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Article

# Behavioural Impact of Captive Management Changes in Three Species of Testudinidae

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**Abstract:** Reptile behaviour and welfare are understudied in comparison with mammals. In this study, behavioural data on three species of tortoises were recorded before and after an environmental change which was anticipated to be positive in nature. The environmental changes differed for each population, but included a substantial increase in enclosure size, the addition of substrate material, and a change in handling procedure. A tortoise-specific ethogram was created to standardise data collection. Focal behaviour sampling was used to collect behavioural data. Changes in the duration of performance of co-occupant interaction and object interaction in the leopard (*Stigmochelys pardalis*) and Aldabra (*Aldabrachelys gigantea*) tortoises were observed following the environmental changes. The Shannon-Weiner's diversity index did not yield a significant increase after the changes but had a numerical increase which was relatively greater for the leopard tortoise group, which had experienced the greatest environmental change. The leopard tortoises also demonstrated changes in a greater number of behaviours compared to the other species, and this was sustained over the study period. However, this included a behaviour indicative of negative affect; aggression. Whilst we are unable to conclude that welfare was improved by the management changes, there are suggestions that behavioural diversity increased, and some promotion of positive social behaviours occurred.

**Keywords:** reptile; tortoise; welfare; diversity index; behaviour; enrichment; ethogram

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## 1. Introduction

In recent times zoos have showed a strong commitment to optimizing the welfare of the animals in their care [1-4] and utilise accreditation scheme membership to showcase this commitment. Many zoos conduct their own welfare research and strive to implement the findings within their premises [5]. Likewise, most zoo accreditation programs, e.g. The Australian Zoo and Aquarium Association, require members to regularly conduct animal welfare assessments [5,6]. Simultaneously there has been a shift in public attitudes towards animals with increasing expectations of high welfare standards [1-3,5].

The welfare of an animal, as described by Webster, can be considered to be '...its capacity to avoid suffering and sustain fitness' [7]. An affective state is defined as a feeling, emotion, or mood such as fear, that motivates an animal to avoid a particular environmental stimulus that is potentially detrimental to their fitness. Affective states can be positive (excitement/joy) or negative (fear/sadness) in valence. Furthermore, these states also vary in motivational intensity or arousal based on the urge to move towards or away from the eliciting stimulus [8]. Determination of welfare state includes considering the number of positive versus negative affective experiences, where 'good' welfare is determined by having more positive experiences, 'poor' welfare determined by having a greater number of negative experiences, and 'neutral' welfare assigned when there are an equal number of positive and negative experiences [9,10]. In practice, welfare is commonly assessed by looking at animal-based or resource-based measures (water, shelter, etc.) [7,11]. However, the former is likely superior being a direct measure of welfare resulting (partially) from

the resource inputs provided [11,12]. There are a number of ways that welfare can be assessed including physiological, immunological, or behaviour-based techniques [9,13,14]. It is common to use multiple modalities in welfare assessment [9,13,14]. In a zoo environment there is a need for methods to be non-invasive, simple, and undemanding on resources [9,13]. As a result, behaviour-based methods are likely to be the most practical and have received the most research focus [13].

There are various models or frameworks which have been used as the basis of welfare assessment tools, including the Five Domains Model [15], the Five Freedoms [16], and the Welfare Quality® protocol [17]. A number of these have been trialled at zoos. However, the Five Domains Model is perhaps the most employed as part of zoological accreditation programs [5,18]. These welfare assessment protocols utilise behavioural observations to infer affective state, but this requires a good understanding of which species-specific behaviours are indicative of differently-valenced affective states [11].

To date there has been a research taxa-bias towards mammals in studies of biology and welfare of animals in zoos [5,11,12,19,20]. Reptiles have been comparatively understudied. A reduced research focus on reptiles may be due to a combination of factors, including difficulties observing wild reptile behaviour, challenges intuitively recognising and interpreting reptile behaviours such as signs of distress, or that reptiles are perceived as less important or less intelligent [1,11,20,21]. Furthermore, a misconception that reptiles are highly tolerant of, and easily adaptable to, suboptimal captive conditions (which is not supported by the literature) may result in the provision of only the most basic husbandry requirements for captive management being considered by some [19,20].

Recently there has been increased research focus on identifying behaviours that may be indicative of welfare state in reptiles [21-30]. However, there remains a dearth of primary studies exploring reptile behaviours, their relation to affective state, and how husbandry practices may modify expression of these behaviours. A key challenge is in identifying indicators that infer positive, as opposed to negative affective states [13]. Given the lack of validated methods to assess reptile welfare it is important that potential tools are explored.

There has been recent interest in using behavioural diversity measures, calculated from behavioural data, to provide an objective insight into the welfare of an individual, or group, of animals by determining how much variation is shown in their behavioural repertoire [31-35]. Greater behavioural diversity is generally accepted as a positive indicator of welfare [31,34,35]. This is based on the assumption that animals displaying varied behavioural repertoires are having their behavioural needs met. Alternately, when diversity is low an animal may be showing reduced overall behaviours due to lethargy or the performance of stereotypies [31, 35].

This study opportunistically investigated changes in the activity budget and behavioural diversity of land tortoises following a change to the captive environment. Testudines were selected as 56% of species in this order are threatened, making the study of captive conditions of high importance to conservation efforts and breeding programmes [23]. The environmental changes were different for each species, but included a substantial increase in enclosure size, added substrates, and a change in handling procedure. Testudines were selected as 56% of species in this order are threatened, making the study of captive conditions of high importance to conservation efforts and breeding programmes [23]. It is suggested that an animal's motivation to interact with environmental enrichment, is positively correlated with welfare [1]. More complex environments allow animals to choose how to interact with their environment, allowing greater control and agency, thus improving welfare [11,19]. Given this, it was hypothesised that the environmental changes would result in a change in behavioural expression, indicative of improved welfare.

## 2. Methods and Methods

### 2.1. Ethics statement

Ethics approval was granted for this research by the Animal Ethics Committee of The University of Adelaide (protocol code S-2021-036), and the research was conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes [36].

### 2.2. Population

This study investigated three established groups of tortoises housed at two locations in South Australia: Adelaide Zoo and Monarto Safari Park. Radiated tortoises (*Astrochelys radiata*; n=5, adult 3 males, 2 females) housed and displayed at Adelaide Zoo, leopard tortoises (*Stigmochelys pardalis*; n=4, adult, all male) not on display and housed at Monarto Safari Park, and sub-adult Aldabra tortoises (*Aldabrachelys gigantea*; n=5, unknown sex), housed and displayed at Adelaide Zoo. Land tortoises were selected as this provided the greatest number of individuals within the same species. The radiated tortoises arrived in the collection in 2018 and had been in their current enclosure since 2020. It is presumed that these individuals were wild caught as they were part of a group of confiscated tortoises. The leopard tortoises arrived in 2009, and had been in their first enclosure, as described in this study, since 2018. These individuals were captive-bred and transported from Auckland Zoo, New Zealand. The Aldabra tortoises arrived in 2017 and had always been housed in the same enclosure. These individuals were captive bred and transported from La Vanille Nature Park, Mauritius. The total number of animals (n=14) and species were determined by the availability within the zoo collection.

### 2.3. Husbandry

Diet for all groups across the study consisted of ad libitum grass hay, and defined portions, fed twice a day, of hard vegetables (e.g. pumpkin, sweet potato, carrot, broccoli, cauliflower), leafy greens (lettuces and endives), Wombaroo herbivorous kangaroo pellets (Wombaroo Food Products, Glen Osmond, South Australia), and a calcium-vitamin D supplement. A minimum of two feeding zones were provided in each enclosure to accommodate all individuals and reduce competition.

All enclosures (Figure 1) were temperature-controlled and were fitted with basking lights and UV lamps. Temperature and humidity were monitored and kept at species-appropriate levels by zookeepers (unbranded generic thermometer / hygrometer, product code: IC7312): accuracy of temperature  $\pm 1^{\circ}\text{C}$ , accuracy of humidity  $\pm 3\%$ .) Enclosures were cleaned and misted daily by keepers.

Prior to the management changes, the radiated tortoise enclosure had substrates of sand and straw. The leopard tortoise enclosure had straw substrate over concrete; the original Aldabra tortoise enclosure had a dirt substrate and a mock rock pool. For diagrams of each enclosure before the environmental change see Appendix A.

### 2.4. Study Design

This study utilised the opportunity of planned changes to the captive management of the three groups by animal management teams at each zoo (Adelaide Zoo and Monarto Safari Park) during the period of this study. These changes were uninfluenced by the research team. The leopard tortoises were moved to a new enclosure that provided a substantial increase in enclosure size and diversity (original 7m<sup>2</sup>), comprising a climate controlled indoor area (13m<sup>2</sup>), a roofed open-air area (30m<sup>2</sup>), and a large uncovered naturally vegetated outdoor area (230m<sup>2</sup>). Depending on weather conditions, the tortoises had access to all areas.

The radiated tortoises received dried leaves as an additional substrate and a marked reduction in the frequency of manual handling. The leaves were plane tree and various species of ficus, selected due to their non-toxicity to tortoises, and general unpalatability. It had been common for the keepers to pick up the tortoises and move them to the feeding

locations when food was offered, and this practice was ceased, allowing tortoises to move around the enclosure with greater choice and control.

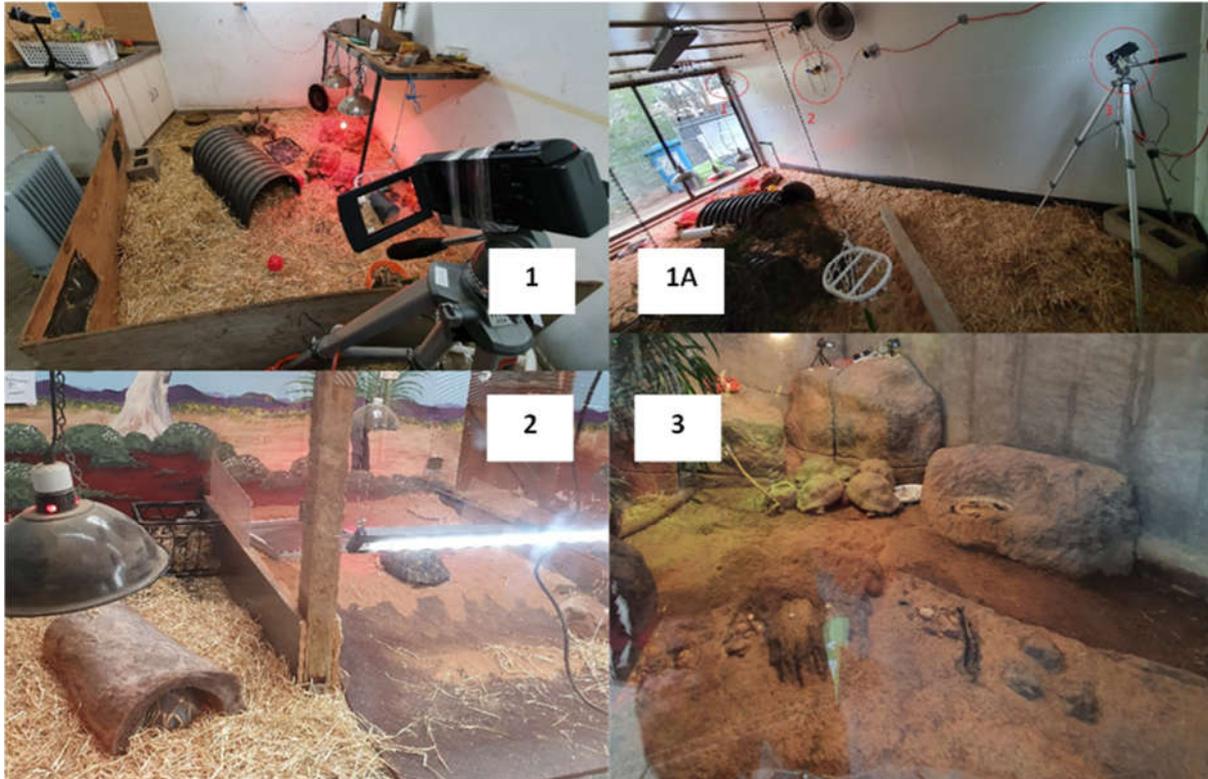
The environmental changes for the Aldabra tortoises were the addition of an enrichment crate filled with straw, and sand added as a substrate to two areas of the enclosure.

### *2.5. Behavioural Data Collection*

Data collection was split into three time points (Figure 2), pre-environmental change (1), post-change (between 10-21 days after change) (2), and approximately seven months (230-250 days) after the environmental change (3). After the environmental change, no behavioural observations were made for at least a week to allow tortoises to habituate to the new environment and reduce the likelihood of confounding results due to an acute stress response, or reaction to novelty. Data for the third time point were only collected for the leopard and Aldabra tortoises; it was considered that additional management changes subsequent to the second time point for radiated tortoises would have confounded interpretation.

Video recordings were taken of animals in all housing locations using camcorders (HC-V180, Panasonic Corporation, Kadoma, Osaka, Japan). In the larger outdoor leopard tortoise enclosure there were occasions when manual data recording was required since the camera's field of view did not capture the full area.

Focal behavioural sampling was performed on every animal in each group using the video footage or direct in-person observation. Each tortoise was viewed for two minutes, and behaviour was continuously sampled. This occurred once every hour between 8AM and 5PM (9 data collection points), over a consecutive three-day period (54 minutes of observation per animal, per time point). For data analysis sampling time points were grouped by time-of-day morning (8-10 AM), midday (10 AM- 2PM), and afternoon between 2PM and 5PM. Behaviour was catalogued using the Zoo Monitor App [37]. The frequency and total duration of each behaviour were recorded for every individual. Only one behaviour could be selected at a time for each individual. The data were compiled by one observer to exclude potential inter-observer variations, following an ethogram with set behavioural descriptions, outlined in Table 1, which was a modified version of an ethogram that has been used in previous studies [8,38,39]. Inter-rater reliability between the observer in the current study and another observer was conducted in an unpublished, and parallel study on the same tortoise groups. The observers reached 80% consensus on identification of the behaviours on a subset of the data.



**Figure 1.** Photographs of enclosures 1 (the original leopard tortoises enclosure), 1A the indoor component of the new leopard tortoise enclosure, 2 radiated tortoise enclosure, and 3 Aldabra tortoise enclosure.



**Figure 2.** Timeline of data collection for the evaluation of tortoise behaviour in response to a change in environment.

**Table 1.** Ethogram used for behavioural analysis [modified from 8,38,39].

Behaviour	Description
Resting	Includes basking, sleeping, or resting on ground with no weight on limbs, for 3 seconds or more. No other activities being performed. All instances of resting are included whether awake or asleep.
Walking	Two or more steps in one direction. One foot removed from ground at a time.
Digging	One or more limbs (front or hind legs) moving substrate. Motion must be repeated twice or more to be counted as digging.
Standing still	Tortoise must be bearing its weight on 1 or more legs for 2 seconds or more
Bathing	Includes submerging whole or part of body for more than 3 seconds
Enrichment and object interaction	Touching or playing with any object provided for play and or enrichment. This includes interaction with food dispensers (ball or similar, NOT a stationary food bowl and NOT the act of eating), using or touching tunnels/hides/ramps/logs/etc. If tortoise is inside a tunnel when set observation period starts and they cannot be reasonably seen, this is counted as individual not observed. However, if the tortoise moves into tunnel during the observation period and remains in there, this is counted as use of tunnel.
Eating/Drinking	Any eating or drinking activity where food/water is consumed, or food is chewed.
Vocalisations	Any audible noises made by the tortoises by nose or throat. This excludes defaecation and/or digestion noises.
Co-occupant interaction	A positive or neutral interaction with another tortoise. This includes climbing, leaning, touching, non-aggressive approach, head bobbing, etc.
Co-occupant aggression	Any negative, aggressive action, or attempted action, towards another occupant including shell ramming, charging, displacement, hooking, aggressive social posturing, scratching, biting.
Stereotypies/Abnormal behaviour	Includes pacing and other repetitive behaviour and interaction with transparent boundaries. Behaviour must be completed 3 times consecutively.
Not Observable	For use only when focal animal is not visible

### 2.6. Data Analysis

In order to explore the effects of the covariates of temperature, species, time of day and timepoint a multivariate General Linear Model (GLM) in the program SPSS was used with the duration of each behaviour of interest being taken as the dependant variable [40]. A Bonferroni correction was applied to account for multiple comparisons.  $P < 0.05$  was taken as the significance level.

Data were then split by species to investigate the applied management change. Given the small sample size for each species, and that the behavioural data were often non-normally distributed data, non-parametric tests were applied (Mann-Whitney or Kruskal-Wallis tests in SPSS).

Shannon Wiener's Diversity Index was used to calculate behavioural diversity before and after the environmental change. The formula for Shannon-Weiner's diversity index is [32]:

$$H = - \sum (p_i * \ln(p_i))$$

$H_{\text{Duration}}$  and  $H_{\text{Rate}}$  [33] were calculated using Excel (Microsoft Corporation, 2021), where  $p_i$  is the duration or frequency, respectively, of  $i$ th behaviour. A higher  $H$  value

represents greater behavioural diversity. Instructions from Snapshot Wisconsin's tutorial [41] were followed to create the spreadsheet. A Kruskal-Wallis test was conducted to determine any statistical significance.  $P < 0.05$  was taken as the significance level. Due to the limited data points for the radiated species, a Wilcoxon test was conducted instead.  $P < 0.05$  was taken as the significance level.

### 3. Results

#### 3.1. Activity budget analysis results

**Table 2.** GLM significant main effects.

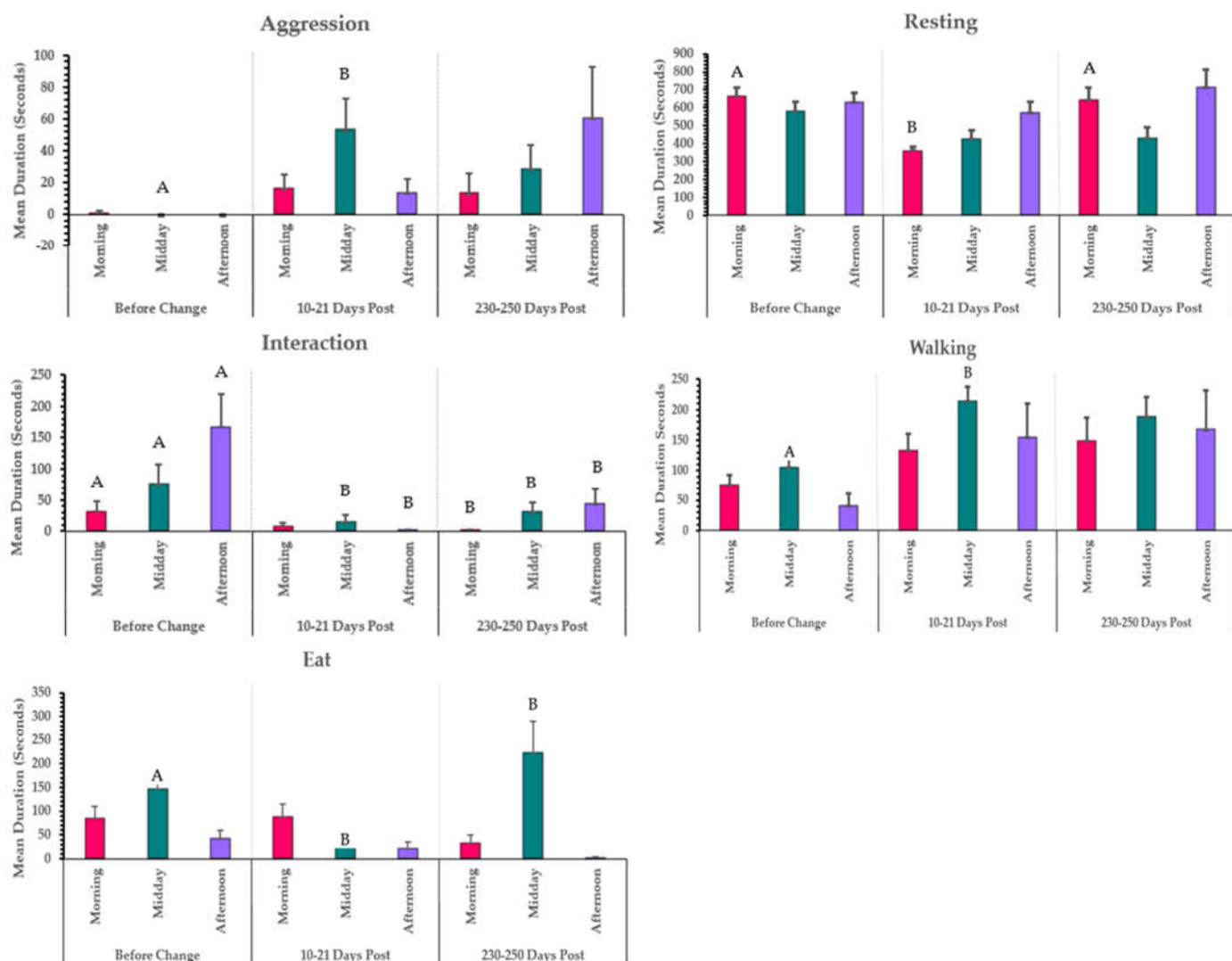
Factor	Behaviour	Df	F-value	Significance
Species	Aggression	1	8.300	0.01
	Object Interaction	1	11.704	0.01
	Eating/Drinking	1	5.649	0.02
	Standing	1	11.136	0.01
	Co-occupant interaction	1	18.758	<0.001
	Abnormal Behaviours	1	5.024	0.03
Time Point	Aggression	2	4.703	0.01
	Object Interaction	2	4.868	0.01
	Resting	2	4.486	0.01
	Standing	2	4.655	0.01
	Co-occupant interaction	2	20.696	<0.001
	Walking	2	6.169	0.003
Temperature	Resting	1	8.223	0.005
	Co-occupant Interaction	1	21.700	<0.001
Time of Day	Aggression	2	31.000	<0.001
	Eating/Drinking	2	6.410	0.003
	Resting	2	6.808	0.002
	Co-occupant Interaction	2	6.522	0.002
	Walking	2	4.390	0.02

The model shows multiple significant behaviours (Table 2).

#### 3.1.2. Temperature and Time of Day Interactions

During the study the median temperature for the radiated tortoises was 25.75 °C, range of 25-26 °C. For the leopard tortoises the median temperature was 23.05 °C, range of 20.1-27.15 °C. The median temperature for the Aldabra tortoises was 28 °C, with range of 25-29 °C. When the group data were combined, temperature had a significant interaction with co-occupant interaction and resting with the former increasing as temperature increased, and vice versa for resting behaviour.

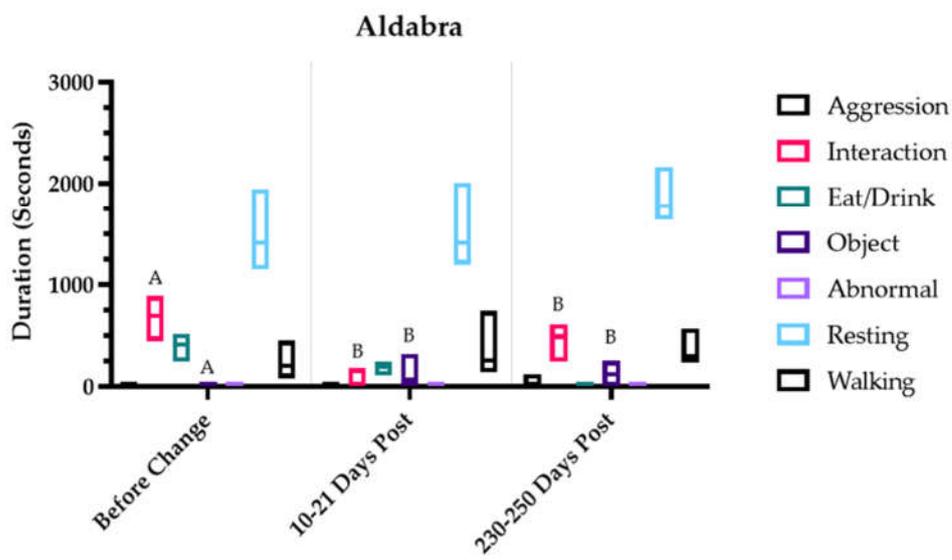
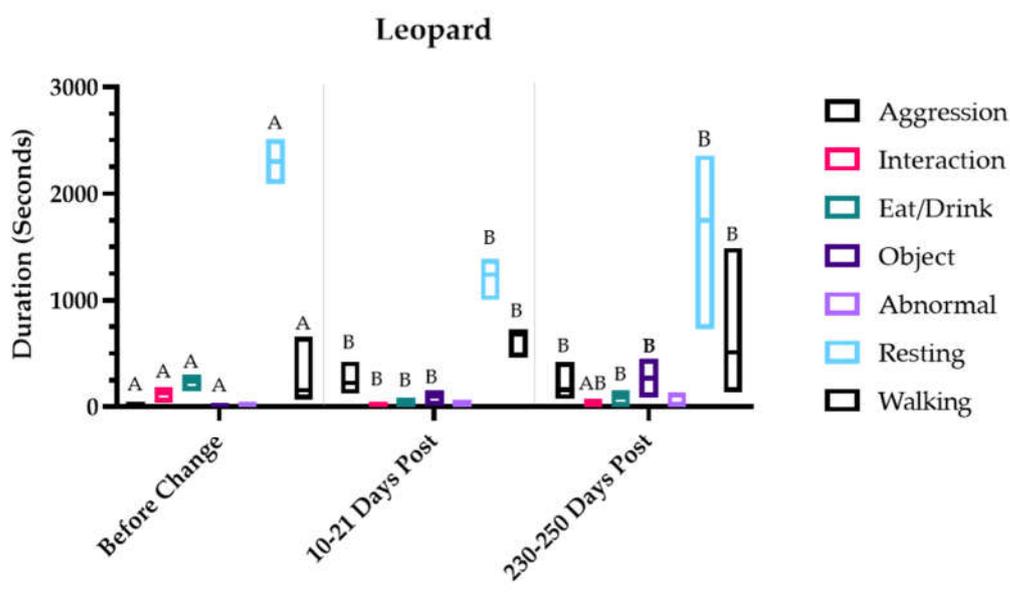
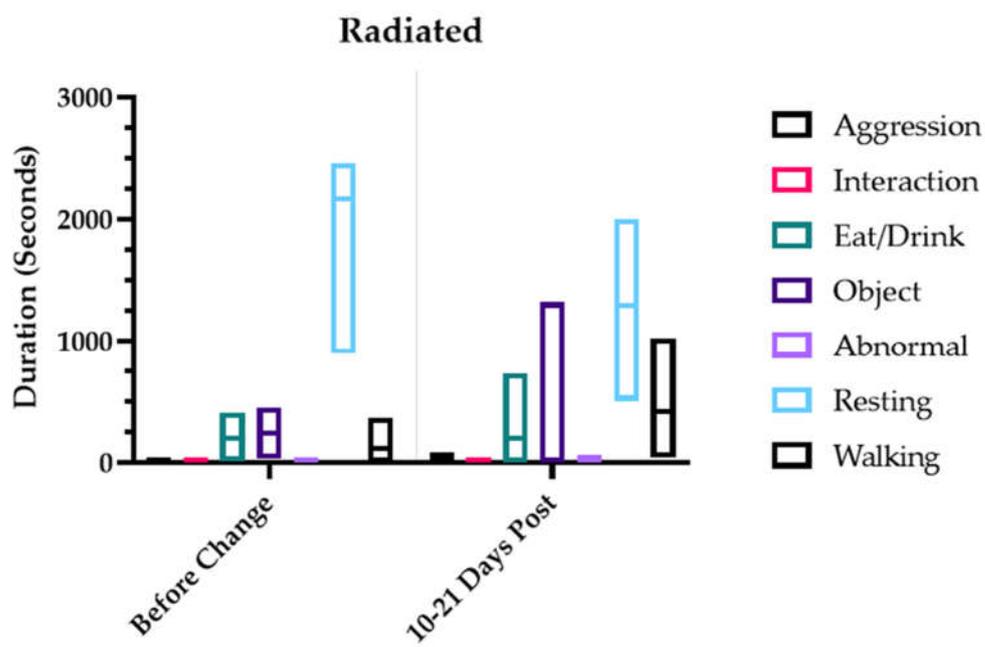
The behaviours aggression, co-occupant interaction, eating and drinking, resting, and walking were influenced by time of day. Specific differences for the combined groups are illustrated in Figure 3.



**Figure 3.** Bar Chart of all significant behaviours separated by time of day at three time points. Pairwise comparisons are indicated with a letter i.e., the letter 'A' over two bars indicates no difference between those time points. Letters 'A' and 'B' over two bars indicates that these are different from each other within the same time of day comparison i.e. morning data compared. "Interaction" refers to co-occupant interaction. Displaying mean and standard error.

### 3.1.3. Species and Time Point Interactions

In the radiated tortoises there were no changes in duration of the observed behaviours following the applied management change. There were differences in aggression, co-occupant interaction, eating and drinking, object interaction, resting and walking in the leopard tortoises. The Aldabra tortoises showed decreased co-occupant interaction and increased object interaction following the change. See Figure 4 for details of direction of effect and pairwise comparisons calculated using the Mann-Whitney/ Kruskal-Wallis test.

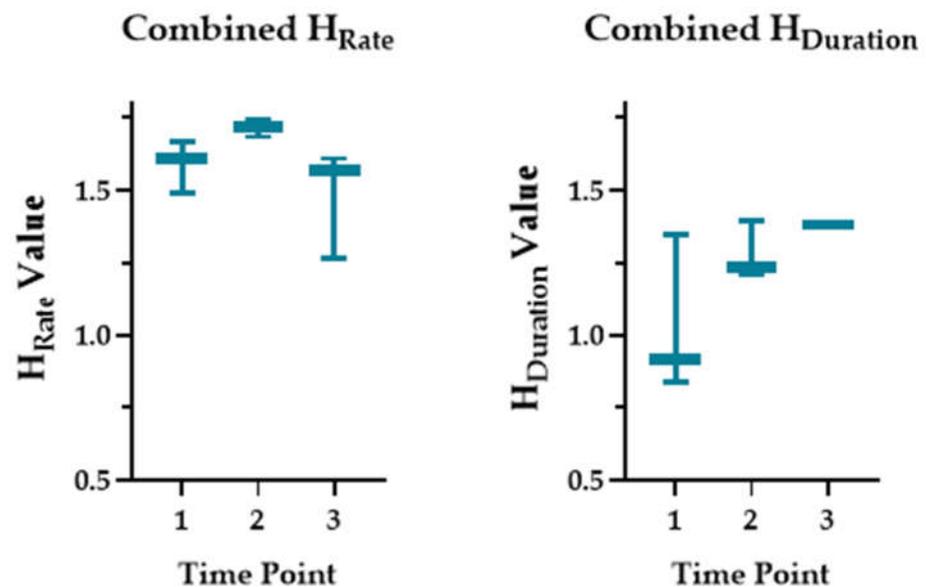


**Figure 4.** Box Plot of Behaviours Separated by Species at Three Time points: (1) Before Environmental Change, (2) 10-21 days Post Change, (3) 6 months Post Change. 'Interaction' refers to 'co-occupant interaction' and 'object' refers to 'object interaction'. Pairwise comparisons are indicated with a letter i.e., the letter 'A' over two timepoints indicates no difference between those time points for the same behaviour. Different letters indicate a difference between time points. Due to additional husbandry changes subsequent to the second time point, there is no third time point for radiated tortoises.

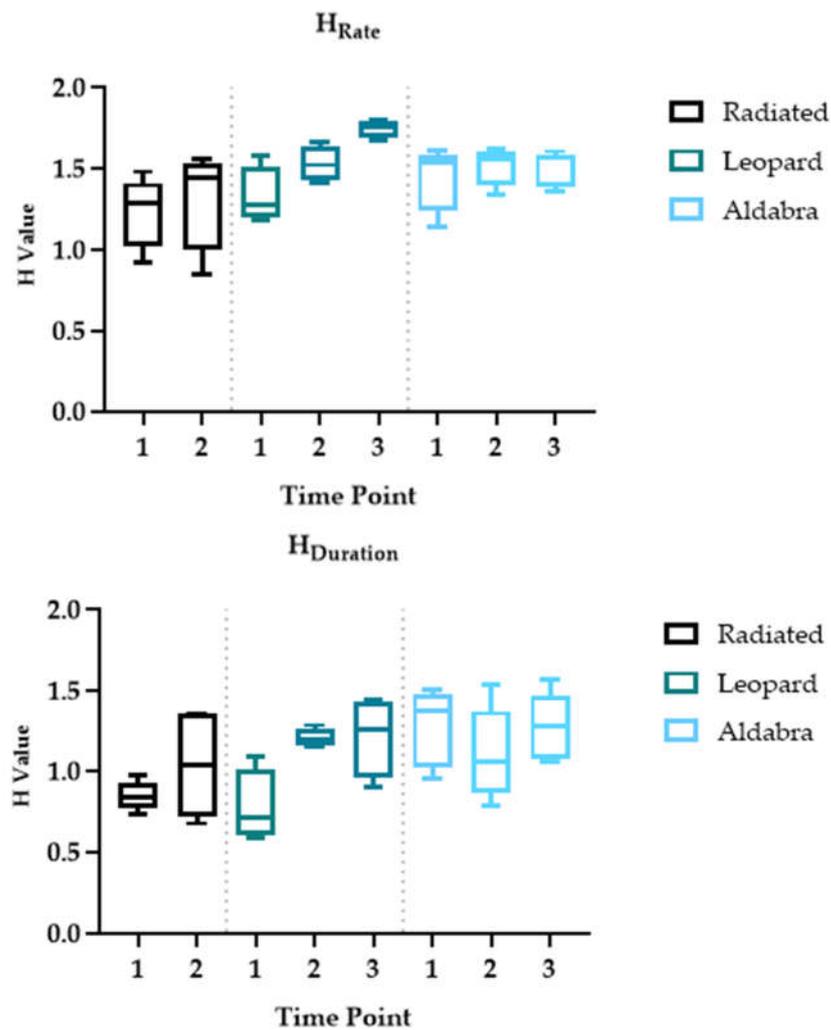
### 3.2. Diversity Index Results

Combined data for the species H values are detailed in Figure 5. The environmental modifications did not elicit a change in  $H_{Duration}$  (Wilcoxon signed rank:  $Z = -1.2136$ ,  $p = 0.55$ ) or  $H_{Rate}$  (Wilcoxon signed rank:  $Z = -0.6742$ ,  $p = 0.5476$ ) in the radiated tortoise population.

Similarly, there were no differences in  $H_{Duration}$  or  $H_{Rate}$  between the time points for the leopard tortoise population (Kruskal-Wallis:  $\chi^2(2) = 0.7$ ,  $p = 0.705$  and  $\chi^2(2) = 0.81$ ,  $p = 0.668$  respectively) or Aldabra population (Kruskal-Wallis:  $\chi^2(2) = 0.51$ ,  $p = 0.775$  and  $\chi^2(2) = 0.039$ ,  $p = 0.981$ ) (Figure 6).



**Figure 5.** Box plots of diversity index  $H_{Rate}$  (left) and  $H_{Duration}$  (right) for all species over three time points (1) Before environmental change, (2) 10-21 days post-change, (3) 230-250 days post-change. Graphs present median, minimum, and maximum value.



**Figure 6.** Diversity index box plots for  $H_{Rate}$  (top) and  $H_{Duration}$  (bottom) separated by species over time (1) Before environmental change, (2) 10-21 days post-change, (3) 230-250 days post-change. Due to additional husbandry changes subsequent to the second time point, there was no third time point for radiated tortoises.

#### 4. Discussion

In this study we report on the impact of environmental changes in three species of testudines based on behavioural data. The management changes did not lead to an overt improvement in welfare but did elicit some changes in individual behaviours that were scored as part of the ethogram. The behaviours where differences were seen included some indicative of positive affect. However, there was also an increase in behaviours that are likely to bring about negative welfare consequences, for example aggression.

A potentially negative behaviour co-occupant aggression, increased in the leopard group. Additionally, object interaction increased, while co-occupant interaction decreased following the management changes in the leopard and Aldabra groups, and these changes were maintained at the longer follow up time point. It could be that the resources introduced gave the tortoises something to compete over, or potentially upset the established social hierarchy. A recent study on tortoise aggression was able to identify a social hierarchical structure which was influenced by tortoise height and aggression levels [29]. There are many factors that have the potential to affect tortoise behaviour. Light and temperature affect the activity levels of tortoises. In summer they experience two daily peaks of activity, in the morning and afternoon [43-45]. In cooler winter conditions they have unimodal activity patterns and long periods of basking is required [45]. Tortoises

thermoregulate behaviourally by moving through areas with different temperature gradients and will restrict movement, seek water, and or seek shade to prevent heat stress [44,46,47]. Temperature may also affect other, non-thermoregulatory, behaviours as a trend of higher social behaviour and lower aggressive behaviour has been linked with higher body temperatures in radiated tortoises [48]. In the current study aggression levels for the leopard species sustained an increase over time, which was not associated with enclosure temperature. This suggests another factor may be influencing this behaviour, or that enclosure temperature may not be an accurate analogue of body temperature. Co-occupant interaction and resting were the only behaviours linked to temperature in the current study, with the latter most likely being a thermoregulatory behaviour. This suggests that during data collection the enclosure temperature remained stable enough to minimally impact behaviour outside of thermoregulatory behaviours. This is unsurprising as the temperatures were controlled at the zoological facility. The impact of the changed management strategy was most strongly seen at midday, with the most significant changes to behaviour seen in Figure 4. Whilst it could be assumed that this is due to temperature differences, this link was not supported.

Increased duration of walking with a decrease in resting in the leopard group could be interpreted as increased exploratory behaviour as a result of the increased space in the new enclosure. However, it would require spatial mapping to determine if this has arisen due to an increase in range traversed, or the making of more frequent smaller trips. Decreases to the behaviour eating and drinking in the leopard group suggest an adverse impact but should be interpreted with care. This may have arisen due to slight variability in management regimes. Whilst, tortoises were fed at approximately the same time each day, this could vary depending on the schedule of the keepers. Compounding this further the tortoises generally ate all their food within a 10-minute period. Unfortunately, this period did not always align with the two minute data collection window for that hour due to the above reasons.

To date the most common method of assessing the behavioural impacts of enrichment items or enclosure changes in reptiles has been ethogram use, to assist in recording changes in behaviour and to create an activity budget [21-23,26,33]. Additionally, there are no studies assessing the welfare implications of reduced space for tortoises, and conversely the welfare improvements to be gained by increasing space for tortoises. This was a novel aspect to the present study. Studies in other reptiles have shown that an increase in space increases both locomotive behaviour and space use [32,49] resulting in increased welfare. In a study on captive adult corn snakes, lower space allocations were found to negatively impact reptile welfare [50]. The snakes housed in larger enclosures were more active and spent 19% of their time fully elongated. Other than spontaneous behavioural observations a series of evoked behavioural tests were also performed to gauge welfare including the novel environment test, novel object test, reverse emergence test, and preference test, the results of which corroborated this finding [50].

In mammals various diversity indexes have been calculated. Generally, these have shown a reduction of behavioural diversity in less complex environments [51] and increased behavioural diversity with greater group size [31]. Time of day has also been shown to impact behavioural diversity [31].

There has been less study of diversity indexes in reptiles and consequently there is no established threshold for adequate behavioural diversity for reptiles in captivity [32,33], although a study on geckos yielded mean index values between 1 and 3, and were interpreted as adequate scores [33]. In the current study there were no differences in the diversity index across time points for any of the species. There was, however, a numerical increase in the score which was comparable with the scoring in the gecko study [33]. This may indicate a positive impact of management changes which perhaps would have attained statistical significance with a greater sample size, or a longer data collection period. Notwithstanding as discussed by Miller et. al., 2020, behavioral diversity indexes are influenced by the complexity of the ethogram used and have been argued to only be comparable when the same ethogram has been used [35]. In the current study, it is noteworthy

that the absolute value for diversity index was remarkably similar across the species after the changes were made, despite the difference in environments across the 3 groups. Yet the relative change in index from before to after the management change was greatest in the leopard tortoises where the most extensive management change occurred.

Whilst our results for the diversity index are inconclusive, there has been substantial recent discussion about the value of behavioural diversity as an indicator of welfare state [34,35,52,53]. Importantly, it may offer a method of gauging positive welfare states in captive reptile populations; a much sought-after goal in welfare science. Usefully it can be calculated using activity budget data as seen in the current study. A caveat attached to use of diversity indexes is that the score does not discriminate between positive and maladaptive behaviours hence the monitoring and knowledge of the types of behaviours being expressed is still required. Although, an increase in stereotypic behaviours typically results in lower diversity [34]. For example, in the current study an increase in aggressive behaviours was seen and this would normally be regarded as a behaviour likely to cause negative affect but may have contributed to an increased index score. Recording an ethogram simultaneously would allow the behavioural type (positive or maladaptive) and diversity to be tracked. Another important consideration is the choice of method used for calculating the index. In the current study there were differences in the index calculated for rate and duration of behaviours. This may be of little concern where a change is imposed and the outcome of interest is the difference in scores but will be more critical if the absolute number is used in decision making around animal welfare.

Limitations of this study include the small sample size due to animal availability. This may have resulted in non-significance of some of the behaviours due to inadequate power. Further, this study cannot be generalised to all reptiles due to variation in species-specific behaviour between taxa [20]. Given the behavioural biology of these species and their relatively ambling demeanour, future studies should consider increasing the length of behavioural sampling, perhaps to 5-10 minutes per animal. Many of the behaviours of interest, such as digging, an exploratory behaviour, occur relatively infrequently and it is possible that they were missed during the relatively small sampling window. Modification to the definition of 'interaction with transparent boundaries' (Table 1. abnormal behaviour) behaviour is also suggested to 'interaction with boundaries', as the tortoises often repeated the same motions on glass as they did on other walls (vertical digging action interspersed with walking). Due to the widely accepted definition of ITB that was included in this study this repetitive behaviour was not included in the abnormal behaviour category of the ethogram when observed. This means that the instances of walking could have been over-reported.

The optimal method for assessing animal welfare in zoological contexts is elusive, although it is envisaged that it will incorporate behavioural observations due to their non-invasive and non-resource intensive nature [54]. Behaviours associated with negative welfare states such as self-harm and abnormal behaviours can be easily observed. However, the more recent shift towards identifying indicators of positive welfare states creates many challenges; the challenge with the most potential for harm is incorrectly interpreting behaviours used to assess welfare. Since, incorrect interpretations allow us to perpetuate the same husbandry and management which is detrimental to welfare [54]. If behavioural diversity is the way forward for assessing reptile welfare, then further research is required. There is a need to establish species baselines, maladaptive behaviour monitoring, and standardise the methodology. Despite these issues the diversity index shows promise as an indicator of welfare at least when data are available before and after a change, in conjunction with ethogram data, to provide an objective measure of management change impact.

**Author Contributions** Conceptualization, Alexandra Whittaker, David McLelland and Jessica Turner; Methodology, Alexandra Whittaker, Jessica Turner and David McLelland; Zoos SA Liaison, David McLelland; Data Curation, Jessica Turner; Formal Analysis, Jessica Turner and Alexandra Whittaker; Writing - Draft Preparation, Jessica Turner; Writing - Review and Editing, Alexandra

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Whittaker, David McLelland and Jessica Turner; Supervision, Alexandra Whittaker and David McLelland; Funding Acquisition, Alexandra Whittaker; Project Administration, Alexandra Whittaker, David McLelland and Jessica Turner. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the NHMRC Code for the Care and Use of Animals for Scientific Purposes, and approved by the Animal Ethics Committee (AEC) of The University of Adelaide (protocol code S-2021\_036).

**Data Availability Statement:** The datasets collected and analysed during the current study are available from the corresponding author upon request.

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## Appendix A Enclosure Diagrams

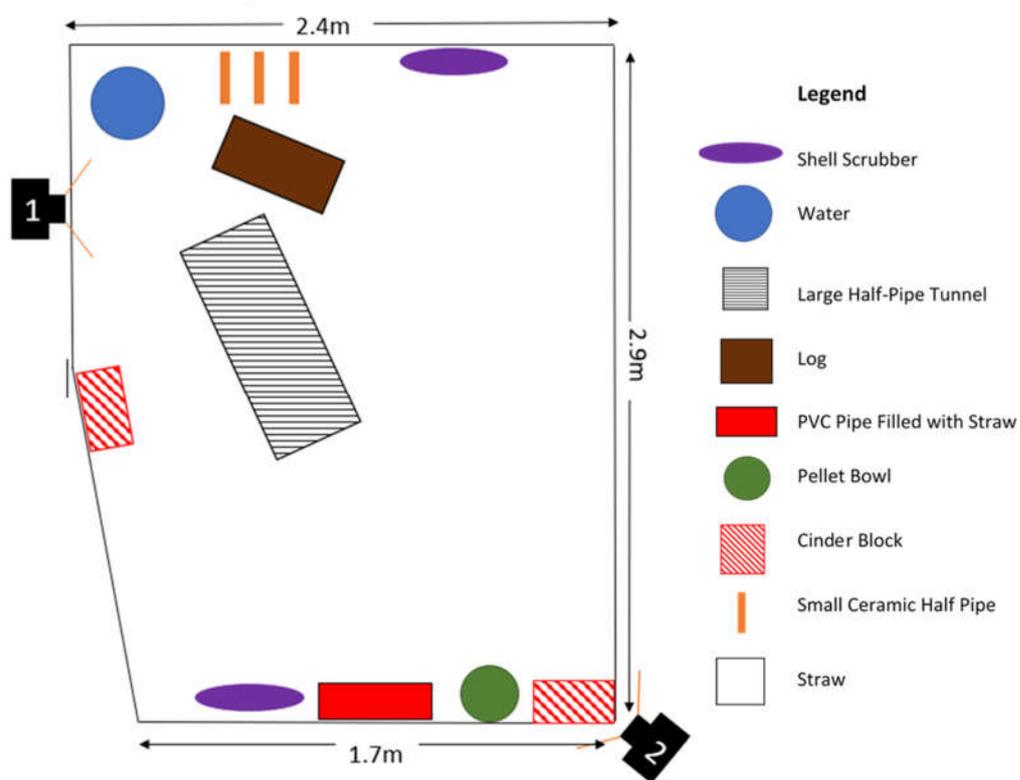
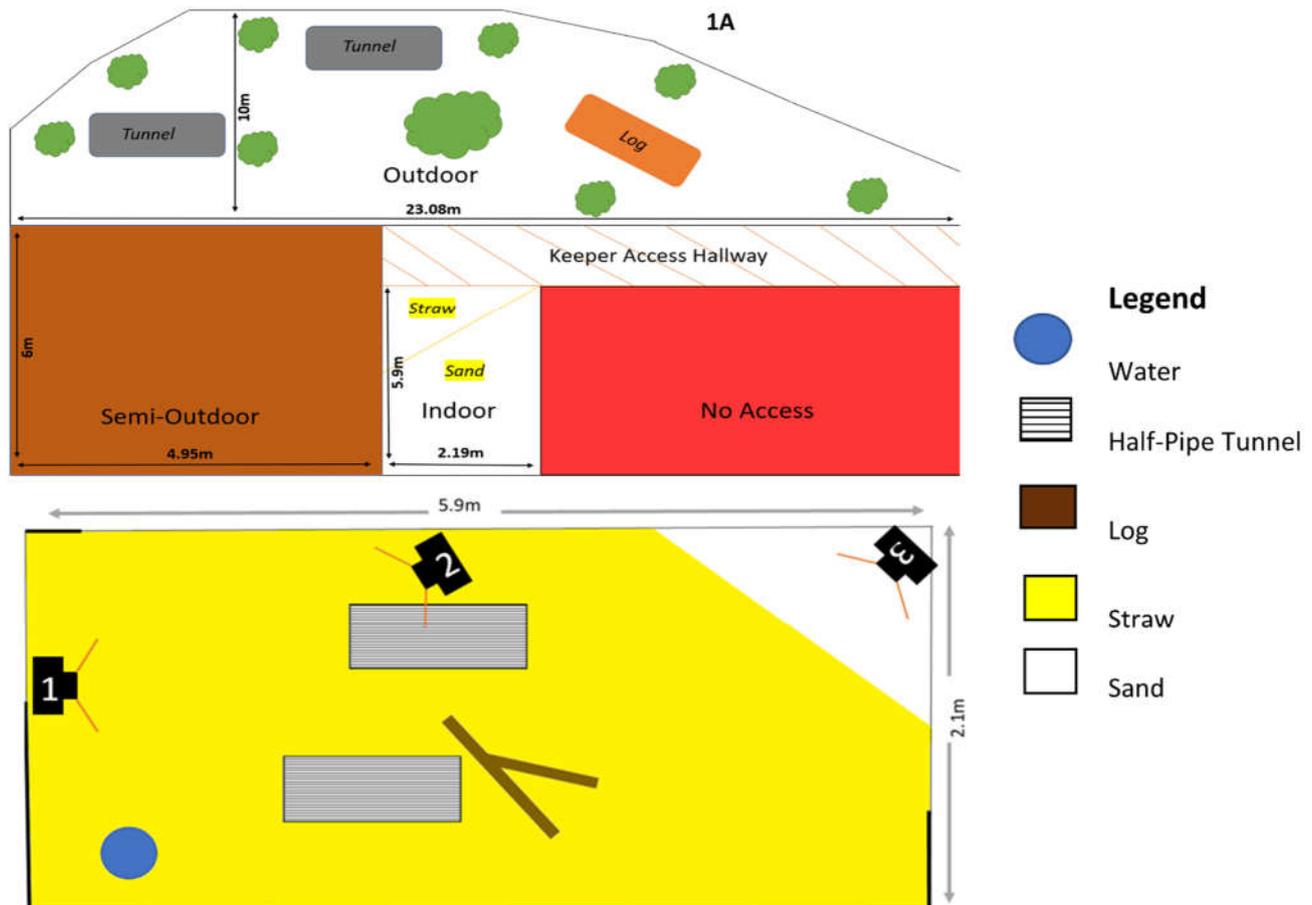
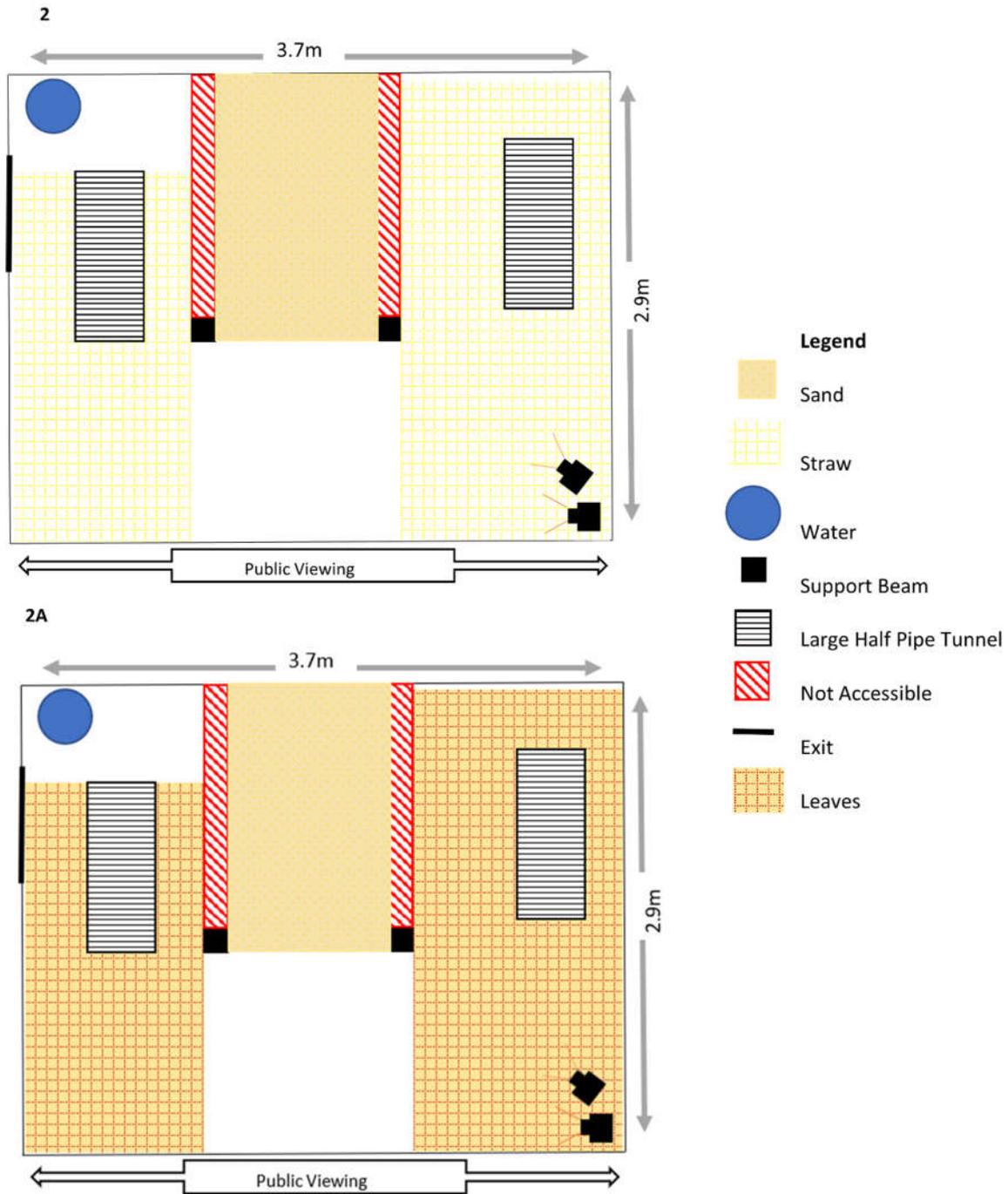


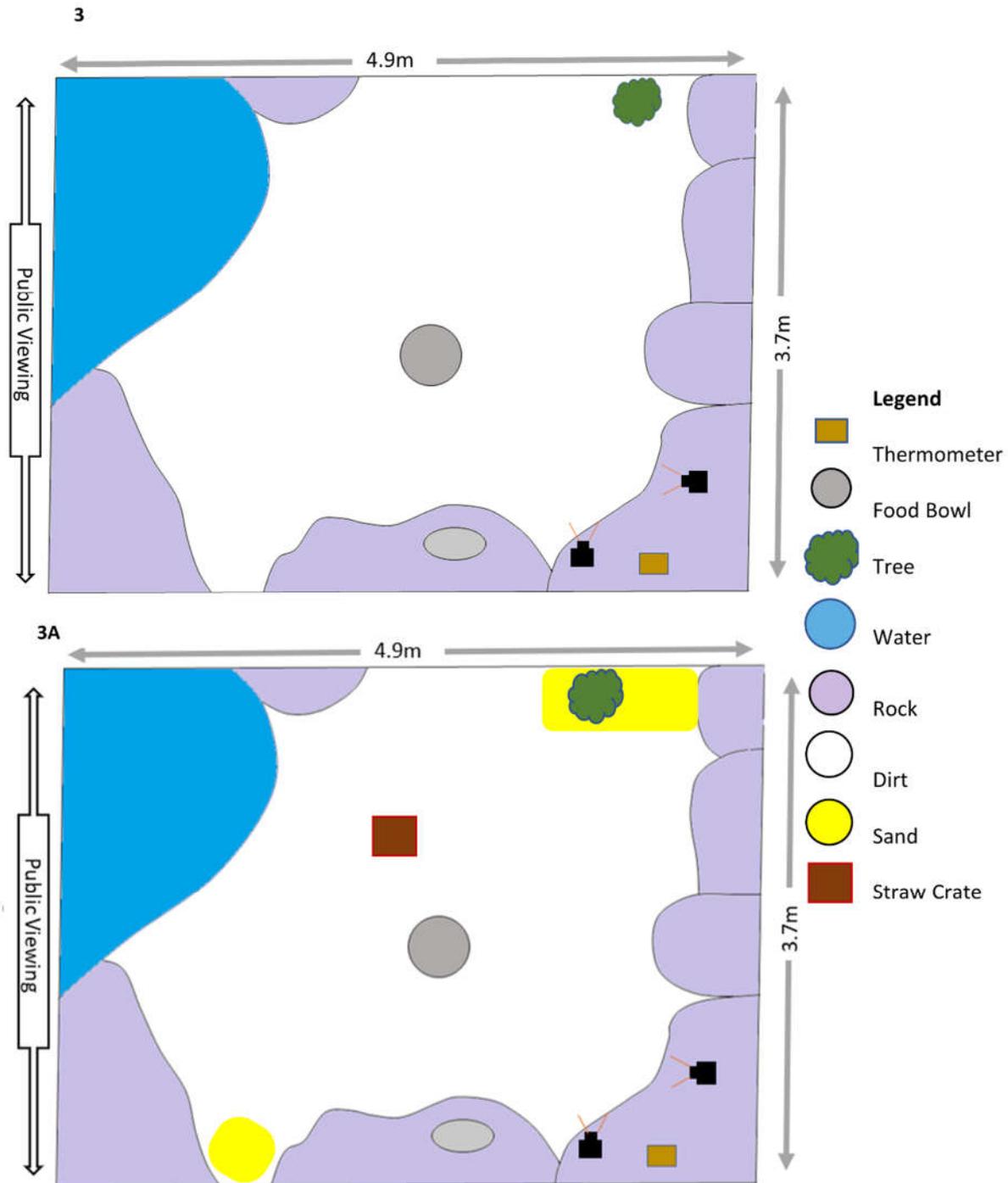
Figure A. Diagram of Enclosure 1 original enclosure for the leopard tortoises.



**Figure B.** Diagram of Enclosure 1A, the new enclosure for the leopard tortoises post change. Top is view of full enclosure, bottom is a detailed diagram of the indoor only section with camera placement.



**Figure C.** Diagram of Enclosure 2 & 2A. Enclosure 2 top is the unchanged enclosure for the radiated tortoises, enclosure 2A bottom is the environmentally changed enclosure for the radiated tortoises, with camera placement.



**Figure D.** Diagram of Enclosure 3 & 3A. Enclosure 3 top is the unchanged enclosure for the Aldabra tortoises, enclosure 3A bottom is the changes enclosure for the Aldabra tortoises, with camera placement.

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