### Reviewer's comments

Interesting observations. Although only one subject is recorded, it is important to have systematic and formal records of behaviours of pet animals too, particularly if understudied. Numerous videos of single episodes of animal play can be found on the internet, but these are rarely accompanied by further study of the behaviour. Here we see a pet adult reptile showing what seems to be locomotor play as seen in wheel running in other mammals. The animal seems to spend the majority of its time outside of the hide walking in the wheel. The animal shows an intrinsic motivation to repeatedly engage with the novel enriching object.

The article adds to existing knowledge as the seemingly playful behaviour of this individual leopard gecko is systematically recorded over a snapshot of its adult life in the absence of formal descriptions of leopard gecko play. The gecko displays wheel-walking comparable to wheel-running in rodent. Is there a possible reason the gecko only walks instead of running?

The authors provide limitations in the discussion in that wheel-running is debatably a stereotyped behaviour. The explanation of the debate would benefit from further discussion (see comment in the discussion). Considering that this is an important criterion that can help to distinguish stereotyped behaviour from play in captive animals (both non-functional repeated behaviours) and the study argues that wheel-walking in the gecko is playful, it would be worth clarifying and explaining the existing debate as well as its relevance to the present study. In addition, more comparisons can be made with the findings from Bashaw et al.'s paper (2016) where exploration is investigated in leopard geckos (see comments in discussion).

The content of the article is mostly clear, although some sentences are too lengthy and rephrasing or separating long sentences would greatly improve the legibility of the text. Please see further edits and comments below throughout the text.

### Article

Play behaviour in non-avian reptiles is poorly understood compared to mammals and birds. No scientifical report exists for the global third most popular non-avian pet reptile leopard gecko (Eublepharis macularius). Leopard geckos are known to react towards novel enrichment items, and anecdotal observation by pet owners report their high activeness and play-like behaviour. Thus contradictory to their small size, they may have high activity levels and enough energy resources to play when given the right environment. An adult leopard gecko kept as a companion animal was provided with a running wheel to examine this theory. Video recordings and a cycle counter attached to the wheel were used to analyze and develop an ethogram of this leopard gecko. The leopard gecko interacted with the wheel up to 36% per measured day, with up to 47 play episodes per measured day. The highest total distance measured by the cycle counter was 560 meters per measured day. This study showed that the leopard gecko conducted locomotion play, meeting five play criteria and capability of high activity levels. The existence of such behaviour by the leopard gecko indicates that given the right environment, locomotion play can be performed even by a small non-avian reptile. Further studies with more samples would be required to conclude results that can become representative of the species, and further investigation of any physiological benefits of exercise by leopard gecko would conclude whether a running wheel should be considered an added enrichment item.

## Introduction

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Play, or play-like behaviours are reported across all classes of vertebrates: mammals and birds, amphibians (frogs) and fishes. [1][2][3][4] In non-avian reptiles (hereafter reptiles), play or play-like behaviours exist. However, few have been reported in scientific literature, and none is mentioned in standard pet/companion handbooks/guides. [51617] Examples of reptile play include monitor lizards (Varanus macraei and Varanus prasinus), crocodilians, Nile soft-shelled turtles (Trionyx triunguis) and thick-toed gecko (Chondrodactylus turneri) engaged in play under captive environment. BIGIIIIIII Of these examples, Monitor Lizards are considered prime candidates for observing play in reptiles due to their large body size and high metabolic rate.[12] They are also considered quite intelligent per several reports of their learning and problem-solving ability.[1]13[14][15] This concept, added to the difficulty in designing a play environment, could be the reason behind the lack of studies in smaller reptile play. No scientific study has been reported to show small insectivore reptiles play, except for the study by Barabanov et al., 2015 showed objective play by thick-toed geckos.[16] In the study, the geckos were placed in a crewless spacecraft and orbited for 30 days in space under <u>microgravity</u>. The study showed the geckos engaging in play behaviour by removing and interacting with a collar worn by the geckos under weightless microgravity conditions. Although the study showing play behaviour by the gecko is scientifically valid, due to the unique experimental environment, it is difficult to translate the finding to a captive environment at a home setting where most pet reptiles are kept, thus calling for further research and different approach in observing play behaviour is required if it were to be applied in a captive environment.

Animal play is divided <u>generally</u> into locomotor/rotational, object, and social play, though all can co-occur, <u>although these are not strict categories as play can overlap in multiple</u> <u>categories</u>.<sup>ILIET2</sup> Locomotor/rotational play is performing intense or sustained locomotor movements, often without any apparent immediate reason or <u>stimulus</u>. Object play is defined as an animal's mouth, paw, push, pull, grasp, lift, hit, carry, and otherwise manipulate objects that seem to provide no immediate benefit, unlike food or nesting material. Social play is defined as when play is directed at conspecifics or other animals taking on the role, at least partially, of a conspecific.<sup>[1]</sup>

Five criteria have been developed to recognize any of the three types of play that can be applied to all animals, including reptiles.

The set of five criteria are:

1. The performance of the behaviour is not fully functional in the form or context in which it is expressed; that is, it includes elements, or is directed toward stimuli, that do not contribute to current survival.

2. The behaviour is spontaneous, voluntary, intentional, pleasurable, rewarding, reinforcing, or <u>autotelic</u> ("done for its own sake").

3. The behaviour differs from the "serious" performance of ethotypic behaviour structurally or temporally in at least one respect: it is incomplete (generally through inhibited or dropped final elements), exaggerated, awkward, or precocious; or it involves behaviour patterns with modified form, sequencing, or targeting.

4. The behaviour is performed repeatedly in a similar, but not rigidly stereotyped, form during at least a portion of the animal's <u>ontogeny</u>.

5. The behaviour is initiated when an animal is adequately fed, healthy, and free from stress (e.g., predator threat, harsh <u>microclimate</u>, social instability), or intense competing systems (e.g., feeding, mating, <u>predator avoidance</u>). In other words, the animal is in a "relaxed field."

In this study, a subject for observation is leopard gecko (*Eublepharis macularius*). Leopard geckos are small <u>nocturnal</u> insectivore reptiles native to deserts of Middle East Asia (India, Pakistan, and Afghanistan) commonly kept in <u>captivity</u> and are the third most popular reptile pet globally.<sup>[19]</sup> In some countries, it is the most popular pet reptile.<sup>[20]</sup> Significance to show whether

**Commented [A2]:** Play has also been systematically recorded in invertebrates:

Galpayage Dona, H. S., Solvi, C., Kowalewska, A., Mäkelä, K., MaBouDi, H., & Chittka, L. (2022). Do bumble bees play?. Animal Behaviour, 194, 239-251. https://doi.org/10.1016/j.anbehav.2022.08.013

Dapporto, L., Turillazzi, S., & Palagi, E. (2006). Dominance interactions in young adult paper wasp (Polistes dominulus) foundresses: A playlike behavior? Journal of Comparative Psychology, 120(4), 394e400. <u>https://doi.org/10.1037/0735-7036.120.4.394</u>

Mather, J. A., & Anderson, R. C. (1999). Exploration, play and habituation in octopuses (Octopus dofleini). Journal of Comparative Psychology, 113(3), 333–338. https://doi.org/10.1037/0735-7036.113.3.333

I am aware that you mention vertebrates, but a more complete and up to date introduction of the literature may benefit from mentioning invertebrate play.

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Commented [A4]: "Object" play?

**Commented [A5]:** How does this show that is was play? Was the purpose of the study to investigate play or was this an anecdotal outcome of another project?

**Commented [A6]:** But being in an enclosed space of also seems to be a captive environment. What's the difference?

**Commented [A7]:** This sentence is too long. Please consider separating into multiple sentences and/or making the content more concise.

Commented [A8]: Change to: "the subject observed is a leopard gecko" Commented [A9]: Change to: "the significance of showing" leopard gecko plays or not may benefit caring for the popular pet by possibly introducing enrichment item(s) to play to improve their <u>welfare</u>.

This study examined a leopard gecko play via video recordings using a running wheel that is commercially available and often used for rodent pets such as hamsters. A running wheel was chosen in this study based on 1) a study showing a wide range of wild animal species used a running wheel at their own will even in the absence of external reward, indicating possible usage for "fun" – i.e., for their own sakes.<sup>[21]</sup> The study revealed that mice, rats, <u>shrews</u>, frogs, and <u>slugs</u> caused the running wheel movement. Although the study could not conclude whether the behaviours shown by the animals were playing, and no wild reptile used the wheel in that study, given the wide range of animals used it, with the right opportunity and observations, there was no reason to reject that a running wheel could serve as a play item for reptiles too. 2) several anecdotal reports are on social media and blogs online where leopard gecko owners claim their geckos used a running wheel repetitively, showing they were "playing" with it. 3) useage of a running wheel could be interpreted as locomotion play.

With a replicable home laboratory setting, this study primarily aims to evaluate whether leopard geckos show any locomotor play behaviour using a running wheel.

# Materials and Methods

### Animal[edit | edit source]

The subject was an adult female, captive-bred leopard gecko (*E. macularius*), the author's companion animal (Figure 1). This gecko is approximately 12 years of age, with a clean medical history and showed no abnormality before and during the study. Its snout-to-vent length is 12 cm and weighs 49 grams.

### Enclosure, the play item, and the feeding pattern[edit | edit source]

The gecko was kept in a wooden enclosure ( $45 \text{cm} \times 45 \text{cm} \times 30 \text{ cm}$  — length × width × height, handmade) containing a water bowl, a wet hide, an artificial rock, a hammock, a dry hide and an artificial bridge. While these enrichment items have been present <u>since pre-studybefore the</u> <u>study</u>, the play item, a running wheel (hereafter, the wheel) (<u>bought from</u> Amazon.co.jp ASIN B08WJWZ175 SANKO Silent Wheel Flat 19 Clear), was newly introduced (Figure 2).

The wheel had a diameter of 19 cm, a circumference of 597 cm, weight of 390 g. The wheel size was chosen as the diameter is longer than the gecko's snout-to-vent length<u>meant minimum</u> minimising stress on the gecko's spine when it bends slightly backwardcurving to walk on the wheel. To consider safety for the gecko, the wheel brand was chosen as it has a solid surface (not wired), preventing from gecko's toes or tail from being trapped. The wheel is had a closed design meaning there is only one entry point to the wheel. The wheel was set up in the enclosure per the manufacturer's instructions.

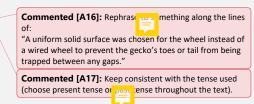
The wheel was equipped with a commercially available cycle counter (bought from a local store which can also be found on Amazon: ASIN: B013E0OMUG *SunDing cycle computer*) to count the distance travelled. The cycle counter uses a small magnet placed on the outer side of the wheel, and when the magnet passes over a sensor, it counts the distance. The magnet used was not from the cycle counter package, which weighed 3.00 g, but a lighter 0.90 g magnet available from a local gadget store was used instead. The lightest magnet sold locally reduced the magnet weight, negatively impacting the wheel weight. The cycle counter was manually validated before usage to confirm that the cycle count sensitivity was the same between the two magnets.

The enclosure was equipped with two heat sources, a panel heater for 24 hours and a heat lamp during the day.

During the entire experiment<u>al</u> period, the temperature during the day was kept at 27-28-degree Celsius<sub> $\tau_i</sub>^1$  nighttime temperature at 24-25 degrees Celsius. Relative humidity was kept at 30-45%. They were consistent since before the start of the study.</sub>

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**Commented [A15]:** Can you specify what the lifespan of the leopard gecko is? Does 12 years mean that the gecko is old?



The gecko was fed every seven days with *LEOPA DRY* (*Meal Replacement pellet designed and sold for leopard gecko, bought from Amazon.co.jp: ASIN: B07SCRY8BN*) using a tweezer, given as much as the gecko would want to eat at one time. The feeding frequency and meal type remained consistent before the study started. The number of pellets eaten was measured to assess if there were any differences in the amount of food consumed.

### Observational Methods[edit | edit source]

To observe the nocturnal leopard gecko, a night vision (940 nm infrared light) motion detection camera (*Xiaomi Mi camera 2K* bought from Amazon.co.jp ASIN: B0BG5K42C2) was used to record the behaviour. The camera was ready to record when the lights went off from 17:45 and continued until 07:00 the next day for a total of 14 days, which were This was considered sufficient to obtain quantitative data for analysis of play behaviour. Pest-After 14 days, the cycle counter was kept for an additional seven days for continuous assessment of the wheel usage.

The camera's motion detection would automatically start the recording when the gecko moves out of its hide. The recorded data and data from the cycle counter were used to analyze the gecko's behaviour, duration, frequencies, and movement distance using the wheel.

### Statistical Analysis[edit | edit source]

Pearson correlation coefficient and p-value were calculated to determine a <u>A Pearson's</u> correlation was assessed to test any correlation between the duration spent on the wheel and the number of play episodes<u>, the /</u>travel distance recorded on the cycle counter to show whether the gecko merely used the wheel as a hide or for locomotion.

### Results[edit | edit source]

The gecko's behaviour pattern of 35 hours of movement recorded over 14 days was analyzed from the video recording and cycle counter. There were six days when the gecko did not come out of its hide (no locomotion action was observed outside of the hide for six days as the gecko remained in its hide).

The video recording showed the gecko took <u>a mean of on average</u> 81 minutes (SD = 89, minimum 11, maximum 247 excluding non-active day) after the lights were out to come out of the shelter and approach the wheel, followed by entry to the base of the wheel inside with its <u>forelimbs</u> first, followed by <u>hindlimbs</u> in a controlled manner. Once inside the wheel, the gecko started walking (Figure 3 and Figure 8 (under supplementary material)), and this occurred every time the gecko reached inside the wheel. The gecko would stop walking to take a rest either on the wheel or by leaving the wheel, going back to the wet hide after a few seconds to a few minutes of continuous walking and would then come back to the wheel to walk again and repeated this behaviour throughout the observed period. No running was observed.

Figure 4 shows the number of play episodes of the gecko on each observation day and its correlation with the gecko's total time engaged in the wheel. The correlation was found to be a moderately positive correlation, r(12) = .53, p = .51 (not significant at p=.05). The number of play episodes is counted as one when the gecko enters then leaves the wheel ultimately (all its limbs were outside the wheel). It showed a mean of 11 play episodes was found (SD = 16, minimum 0, maximum 47) per observed day. The total engagement time is defined as the duration of the gecko in the wheel per observed day. It showed a The gecko spent mean an average of 133 minutes (SD = 188, minimum 0, maximum 525) on the wheel.

The odometer display from the cycle counter showed a mean distance of 124 meters (SD = 177, minimum 0, maximum 560) per measured day. No behaviour observations were present that triggered the wheel's movement other than walking inside the wheel. The distance measured on the cycle counter and total time engaged in the wheel (Figure 5) found-showed a strong positive correlation, r(12) = .99, p = .00001 (significant at p=.05). Table 1 summarizes noteworthy behavioural observations at each measured day and lists distance measured on the cycle counter.



The <u>activity budget</u> was determined based on the analysis of the video recordings. It showed 82% <u>of the time recorded (9426 minutes)</u> resting in the wet hide, 18% (2094 minutes) active, of which 16% (1862 minutes) were interaction with the wheel, and 2% (232 minutes) spending time outside the wheel/hide during the eight days which showed activity outside of the wet hide (Figure 6).

A negligence budget was observed interactingThe gecko interacted with an enrichment item artificial rock (0.026%/3 minutes) and included within the 2% time outside the wheel/hide. No interaction with other enrichment items was observed. Figure 7 shows the activity budget for Day 2, which was the most active day observed. It showed 61% (875 minutes) resting in the wet hide, 39% (565 minutes) active, of which 36% (525 minutes) were interaction with the wheel and 3% (40 min) spending time outside the wheel/hide. No interaction with other enrichment items was observed on Day 2.

Feeding frequency of every seven days was kept, except during the <u>ecdysis</u> from Day 10-13; feeding conducted on Day 12 showed little food consumed (two pellets); therefore, on Day 14, another attempt at feeding was conducted. On Day 21, the last feeding occurred during the study observation period. Table 1, Behavioral/general observation comment column, notes the feeding timing and consumed number of pellets.

### Discussion[edit | edit source]

Let the observation results be applied to the five criteria for classifying play to determine if the leopard gecko played using the wheel.

#### Criteria 1:

Walking on the wheel is by no means biologically linked with the gecko's immediate survival, as the gecko has survived for the past 12 years without it. The repetitive interaction with the wheel does not serve any immediate function either.

### Criteria 2:

The gecko was not forced or trained to use the wheel, nor were any rewards given. The gecko repeatedly walked on and off the wheel during the observed period, <u>understanding showing an ability how to leave the wheel voluntarily</u>. The number of play episodes, total engagement time in the wheel and the total distance walked on the wheel showed large variances within the 14 days observation period, <u>translating</u>. This suggests that that the interaction with the wheel itself was voluntary, intentional, spontaneous, and performed for its own sake.

### Criteria 3:

While walking is a natural behaviour, voluntarily doing so on the wheel rather than purely walking on the enclosure's substrate could be interpreted as a modified behaviour. The total engagement time in the wheel / total distance walked on the wheel could be exaggerated behaviour compared to low interaction levels with other enrichment items.

#### Criteria 4:

This criterion is possibly the most debatable. There is an ongoing debate about whether wheel running in mice is stereotypical behaviour.<sup>[22]</sup> At least some mice and rats are known to show pathologically stereotypical behaviours with wheel running, such as continuing to use the running wheel until starvation.<sup>[23]</sup> A study by Meijer & Robbers 2014 showed wild mice also used a running wheel, suggesting wheel running in mice is not stereotypic.<sup>[21]</sup> although some authors disagree with this conclusion as abnormality arises from a normal state; hence wild mice could show stereotyped behaviour.<sup>[24]E3</sup> To date, wheel running in leopard geckos is not scientifically reported; thus, it cannot be compared with studies from mice. However, a similar behaviour seems to be occurring, although the gecko only showed walking and not running.

Nevertheless, at least for the gecko of this study, during the observation period, the gecko repeatedly interacted with the wheel voluntarily. The duration of interaction with the wheel

**Commented [A31]:** This is very interesting! From the photo we can see that the gecko has plenty of space to walk in and avoid the wheel, yet the animal chooses to approach and use the wheel repeatedly. I think it would make this even stronger if you can be rovide the surface area of the wheel that can be use to valk on as well as the remaining remaining surface area of one enclosure where the gecko can walk. From the photo we can see that the gecko has plenty of space to walk in and avoid the wheel, yet the animal chooses to approach and use the wheel, the animal chooses.

**Commented [A32]:** Add that walking in the wheel did not result in gaining any reward eed d for immediate survival such as gaining food, mate o the er.

**Commented [A33]:** Perhaps add that walking typically serves the same hal to move between locations, however wheel w light does not transport the animal anywhere. Furthermore, the wheel is plain and white coloured, with no landmarks, thus it does not mimic movement between locations either.

**Commented [A34]:** How and why does the variance show that the behaviour was voluntary, intentional and spontaneous?

You could include for formation about the variance under criterion 4. The variability in the duration or play bouts within and between days suggests that the behaviour is not strictly rigid. Although, it would be even better to find motor patterns or related characteristics (such as duration and/or distance) that differ from usual walking as per criterion 3.

**Commented [A35]:** Rephrase this to be more firm. Avoid using "could". We can see from the data that the animal spends the vast majority of its time outside the hide on the wheel rather than moving in any provide the enclosure and, as you say, any interaction the enclosure her enrichment items is much rarer.

**Commented [A36]:** Stereotypy is mostly observed in suboptimal living conditions. Therefore, if wild animals engaged in wheel running it supports the argument that the activity is not encoupyed. You may also add that the captive environment have be driving the gecko to engage in wheel walking to keep somulated, as the enclosure is more limiting compared to the wild.

**Commented [A37]:** This is a bit unclear. Either expand the argument to explain what you mean by "abnormality arises from a new state", or perhaps refer the reader to the literature aready shows debates on the topic. e.g. see: Eilam, D., Zor, R., Szechtman, H., Hermesh, H., 2006. Rituals, stereotypy and compulsive behavior in animals and humans. Neuroscience & Biobehavioral Reviews 30, 456–471. https://doi.org/10.1016/j.neubiorev.2005.08.003

**Commented [A38]:** Is there walking and no running because the people is old? Why did the gecko not run? Does this species in the wild or even in captivity?

differed across the observation period. Its feeding patterns and daytime resting patterns did not change. Post-feeding and pre, during and post-shedding (ecdysis) behaviour directly translated into cease of the interaction with the wheel, which was an expected response from a physiological point of view as during digestion and shedding, reptiles are known to be less active in prioritizing their energy expenditure for these physiological events.<sup>[26]</sup> Therefore, based on the gecko's behaviour, it was not behaving pathologically stereotyped.

#### Criteria 5:

The gecko had no known or present health issues, fed with unchanged frequency before the wheel introduction with food quantities as much as she wanted and no changes to its weight significantly. The temperature/humidity conditions also stayed consistent. Handling was avoided to reduce potential stress on the gecko during the observational period other than at the start and end of observation to weigh the gecko. The gecko displayed typical healthy behaviour.

Based on the above, the study data shows that the gecko's behaviour meets the five play criteria.

The highest total engagement time in the wheel / total distance walked on the wheel was in the first two days of the wheel introduction, indicating novelty response was present. However, the gecko continued to use the wheel when it was not under any physiological event, such as post-feeding digestion; thus, the gecko continued to show some motivation to interact with the wheel. The gecko is known to react to new enrichments, and this study showed that a gecko of older age can still show such a reaction to a new enrichment and learn to use it.<sup>[27]28]</sup>

The by-product of locomotion play, especially using a running wheel designed to be walked or run by an animal, is that the animal will get a certain level of exercise. While voluntary wheel running by mice has shown some benefit to their health by increasing the average or median lifespan of mice by up to 17% and protection from sarcopenia,<sup>[29]30]</sup> no such study exists in leopard geckos thus physiological benefits, if any, of voluntary exercise in leopard gecko is a topic for further investigation.

TheA surplus energy theory suggests that animals engage in play, especially locomotion play, when they have excess energy that can be expended without impacting their survival.<sup>[1]</sup> Captive animals, including leopard geckos, kept in limited space that is well fed, should have enough energy to spare and thus would be a candidate reptile to engage in play behaviour. Unfortunately, there are no explicit scientific references on wild leopard gecko behaviour or their activity budget, so this study data cannot be compared against the conditions of the wild animal. Although the mean play activity budget of 16% does not surpass published work on Nile softshelled turtle,<sup>[10]</sup> it surpasses some mammals shown to have ~10% play activity budget.<sup>[10]</sup> The walking distance of <sup>[560</sup> meters on one of the peak days observed surpasses daily movement distance data on similar-sized diurnal lizards,<sup>[11]</sup> indicating the leopard gecko is likely displaying exaggerated behaviour (criterion 3), has the necessary spare energy to engage in play behaviour.

Zieliński (2023) showed that even with 45 min of observation, <u>post-addition of after</u> enrichment items <u>were provided</u> to leopard geckos that were kept under low enrichment enclosures showed a higher frequency of enrichment interaction than those geckos kept

under biotope terrariums which are considered as high enrichment environment. The study also showed that even a leopard geckos from biotope terrariums increased exploration when a new enrichment item was introduced,<sup>221</sup> indicating the leopard gecko<u>s</u> kept under high enrichment enclosure still have additional energy to be expended for interaction interacted with enrichment enrichment novel enriching object.

The distance walked on the wheel would be unlikely to be met in any typical enclosure environment at home setting, and therefore a running wheel may be an enrichment item to be considered from an animal welfare point of view if voluntary locomotion activity such as walking could be proven beneficial. Although this does not mean the geckos should be kept in small spaces, having a large space enclosure may not be possible for many pet owners, and it is indeed not a strict requirement to do so. The most common enclosure is a 10-gallon (38 litres) terrarium for keeping a single leopard gecko, and it is suggested by the two best sellers of Commented [A39]: Interesting!

**Commented** Consider splitting the sentence.

Commented [A41]: However, you could still compare the time budgets on exploration by leopard geckos found here in Bashaw et al. (2016) paper (https://doi.org/10.1016/j.applanim.2016.08.003) that you have cited above. Figure 1 in their paper shows that the geckos interacted 20% of time with any prichment object. Your findings reflect similar value to prichment object. Your findings reflect similar value to prichment object 16% of its time on the wheel. I would also comment on time allocated to locomotor activity alongside novel object interaction.
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<b>Commented [A44]:</b> You test this specifically so you don't know if additional en
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leopard gecko pet guides in Amazon.<sup>[5][6]</sup> A running wheel could fit into an enclosure of this size if the enclosure has enough height. A caution here is that a wheel cannot be introduced to an enclosure where multiple leopard geckos are kept to avoid unintentional injury, such as when one leopard gecko uses the wheel. If another gecko gets too close to the moving wheel, it could accidentally go into contact with the moving wheel. This caution aligns with the manufacturer's instructions for the running wheel for pet rodents.

The limitations of this study must be emphasized. Firstly, the findings are based on a single leopard gecko: thus, this study alone cannot be generalized to this species. Further studies using a higher number of animals under different life-stage and sex of the animal is required. Secondly, the study's measurements are taken in a relatively short-term, primarily assessing play behaviour. A long-term study to determine any changes in activity budget and walking distances is required to determine any changes in seasons, lighting hours, temperature, and feeding patterns. In addition, the walking distance measured on the wheel does not directly translate to field walking distance because the friction and weight of the wheel affect performance, at least in mice,<sup>[32]</sup> but logically applicable to any animals' locomotion. Also, the wheel would sometimes rock when the gecko stopped walking. Sometimes the rocking occurred right when the magnet attached to the wheel was over the cycle counter sensor, leading to overcounting the cycles; thus, using the cycle counter alone would not provide an accurate distance walked. However, the walking distance strongly correlated with the duration spent on the wheel. Thus, the cycle counter distance can indicate the gecko's interaction level with the wheel. Thirdly, this study cannot assess the enclosure size and its effect on wheel introduction. Finally, to the author's knowledge, no scientific observations of wild leopard geckos' activity levels have been reported; thus, it cannot be determined that the activity levels shown in this study are within the range of the species.

### Conclusions[edit | edit source]

The study found the leopard gecko voluntarily learned to walk on the running wheel at its desired duration, repeating this behaviour throughout the 14 days observed, yet not to the extent of pathological stereotypy behaviour. It met the play behaviour criteria; thus, it is safe to conclude that the leopard gecko played. Specifically, it falls under locomotion play. Whether this is a common feature among the specie or within similar-sized reptiles remains to be explored.

The study also revealed the possibility of using a running wheel as an item in investigating the exercise physiology of leopard geckos. The introduction of the wheel, given that it is safely set up and a leopard gecko is singly kept may serve as an enrichment item for the leopard gecko. Long-term observations with multiple leopard geckos would be required to confirm its benefits or appropriateness from an enrichment point of view.

**Commented [A46]:** Would you be able to provide an approximation of the error? For example, was this a matter of an extra 2 cycle see upared to X total cycles? At the moment we do not we to what extent the wheel was overestimated. How can use cycle counter be comparable across play episodes/de

**Commented [A47]:** Why does this matter? Do you mean, if the whee not up most of the space of the enclosure, then the gecko v as h sed to use the wheel rather than other areas of the enclosure?