



The effect of environmental enrichment on *Salvator merianae* (Squamata: Teiidae) under captivity conditions

Efecto del enriquecimiento ambiental en *Salvator merianae* (Squamata: Teiidae) en condiciones de cautiverio

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ABSTRACT

Environmental enrichment seeks to improve the quality of care for animals in captivity through the constant generation of new sources of stimuli to simulate a complex and changing environment. *Salvator merianae* is a species of large lizard whose native distribution covers the subtropical and humid zones of southeastern South America. The study was carried out in the Experimental Lizard Hatchery belonging to the Facultad de Agronomía y Zootecnia from Universidad Nacional de Tucumán, UNT for its initials in Spanish. Two pens were used, a control group R1 (Enclosure 1) and an experimental group R2 (Enclosure 2), where data were taken without enrichment (R2 W/O-E) and with enrichment (R2 WE). An ethogram was used to record the different behaviors that were then grouped into eight categories to evaluate how animals spend their time. Behaviors were recorded on video, the applied technique was the focal animal sampling with instantaneous recording, the extracted data were exported into individual spreadsheets. The Landau index was calculated to determine the existence of hierarchies. The data suggest that the modification of the enclosure conditions has the capacity to alter the behavioral profiles. Only a few behavioral categories showed significant differences. No significant differences were found, in the frequency of the behavioral categories, between males and females. There was a decrease in the frequency of reproductive behavior in males in R2. There was a non-linear hierarchy among the individuals. A decrease in the chases was observed among individuals in R2.

Keywords — Behaviour, ethogram, enclosures, welfare, reptiles.

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RESUMEN

El enriquecimiento ambiental busca mejorar la calidad del cuidado de los animales en cautiverio mediante la generación constante de nuevas fuentes de estímulos, para simular un ambiente complejo y cambiante. *Salvator merianae* es una especie de lagarto de gran tamaño cuya distribución nativa abarca las zonas subtropicales y húmedas del sureste de América del Sur. El estudio se realizó en el Criadero Experimental de Lagartos perteneciente a la Facultad de Agronomía y Zootecnia de la UNT. Se utilizaron 2 corrales, un grupo de control R1 (Recinto 1) y uno experimental R2 (Recinto 2) donde se tomaron datos sin enriquecimiento (R2 W/O-E) y luego con enriquecimiento (R2 WE). Se elaboró un etograma para registrar los distintos comportamientos que luego se agruparon en ocho categorías para evaluar cómo los animales emplean su tiempo. Se realizaron filmaciones, a las que se les aplicó la técnica de muestreo animal focal con registro instantáneo y los datos extraídos se volcaron en planillas individuales. Se calculó el índice de Landau para determinar la existencia de alguna jerarquía. Solo “Refugio”, “Otros” y “Termorregulación” mostraron diferencias significativas antes y después del enriquecimiento. No hubo diferencias significativas en la frecuencia de las categorías comportamentales entre machos y hembras. Hubo una disminución en la frecuencia del comportamiento reproductivo en machos en el R2. No se registró jerarquía de tipo lineal entre los individuos. Hubo una disminución de las persecuciones entre los individuos en R2.

Palabras clave — Comportamiento, etograma, recintos, bienestar, reptiles.

INTRODUCTION

The term “environmental enrichment” refers to a wide spectrum of practices that involve modifying the environment where animals are housed to provide them with opportunities to perform typical behaviors, with the ultimate goal of increasing the welfare of animals in captivity (Broom, 1991; Shepherdson, 1994, 1998, 2013; Young, 2003; Tarou and Bashaw, 2007; Hosey, 2009). The term animal welfare has different definitions, but we can generalize it by indicating that it is the science that studies the quality of life of animals, being its two main objectives to achieve and evaluate: 1) the maintenance of good physical health conditions, and 2) good mental and emotional health (Newberry, 1995; Young, 2003; Swaisgood, 2007; Watanabe, 2007; Whitham and Wielebnowski, 2013). Although the practice of evaluating animal welfare, and the incorporation of techniques for its improvement, is currently very important in zoos and aquariums (Young, 2003; Hosey, 2009; Shepherdson, 2013; Whitham, 2013) it has its origins in the livestock farming industry. The concept of the Five Freedoms (ie. absence of discomfort) emerged based on a report presented by the Brambell Committee (Brambell, 1965; Farm Animal Welfare Council, 1992), and now are used as the base for legislation and regulations that govern not only farm operations and labs, but also zoological institutions (Young, 2003; Aparicio, Vargas, Prieto, 2005; Hosey, 2009; Córdova Izquierdo et al., 2009; Barber, Lewis, Agoramoorthy and Stevenson, 2010; Kagan and Veasey, 2010).

Because animal welfare is based on good physical and mental health, various methods have been used to evaluate it. However, they can be grouped into two main types: indicators based on the environment and indicators based on the individual; the latter type of indicators provides more direct information on the animal's well-being. (Dawkins, 2006; Manteca, Amat, Salas and Temple, 2016; Benn, McLelland and Whittaker, 2019). This information can be obtained by using physiological parameters (hormone measurements and other substances present in the blood) or behavioral measures (use of time, changes in frequency of behaviors, abnormal behaviors, excessive aggression, and apathy, among others (Broom, 1991; Swaisgood, 2007; Hosey, 2009; Hill and Broom, 2009; Kagan and Veasey, 2010; Manteca et al., 2016).

Measuring animal welfare is a complex task, an analysis of the average time that the animal spends performing different behaviors (activity budget) can be quantitatively measured and compared to wild populations or with other populations in captivity. These comparisons can be used to evaluate welfare and also the effect of enrichment (Young, 2003; Hosey, 2009; Munita, Kagan and Veasey, 2010; Shepherdson, 2013; Tadich and Briceño, 2016; Beaudin Judd, Weladji, Louis Lazure and Paré, 2019; Kamaluddina, Matsudab, Munir Md-Zaina, 2020). Another important measure used is to evaluate the presence and frequency of repetitive abnormal behaviors or stereotypes (rate of abnormal repetitive behaviors -ARB), once present, they are very difficult to eliminate (Mason, Clubb, Latham and Vickery, 2007). Well-being measures also include exploration, play, species-specific behaviors, and behavioral diversity (Hosey, 2009; Shepherdson, 2010, 2013).

Most of the publications on environmental well-being and enrichment are focused on mammals and birds (Shepherdson, 1994, 1998; Young, 2003; Hosey, 2009); however, in recent years the number of studies centering on reptiles and amphibians has increased (Warwick, 1990; Hayes, Jennings and Mellen, 1998; Fleming, 2007; Burghardt, 2013; Warwick, Arena, Lindley, Jessop and Steedman, 2013; Eagan, 2018; Benn et al, 2019). Improvements have been recorded in their well-being, play, and even learning (Burghardt, 2013). Enrichment activities have improved welfare of turtles (Burghardt, Ward and Rosscoe, 1996; Therrien, Gaster, Cunningham-Smith and Manire, 2007; Mehrkam and Dorey, 2014) but other taxa have not been well studied (Swaisgood and Shepherdson, 2005; Burghardt, 2013). However, other authors have found no evidence that enrichment affects the behavior of reptiles (Rosier and Langkilde, 2011). These authors warn that more research is required on the effectiveness of the parameters used to evaluate the effects of enrichment, especially when it comes to animals less phylogenetically related to humans.

Commonly known as *Iguana overa*, *Salvator merianae* is one of the largest terrestrial lizards in the American continent, measuring up to 145 cm in length from nose to tail, and weighing up to 8 kg in males, those being larger than females (FWC bioprofile for the Argentine black and white tegu (*Tupinambis merianae*) (Winck and Cechin 2008; Harvey, Ugueto and Gutberlet, 2012; McEachern, Yackel Adams, Klug, Fitzgerald and Reed 2015). Its native distribution covers the subtropical and humid zones of southeastern South America (Jarnevich et al., 2018). It presents a well-marked annual activity pattern alternating a period of activity, which spans

from August to April and includes a peak of maximum activity between November and December, with a long period of hibernation in underground burrows (Hall, 1978; Winck and Cechin, 2008; Montaña et al., 2013; McEachern et al., 2015). Its reproductive period extends from September to the end of February and the females exhibit complex parental behaviors (Noriega, Fogliatto, Mignola and Manes, 1996; Manes, Ibañez and Manlla, 2003). Nesting occurs during October and the young are born between January and February.

S. merianae has always been hunted by native populations of Argentina for both subsistence and commercial reasons. Its leather is highly valued and is exported as a raw material for the manufacture of footwear and fine leather goods (Porini, 2006); its meat is also used for consumption (Caldironi and Manes, 2006). In 1988 in Argentina, the “*Tupinambis* Commission” was created to develop a sustainable management plan and later, during the 90s, commercial and experimental hatcheries were created in various parts of the country, to develop and then share techniques for their breeding in captivity as a sustainable alternative to the exploitation of wild populations.

The objective of this study is to evaluate the welfare of *S. merianae* specimens in a hatchery system and determine the influence of environmental enrichment on the variability and types of behaviors in individuals. To do this, we used four behavioral indicators to assess well-being: exploration, species-specific behaviors (thermoregulation, hunting), behavioral diversity, and ARB. A better welfare was assumed if exploration was increased, time spent on species-specific behaviors increased, behavioral diversity increased, or ARB frequency decreased, in the presence of enrichment.

MATERIALS AND METHODS

Subjects of study.— Data were collected from 13 individuals of *S. merianae* belonging to the Lizard Experimental Hatchery in the Developmental Biology Department of the Facultad de Agronomía y Zootecnia from the Universidad Nacional de Tucumán during the 2017, from September to November, this being the most active period.

Workplace.— The hatchery encompasses 800 m² made up of 10 pens to separate the different categories of animals by age, 12 nesting enclosures, a *lazaretto*, a food preparation room, a classroom-laboratory, a meeting room, and an incubation room equipped with 3 incubators.

The enclosures are open air, with a dirt floor covered in grass, an underground shelter, two feeders, and one or more water bowls depending on the size of the pen. Animals are fed, *ad libitum* with a diet especially designed for production purposes by Vega, Parry and Manes (2000).

Methodology.— The data were collected from 2 pens, each one housing a reproductive group made up of randomly selected individuals, all weighing over 2 kg.

The studied groups were: a control group R1 (Enclosure 1) comprised of two males and four females and an experimental group R2 (Enclosure 2) formed by two males and five females. In R2, data were taken without enrichment (R2 W/O-E) and later with enrichment (R2 WE), with a 20-minute difference between both measurements.

The enrichment activities were carried out twice a week in September and October, and 3 times a week in November. Applied enrichment consisted of the following: 1) Alimentary: change of the diet incorporating fruit and live prey, change of food presentation; 2) Structural: adding climbing logs, a pool with water and a sandbox; 3) Olfactory: perfumes and grasses with odors and 4) Interactive: balls, dog toys and an elastic band with a piece of food to pursue. Often two or more activities were implemented together on the same day (Figure 1).

Four 20-minute sessions were carried out and recorded per sampling day, with a 10-minute pause between each one and alternating between the two enclosures using a Canon HD Vixia HF R72 57 X camera. Observations were carried out from 9:30 a.m. to 4 p.m. By reviewing the footage using the focal animal technique with instantaneous recording, the behaviors of each individual were recorded on spreadsheets, at 30-second intervals. The 21 defined and identified behaviors were grouped into seven different categories: Physiological needs, Thermoregulation, Aggressive interaction, Exploratory activity, Out of sight, Shelter, Other (Table 1). To determine the presence of hierarchy, sociometric matrices were constructed based on the chase and flight events -defined in the Aggressive Interaction category.



Fig. 1. Examples of different environmental enrichment activities carried out in the R2.

Fig. 1. Ejemplos de diferentes actividades de enriquecimiento ambiental realizadas en el R2.

Data analysis.— To analyze the data, the Mann-Whitney and Wilcoxon tests were applied using the software PAST (PAleontological STatistics) version 3.25.

In the case of the dominance matrices, the dominance coefficient and the Landau index were also calculated.

RESULTS

This study involved 200 hours of observation and a total of 42.87 hours of footage over 28 days. At the end of the video analysis process, the total number of spreadsheets set up was 745, totaling 186.25 tabulated hours with an average of 14.25 hours dedicated to each individual. As a result of these observations and together with contributions taken from the bibliography (Lopez and Abe, 1999), an ethogram was created for the studied species (Table 1).

Table 1. Behaviors and behavioral categories.

Tabla 1. Comportamientos y categorías de comportamiento.

| Category | Behavior | Definition |
|----------------------------|----------------------|----------------------------------------------------------------------------------------------------------------------|
| Physiological needs | feed | Consume food |
| | drink | Consume water from recipient |
| | defecate | Fecal excretion |
| Thermoregulation | in the sun | Staying motionless in the sun |
| | in shadows | Staying motionless in the shadow |
| Aggressive interaction | bite | An individual holds another tightly with its mouth |
| | agonistic behavior | An individual turns from side to side arching its body and sometimes opening its mouth |
| | escape | An animal runs away from another when it is chased |
| | chase | An animal head straight for another quickly |
| Male reproductive behavior | biting tale | An animal holds another by the tail |
| | biting nape | An animal holds another by the back of the head |
| | mark | The animal walks dragging its hind legs (where the femoral glands are) from side to side against the ground |
| | scrub | An animal rubs its body with another individual sometimes using one of its legs |
| | parallel position | One animal is placed side by side in parallel and touching the sides |
| | mount attempt | An animal tries to get on top of another individual |
| | copulation | The male tucks his tail under that of the female while he holds her with one of the front legs and copulation occurs |
| | hook movement | Alternate upward movement of hind legs |
| | snort | Emission of vocalization externally evidenced by contractions in the neck area |
| | Exploratory activity | explore |
| jump | | An animal rises from the ground pushed with its legs to try to reach something or climb somewhere |
| climb | | An animal is attached to some structure and uses its legs to climb on it |
| sniff | | Quickly inserting and sticking the tongue out in the vicinity of an animal or object |
| Out of sight | out of sight | The animal is not visible anywhere in the film frame and it is not recorded that it is in the shelter |
| Shelter | shelter | The animal is inside the shelter |
| Other | other | Performing behaviors that are not previously defined in the ethogram |

Behavior frequency.— From the datasheets, the frequencies of behaviors recorded for each of the different behavioral categories were obtained, discriminating by enclosures and enrichment activity (Figure 2). Differences between sexes were not significant.

The behavioral category “Shelter” was the most frequent in the 2 enclosures (R1 and R2), with or without enrichment. This behavior is more frequent in females in both enclosure and decreases when enrichment is applied (R2) only in males (43% to 29%). On the contrary, “Thermoregulation” activities are more frequent in males than in females, and they decrease with enrichment only in females (25% to 17%). In the “Other” category, the appearance of new behaviors was recorded, and the increase was present in both females and males when environmental enrichment was incorporated (1% to 5%). “Physiological Needs” occupied a small proportion of the total time, increasing with enrichment and without important differences between males and females. “Agonist Interactions” and “Exploratory Activity” were carried out in higher proportions by males in both R1 and R2, increasing with enrichment.

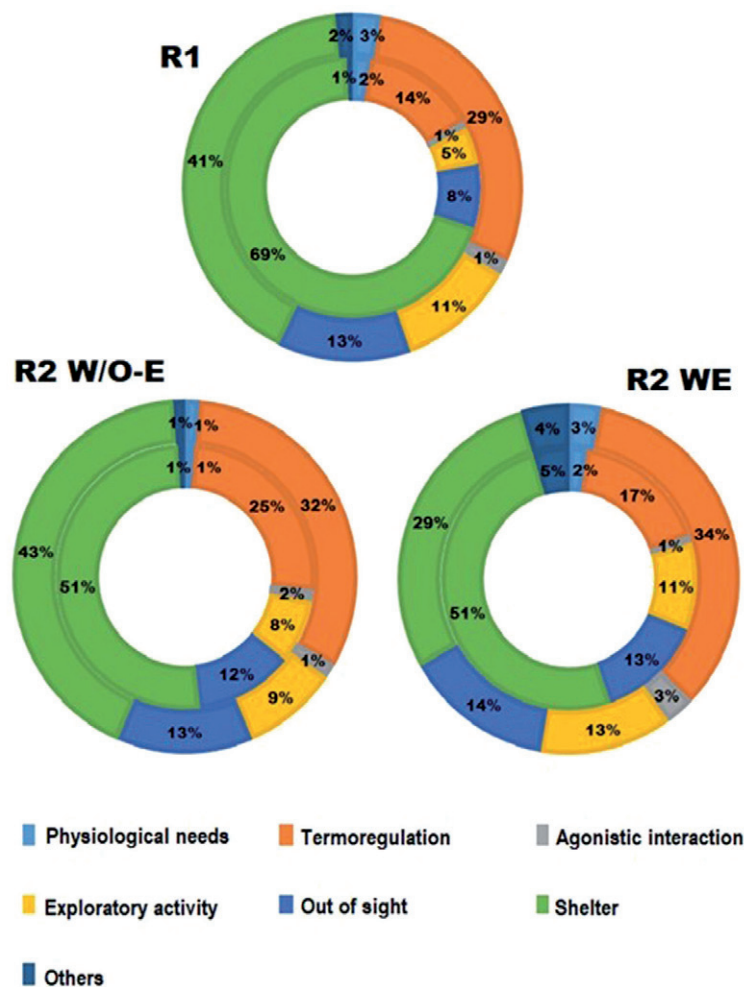


Fig. 2. Comparison of cumulative proportions of behavioral categories in R1 and R2, with and without enrichment (inner circle: females, outer circle: males).

Fig. 2. Comparación de proporciones acumuladas de categorías de comportamiento en R1 y R2, con y sin enriquecimiento (círculo interior: mujeres, círculo exterior: hombres).

Finally, the “Out of Sight” category did not present differences between males and females and only increased slightly with the incorporation of enrichment (Figure 2).

The Mann-Whitmann test comparing the data from R1 and R2 only gave a significant difference for the “Physiological Needs” ($U = 485$, $p = 0.009$) and “Shelter” ($U = 460$, $p = 0.001$) categories, regardless of sex. To analyze the effect of enrichment, R2W/O-E and R2WE were compared using the Wilcoxon test. A significant difference was found in the categories of “Thermoregulation” ($W = 97$ $p = 0.003$), “Shelter” ($W = 87.5$ $p = 0.025$) and “Other” ($W = 75$ $p = 0.038$).

The frequency of reproductive behaviors was higher in enclosure 1, peaking in October. In the case of R2, much lower values were observed throughout the sampled period, although it followed the same pattern: hitting a minimum in September, reaching its peak also in October and decreasing again in November (Figure 3).

Sociometric matrices and dominance coefficient (CD) by enclosure.— Once the sociometric matrices were constructed (Figure 4), the dominance coefficient was calculated using the formula $CD = \text{Gained} / (\text{Gained} + \text{Lost}) * 100$ (Lehner, 1998).

The results of the calculations results were:

- R1: F1, F2, F3 = 0; MWP = 25%; F5 = 84.3% and MWM = 90%
- R2W/O-E: F4 and F5 = 0; F2 = 10%; F1 = 33.3%; F3 = 97.3%; MYP = 56.5% and MWM = 57.8%
- R2WE: F1, F2, F5 = 0; F4 = 20%; F3 = 90.9%; MYP = 23.8% and MWM = 100%

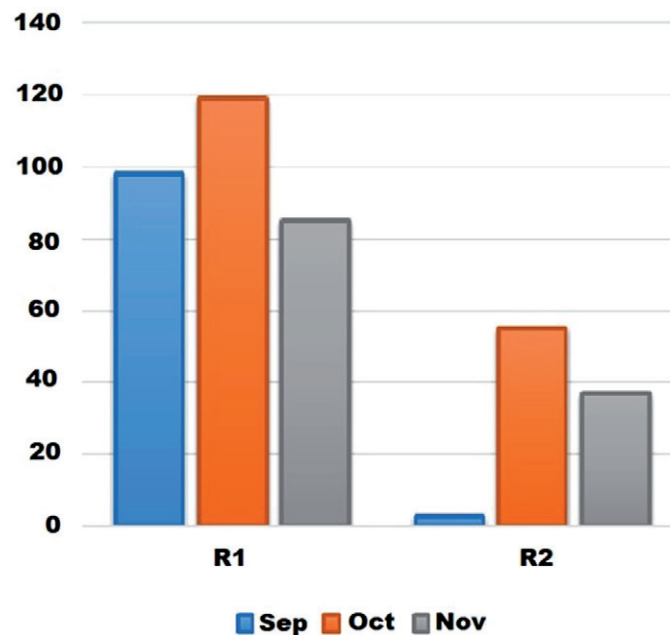


Fig. 3. Comparison in the frequency of Reproductive Behavior in Males between R1 and R2 during the 3 months of the experiment.

Fig. 3. Comparación en la frecuencia del Comportamiento Reproductivo en Machos entre R1 y R2 durante los 3 meses del experimento.

| | MWM | F4 | MWP | F1 | F2 | F3 | W | L |
|-----|-----|----|-----|----|----|----|----|----|
| MWM | | 5 | 22 | 5 | 5 | 0 | 37 | 4 |
| F4 | 0 | | 1 | 3 | 23 | 0 | 27 | 5 |
| MWP | 4 | | | 1 | 3 | | 8 | 23 |
| F1 | | | | | 0 | 0 | 0 | 9 |
| F2 | | | | | | 0 | 0 | 31 |
| F3 | | | | | | | 0 | 0 |

| | MWM | F3 | MYP | F4 | F5 | F2 | F1 | W | L |
|-----|-----|----|-----|----|----|----|----|----|----|
| MWM | | | 16 | 1 | | | | 17 | 0 |
| F3 | | | | 3 | | 7 | | 10 | 1 |
| MYP | | | | | 5 | | | 5 | 16 |
| F4 | | 1 | | | | | | 1 | 4 |
| F5 | | | | | | | | 0 | 5 |
| F2 | | | | | | | | 0 | 7 |
| F1 | | | | | | | | 0 | 0 |

| | F3 | MWM | MYP | F1 | F2 | F5 | F4 | W | L |
|-----|----|-----|-----|----|----|----|----|----|----|
| F3 | | 4 | 0 | 4 | 3 | 6 | 19 | 36 | 1 |
| MWM | 1 | | 10 | 5 | 5 | 0 | 1 | 22 | 16 |
| MYP | 0 | 8 | | 1 | 1 | 0 | 3 | 13 | 10 |
| F1 | 0 | 3 | | | | 2 | | 5 | 10 |
| F2 | | 1 | | | | | | 1 | 9 |
| F5 | | | | | | | | 0 | 8 |
| F4 | | | | | | | | 0 | 23 |

Fig. 4. Tables of sociometric matrices for each enclosure R1 and R2, with (R2 WE) and without enrichment (R2 W/O-E). MWM: male without mark, MWP: male white point, MYP; male yellow point, F: female.

Fig. 4. Cuadros de matrices sociométricas para cada recinto R1 y R2, con (R2 WE) y sin enriquecimiento (R2 W/O-E). MWM: macho sin marca, MWP: macho punto blanco, MYP; punto amarillo macho, F: hembra.

Landau index by enclosure.— By calculating Landau's index, the following values were obtained for each enclosure: R1 = 0.16; R2W/O-E = 0.54 and R2WE = 0.39. None of the enclosures showed a linear hierarchy since all the values were substantially less than one (linear hierarchy).

It is observed that there is a clear dominance of one male over another. MWM dominates MWP in R1, and MWM dominates MYP in R2, with and without enrichment. Furthermore, in this enclosure, the number of interactions between males was similar with and without enrichment (Figure 4). In the case of females, it is similar; one female has a higher dominance coefficient than the others, F5 in R1, and F3 in R2W/O-E and R2WE. The interaction graphs (Figure 5) reflect the recorded chases, in R2 it is observed that in the absence of enrichment there is a complex network of interactions and with the introduction of enrichment, there was a marked decrease in interactions among individuals

DISCUSSION

Reptiles are frequently seen as simple animals from a behavioral point of view; many of the parameters used to measure well-being, such as signs of pain or suffering, are

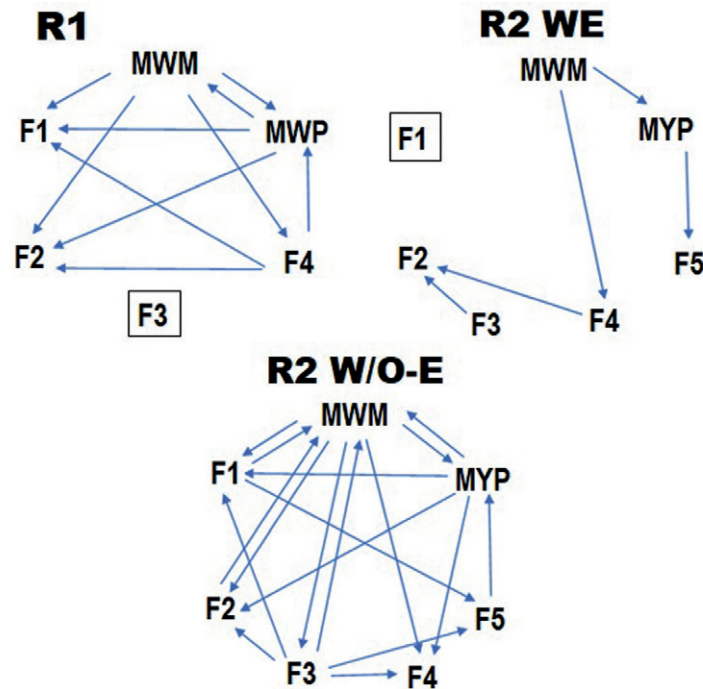


Fig. 5. Representation of the interactions between individuals in each enclosure, R1 and R2, with (R2 WE) and without enrichment (R2 W/O-E) based on the recorded chase and flight events. MWM: male without mark, MWP: male white point, MYP; male yellow point, F: female.

Fig. 5. Representación de las interacciones entre individuos en cada recinto, R1 y R2, con (R2 WE) y sin enriquecimiento (R2 W/O-E) en base a los eventos de persecución y huida registrados. MWM: macho sin marca, MWP: macho punto blanco, MYP; punto amarillo macho, F: hembra.

still difficult to recognize and evaluate in this group (Warwick *et al.*, 2013; Benn *et al.*, 2019). However, reptiles can display abnormal behaviors indicating stress (Warwick, 1990; Warwick *et al.*, 2013). Studies on the application of environmental enrichment and its results in reptiles are less abundant than in mammals and are also quite taxonomically dispersed (Burghardt *et al.*, 1996; Rosier and Langkilde, 2011; Burghardt, 2013; Eagan, 2018; Benn *et al.*, 2019).

During this experiment, much emphasis was placed on structural enrichment, adding several logs, a pool and a sandbox inside the pen; however, the animals seemed to prefer to use the roof of the shelter for basking. The increased interest that the logs aroused was to sniff and scratch the loose bark pieces during the first days, while they only showed interest in the pool on very hot days. On the other hand, the sandbox was useful as a place to hide food, which they actively looked for by digging it out (Figure 1).

A behavioral measure of well-being that increased with enrichment was behavioral diversity represented in the “Other” category of the ethogram (Figure 2). This category includes behaviors such as digging, which was not registered in the initial ethogram (Table 1), but that is part of their behavior under natural conditions (Montaño *et al.*, 2013).

In both enclosures with and without enrichment, the most frequent behaviors were “Shelter” and “Thermoregulation” (Figure 2). The latter is a very important

behavior in reptiles and the time they spend thermoregulating is strongly related to active behaviors: foraging, reproduction and feeding (Rocha, Vrcibradic, Kiefer, de Menezes and da Costa Siqueira, 2009). Enrichment produces changes in the thermal behavior of some reptiles (Bashaw, Gibson, Schowe and Kucher, 2016). In this case, a decrease in this activity is observed during enrichment, both when compared with R1, and R2WE. This could indicate that they do not need to spend much time thermoregulating; thus, they can spend more time on another activity. Thermoregulatory studies in reptiles seem to indicate that active lizards carefully thermoregulate when they emerge and are therefore active at body temperatures that lead to maximal locomotor performance. However, they rarely display peak levels of activity, suggesting that they are in a state of “always ready” to act and respond quickly in situations of predator escape, dominance fights, or close-range pursuit (Hertz, Huey and Garland, 1988; Rocha et al, 2009). This implies that they have more energy available than they choose to use, so the decreased time spent in thermoregulation in response to enrichment, would not affect their thermal balance. Appropriate thermoregulation is another indicator of well-being in ectothermic animals (Benn et al, 2019).

In neither of the two enclosures (R1, R2) did the specimens show any abnormal behaviors such as stereotyped movements, tail autotomy, anorexia, or a state of continuous aggression, recognized as behaviors indicating poor well-being (Broom, 1991; Hayes et al., 1998; Warwick et al., 2013; Shepherdson, 2013; Benn et al., 2019), all of which indicate that in general the captivity conditions in the hatchery are good.

Based on the application of enrichment in mammals, an increase in exploratory activity and a decrease in resting time or sedentary lifestyle would be expected (Refuge in this study). In *Salvator*, only a decrease in the time spent in the refuge was observed (Figure 2), unlike what occurs in *Eublepharis macularius*, in which enrichment did not affect this behavior (Bashaw et al., 2016). “Exploratory activity” did not change significantly with enrichment, which could be explained by two possibilities: 1) the quality of the sites in terms of exploration opportunities is very similar; they are large open-air sites with water and shelter available; and, 2) that the changes in exploratory behavior in the enrichment enclosure have been masked in the “Other” category (Figure 2) where behaviors such as digging to find food and scratching logs that imply some types of exploratory behavior were recorded (sniff, sniffing while moving).

The reproductive period of *Salvator* lizards extends from September to the end of February but territoriality and aggression begin first in males (September). In females, aggressiveness appears at the end of October coinciding with the start of egg-laying (Fitzgerald, Chani and Donadio, 1991; Chani, 1995); females also exhibit complex parental behaviors (Noriega et al., 1996; Manes, Ibañez and Manlla, 2003). In the two pens analyzed in this study, a marked decrease in reproductive behavior was observed in enclosure R2 with respect to the control group (Figure 3). There is a possibility that the enrichment activities applied would have distracted the animals, thus reducing the overall proportion of time spent on reproductive behaviors. These circumstances likely affected in some way the interactions between individuals. Figure 3 shows that, despite the smaller number of reproductive behaviors in R2, they

followed the same pattern as in R1, peaking in October, which coincides with this species' natural behavior (Fitzgerald *et al.*, 1991).

Landau's index analysis showed that none of the studied groups had a linear hierarchy, although there is evidence of a dominance coefficient. In *Salvator* dominance is size-dependent, with larger individuals behaving more aggressively and displacing smaller ones (Fitzgerald *et al.*, 1991; Chani, 1995; Herrel *et al.*, 2009). Aggression has also been observed to decrease when they are housed in large and familiar spaces (Herrel *et al.* 2009). The marked decrease in persecutions by introducing enrichment in our enclosures (Figure 5), is of particular interest within an animal farming context. It would contribute to facilitating the coexistence of individuals living in the same enclosure, potentially reducing stress on animals and the need to treat injuries resulting from these aggressive interactions.

There is some controversy as to whether the parameters currently used to evaluate the success of environmental enrichment tasks are suitable for their use in reptiles, amphibians and other organisms phylogenetically further removed from mammals (Rosier and Langkilde, 2011), considering that such parameters were not designed for these animal groups. As environmental enrichment is one of the best tools available to try to improve the level of welfare of animals in captivity, it is very important to rely on empirical evidence that demonstrates the effectiveness of the different techniques for different situations and animal groups.

Also considering the low volume of behavioral data of *S. merianae*, future experiments could be carried out to monitor the behavioral patterns of individuals throughout the entirety of their activity period, to have a better perspective of the full range of behaviors of which this species is capable, information that would also help in designing more appropriate enrichment techniques that produce more apparent results.

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CONFLICT OF INTEREST

There is no conflict of interest

REFERENCES

- Aparicio, M. A., Vargas, J. G., Prieto, L. (2005). Consideraciones sobre el bienestar animal. (PDF) CONSIDERACIONES SOBRE EL BIENESTAR ANIMAL (researchgate.net).
- Barber, J., Lewis, D., Agoramorthy, G., Stevenson, M. F. (2010). Setting standards for evaluation of captive facilities. In D. G. Kleiman, K. V. Thompson and C. K. Baer. (Eds.), *Wild Mammals in Captivity: Principles and Techniques for Zoo Management* (pp. 22-34). Chicago, United States: University of Chicago Press.
- Bashaw, M. J., Gibson, M. D., Schowe, D. M., Kucher, A. S. (2016). Does enrichment improve reptile welfare? Leopard geckos (*Eublepharis macularius*) respond to five types of environmental enrichment. *Applied Animal Behaviour Science*, 184, 150-160. <https://doi.org/10.1016/j.applanim.2016.08.003>
- Beaudin Judd, J., Weladji R. B., Louis Lazure, L., Paré, P. (2019). Activity budget and spatial distribution of Bennett's wallabies (*Macropus rufogriseus*) in open versus closed exhibit designs. *Zoo Biology*, 38(3), 258-265. <https://doi.org/10.1002/zoo.21486>
- Benn, A. L., McLelland, D. J., Whittaker, A. L. (2019) A review of welfare assessment methods in reptiles, and preliminary application of the Welfare Quality® protocol to the pygmy blue-tongue skink, *Tiliqua adelaidensis*, using animal-based measures. *Animals*, 9(1), 27. <https://doi.org/10.3390/ani9010027>
- Brambell, F. W. R. (1965) Report of the Technical Committee to Enquire into the Welfare of Animals kept under Intensive Livestock Husbandry Systems. Cmnd. 2836. H.M.S.O., London.
- Broom, D. M. (1991). Animal welfare: concepts and measurement. *Journal of animal science*, 69, 4167-4175. <https://doi.org/10.2527/1991.69104167x>
- Burghardt, G. M. (2013). Environmental enrichment and cognitive complexity in reptiles and amphibians: Concepts, review, and implications for captive populations. *Applied Animal Behaviour Science*, 147, 286-298. <https://doi.org/10.1016/j.applanim.2013.04.013>
- Burghardt, G. M., Ward, B., Rosscoe, R. (1996). Problem of Reptile Play: Environmental Enrichment and Play Behavior in a Captive Nile Soft-Shelled Turtle, *Trionyx triunguis*. *Zoo Biology*, 15, 223-238. [https://doi.org/10.1002/\(SICI\)1098-2361\(1996\)15:3<223::AID-ZOO3>3.0.CO;2-D](https://doi.org/10.1002/(SICI)1098-2361(1996)15:3<223::AID-ZOO3>3.0.CO;2-D)
- Caldironi, H. A., Manes, M. E. (2006). Proximate composition, fatty acids and cholesterol content of meat cuts from tegu lizard *Tupinambis merianae*. *Journal of Food Composition and Analysis*, 19(6-7), 711-714. <https://doi.org/10.1016/j.jfca.2005.09.005>
- Chani, J. M. (1995). Comportamiento agresivo y jerarquías por tamaño en *Tupinambis teguixin* (Sauria: Teiidae). *Acta Zoológica Lilloana*, 43, 81-86.
- Córdova Izquierdo, A., Ruiz, Lang, C. G., Saltijeral Oaxaca, J. A., Xolalpa Campos, V., Cortés Suárez, S., Méndez Mendoza, M., Huerta Crispin, R., Córdova Jiménez, M. S., Córdova Jiménez, C. A., Guerra Liera, E. (2009). Importancia

- del bienestar animal en las unidades de producción animal en México. Redvet Revista Electrónica de Veterinaria. <http://www.veterinaria.org/revistas/redvet/n121209/120910.pdf>
- Dawkins, M. D. (2006). A user's guide to animal welfare science. *Trends in Ecology and Evolution*, 21, 77-82. <https://doi.org/10.1016/j.tree.2005.10.017>
- Eagan, T. (2018). Evaluation of Enrichment for Reptiles in Zoos, *Journal of Applied Animal Welfare Science*, 22(1), 69-77. <https://doi.org/10.1080/10888705.2018.1490182>
- Fitzgerald, L. A., Chani J. M., Donadio O. E. (1991). *Tupinambis lizards* in Argentina: Implementing management of a traditionally exploited resource. In Robinson, J. and K. Redford. (eds.), *Neotropical Wildlife: Use and Conservation* (pp. 303-316). Chicago, USA: University of Chicago Press.
- Fleming, G. J., (2007). Reptile behavioral problems, enrichment, and training. *Proceedings Natural American Veterinarie Conference*, 21, 1539-1541.
- Hall, B. J. (1978). Notes on the husbandry, behaviour and breeding of captive tegu lizards. *International Zoo Yearbook*, 18, 91-95. <https://doi.org/10.1111/j.1748-1090.1978.tb00229.x>
- Harvey, M. B., Ugueto, G. N., Gutberlet, R. L. (2012). Review of Teiid Morphology with a Revised Taxonomy and Phylogeny of the Teiidae (Lepidosauria: Squamata). *Zootaxa*, 3459 (1-156).
- Hayes, M. P., Jennings, M. R., Mellen, J. D. (1998). Beyond mammals: environmental enrichment for amphibians and reptiles. In Shepherdson, D.J., Mellen, J.D., Hutchins, M. (Eds.), *Second nature: environmental enrichment for captive animals* (pp. 205-234). Washington DC, USA: Smithsonian Institution Press.
- Herrel, A., Andrade, D., Carvalho, J., Assis, A., Abe, A., Navas, C. (2009). Aggressive Behavior and Performance in the Tegu Lizard *Tupinambis merianae*. *Physiological and Biochemical Zoology*, 82(6), 680-5 DOI:10.1086/605935
- Hertz, P., Huey, R., Garland, T. (1988). Time Budgets, Thermoregulation, and Maximal Locomotor Performance: Are Reptiles Olympians or Boy Scouts? *Integrative and Comparative Biology*, 28(3), 927-938 <https://doi.org/10.1093/ICB/28.3.927>
- Hill, S. P., Broom, D. M. (2009). Measuring Zoo animal welfare: theory and practice. *Zoo Biology*, 28, 531-544. <https://doi.org/10.1002/zoo.20276>
- Hosey, G., Melfi, V., Pankhurst, S. (2009). *Zoo animals: behaviour, management and welfare*. Oxford University Press.
- Jarnevich, C. S., Hayes, M. A., Fitzgerald, L. A., Yackel Adams, A. A., Falk, B. G., Collier, M. A. M., Bonewell, L., Klug, P. E., Naretto, S., Reed, R. N. (2018). Modeling the distributions of tegu lizards in native and potential invasive ranges. *Scientific Reports*, 8, 10193. <https://doi.org/10.1080/10888705.2021.1910032>
- Kagan, R., Veasey, J. (2010). Challenges of Zoo Animal Welfare. In D. G. Kleiman, K. V. Thompson and C. K. Baer. (Eds.), *Wild Mammals. In Captivity: Principles and Techniques for Zoo Management* (pp: 11-21). Chicago, USA: University of Chicago Press.
- Kamaluddina, S. N., Matsudab, I., Munir Md-Zaina, B. (2020). Activity Budget and Postural Behaviours in Orangutans on Bukit Merah Orang Utan Island for As-

- sessing Captive Great Ape Welfare. *Journal of Applied Animal Welfare Science*, DOI: 10.1080/10888705.2021.1910032.
- Lehner, P. N. (1998). *Handbook of ethological methods* (2nd. Ed). Cambridge University Press.
- Lopes, H. R., Abe, A. S. (1999). Biología reproductiva e comportamiento do teiú, *Tupinambis merianae*, em cativero (Reptilia, Teiidae). In Fang, T.G., Montenegro, O.L., Bodmer, R.E. (Eds.) *Manejo y Conservación de Fauna Silvestre en América Latina*. pp. 259-272. La Paz, Bolivia: Instituto de Ecología.
- Manes, M. E., Ibañez, M. A., Manlla, A. (2003). Factores físicos y conductas de nidificación de lagartos *Tupinambis merianae* en cautiverio. *Revista Argentina de Producción Animal*, 23, 119-126.
- Manteca, X., Amat, M., Salas, M. D., Temple, D. (2016). Animal-based indicators to assess welfare in zoo animals. *CAB Reviews*, 11(10) 10.1079/PAVSN-NR201611010
- Mason, G., Clubb, R., Latham, N., Vickery, S. (2007). Why and how should we use environmental enrichment to tackle stereotypic behaviour? *Applied Animal Behaviour Science*, 102, 163-188. <https://doi.org/10.1016/j.applanim.2006.05.041>
- McEachern M. A., Yackel Adams A. A., Klug P. E., Fitzgerald L. A., Reed R. N. (2015). Brumation of introduced *Tupinambis merianae* (Squamata: Teiidae) in southern Florida. *Southeastern Naturalist*, 14, 319-328. <https://doi.org/10.1656/058.014.0207>
- Mehrkam, L. R., Dorey, N. R. (2014). Is preference a predictor of enrichment efficacy in Galapagos tortoises (*Chelonoidis nigra*)?. *Zoo Biology*, 33, 275-284. <https://doi.org/10.1002/zoo.21151>
- Montaño, R.R., Leny Cuéllar R, Fitzgerald L.A., Mendoza F, Soria F, Fiorello C.V., Deem S.L., Noss A.J. (2013). Activity and Ranging Behavior of the Red Tegu Lizard *Tupinambis rufescens* in the Bolivian Chaco. *South American Journal of Herpetology*. 8(2), 81-88. <https://doi.org/10.2994/SAJH-D-13-00016.1>
- Munita, C., Tadich, T. A., Briceño, C. (2016). Comparison of behavioral sampling methods to establish a time budget in a captive female cheetah (*Acinonyx jubatus*). *Journal of Veterinary Behavior*, 13, 1-5. <https://doi.org/10.1016/j.jveb.2016.03.003>
- Newberry, R. C. (1995). Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science*, 44(2-4), 229-243. [https://doi.org/10.1016/0168-1591\(95\)00616-Z](https://doi.org/10.1016/0168-1591(95)00616-Z)
- Noriega, T., Fogliatto, O., Mignola, L., Manes, M. E. (1996). Ciclo biológico y patrones de comportamiento en una población de iguanas overas *Tupinambis teguixin* (L) (Sauria, Teiidae) adaptada al cautiverio. *Revista Agronómica del Noroeste Argentino*, 28,109-127.
- Porini, G.M. (2006). Proyecto *Tupinambis*: Una Propuesta para el Manejo de *Tupinambis rufescens* y *T. merianae* en la Argentina. *Manejo de fauna silvestre en la Argentina: Programas de uso Sustentable*. Pp. 65-75. Dirección De Fauna Silvestre, Secretaría de Ambiente y Desarrollo Sustentable. Buenos Aires, Argentina.

- Rocha, C. F. D., Sluys, M. V., Vrcibradic, D., Kiefer, M. C., de Menezes, V. A., Da Costa Siquiera, C. (2009). Comportamento de termorregulação em lagartos brasileiros. *Oecologia Australis*, 13(1), 115-131. <https://doi.org/10.4257/OECO.2009.1301.09>
- Rosier, R. L., Langkilde, T. (2011). Does environmental enrichment really matter? A case study using the eastern fence lizard, *Sceloporus undulatus*. *Applied Animal Behaviour Science*, 131, 71-76. <https://doi.org/10.1016/j.applanim.2011.01.008>
- Shepherdson D. J. (1994). The role of environmental enrichment in the captive breeding and reintroduction of endangered species. In Olney P. J. S., Mace G. M., Feistner A. T. C. (Eds.). *Creative conservation*. Dordrecht, Netherlands: Springer. https://doi.org/10.1007/978-94-011-0721-1_8
- Shepherdson, D. J. (1998). Tracing the path of environmental enrichment in zoos. In Shepherdson, D.J., Mellen, J.D. Y Hutchins, M. Washington (Eds.). *Second Nature: environmental enrichment for captive animals* (1-12). DC, USA: Smithsonian Institution Press.
- Shepherdson, D. (2010). Principles of and research on environmental enrichment for mammals. In D. G. Kleiman, K. V. Thompson and C. K. Baer. (Eds.), *Wild Mammals in Captivity: Principles and Techniques for Zoo Management* (pp. 62-67). Chicago, United States: University of Chicago Press.
- Shepherdson, D. J. (2013). Enriquecimiento ambiental. In Irwin M., Stoner J. B. y Cobaugh A. M. (Eds.). *Zookeeping: una introducción a la ciencia y la tecnología* (pp. 475-487). Buenos Aires, Argentina: Fundación Temaikén.
- Swaigood, R., Shepherdson, D. (2005). Scientific approaches to enrichment and stereotypies in zoo animals: What's been done and where should we go next? *Zoo Biology*, 24, 499-518. <https://doi.org/10.1002/zoo.20066>
- Swaigood, R. R. (2007). Current status and future directions of applied behavioral research for animal welfare and conservation. *Applied Animal Behaviour Science*, 102, 139-162. <https://doi.org/10.1016/j.applanim.2006.05.027>
- Tarou L, Bashaw M. (2007). Maximizing the effectiveness of environmental enrichment: Suggestions from the experimental analysis of behavior. *Applied Animal Behaviour Science*, 102(3-4), 189-204. <https://doi.org/10.1016/j.applanim.2006.05.026>
- Therrien, C. L., Gaster, L., Cunningham-Smith, P., Manire, C. A. (2007). Experimental evaluation of environmental enrichment of sea turtles. *Zoo Biol.*, 26, 407-416. <https://doi.org/10.1002/zoo.20145>
- Vega Parry, H. E., Manes, M. E. (2000). Alimentación de lagartos overos *Tupinambis merianae* con subproductos avícolas. *Revista Argentina de Producción Animal*, 20(2), 135-143.
- Warwick, C. (1990). Reptilian ethology in captivity: Observations of some problems and an evaluation of their aetiology. *Applied Animal Behaviour Science*, 26, 1-2. [https://doi.org/10.1016/0168-1591\(90\)90082-O](https://doi.org/10.1016/0168-1591(90)90082-O)
- Warwick, C., Arena, P., Lindley, S., Jessop, M., Steedman, C. (2013). Assessing reptile welfare using behavioural criteria. In *Practice*, 35: 123-131. <https://doi.org/10.1136/inp.fl197>

- Watanabe, S. (2007). How animal psychology contributes to animal welfare. *Applied Animal Behaviour Science*, 106, 193-202. <https://doi.org/10.1016/j.applanim.2007.01.003>
- Whitham, J. C., Wielebnowski N. (2013). New directions for zoo animal welfare science. *Applied Animal Behaviour Science*, 147, 247-260. <https://doi.org/10.1016/j.applanim.2013.02.004>
- Winck, G., Cechin, S. (2008). Hibernation and emergence pattern of *Tupinambis meriana* (Squamata: Teiidae) in the Taim Ecological Station, southern Brazil. *Journal of Natural History*, 42, 239-247. <https://doi.org/10.1080/00222930701828667>
- Young, R. J. (2003). *Environmental Enrichment: An Historical Perspective In Environmental Enrichment for Captive Animals*. Oxford, UK: Blackwell Science <https://doi.org/10.1002/9780470751046.ch1>