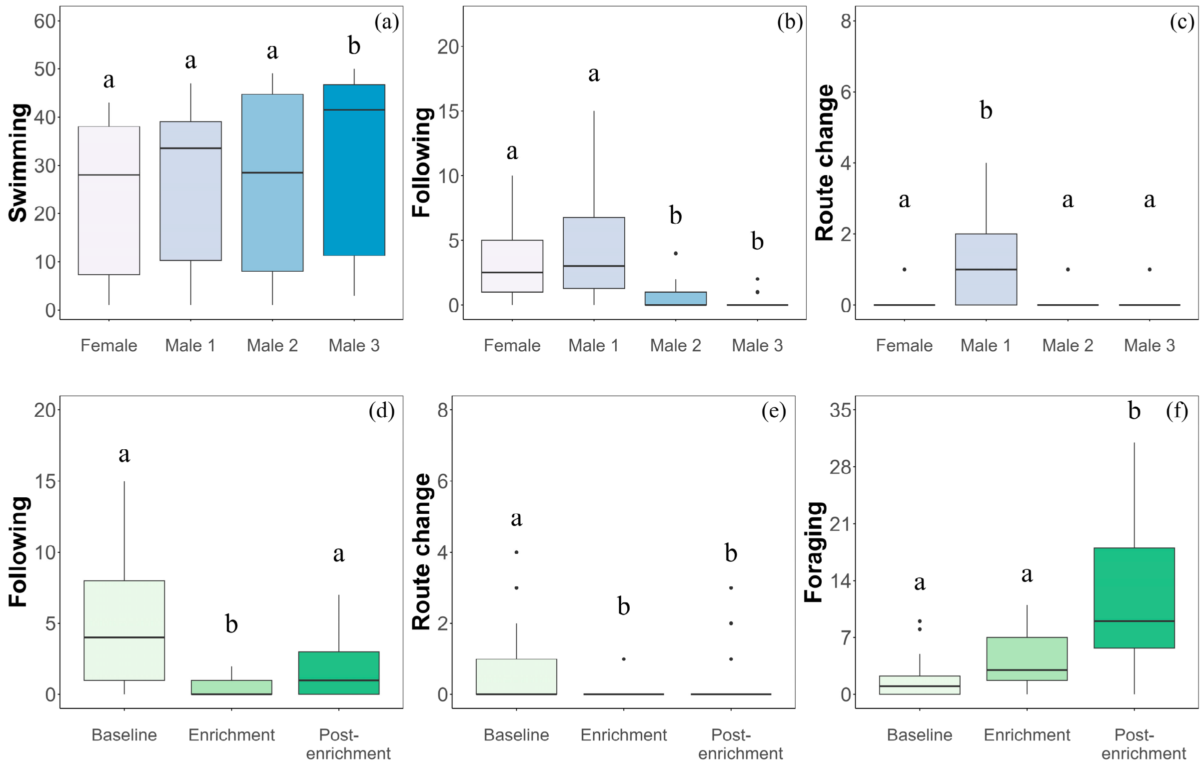

Figure 3. Results from the GLMM assessing the variation in ray behavior according to the type of enrichment (vegetables, ice, substrate, and container), the individual (males 1, 2, 3, and female), and the study period (baseline, enrichment, and post-enrichment). Boxplots: The dark line in the center of the box represents the median, while the lower and upper edges of the box represent the first and third quartiles. The whiskers depict the range of the data, excluding outliers (shown as black dots). Different superscript letters represent statistically significant differences. Behaviors varying in function of the individuals are: (**a**) Swimming; (**b**) Following, and (**c**) Route change. Behaviors varying in function of the study period are: (**d**) Following, (**e**) Route change, and (**f**) Foraging.

Table 3. Spread Preference Index (SPI) of each cownose ray in quadrant 1 (SPI_Q1) and quadrant 2 (SPI_Q2). Positive values (SPI > 0) signify a preference for the quadrant, whereas negative values (SPI < 0) indicate an avoidance of it. A value of zero denotes indifference towards any quadrant; that is, cownose rays use both quadrants equally.

Individual	Period	Preference_Q1	Preference_Q2	Availability_Q1	Availability_Q2	SPI_Q1	SPI_Q2
Female	Before	0.99	0.01	0.5	0.5	0.49	−0.49
Female	During	1	0	0.5	0.5	0.50	−0.50
Female	After	0.98	0.02	0.5	0.5	0.48	−0.48
Male 1	Before	0.93	0.07	0.5	0.5	0.43	−0.43
Male 1	During	0.98	0.02	0.5	0.5	0.48	−0.48
Male 1	After	0.97	0.03	0.5	0.5	0.47	−0.47
Male 2	Before	0.64	0.36	0.5	0.5	0.14	−0.14
Male 2	During	0.85	0.15	0.5	0.5	0.35	−0.35
Male 2	After	0.66	0.34	0.5	0.5	0.16	−0.16
Male 3	Before	0.34	0.69	0.5	0.5	−0.19	0.19
Male 3	During	0.71	0.29	0.5	0.5	0.21	−0.21
Male 3	After	0.38	0.62	0.5	0.5	−0.12	0.12

Similarly to the quadrants, the rays utilized the different depths of the enclosure differently (F = 338.63, $p < 0.001$, N = 3, DF = 2). The rays more frequently used the shallower depths of the aquarium (1 m), followed by the surface; the deepest part of the tank (6 m depth) was utilized less by the rays. Regarding environmental enrichment, the rays showed greater interaction with the vegetables, but significant differences were observed only between the use of the substrate and the ice block, with the rays interacting much more with the substrate (Figure 4). The rays utilized the ice block the least (Figure 4).

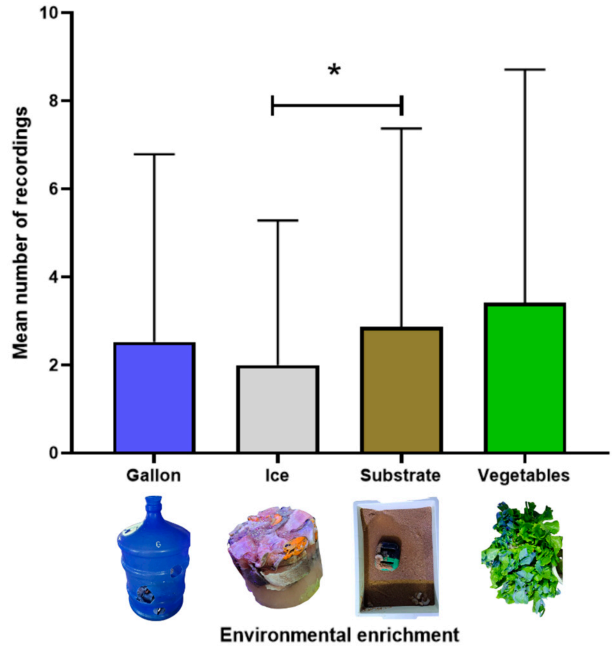

Figure 4. Mean number of records (with error bars) of the use of each enrichment item offered to the rays. The asterisk represents a significant statistical difference between the two environmental enrichment items ($p < 0.05$).

4. Discussion

The use of food enrichment stimulated the swimming of the rays. It modified the display of some behaviors but decreased foraging while providing items, partially corroborating the study's initial hypothesis. There were behavioral differences related to the type of food item, with intra- and interspecific biting behaviors occurring more when vegetables were offered and colliding behavior occurring more with the ice block item. Male 1 was the ray most behaviorally differentiated from the others, exhibiting more following, route change, and intraspecific biting behaviors. The female exhibited more interspecific biting and following behaviors, while male 3 exhibited more swimming behavior.

Food enrichments are typically used to increase the display of foraging behaviors, as animals exhibit this behavior at a high rate in nature [46,47]. Animals often display contrafreeloading when kept under human care (when the animal prefers to forage rather than consume food offered in a feeder), demonstrating that foraging is important for their daily routine [48,49]. In the present study, foraging significantly decreased while offering food items, resembling the baseline phase's exhibition levels. The enrichment items were offered in pairs, only in spots in the middle of quadrant 1, to avoid or decrease competition. The rays interacted with the items and, due to an apparent excitement, swam around the items for long periods (Supplementary Material S1). They did not forage through the tank during these periods but headed directly for the items. Thus, there was a recorded decrease in foraging but increased swimming and interaction with the enrichment items. After removing the items in the post-enrichment phase, the rays began to forage statistically significantly more throughout the tank, perhaps in search of items like those offered, indicating that the effects of food enrichment were beneficial for the fish. This effect has been observed in studies with other species, like the rock bream fish *Oplegnathus fasciatus* [50], greater rhea *Rhea americana* [51], and pine martens *Martes martes* [52]. It is important to state that the food present in the environmental enrichment items was completely ingested for almost the entire experiment and that since food enrichment was offered, observation times occurred during the feeding period already established by the São Paulo Aquarium as a sudden change in the feeding period can negatively affect the rays, causing frustration and food rejection [53]. Studies examining the effects of food enrichment on the behavior of rays, sharks, and even bony fish, particularly those informing about post-enrichment effects on fish behavior, are rare or non-existent in the scientific literature. Therefore, we recommend further research to address this significant knowledge gap.

The following behavior was more exhibited during the baseline period, especially by male 1 and female, decreasing significantly during the enrichment phase and increasing during the post-enrichment phase. This behavior may be linked to reproduction, such as when a male interested in a female follows her to assess her receptiveness for copulation [54]. Additionally, this behavior is naturally displayed by this species of ray, which is typically observed in the wild in large schools [55]. Thus, it is likely that with the placement of enrichment items in the tank and the high interaction of the rays, the swimming behavior in schools and/or the sexual appetite decreased. When the enrichment was removed from the tank, the rays increased solitary foraging, significantly increasing the following behavior. Since copulation was observed between male 1 and the female during the study (not during the behavioral recording sessions), it is quite likely that the following behavior indeed serves a reproductive function in this context. This reinforces the idea that the male followed the female to assess her receptivity for mating, as mentioned earlier. Further supporting this idea, we have the increased display of the route change behavior, primarily exhibited by male 1. This behavior was consistently observed when male 1 perceived the approach of other males towards the female. At these times, male 1 would abruptly change his swimming route and chase after the other individuals until he expelled them from the area where the female was located. This behavior reinforces male 1's sexual interest and defense of the female.

It is interesting to note that the use of food enrichment with vegetables increased agonistic behaviors, both intra- and interspecific, among the rays, mainly from the female and male 1, and the use of food enrichment with the ice block increased the records of colliding behavior. These results show increased competition among the rays for the items, corroborating that they stopped swimming in schools because they were competing for the enrichment items. Food disputes have been recorded in Aquaria before, leading to an increase in agonistic behaviors [56,57]. Again, it is important to highlight that the number of food enrichment items offered was always in pairs, and smaller than the number of ray individuals in the tank. However, the rays were attracted by the feeding frenzy of other individuals at the items and ended up biting them to interact with them. Therefore, we suggest that the items be offered in large quantities, preferably at different points in the tank, to decrease the chance of agonistic behaviors and animal injuries. The increase in agonistic behavior and collisions with these two environmental enrichment items may be related to food particles that became dislodged during the interaction, remaining suspended in the water column and stimulating the simultaneous capture by multiple individuals. This phenomenon was observed at various points during data collection and may represent only an artifact of the experiment.

Regarding the differential use of quadrants and depths, the rays used the quadrant with a depth of 1 m (quadrant 1) more frequently and the quadrant with a depth of 6 m less frequently (quadrant 2) in all phases of the study (baseline, enrichment, and post-enrichment). This differential use was linked to routine feeding and enrichment items, which were always placed in quadrant 1. Thus, the rays extensively utilized the area, always searching for food. Quadrant 2, although relatively well used by the rays, was where the nurse sharks (*Ginglymostoma cirratum*) mostly stayed. These benthic animals can be predators or competitors of the rays [58], and most of the time, the rays used quadrant 2, swimming near the surface or, at most, to depths of up to 1.5 m. This result indicates that environmental enrichment influences the tank's use by the rays, and it is suggested that the items be evenly distributed across the quadrants to encourage the complete use of the tank. Another reason for the rays' use of quadrant 2 was due to agonistic interactions between males in quadrant 1. Due to the males' sexual interest in the female, males 1 and 2 expelled male 3 from quadrant 1 when he approached the female, causing him to occupy quadrant 2 temporarily. In this way, quadrant 2 was used, but as previously mentioned, swimming took place mainly on the surface of quadrant 2, not the bottom.

Although it did not increase foraging during the use of the items, the use of food enrichment proved interesting for the rays. The interaction with the items was high (less so with the ice block, but still with good interaction), and swimming activity was stimulated. As agonistic behaviors also increased while using the items, adjustments to the number of items offered together and their arrangement in the tank are necessary. It is important to consider individuality when evaluating enrichment items for this species, as behavioral responses differed among individuals in several categories.

5. Conclusions

Overall, the findings suggest that enrichment items effectively stimulate natural behaviors in cownose rays, such as swimming, and were shown to be very attractive to them. Additionally, after the removal of items from the tank, as foraging was stimulated, we concluded that environmental enrichment had a positive effect on stimulating this behavior in this phase. This demonstrates that the rays were motivated to exhibit this behavior in searching for food throughout the environment, advocating for the integration of enrichment items into species management protocols to enhance welfare.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jzbg5020023/s1, Video S1: Cownose rays interaction with a tray with substrate and food.

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Data Availability Statement: The original data presented in the study are openly available in the Mendeley Data Repository at doi:10.17632/vxgdnw5ppx.1.

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