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Article

Novel Food-Based Enrichment Increases Captive Cownose Stingray (*Rhinoptera bonasus*) Engagement with Enrichment Item

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Abstract: Environmental enrichment can provide captive animals with stimuli that increase physical and mental activity. We investigated the extent to which a novel enrichment item, defined as enrichment that individuals have never experienced prior to the study, engages captive cownose stingrays (*Rhinoptera bonasus*). We hypothesized that stingray interactions with the enrichment item are associated with the novelty of the enrichment item (i.e., time since presentation of the enrichment item, number of weeks exposed to the enrichment), the presence of food, and the number of human visitors at the stingray exhibit. The novelty of the enrichment item and the presence of food were most strongly associated with stingray interactions with the enrichment item. Although stingrays engaged with the enrichment throughout a 5-week period, interactions decreased as the amount of time the enrichment item remained in the water increased during a 60 min period and interactions decreased as the weeks progressed. Furthermore, stingrays had increased interactions when food was present inside the enrichment item. The number of human visitors had a weaker association with stingray interactions with the enrichment item, but during the food-based trials, there were increased interactions with the enrichment when more visitors were present. Our findings suggest that variation in enrichment routines (to maintain novelty) and variation in an enrichment item's use of food may help continue engagement with enrichment items.

Keywords: animal welfare; aquarium; behavioral enrichment; captivity; elasmobranch; fish; zoo visitors

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1. Introduction

Environmental enrichment can provide stimuli that increase the physical and psychological welfare of captive animals [1–3]. Generalized goals for enrichment programs often include an increase in behavioral diversity, activity levels, frequency of natural behaviors, and engagement with an enclosure, as well as a decrease in stereotypical behavior [4–6]. Environmental enrichment can be visual, tactile, auditory, gustatory, or cognitive, and may involve social interactions with conspecifics or other species (including humans), as well as training programs with zoo and aquarium staff [7,8].

Understanding a captive animal's behavioral repertoire, life history, and use of its enclosure is critical in the calibration of an environmental enrichment program to the specific needs of different species or individuals [9–11]. Naturalistic environmental enrichment programs aim to increase the similarity between a captive animal and a free-ranging animal's behavioral activity budgets, while behaviorally engineered enrichment programs aim to increase the overall behavioral repertoire of captive animals [3,5]. Living prey items and the incorporation of substrates free-ranging animals would encounter can provide more naturalistic conditions within an enclosure [12,13]. Plastic pipes, Kong® toys, electronic devices, and non-toxic art materials can also be applied to enclosures to increase stimulation opportunities [1,12,14].

Characteristics of the enrichment item (e.g., the presence of food, the strength of the stimulus, the frequency that the enrichment is available, the behaviors the enrichment elicits) can influence how often an animal interacts with an enrichment item [15,16]. Furthermore, an individual's prior experience with similar environmental enrichment items may impact the rate of habituation to the enrichment item [17], given that as the amount of time animals are exposed to environmental enrichment increases, the novelty of the environmental enrichment can decrease [15,18].

Quantifying responses to environmental enrichment is important for assessing the appropriateness of enrichment items and routines in the context of animal welfare [19,20]. Approximately 75% of the published research on the welfare of zoo animals has focused on mammals [21], indicating a need for more taxonomic representation in research, including fish [22]. Although there has been an increase in enrichment studies, much of the work focuses on laboratory animals, and most studies focus on mammals and birds [23]. Nonetheless, a meta-analysis involving 82 aquatic species found that overall environmental enrichment had a positive impact on animal welfare [24].

In addition to enrichment, the behavior of captive animals representing a range of taxa can be impacted by the presence, volume, or noise level of human visitors [25]. However, behavioral responses vary greatly, from positive to negative to no perceived behavioral change [26,27]. Behavioral responses may be species-specific [28] or at the level of the individual animal [25,29,30], but there may also be confounding factors such as weather that also impact behavioral responses [31,32].

The objective of this study was to investigate the degree to which captive cownose stingrays (*Rhinoptera bonasus*) interacted with a novel enrichment item, defined as enrichment that individual stingrays had never experienced prior to this study. Cownose stingrays are benthic, suction feeders with morphological and sensory adaptations for capturing and extracting food [33]. Cownose stingrays consume a diverse and generalist diet, typically focused on benthic invertebrates such as crustaceans, bivalves, gastropods, polychaetes, and amphipods [34–36]. Free-ranging cownose stingrays can vary greatly in their home range size (<1 to >70 km^2) and daily distances traveled (<1 to >8 km), and they often live in groups of dozens to hundreds of individuals [37–39]. In captivity, food may serve as a reward that provides individuals the opportunity to exhibit agency and motivate individuals to interact with environmental enrichment [40]. Furthermore, food enrichment in captive cownose rays may increase natural behaviors, such as foraging [41].

We hypothesized that stingray interactions with the enrichment item are associated with the novelty of the enrichment item (in that the animals had no prior exposure to this enrichment item), the presence of food in the enrichment item, and the number of human visitors at the stingray exhibit. To examine the impact of novelty, we predicted that the number of times the stingrays interacted with the enrichment item would decrease from the first week to the last week of the study. We also predicted that the number of times the stingrays interacted with the enrichment item would decrease with time during each weekly exposure to the enrichment item. Furthermore, we predicted that there would be more interactions when the enrichment item contained food. Finally, we predicted that there would be a decrease in stingray touches as the number of human visitors at the exhibit increased, given that the human visitors often provided food to the stingrays.

2. Materials and Methods

2.1. Study Subjects and Location

We conducted our study at the Stingray Cove exhibit at the Memphis Zoo in Memphis, Tennessee, USA in September and October 2021. Living Exhibits Incorporation operated the seasonal exhibit with the animals onsite from late March until early November 2021, after which Living Exhibits Incorporation transported the animals to a winter location. In the 2021 season, the exhibit was open to the public from early April to late October.

Living Exhibits Incorporation's protocol allowed visitors to directly interact with the stingrays. After paying an entrance fee, visitors could touch the dorsal side of the stingrays, and for an additional fee, visitors could feed the stingrays shrimp, squid, or capelin. The touch pool was a 20,000-gallon saltwater pool (54 feet long × 26 feet wide × 24 inches deep) that included a waterfall and gravel substrate (Figure 1). This exhibit contained 43 cownose stingrays (*Rhinoptera bonasus*), the focus of the study, as well as 4 bamboo sharks (*Chiloscyllium punctatum*) and 3 southern stingrays (*Hypanus americanus*).

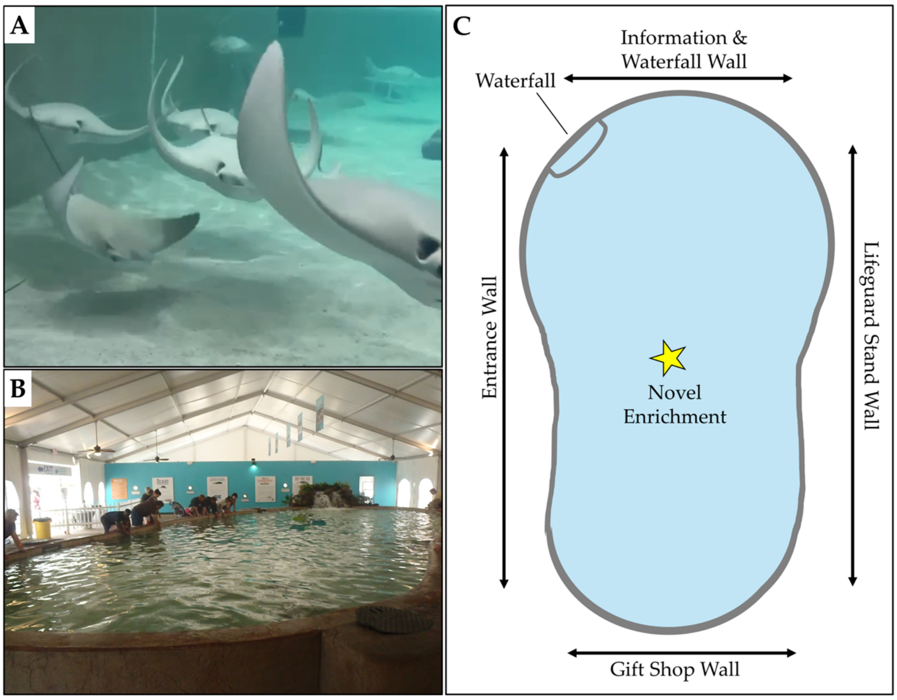

Figure 1. Stingray Cove at the Memphis Zoo housed bamboo sharks (*Chiloscyllium punctatum*), southern stingray (*Hypanus americanus*), and (**A**) cownose stingray (*Rhinoptera bonasus*), the focus of the study. The exhibit allowed visitors to (**B**) interact with the animals, and visitors had access to the entire perimeter of the pool. For each behavioral trial, the novel enrichment item was introduced from the lifeguard stand wall and then placed in the (**C**) center of the exhibit, as indicated by the star. Photo credits: Mia Harris (**A**) and Living Exhibits Incorporation (**B**).

At the time of this study, there was ongoing sensory and food-based enrichment that was in place prior to the start of this study. These ongoing enrichment practices continued throughout this study. Such ongoing, prior enrichment included sensory enrichment in the form of bubblers, artificial kelp composed of grade felt, plastic toy rings, rock structures that were rearranged daily, hula hoops, and other obstacles. These items provided stingrays with environmental surfaces that varied in texture, density, color, and size. Ongoing, prior food-based enrichment included food-filled Kong® toys, Holee Molee food ball feeders, whole fish, ice blocks that contained food, polyvinyl chloride (PVC) puzzle feeders, clams, feeding from human visitors, and feeders that sank, remained suspended within the pool water column, or floated. The Kong® toys required the stingrays to push the toy against a pool wall before participating in a jetting behavior to suction the food out of the toy. The ongoing enrichment provided opportunities for individuals to engage with a myriad of enrichment items; therefore, the novel enrichment that was the focus of this study was never the sole enrichment item available within the pool.

Our design of the novel environmental enrichment item for this study, hereafter referred to as the jellyfish toy, followed the Living Exhibits standards and protocols for all enrichment items present in the exhibit. Behavioral data collection involved observations of the animals in their pool exhibit, the entirety of which was open to the public.

2.2. Enrichment Item

We created the jellyfish toy, which included both sensory and food enrichment, as a novel enrichment item with which none of the stingrays had prior interactions. The jellyfish toy was novel to all animals (stingrays and sharks) in the exhibit. The jellyfish toy consisted of a hula hoop that had four segmented polyethylene-foam pool noodles wrapped and evenly spaced around its rim (Figure 2). In between each pool noodle segment, there were three felt, sensory tentacles with a pellet weight attached to each tentacle. The purpose of the pellet weights was to reduce the speed at which the enrichment item drifted from the center of the pool. The middle tentacle was the longest (30 cm) and had the heaviest pellet weight (150 mg), while the outer two tentacles were shorter (15 cm) and had lighter pellet weights (75 mg). Two nylon cords stretched from each pool noodle segment to the opposing pool noodle segment, with the two cords meeting in the middle of the jellyfish. At this intersection, another nylon cord held the double-netted Kong® ball, which was suspended within the exhibit water column, where it remained underwater but did not touch the floor of the pool. During trials when food was absent, the Kong® ball remained empty. During trials when food was present, we filled the Kong® ball with shrimp, squid, and capelin (the same food items as what visitors could purchase to feed the animals). To access the food, stingrays suctioned items through the openings in the Kong® ball netting. Stingrays did not have access to the jellyfish toy outside of the research sessions. The design of the jellyfish toy encouraged the stingrays, who are benthic, suction feeders, to continuously swim during interactions with the enrichment and engage with the Kong® ball well below the surface of the water. However, stingrays were unable to immediately deplete the food within the Kong® ball, which allowed for the consistent presence of food throughout the food-based enrichment treatment.

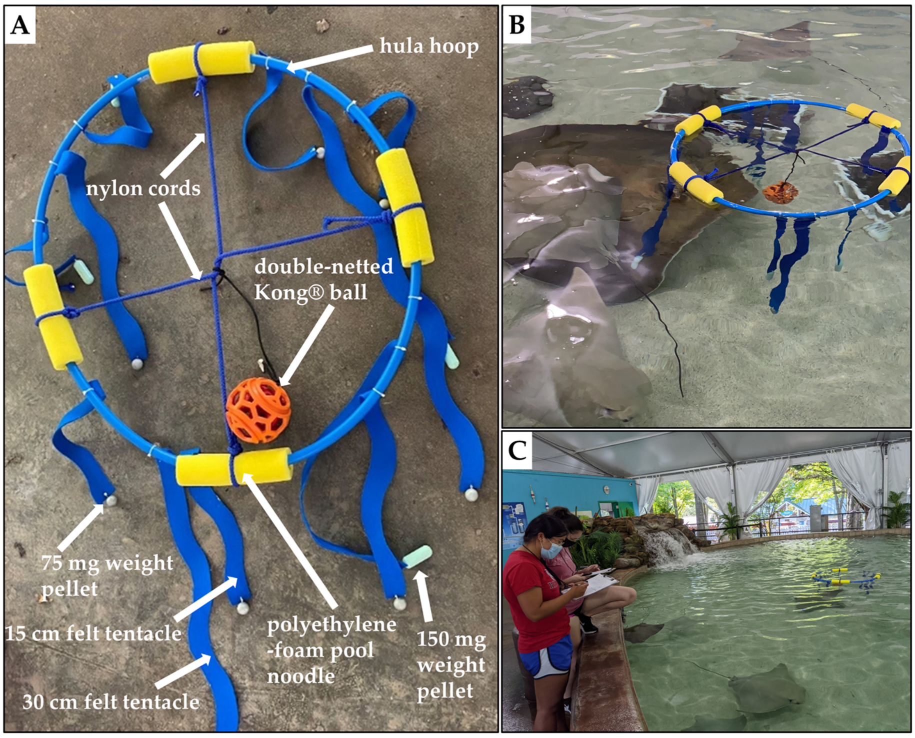

Figure 2. The jellyfish enrichment toy (**A**) consisted of a floating hula hoop supported by polyethylene-foam pool noodle segments. A cord suspended the double-netted Kong® ball in the middle of the hula hoop. Weighted felt tentacles varying in length between 15 cm to 30 cm were tied to the hula hoop ring. (**B**) All animals within the exhibit had access to the enrichment, but (**C**) the focus of the study was on the use of the enrichment by the cownose stingray. Photo credits: Mia Harris.

2.3. Data Collection

We recorded data in two-hour blocks for five weeks (every Wednesday from 1:30 to 3:30 p.m. from 29 September to 27 October 2021), for a total of 10 h. Within each 2-h data block, there were two 60 min treatments: (1) during the first 60 min of data collection, the jellyfish toy did not include food within the Kong® ball; and (2) during the second 60 min, the jellyfish toy contained food within the Kong® ball. Given that the focus of this study was to examine how the stingray responded to a novel enrichment item, 10 h throughout a 5-week period, keeping the time-of-day and day-of-the-week consistent, allowed us to examine the extent to which the novelty of the new enrichment item may or may not decline.

At the start of each 60 min data collection period, we placed the jellyfish toy in the middle of the pool (Figure 1). Starting at this location allowed the toy to slowly drift without becoming tangled with the ongoing daily enrichment items and prevented it from being caught against the perimeter of the pool. After the first hour, we removed the jellyfish toy from the pool and filled the double-netted Kong® ball with food to start the second 60 min period of data collection.

During each of the two 60 min treatments (no food and food present in the jellyfish toy), we counted the number of times stingrays interacted with the jellyfish toy during a 1 min period. We defined an interaction as swimming directly underneath the jellyfish toy or touching the jellyfish toy, and a single interaction consisted of a stingray approaching the jellyfish toy, interacting, and then exiting the immediate location of the jellyfish toy. We did not identify individual stingrays, so we recorded all interactions that occurred during each 1 min period. Therefore, it was possible for a stingray to interact with the jellyfish toy multiple times during a 1 min period by exiting the jellyfish area and returning for another interaction. The number of stingrays in the study remained consistent across all weeks of the study.

At the start of each minute, we recorded the total number of human visitors present at the exhibit. If a human visitor remained at the exhibit for multiple minutes, we included this individual visitor in each subsequent count at the start of each minute. Researchers included themselves in the visitor tallies recorded each minute.

At the end of the food-treatment data session, we retrieved the jellyfish toy and qualitatively determined if the amount of food in the toy changed (i.e., the stingray successfully accessed the food from inside the Kong®) by visually examining the pockets of space in the double-netted ball and touching the inside of the toy to determine how tightly packed the remaining food was inside of the toy. We did not quantify the amount of food present at the start or finish of the data collection period.

Although the focus of this study was on the cownose stingray, we also recorded when individuals from the two other species in the exhibit (southern stingray and bamboo shark) interacted with the novel enrichment item.

2.4. Data Analysis

We used generalized linear models (GLMs) with the Poisson distribution to determine if there was a relationship between the number of stingray interactions with the jellyfish toy and the week of study (1–5 weeks), the amount of time (minutes) the jellyfish toy had been in the pool during the 60 min data session (1–60 min), the presence of food within the jellyfish toy (no or yes), and the number of human visitors at the exhibit at the start of each minute (2–30, including the researchers collecting data). We built models using these variables, a combination of these variables, and interactions among the variables, and then determined the Akaike's information criteria (AIC) for each to determine the best model. We conducted all statistical analyses using R statistical software version 4.4.1 [42].

Because time of day can potentially impact the number of visitors present, and our non-food treatments occurred during the first hour of each week's data session, we used a Wilcoxon test to determine if there was a difference in mean visitor numbers between the non-food and food sessions across the five weeks of study. For all analyses, we determined statistical significance at $p \leq 0.05$.

3. Results

Overall, the cownose stingrays interacted with the jellyfish toy anywhere from 0 to 30 interactions/minute (mean ± SE: 6.4 ± 0.2). All variables tested in the GLM model predicted touches per minute by the stingrays (Table 1). The minutes the jellyfish toy had been in the pool during a data session, the week of trial, and the presence of food had the strongest influence on interactions with the jellyfish toy per minute ($p < 0.001$ for all). The number of visitors at the exhibit also had a relationship with the number of stingray interactions with the jellyfish toy, albeit a weaker relationship ($p = 0.040$). The interaction among three variables (minutes enrichment in the pool, week of study, and food presence) had the lowest AIC value, indicating the best fit (Table S1).

Table 1. Results for generalized linear model with Poisson distribution.

	Estimate	Standard Error	Z-Value	*p*-Value
Intercept	2.42	0.051	46.95	<0.001
Food presence (yes/no)	0.41	0.033	12.51	<0.001
Week of trial (1–5)	−0.18	0.012	−14.77	<0.001
Min. enrichment in pool (1–60)	−0.012	0.00095	−12.49	<0.001
Number of visitors (2–30)	0.0055	0.0027	2.05	0.040

There were 7 other individuals in the pool during the study period (4 bamboo sharks and 3 southern stingrays). The bamboo sharks and southern stingray approached the jellyfish toy (Figure 2B shows a photo of a southern stingray with the cownose stingray), but these interactions with the toy were minimal (bamboo sharks: <3% of the study time; southern stingrays: <10% of the study time). These 7 individuals did not appear to impact the use of the jellyfish toy by the 43 cownose stingrays.

3.1. Novelty of the Enrichment Item

Interactions with the jellyfish toy decreased both as the jellyfish toy remained in the pool during each 60 min treatment (Figure 3A) and as the weeks progressed (Figure 3B) when food was present in the jellyfish toy. Within a 60 min data period, when food was present, interactions/minute (mean ± SE) were 12.4 ± 1.1 during the first 10 min versus 5.3 ± 0.4 during minutes 51–60. Across the five weeks of the study, when food was present, stingray interactions with the jellyfish toy (mean ± SE) went from 10.9 ± 0.9 interactions/minute (min = 0; max = 30) during the first week to 4.3 ± 0.4 interactions/minute (min = 0; max = 15) during the fifth week.

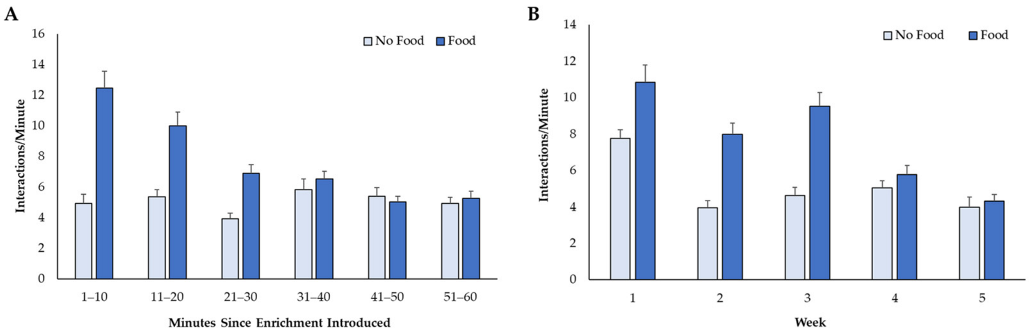

Figure 3. Stingray interactions per minute (mean ± SE) with the enrichment (**A**) decreased as the amount of time the enrichment was present in the water increased during the individual trials. The interactions per minute (**B**) also decreased as the number of weeks of trials increased, specifically when food was present inside the enrichment.

3.2. Food Enrichment

Across the 5-week period, mean (SE) interactions/minute with the jellyfish toy were 5.1 ± 0.7 interactions/minute when no food was present in the jellyfish toy versus 7.7 ± 1.2 interactions/minute when food was present.

3.3. Human Visitors

The number of human visitors at the exhibit at a given time varied from 2 to 30 individuals, but 69.6% of the time there were ≤10 visitors present. The mean number of visitors across each 60 min data period was similar for weeks 1, 2, 4, and 5. During the third week, which coincided with a holiday for local schools, visitor attendance was greatest. The number of visitors present at the exhibit did not differ between the non-food enrichment and the food enrichment treatments (V = 8, n = 5, p = 1.0; Figure 4A).

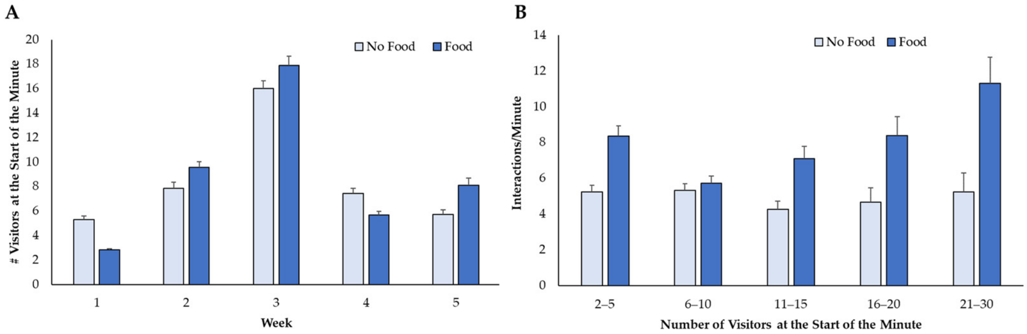

Figure 4. The number of visitors at the start of every minute (mean ± SE) at the stingray exhibit (**A**) peaked at week 3, but there was no difference in the number of visitors during the no-food and food enrichment trials. Overall, the stingrays interacted with enrichment (**B**) when more visitors were present at a given time, but during the food trials.

Of the variables tested, the number of visitors had the weakest association with the number of interactions that the stingray had with the enrichment item (p = 0.040). Overall, interactions with the jellyfish toy increased when more visitors were present, especially during the food enrichment (Figure 4B).

4. Discussion

We found support for the hypothesis that stingray interactions with enrichment are associated with the novelty of the enrichment item, the presence of food in the enrichment item, and the number of human visitors at the stingray exhibit. While all tested variables were associated with stingray interactions, the greatest associations were with time since the presentation of the enrichment item, the number of weeks exposed to the enrichment, and the presence of food. The number of visitors at the exhibit at a given time also was associated with the stingrays' interactions with the enrichment item, but the association was not as strong, and it was primarily associated with the trials when the jellyfish enrichment toy contained food.

In our study, stingray interactions with the jellyfish toy decreased with both (1) time since the jellyfish toy was introduced into the water during each 60 min trial and (2) throughout the five weeks of study. Our study kept the enrichment trials at 60 min, and the non-food trial always preceded the food-based enrichment trial. Given that variability in the amount of time for which the enrichment is provided can help maintain interest [18], we suggest that future studies examine if the novelty of the enrichment item can be maintained for a longer period when there is variability in the enrichment schedule. We acknowledge that our study covered 10 h over a 5-week period, but given that our main focus was to examine the stingrays' interactions with a novel enrichment item, this 5-week period allowed us to determine that engagement with the enrichment item was greatest at the start of the study and at the start of the individual 1 h data collection periods, and greatest when food was present in the enrichment item. By collecting data every Wednesday during the same time of day, we were able to minimize other confounding variables associated with diurnal behavioral patterns in the stingrays, husbandry routines, and visitor presence.

Overall, the presence of food in the jellyfish toy was associated with greater interactions with the jellyfish toy by the stingray. Furthermore, the presence of food resulted in more interactions with the jellyfish toy especially during the first 30 min of the trials, and the first three weeks of the study. As the trials approached 60 min and the last couple weeks of the study, interactions with the jellyfish enrichment toy were similar between trials when food was present and when food was absent. Although interactions declined with time when food was present, such food may be beneficial in increasing engagement with the enrichment. Food enrichment for cownose stingrays can increase swimming behavior even after completion of the enrichment [41]; however, we did not record post-enrichment behavior in the current study.

We found that cownose stingray interactions with the jellyfish enrichment toy were greater when human visitor numbers were higher, but this finding primarily occurred when the enrichment item contained food. These findings were not what we had expected, as we predicted that the stingrays would engage less with the enrichment when more visitors were present due to the visitors often providing food to the animals. Visitor presence may unintentionally promote unsettled or exploratory active behaviors in cownose stingrays, thus increasing engagement with enrichment items; however, further study is needed to test this hypothesis. In a separate study of four stingray species (southern stingray, *Dasyatis americana*; Atlantic stingray, *Dasyatis sabina*; blue-spotted mask ray, *Neotrygon kuhlii*; fiddler ray, *Trygonorrhina fasciata*), activity levels increased with visitor volume, but behavioral changes were not consistent across individual stingrays [25].

Such individual variation also has been shown in swimming behavior in response to food enrichment [41]. This variation can also impact how often individuals interact with the enrichment item with some animals engaging with the jellyfish enrichment toy more than their conspecifics, impacting touch counts. We did not record data on the 43 individual cownose stingrays separately, but we suggest that, when possible, examination of patterns in individual fish can help better understand how enrichment shapes universal as well as individual behavioral responses. Furthermore, there were seven other animals in the exhibit (four bamboo sharks and three southern stingrays). Although we did not focus on these two species in this study, there was minimal interaction with the enrichment item compared to the cownose stingray. However, such findings do not indicate the enrichment was not appropriate for these species; the mere number of cownose stingrays in the exhibit (43 individuals) allowed for more cownose stingray interactions with the jellyfish enrichment toy.

The incorporation of environmental enrichment for captive animals has expanded across disciplines and industries (e.g., wildlife rehabilitation, zoological institutions, pet ownership, livestock, aquaculture) [43,44]. However, the most common recipient of such programs and the subject of behavioral studies are mammals, leaving a gap in research that considers the welfare needs of other taxa, including fish [2,23,27,45,46]. Recently, a growing body of research focused on aquatic animal welfare has explored the different applications of environmental enrichment on fish [24,43]. To date, it has been demonstrated that some captive fish have increased cognitive ability and neuroplasticity when exposed to environmental enrichment [47] and demonstrate memory retention [48]. From learning and acquiring information to anticipating material outcomes from specific behaviors, fish can exhibit agency and preferences as unique individuals [46,49]. Rainbow trout (*Oncorhynchus mykiss*) were able to differentiate various color stimuli for food, respond to alterations in reward patterns, and discriminate between shape versus color stimuli; and fish that were introduced to environmental enrichment as juveniles had higher performance scores [50]. Juvenile black rockfish (*Sebastes schlegelii*) introduced to habitat and social environmental enrichment took less time to solve a T-maze test than juveniles without such enrichment [51].

Captive Vermiculate River stingray (*Potamotrygon castexi*) used water as a tool to extract food from a tube [40]. Furthermore, some reticulated freshwater stingray (*Potamotrygon falkneri*) observers can learn from their peers [52]. Therefore, environmental enrichment can have positive impacts on fish.

In summary, we found that a novel enrichment item, defined as enrichment that the individuals had never encountered prior to the study, engaged the stingrays most when it was novel (the earlier weeks of the study and at the start of the individual treatment periods); however, the stingrays continued to interact with the enrichment during the 5-week period and throughout the 60 min treatment periods. Such interactions were greatest when food was present in the enrichment item. Therefore, we encourage the use of environmental enrichment, including items that are novel to individuals, as well as enrichment that periodically includes food. Moving forward, given that enrichment in fish is understudied, behavioral responses to species-appropriate enrichment should continue to be monitored to offer positive experiences to the animals [11,53,54]. Furthermore, increased usage of technology for enrichment purposes also can help focus on the enrichment appropriate to the species and individual animal [55].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jzbg5040037/s1, Table S1: Model results, from lowest to highest AIC, to examine five variables; Table S2: Data set for the study examining the number of cownose stingray interactions with a novel enrichment item.

Author Contributions: Conceptualization, M.C.Y.H., H.F., S.M., A.D.F. and S.A.B.; methodology, M.C.Y.H., H.F., S.M., A.D.F. and S.A.B.; formal analysis, M.C.Y.H. and S.A.B.; investigation, M.C.Y.H. and H.F.; resources, S.M., A.D.F. and S.A.B.; data curation, M.C.Y.H., H.F. and S.A.B.; writing—original draft preparation, M.C.Y.H. and S.A.B.; writing—review and editing, M.C.Y.H., H.F., S.M., A.D.F. and S.A.B.; visualization, M.C.Y.H. and S.A.B.; supervision, S.A.B.; project administration, M.C.Y.H., H.F., S.M., A.D.F. and S.A.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to the enrichment item following pre-determined protocols for Living Exhibits Inc. and all data collection conducted from the public area of the exhibit.

Data Availability Statement: All data are available in the Supplementary Materials (Table S2).

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Conflicts of Interest: The authors declare no conflicts of interest.

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