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Inclusive Enrichment for Dragons: Behavioral Responses of Amputee and Non-Amputee Individuals of Bearded Dragons *Pogona vitticeps* to Different Food Enrichment Items

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Abstract: Studies on environmental enrichment for captive bearded dragons (*Pogona vitticeps*) are still very scarce in the literature, but they are of the utmost importance in the search for quality of life for these animals when they are under human care. Environmental enrichment items should be inclusive, allowing disabled animals to explore them and providing good experiences. Therefore, this study aimed to evaluate food enrichment strategies to increase the foraging behavior of captive non-disabled and disabled bearded dragons. This work was carried out with six adult bearded dragons, three of which had one limb amputated and three that had all limbs, kept at the São Paulo Aquarium. Live kingworm larvae (*Zophobas morio*) were offered as food in three different levels of enrichment challenge (low, medium, high). Behavioral data were collected using focal sampling with instantaneous recording, with a time interval of every 30 s. Results showed that the more complex the food enrichment challenge, the greater the frequency of bearded dragon foraging. In addition, both amputees and non-amputees responded similarly to the enrichment items, differing primarily in their activity levels, where amputee individuals showed more tendencies toward 'stationary' and 'lying down' behaviors. More studies with reptiles should be encouraged so that good management practices become daily protocols for a better quality of life and for rescuing the natural behaviors of animals kept under human care, even those with anatomical disabilities.

Keywords: amputated animals; food enrichment; insectivorous lizards; use of enclosure; welfare

1. Introduction

Central bearded dragons (*Pogona vitticeps*, Agamidae, Reptilia) are omnivorous lizards from Australia, commonly kept as pets or in zoos [1,2]. This species can opportunistically prey on insects and small vertebrates, but also consume vegetables and fruits [3,4]. They are diurnal, normally observed basking on the ground, bushes, rocks, trees, and fences, being skillful climbers [5]. In captivity, it is essential to provide climbing structures that

allow for the use of the environment in three dimensions [2]. Males are not social and can seriously injure or kill other males if housed together, whereas females are more tolerant and can live in small groups [6,7]. In response to social threats or to the introduction of new individuals, bearded dragons exhibit a range of behaviors as threat responses, such as head bobbing, beard darkening, body flattening, and a distinctive arm-waving gesture [8]. These behaviors can escalate into a physical fight if neither of the two dragons withdraws. If kept together, however, social learning can occur [9,10].

Individuals under human care can originate from the wild or from captive breeding [11–13]. In both cases, the way the animals are captured and managed, such as whether the lizards are housed alone or in groups, the number of individuals per enclosure, and how they are transported, can result in physical traumas, such as injuries or mutilations, due to fights and high stress levels [14]. Limb or tail amputation are examples of common physical traumas observed in bearded dragons, and disabled individuals may end up on display for environmental education or breeding activities in zoos and aquariums [15].

Regardless of the animal's physical condition, it is essential to seek management that guarantees stimuli capable of promoting positive affective experiences that can increase levels of well-being [16]. The search for well-being, within the 24/7 concept (this concept postulates that humans should provide quality of life across the entire lifespan of the animal under their care) [17], must be reinforced not only to meet the biological and ethological needs of the bearded dragons but also to offer greater possibilities for positive experiences in the face of the history and life experiences lived by the individuals [17]. Environmental enrichment techniques are tools capable of motivating the expression of appetitive and consummatory behaviors in animals and therefore play a fundamental role in promoting positive experiences that enhance the well-being of animals kept in artificial environments [18].

Normally, the environmental enrichment items offered to animals are designed for fully able-bodied individuals (hereafter non-amputee), with few studies evaluating the ability of physically disabled animals (hereafter amputee) to use the environmental enrichment items [19,20]. Environmental enrichment should not only be inclusive for physically disabled animals, but also for individuals with psychological/neurological disabilities, and this has been tested with promising results [21].

There is very little scientific information on enrichment techniques applied to bearded dragons, and none for physically disabled animals. However, environmental enrichment has been tested on other species of lizards and reptiles with promising results. For example, Leopard geckos (*Eublepharis macularius*) were provided with five types of environmental enrichment (sensory, physical, and dietary) and interacted with all of them, enhancing behavioral diversity and modifying thermal behavior rates, thus increasing their welfare [22]. In another study focusing on physical and dietary enrichment items, leopard geckos frequently interacted with the items, especially physical ones that could be buried in terrariums, showing that such items were preferred by the animals [23]. A recent review on the effects of environmental enrichment on reptiles kept under human care in Europe reported mostly positive effects for the animals, including in management practices such as capture and handling, and in educational activities with the public [24]. Thus, environmental enrichment should be mandatory for reptiles, although the provision of it is still rare [25].

Given the scarcity of studies on the effects of environmental enrichment for reptiles, especially those evaluating these effects on disabled animals, this study aimed to evaluate the effects of different challenge food enrichment items, offering varying levels of difficulty, on the behavioral responses of amputee and non-amputee bearded dragons under human care. We hypothesized that the more challenging items would stimulate more foraging behavior in non-amputee lizards compared to amputee lizards, and that amputee lizards would prefer the less challenging enrichment items due to their physical limitations.

2. Materials and Methods

The present study was conducted at São Paulo Aquarium (São Paulo, Brazil; 23°35′36.5″ S 46°36′51.1″ W). Six adult female bearded dragons were studied, already acclimated to an enclosure with an area of 8.42 m^2 (kept in this enclosure for more than six months). Three individuals had amputated limbs and tails (group amputee; Figure 1A), while three individuals had all four limbs and tails (group non-amputee; Figure 1B). The animals were kept together, in a single enclosure, with no other species or interspecific interaction, except with humans during daily visits and care. The animals were handled, and the enclosures were maintained by the institution staff. All the food used in the enrichment items was part of the animal's diet, established by the institution [diet: vegetables, fruit, and live invertebrates: mealworm and kingworm larvae (*Tenebrio molitor* and *Zophobas morio*), and adults of speckled cockroaches (*Nauphoeta cinerea*), dubia cockroaches (*Blaptica dubia*), and Jamaican field crickets (*Gryllus assimilis*)]. Live prey was chosen as the main food for the enrichment items for being very attractive to the animals.

Figure 1. Amputee (**A**) and Non-amputee (**B**) bearded dragons kept by the São Paulo Aquarium. Image credits: João Eisenberg.

Three different levels of food enrichment challenges were offered to the bearded dragons: low challenge (bowl: live kingworm larvae offered directly into the bowl feeder; Figure 2A); medium challenge (maze: live kingworm larvae mixed with foliage in a silicone maze; Figure 2B); high challenge: (bottle: live kingworm larvae placed inside a pet bottle with holes to allow the larvae to escape and consumed by the lizards; Figure 2C). Food enrichment was offered randomly on alternate days and weeks over 12 weeks, from July to October 2022. All enrichment items were offered in quantities equal to the number of animals to avoid disputes and the occurrence of agonistic behavior.

Figure 2. Enrichment items with varying levels of difficulty offered to the amputee and non-amputee bearded dragons at São Paulo Aquarium: (**A**) Low challenging enrichment: bowl; (**B**) Medium challenging enrichment: maze; (**C**) High challenging enrichment: bottle. Image credits: João Eisenberg.

All the animals had their behaviors recorded using scan sampling with instantaneous recording, with a time interval of 30 s [26], for 45 min, starting when the enrichment items were placed inside the enclosure. As one of the animals died from natural causes in the last two weeks of the experiment, the amount of data collected for the individuals was different. A period of preliminary observations was applied to build the ethogram used in the present study (Table 1).

Table 1. Ethogram of the bearded dragons (*Pogona vitticeps*) used to evaluate the effects of food enrichment on their behavior, built based on 10 h of ad libitum preliminary observations.

Behavior (Acronym)	Description
Stationary (ST)	Animals were considered to be stationary or inactive if they were standing still in a standard position (trunk and head slightly arched), with all four/three legs on the ground; in a standard position with their head turned to either side; in a standard position moving one of their limbs, body, or head; in a standing position, with only their hind legs on the ground and their front legs resting on stones in the enclosure; animals could be sleeping or not.
Verticalizing trunk and head (VT)	Animals moved their heads and trunks upright continuously and repetitively over a short period of time.
Locomotion (LO)	Animals that were walking or running around the enclosure; jumping from one rock to another or climbing the different reliefs that make up the enclosure's landscape.
Lying down (LD)	Animals were lying down if they had their entire body, limbs, and head resting against the ground, which could be turned to either side; animals could be sleeping or not.
Foraging (FO)	Animals that were interacting with the prey or the enrichment in some ways were considered to be foraging; they could be watching the prey, chasing the prey or feeding on it, or interacting directly with the enrichment in an attempt to capture its food.
Fighting (FI)	Animals were fighting if they were in direct combat with another animal in the enclosure, either for food or dominance; these animals showed an attack posture through biting and pawing.
Digging (DI)	This category included animals that were excavating the soil, using both forelimbs, in the enclosure.
Licking (LI)	Animals that were tasting the objects that make up the landscape of the enclosure, such as stones, cacti, or the soil itself, with their tongues.
Inflating the throat pouch (IP)	This category encompassed animals positioned normally, with expanded throat pouches.
Scrubbing (SC)	Animals found rubbing part of their body, head, or limbs against some object that makes up the landscape of the enclosure, such as stones and cacti.
Not visible (NV)	Animals that were out of sight of the researcher.
Other behaviors (OT)	All the other behaviors observed that had a lower incidence and relevance in relation to the study's objective: defecating, yawning, and falling from enclosure's structures.

For the statistical analysis, we assessed collinearity between the variables using the Variation Inflation Factor (VIF). All VIF results were found to be less than 2, suggesting some degree of collinearity but not significant enough to impact the subsequent analyses [27]. After that, Generalized Linear Mixed Models (GLMMs) were built, with bearded dragons' behaviors used as response factors, while physical condition (amputee and non-amputee), enrichment items [enrichment with low (bowl) × medium (maze) × high (bottle) challenges] were used as fixed variables. Weeks were used as a random variable. For the significant results, Tukey's post hoc tests were used. All analyses were conducted in the Minitab 19 software, with a significance level of 95%.

This research was authorized by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics of the São Paulo University (USP) (protocol number 1921190422).

3. Results

A total of 8176 behaviors exhibited by the bearded dragons were recorded during the study over 27 h of behavioral recordings. The behaviors most often exhibited by the animals, in both conditions, were "stationary", "lying down", and "foraging" (Figure 3).

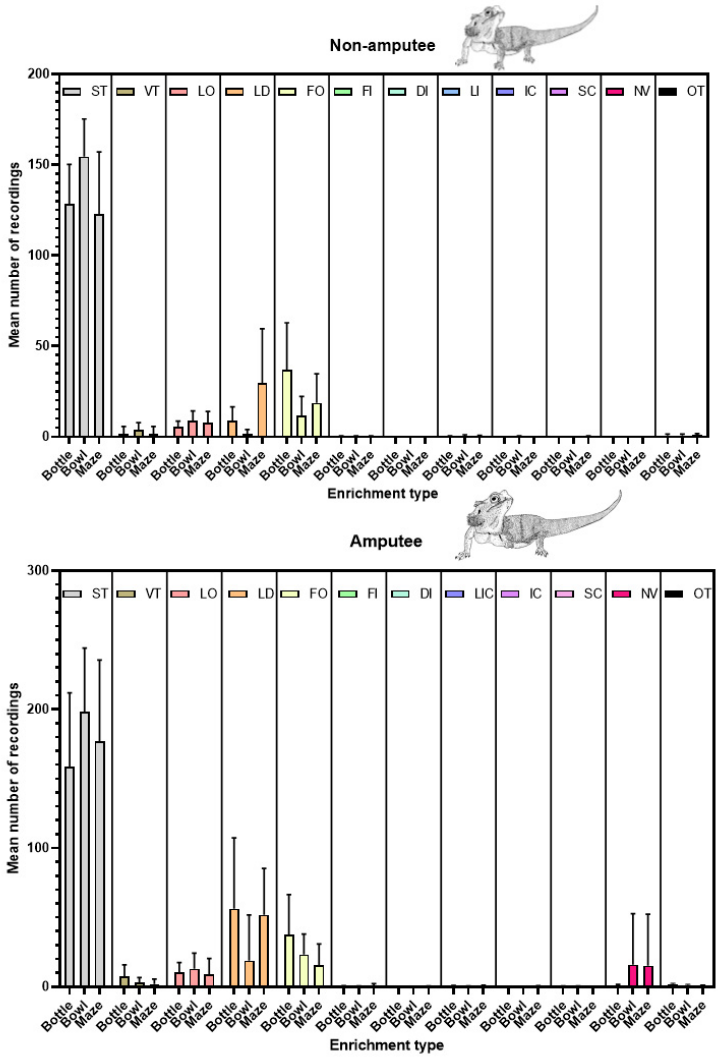

Figure 3. Most recorded behaviors for amputee and non-amputee bearded dragons, according to the environmental enrichment items offered.

Of the 10 behaviors, only three were influenced by the type of enrichment (bowl, maze, and bottle), and the conditions of the individual (amputee and non-amputee). The display of "stationary" and "lying down" behaviors varied significantly only between the condition of the individuals, with amputees displaying more of both behaviors ($F = 9.39$, $p = 0.005$; $F = 8.03$, $p = 0.009$, respectively; Figure 4A,B). "Foraging" varied according to the enrichment presented, and both amputees and non-amputees foraged more with the bottles than with the bowls and the maze ($F = 4.09$, $p = 0.027$; Figure 4C). All the other behaviors were not influenced by the explanatory variables analyzed.

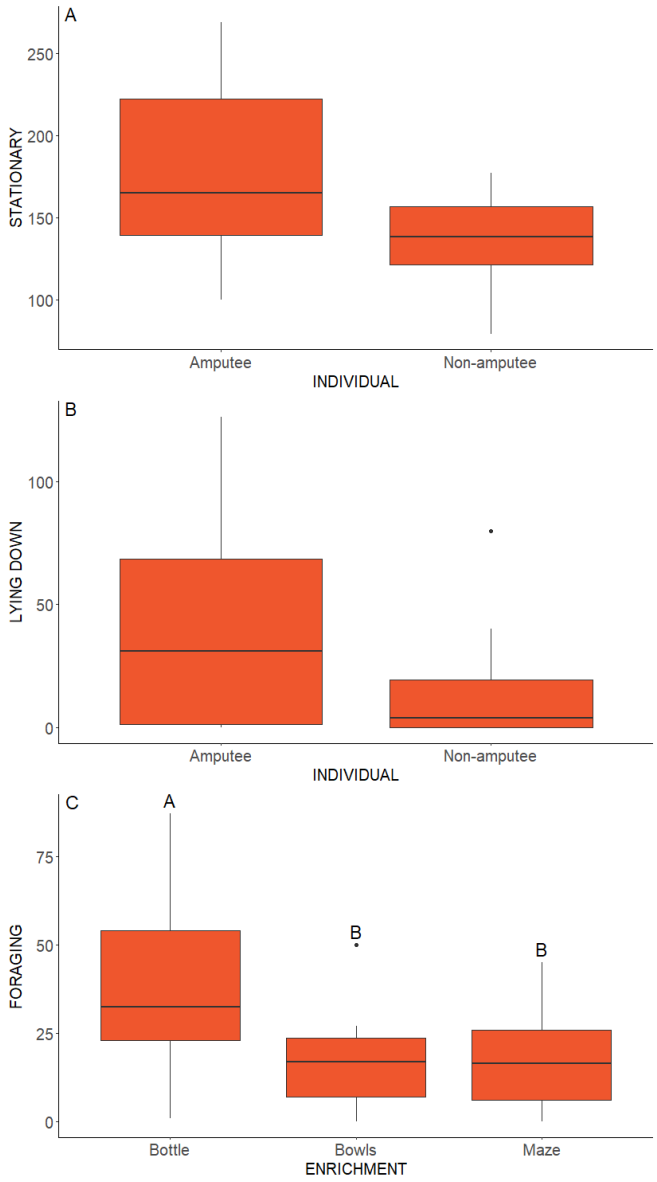

Figure 4. GLMM results of the variation in the behavior of the bearded dragons, according to the condition of the individual (amputee and non-amputee) and the type of enrichment (bottle, bowl, maze): (**A**) Behavior "stationary"; (**B**) Behavior "lying down"; (**C**) Behavior "foraging" (different superscript letters indicate statistically significant differences; the black dot in B represent an outlier).

4. Discussion

The results presented here partially corroborated our hypothesis, that the responses to the enrichment items would be different depending on the physical condition of the lizards and on the challenges posed by the different enrichment items, with the more challenging item stimulating more foraging in the non-amputee animals compared to the amputees and with the amputees preferring the less challenging enrichment item, due to their physical limitations. The more challenging item (plastic bottle with food inside) did stimulate more foraging activities for both amputee and non-amputee individuals.

Overall, amputees and non-amputees behaved similarly when presented with different enrichment items. Only two behaviors differed between the two conditions, with both "stationary" and "lying down" behaviors being expressed more often by the amputee animals. "Stationary" was exhibited more often with the enrichment bowl, when the animals were feeding in the feeder, where food was normally offered, while "lying down" was exhibited less often with the bowl.

Even when interacting with the enrichment items, the animals varied between moments of activity and interaction and moments of inactivity. Both behaviors reflect the lizards' habit of remaining inactive for long periods, especially when in areas of optimal thermoregulation [28,29]. It is interesting to note that the "lying down" behavior may reflect comfort and security, since the animals are not in a state of alertness at these times [30,31] or may reflect trauma, agonistic interaction, or thermal discomfort (low temperatures) [32]. The overall behavioral responses of amputees and non-amputees suggest a high level of well-being.

If we consider that the bottle was the most challenging enrichment item and the most used by all the animals, we corroborate the idea that animals would use the items that provide them the best affective state, that is, the most positive experiences [31]. In addition, this result also supports the idea that reptiles respond to enrichment like carnivorous mammals, benefiting from greater behavioral opportunities, such as hunting and catching their prey [31]. Thus, bottles with live prey motivated the expression of the appetitive and consummatory behaviors of the bearded dragons, possibly enhancing their welfare [18].

Although bearded dragons are solitary animals, the structuring of a group of only females in the present study did not favor the occurrence of agonistic behaviors after the establishment of the hierarchy among the individuals. Appropriate social groups with suitable environmental conditions in terms of size, complexity, and availability of resources allow individuals to choose whether to spend time together or apart, and this can have a positive impact on well-being [17]. The low recording of agonistic interactions may indicate that the enclosure environment was satisfactorily meeting the needs of the animals, providing enough shelters, climbing structures, thermoregulatory sites, etc., to the animals, which decreased disputes among the individuals. It is interesting to note that although the animals received enrichment items in a quantity compatible with the number of individuals, we often recorded the animals sharing the enrichment items, with few disputes occurring in these contexts, signaling an improvement in their social relationships.

Finally, zoos play an important role in dialog with society since they can reach enormous audiences annually (around 700 million visitors a year) [33]. This makes them a real platform for encouraging their audiences to engage with important messages such as biodiversity conservation, planetary health, human well-being, and sustainable living [34]. When an institution such as a zoo or aquarium puts on display a disabled animal and associates this with welfare and educational information, it is playing this very important role [35,36]. Therefore, these institutions need to be concerned about the quality of life of animals with some kind of physical disability, especially so that appropriate messages about problems like mistreatment, animal welfare, and inclusion (thinking about enrichment activities) are passed on to the visiting public.

It is important to note that our study has some limitations that may hinder the generalization of the results, so they should be interpreted with caution. Firstly, the sample size of the study was small, with only six individuals. All the animals currently housed at the São Paulo Aquarium were used in the study, making it impossible to increase the sample size at this location. Despite its small size, our sample is like those in most studies on environmental enrichment conducted in zoos and aquariums [25]. Conducting the experiment with more individuals in both conditions (amputee vs. non-amputee) could yield more robust results. Secondly, the animals were housed together in the same enclosure. Male bearded dragons are typically kept alone due to their aggressiveness, but small groups of individuals can be kept together, especially groups of females [1,5,6]. In our study, only females were housed together, which was the institution's standard practice. However, the behavioral data indicated low levels of aggression among the animals and positive interactions during the use of enrichment items after six months together. Thus, it is important to conduct observations early in the animals' cohabitation so that protective measures can be taken if serious fights occur. Nonetheless, it would be ideal to evaluate the enrichment items for animals housed individually to determine if the results would differ from the conditions in the present study due to an individual influencing the behavior of

another (dominance). Collecting data on bearded dragons kept alone will help to understand how each individual responds to the environmental enrichment stimuli offered. In our study, the individuals were kept together in the same enclosure and data were collected using scan sampling. This type of sampling does not allow us to individualize behavioral responses and does not allow us to understand how individuals influenced the responses of other individuals. One way of measuring the effects of an individual on the behavioral responses of others would be to insert the individual as a random factor in the GLMMs. To achieve this, the data must be collected in a focal manner. Then, as a final suggestion, behaviors should be recorded in a focal manner to allow an analysis of how each individual responds to the different environmental enrichment items offered, especially if the animals are kept together.

5. Conclusions

In conclusion, the food enrichment items offered to bearded dragons proved to be inclusive, with the behavioral responses proving to be very similar between the amputee and non-amputee animals, even when the items offered were the most challenging. We, therefore, suggest that plastic bottles with live prey inside should be offered regularly to this species, both to disabled and non-disabled animals. The welfare program of zoological institutions should include items that can be used by lizards with different physical conditions, if they are tested for safety and accessibility. Including disabled animals in the enrichment routine could improve their quality of life, both physically and psychologically, by offering the possibility of more positive experiences.

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Data Availability Statement: The original data presented in the study are openly available in Mendeley Data Repository at doi: 10.17632/c3t4bwkpc4.1.

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