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Article

An Improvement in Enclosure Design Can Positively Impact Welfare, Reduce Aggressiveness and Stabilise Hierarchy in Captive Galapagos Giant Tortoises

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Abstract: The interest in the welfare of zoo animals, from both the public and the scientific community, has long been biased towards mammals. However, growing evidence of the complex behavioural repertoires of less charismatic animals, such as reptiles, reveals the necessity to better comply with their welfare needs in captivity. Here, we present the effects of an enclosure change towards a more natural habitat in captive Galapagos tortoises (*Chelonoidis* spp.) held at ZSL London Zoo. Using behavioural observations, we found that the tortoises habituated to their new enclosure in six days. This represents the first quantification of habituation latency to a new enclosure in a reptile model to our knowledge—which is important information to adapt policies governing animal moves. The tortoises expressed time budgets more similar to those of wild individuals after their transition to the new enclosure. Interestingly, the hierarchy between the individuals was inverted and more stable after this change in environment. The tortoises interacted less often, which led to a decrease in the frequency of agonistic encounters. We also found that higher ambient sound volume was associated with increased likelihood of interactions turning into fights. Taken together, our results demonstrate the potential of appropriate enclosure design to improve reptile welfare.

Keywords: agonistic interactions; animal welfare; enclosure change; Galapagos tortoises; habituation; zoo-based management

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1. Introduction

Galapagos giant tortoises (*Chelonoidis* spp.) have a particularly intricate evolutionary history, with no clear consensus about the taxonomic rank of the 15 evolutionarily distinct units described so far [1–3], mainly because of human-driven hybridisations, extinctions and translocations that broke historic barriers between taxa, as well as a relatively recent divergence of forms [4,5]. Regardless of the division, the Galapagos giant tortoise complex is undoubtedly at risk of extinction in the wild [6]; three of the recognised evolutionarily distinct units are considered extinct, and the rest are all classified as threatened (vulnerable to critically endangered) with an estimated total of 35,035 tortoises left in the wild, all populations considered together [7]. The complex has suffered from overexploitation, as well as competition and predation from invasive species (at least 1579 alien species have been introduced to the Galapagos [8]). Poaching and habitat destruction have also been ongoing issues since the 1700s [9], with mass mortalities caused by poaching still occurring infrequently [10]. Climate change also presents a new threat to the group [11]. The coordinated conservation and restoration efforts of Galapagos tortoises sensu lato, i.e., encompassing all subspecies [5], began 60 years ago [7], and ex- and in-situ conservation eventually prevented these tortoises from becoming extinct thanks to the conjunction of a successful captive breeding and rearing program at the Charles Darwin Foundation [12,13] and 82 zoo collections around the world [14] alongside legal protections, habitat restoration, invasive species control and local community engagement [7].

However, the maintenance of Galapagos tortoises in captivity has been associated with issues that could undermine their welfare, such as abnormal growth patterns [15], impaired breeding [16] and deviation from natural behaviour [17]. High aggressivity in captive Galapagos tortoises was recently reported at ZSL London Zoo [18]. A large proportion of this aggression was shown to be caused by interactions with visitors in 'encounter experiences' that did not account for fixed action pattern behaviours and was reduced by the cessation of this activity. However, residual aggression remained at higher than natural levels ([18], authors pers. obs.). Agonistic behaviour is expected in both wild and captive Galapagos tortoises, but is most often observed between males, at low frequency (<1% of the time), and to express dominance [19]. Conversely, the hyperaggressive animals at ZSL were the females—they engaged in agonistic behaviours almost four times more often than is expected in males [18]. Although they have long been considered solitary, Galapagos tortoises form social groups of two to three tortoises in the wild [20], organised in linear hierarchies [19]. At ZSL, two of the three individuals were shown to share the same hierarchical rank, which was posited to contribute to the high occurrence of fights. Conflicts over resources were also proposed as a potential explanation for aggression in these animals [18]. Generally, in reptiles, high levels of aggressivity can be engendered by high population densities and competition for resources [21], courtship [22], seasonality [23], stress [24] or sub-optimal environments in captivity [25]. The latter context could be a manifestation of compromised welfare, as in many other taxa [26]. However, the literature on aggression being a result of sub-optimal captive environments is scarce for reptiles and few tools have even been developed for its evaluation [27,28].

Environmental enrichment, available space and resources, and natural settings contribute to the well-being of a wide range of animal taxa [29]. The three tortoises in question had grown substantially since arriving in their original enclosure at ZSL, so it was suspected that these were limiting factors for their welfare. Consequently, these individuals were moved to a new, bigger enclosure with environmental enrichments inspired by the Galapagos islands and the biology of wild tortoises (Figure S1; see Section 2 for a full description). The behavioural and environmental monitoring that accompanied the animals' move as a matter of institutional policy provided us with the opportunity to investigate whether this enclosure change influenced their behaviour, especially aggression and hierarchy. This information was essential to inform both ongoing management of focal animals, but also future husbandry and management decisions for captive reptiles more generally. For example, determining the duration of the habituation period to a new enclosure is crucial for zoos to be able to restrict the number of stimuli during that time, and thus reduce the general stress levels of their animals [30]. Yet, to our knowledge, no literature is available on the habituation length of any species to new enclosures. A standard two-week habituation period is often implemented at ZSL, but has no empirical foundation. Therefore, we measured the tortoises' latency to habituate to their new enclosure to provide an evidence base for determining future habituation periods. As a measure of welfare, we tested whether the tortoises had time-budgets closer to those recorded in the wild after the enclosure change [31]. We also monitored the interactions between the tortoises to determine whether the enclosure change was a successful strategy for lowering aggressiveness levels within the group, and if the hierarchy was modified by the enclosure change. Finally, we explored factors that could determine the outcome of an interaction. Indeed, little is known about the stimuli that drive interactions to become peaceful or agonistic. Overall, our results add to our understanding of the welfare of captive reptiles and of the social biology of Galapagos giant tortoises, and provide avenues to improve the ex-situ management protocols for Galapagos tortoises.

2. Materials and Methods

2.1. Study Subjects

We studied the three sibling 25-year-old female *Chelonoidis nigra* sensu lato, representing hybrids between island forms, from ZSL London Zoo (ZSL), UK, registered in the Species360 Zoological Information Management System (ZIMS) under the Global Accession Numbers MIG12-27565167 (Tortoise A), MIG12-27565168 (Tortoise B) and MIG12-29859386 (Tortoise C). The latter arrived at ZSL in 2009, three years before its conspecifics, and was previously identified as dominant, while the others shared an equal position within the hierarchy [18]. Each tortoise could easily be identified from the unique pattern of scuffs on its scutes and from existing colour marks [18].

2.2. Experimental Design and Data Acquisition

The behavioural repertoire and sequence of aggression in female Galapagos tortoises was described in detail in a previous article concerning the focal individuals for this study [18], and we used these to develop our ethogram (Table 1). Behavioural data were recorded with timelapse cameras (Plotwatcher Pro Day 6 Outdoors and Crenova PH760) covering most of the enclosure space and taking images every 30 s from 0700 h to 1900 h. Recording outside of this time range was unnecessary, as Galapagos giant tortoises remain inactive at night ([32], authors pers. obs.). All camera footage was obtained using camera traps, so the animals could be filmed without the presence of human camera-people affecting behaviour. Videos were watched using Gamefinder (Outdoor 6) and Windows Media Player: time spent expressing each behaviour in the ethogram, as well as the outcome of each interaction were manually recorded by a unique and trained observer in focal sampling.

Table 1. Ethogram used for the time-budgets and description of the possible nature of interactions.

State Behaviour	Definition	Classification
Eating	Consuming food or water	Active
Walking	Moving, in locomotion	
Water bathing	Being at least partially in the pond	
Mud bathing	Being at least partially in the mud wallow	Inactive
Heat bathing	Resting under a heat station	
Resting	Lying on the floor, stationary	
Out-of-sight	Not visible from any camera	
Interaction	**Two animals face each other, close enough to touch**	**Classification**
Peaceful	Both individuals keeping their head out at resting level, no biting attempt	Non-agonistic
Intimidation	One individual raising head higher than resting level, the other retracting head in submission, no biting attempt	Agonistic
Fight	Both individuals raising head higher than resting level and trying to bite each other	

The tortoises were initially filmed for five days in March 2021, while still living in the "old" enclosure where they had been kept since their arrival at ZSL (see details in [18]). This represented novel footage, as opposed to re-use of footage from Freeland et al. [18]. No visitors were present at the time, as the zoo was closed in the context of the COVID-lockdown, and (due to seasonally low external temperatures) the tortoises were confined to their indoor enclosure rather than having access to their outdoor paddock, which reflected the conditions of their care for the vast majority of the year, and matched the conditions used by Freeland et al. [18]. In October 2021, the tortoises were transferred into a larger enclosure, with vegetation structure, substrate, humidity and temperature modelled on those characterising the Galapagos islands, and were filmed for the 10 days following this transition (Table 2, Figure S1). Animals were moved to the new exhibit under operant conditioning (via target training) instead of manual restraint. For two weeks after the move, including the 10 days of filming, the "new" exhibit remained closed to the public, so that the tortoises could acclimatise to their new surroundings, although a small number of people were permitted access for relatively short periods of time for animal husbandry, maintenance and repair, and VIP viewing. The numbers and types of visitors, and their arrival and departure times, were recorded in a contemporaneous log. The volume of the noise in the new enclosure was recorded using a decibel-meter (XL2, NTiAudio) and averaged over five-minute intervals. The auditory system of Galapagos tortoises being poorly studied, we measured the ambient noise volume in Z-weighting (i.e., same weighting for all frequencies across the audio spectrum).

Table 2. Main differences between the old and new enclosures.

Characteristic	Old Enclosure	New Enclosure
Total area	~68 m^2	~350 m^2
Humidity	Not regulated	Regulated
Temperature	Not regulated	Regulated
Possibility to hide from visitors	No	Yes
Number of sand pits	0	1
Number of mud wallows	0	1
Number of heat stations	1	3
Number of water ponds	1	2
Live plants	No	Yes

2.3. Statistical Analyses

All analyses were conducted in the R environment 4.1.0 [33]. Graphical representations were carried out using the 'ggplot2' [34] and 'ggpubr' packages [35]. Our data and code are available at Git repository: https://github.com/LeaFieschiMeric/aggressivity_in_galapagos_tortoises.git (accessed on 10 August 2022).

2.3.1. Latency to Habituation

The duration of the habituation period to the new enclosure was determined through a change-point analysis, conducted separately for each tortoise. We approximated days of substantial changes in activity levels using binary segmentation. Because activity levels were relatively stable in the old enclosure, we considered the tortoises to be habituated after the last significant change in activity level in the new enclosure. This analysis was conducted using the 'changepoint' R package [36]. Our data complied with the assumptions of the test: the error structures were normally distributed and without autocorrelation.

2.3.2. Effect of the Enclosure Change on Time-Budgets and on Interactions

Time-budgets (i.e., proportion of time devoted to each activity in the animal's behavioural repertoire) and interactions were compared between enclosures once tortoises were habituated to their environment, thus only data recorded after the estimated habituation period was used for the new enclosure. Paired-samples t-tests were conducted to compare the average daily time spent expressing each behaviour in the old and new enclosures. The occurrence of each type of interaction was compared between enclosures using t-tests. Cohen's d was used as the effect size statistic. A Z-test was carried out to determine if the proportion of interactions resulting in fights was different between enclosures.

2.3.3. Rearrangement and Stability of the Hierarchy among Individuals

To determine whether the change in enclosure affected the pre-established hierarchy between the tortoises, Elo rating scores were computed using the 'Elorating' R package [37].

Briefly, the Elo system enables ranking of individuals according to their propensity to win agonistic interactions (recognised through the displacement or retraction of the head of the submissive individual) in the context of the same propensity of the losing individual. We used a starting value of 1000 and intimidating interactions were given more weight (k = 200) than fights (k = 100) to reflect stronger dominance when the submissive individual does not even engage in aggression (Table 1), and to be consistent with the previous study on that same population of tortoises [18]. We calculated estimates of Elo-ratings and of stability indices separately for both enclosures, and at intervals throughout the habituation period.

2.3.4. Determinants of the Outcome of an Interaction

We explored factors that could determine the outcome of an interaction: the presence of visitors, the ambient sound volume in the enclosure, the time of day, the proximity to food and other resources, and the identity of the individuals involved in the interaction. To this end, we used data from the new enclosure after the tortoises were habituated, as some of our explanatory variables of interest could not be recorded in the old enclosure. A full generalized linear model with a binomial error distribution and a logit link-function [38] was fitted with the outcome of the interaction (fight vs. peaceful or intimidation) as the response and the following fixed effects: the time of the day (morning, afternoon, evening), the number of visitors present in the enclosure within five minutes preceding the interaction, the average volume of ambient noise within five minutes preceding the interaction, the proximity to a resource (food, water pond, mud wallow, or heat station), the identity of individuals involved in the interaction and the date. Coefficients associated with each predictor were tested using Wald tests, and were considered significant if associated with a p-value below a 0.05 threshold. Odds ratios were used as effect size statistics.

3. Results

3.1. Individual Latency to Habituation

The control charts and change point analyses on individual activity levels suggest that the tortoises were all habituated to their new enclosure after six days (Figure 1). The average daily activity level of the habituated tortoises increased between the old (mean ± SD = 121 ± 40.3 min) and the new enclosures (mean ± SD = 227 ± 49.4 min).

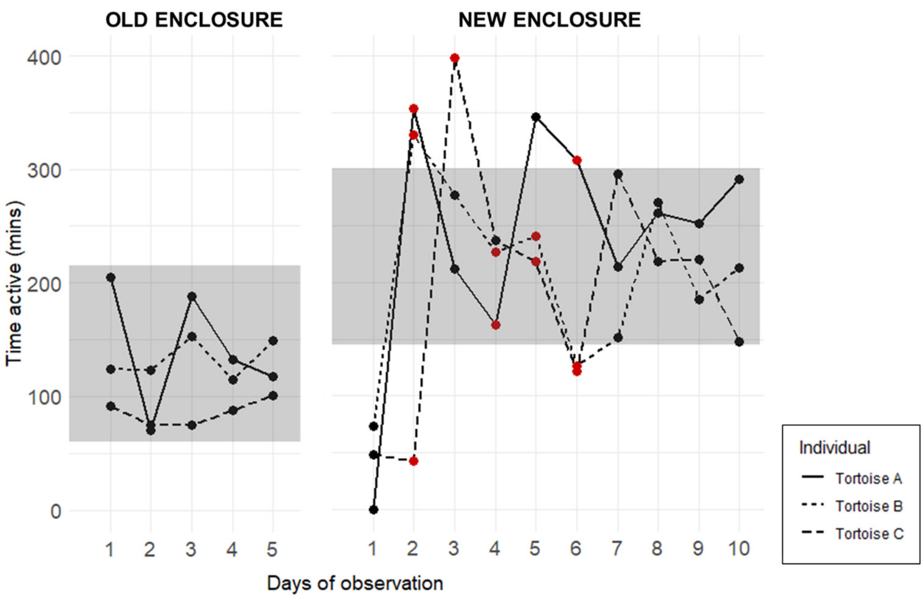

Figure 1. Individual control charts for activity levels in each enclosure. Days of substantial change in activity (identified by the change-point analysis) are coloured in red. The grey interval delimits the range of variation in individual responses in habituated individuals, for both the old and the new enclosures. All three tortoises were habituated to the new enclosure after six days.

3.2. Effect of Enclosure Change on Time-Budgets and Aggressivity in Habituated Tortoises

After the habituation period, the tortoises spent significantly more time active in the new enclosure compared to the old one (t = 5.839, p-value = 0.028), principally because they spent more time eating (Figure 2). They spent significantly less time resting on the ground (t = −24.190, p-value = 0.002) but significantly more time resting in the mud wallow (t = 4.696, p-value = 0.042), a resource that was not available in the indoor area of the old enclosure (Table 3). All individuals tended to spend more time heat-bathing and less time water-bathing in the new enclosure (Figure 2). The effect sizes indicate that the magnitude of the effect of the enclosure change was similar across the behaviours, except for eating (Table 3).

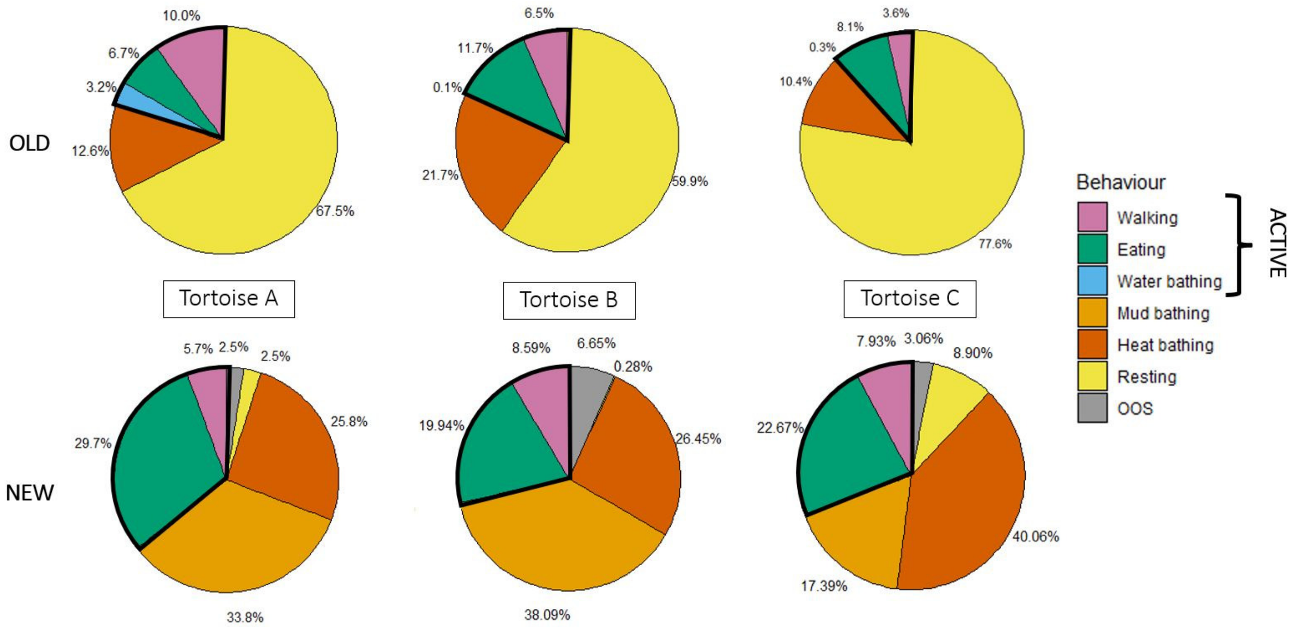

Figure 2. Pie charts representing daily time-budgets in the old (above) and the new (below) enclosures, in habituated individuals. The average proportion of the total observation time (0700 h to 1900 h) spent expressing each behaviour is indicated. The bold section encompasses "active" behaviours.

Table 3. Results from paired-samples t-tests, to determine the effect of the enclosure change on the average daily time spent expressing each behaviour (time-budgets).

Behaviour	t	df	p-Value	Cohen's d
Walking	0.269	2	0.813	0.313
Eating	3.592	2	0.069	1.682
Water bathing	−1.208	2	0.350	−1.000
Mud bathing	4.696	2	**0.042**	1.680
Heat bathing	2.187	2	0.160	1.494
Resting	−24.19	2	**0.002**	−1.799
Active	5.839	2	**0.028**	1.667
Inactive	−12.481	2	**0.006**	−1.753

Significant differences are indicated by bolded p-values. The effect sizes were calculated using Cohen's d formula: $d(x) = \frac{mean(x)_{NEW\ ENCLOSURE} - mean(x)_{OLD\ ENCLOSURE}}{sd(x)}$.

Once habituated, the tortoises tended to have fewer fights (Figure 3). However, this decrease in agonistic interactions resulted from the significant reduction in interactions in general (Table 4). In fact, the occurrence of peaceful interactions significantly decreased, and the proportion (as opposed to the absolute number) of agonistic interactions was significantly higher in the new enclosure (X = 9.822, p-value < 0.002).

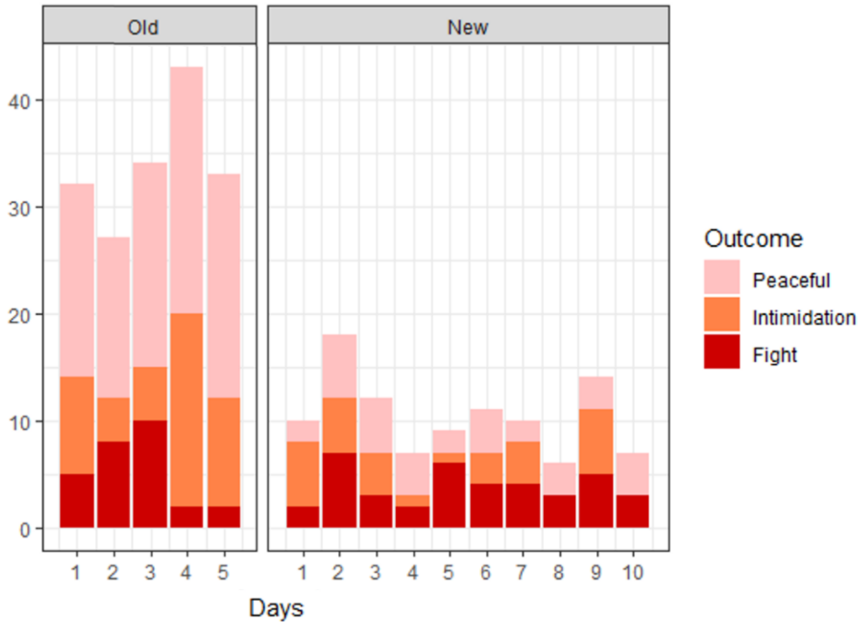

Figure 3. Barplot of the daily occurrence of interactions between tortoises, stacked by interaction type (see details in the ethogram, Table 1), in the old and the new enclosures.

Table 4. Results from unpaired *t*-tests conducted to investigate the effect of the enclosure change on the average daily occurrence of each type of interaction, in habituated individuals.

Interaction	*t*	df	*p*-Value	Cohen's *d*
Fight	−0.886	7	0.405	−0.602
Intimidation	−1.005	5	0.361	−0.840
Peaceful	−10.258	7	**<0.01**	−1.837
Total interactions	−7.350	7	**<0.01**	−1.136

Significant differences are indicated by bolded *p*-values. The effect sizes were calculated using Cohen's *d* formula: $d(x) = \frac{mean(x)_{NEW\ ENCLOSURE} - mean(x)_{OLD\ ENCLOSURE}}{sd(x)}$.

3.3. Dynamics of Variation in the Dominance Hierarchy

The dominance hierarchy was inverted by the enclosure change; the individuals with the lowest (Tortoise B) and the highest (Tortoise C) hierarchical ranks in the old enclosure, respectively, became the most and the least dominant in the new enclosure. Interestingly, the hierarchy gained stability over the enclosure change, although it was most stable during the habituation period. Once habituated to the enclosure (whether in the old or the new one), Tortoise A shared very close Elo-rating values with the most dominant individual (Figure 4, Table 5).

Table 5. Average Elo-ratings per individual, calculated for each period of interest: the old enclosure and the new enclosure during and after habituation.

Individual	Old Enclosure	New Enclosure [D0-D6]	New Enclosure [D7-D10]
Tortoise A	1062	991	1105
Tortoise B	773	1250	1129
Tortoise C	1165	759	766
Stability	0.250	0.923	0.667

The higher the Elo rating, the more dominant the individual. Intimidations were attributed twice as much weight as fights, because they were considered to result from a stronger dominance over the recipient tortoise (see details in the ethogram, Table 1).

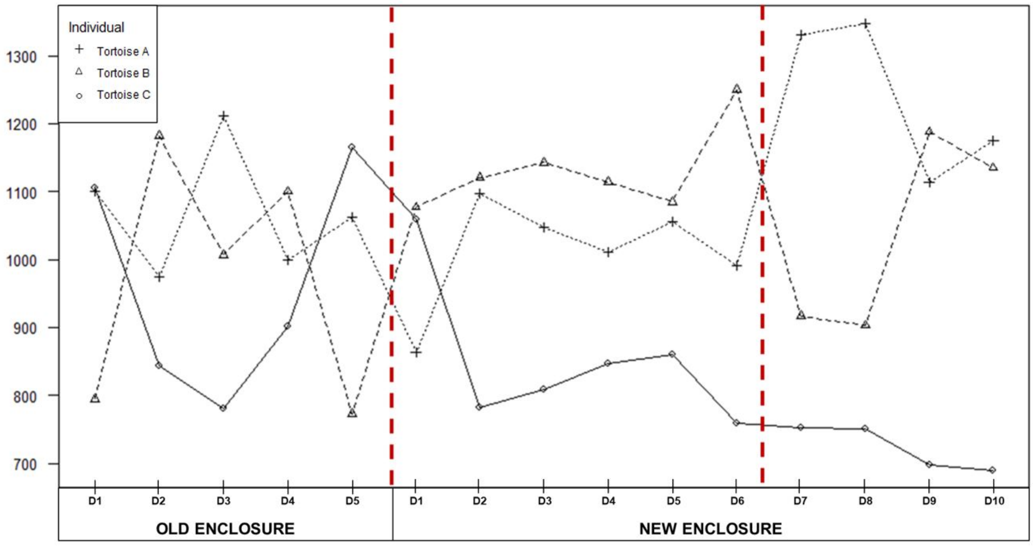

Figure 4. Elo ratings of the aggressive interactions between tortoises before and after the enclosure change. The higher the Elo rating, the more dominant the individual. Intimidations were attributed twice as much weight as fights, because they were considered to result from stronger dominance over the recipient (see Table 1). The first point for each line represents the rank differential that has been generated from one day of inter Elo-ratings per tortoise through time. The dotted vertical lines delimit the three periods for which average Elo-ratings were computed (see Table 5).

3.4. Determinants of the Outcome of an Interaction

Although the time of day, identity of the individuals involved, presence of visitors, proximity to a resource and date did not significantly determine the outcome of the interactions, the volume of the ambient noise did. Higher values of noise were associated with a higher likelihood of an interaction escalating into a fight. Although the odds-ratios suggest a strong relationship between the outcome of the interaction and the proximity to a resource, it should be considered carefully as most of the interactions happened in close proximity to a resource, which likely inflated this effect size. The rest of the odds-ratio indicate that the volume of ambient noise and the number of visitors affected the outcomes of the interactions with a similar magnitude (Table 6).

Table 6. Estimates of the coefficients associated with the potential determinants of the outcome of an interaction (fight vs. peaceful or intimidation), with their associated *z*- and *p*-values.

Fixed Effect	Estimate	*z*-Value	*p*-Value	Odds-Ratio [95%CI]
Intercept	−47.13	−0.019	0.985	
Daytime (Morning)	−0.647	−0.491	0.624	0.524 [0.040; 6.933]
Daytime (Mid-day)	0.006	0.007	0.995	0.994 [0.144; 6.852]
Pair (B–C)	−1.033	−0.977	0.328	0.356 [0.045; 2.826]
Pair (C–A)	−1.849	−1.443	0.149	0.157 [0.013; 1.942]
LZeq	0.425	1.970	**0.049**	1.530 [1.002; 2.336]
Visitors	0.172	0.842	0.400	1.188 [0.796; 1.772]
Resource	18.700	0.008	0.994	13.22×10^7 [0; Inf]
Date	−0.034	−0.074	0.941	0.967 [0.392; 2.381]

Odds-ratios are provided with their 95% confidence intervals as effect sizes. We tested the effect of the time of the day (daytime, contrasted against the late afternoon), the average volume of ambient noise within five mins preceding the interaction (LZeq), the total number of visitors in the enclosure within five mins preceding the interaction (Visitors), the identity of the individuals interacting (Pair, contrasted against the pair B–A) and the proximity to a resource on the outcome of each interaction in the new enclosure. The date could not be included as a random effect because of the low number of levels after habituation (four days) and was therefore included as a fixed effect.

4. Discussion

Determining the efficiency of enclosure modifications on animal welfare is critical for zoos and aquaria. Although substantial literature is available to inform enrichment and management strategies in mammals, and especially in primates, data are much more restricted in less traditionally charismatic classes such as amphibians or reptiles [39,40]. Here, our results indicate that after a short habituation window, welfare can be improved, and aggressiveness reduced when giant tortoises are moved to a more resource-rich, naturalistic enclosure.

4.1. Welfare Implications of Enclosure Switching

To offset the sub-optimal welfare resulting from the high homogeneity and predictability of the environments of captive animals, enrichments (i.e., the provision of specific stimuli necessary for their well-being) are widely used in zoos and livestock farming [41,42]. Although beneficial in the long term, these environmental modifications can also generate temporary stress subsequent to the change, as seen in a large number of taxa, from cheetahs to capuchin monkeys [43,44] but the duration of this period of increased sensitivity is rarely quantified (for an example, see Little and Sommer [45]). To improve the welfare of animals undergoing such a transition, zookeepers at ZSL restrict the number of potentially stressful stimuli (e.g., limit the number of visitors to have access to the enclosure) for a period (typically two weeks as a default) following an important environmental change, to reduce the general stress levels of their animals and avoid sensitisation from occurring instead of habituation. Here, we find that (for these individuals of this species at least and based on behaviour alone) this choice is conservative as all tortoises were habituated to their new enclosure after only six days according to their activity levels. Further study of more individuals and species, and ideally using additional markers of welfare, such as stress hormone analysis, is needed before more general recommendations can be made, and our results should not be used in isolation to determine habituation periods.

Animal welfare can be evaluated through a multitude of indicators, from blood cortisol measures to body condition scores [46]. Here, we used a non-invasive and low-cost (other than time spent encoding footage) method that has already been applied in many animal taxa to compare the time invested in each behaviour with the time-budgets measured in wild individuals [47–49]. Wild Galapagos tortoises are reported to spend 40% of their daylight hours resting, 20% feeding, 10% moving, and less than 5% drinking [31]. Our results demonstrate that after the tortoises were transferred to their new enclosure, their time-budgets became closer to those of wild individuals, thus indicating that their welfare improved: once habituated, they spent 24% of their time feeding (vs. 8% in the old enclosure) and 7.4% of their time walking (vs. 6.7% in the old enclosure). Resting was harder to evaluate in our case because the tortoises spent time resting in a resource that was not available in the indoor area of their old enclosure (the mud wallow), so this measure is not valid for comparison. Interestingly, transfer to a new enclosure has various effects in other species: while it leads to increased locomotion in langurs [45], changes in enclosure decrease locomotion in Amur tigers [50] and have individual-dependent effects in pandas [51]. This shows the importance of the comparison to species–specific time-budgets from wild conspecifics when evaluating the welfare of captive animals.

4.2. Successful Reduction in the Occurrence of Agonistic Interactions

In addition to the general welfare improvement, the change in enclosure was associated with a slight reduction in the total number of aggressive interactions, showing that the strategy adopted by ZSL was successful in reducing fights, as originally intended. Interestingly, this decrease resulted from a significant reduction in the total occurrence of interactions; the proportion of agonistic encounters actually increased in the new enclosure. Indeed, with the new enclosure being much larger than the old one and containing duplicates of resource types, the tortoises have more opportunity to isolate from conspecifics. It is therefore probable that they avoid interaction when they can, and mostly interact when they need to settle a dispute, thus through aggression. Freeland et al. [18] identified a series of posturing behaviours that occur before physical aggression and often lead to a non-violent resolution of disputes. The present findings strongly suggest that given the opportunity, giant tortoises will avoid any interaction in preference to even using these posturing behaviours; it is also possible that more subtle posturing exists that has not yet been identified. The reduction in the number of aggressive interactions is the most important metric for welfare here, and it is important to highlight that low levels of agonistic interactions are part of the natural social repertoire of this species. Thus, complete elimination of this behaviour should not be a goal.

The residual fighting interactions in the new enclosure are likely associated with the reorganisation of the hierarchy within the group. Indeed, aggressiveness is a behaviour involved in the establishment of dominant relationships and is commonly increased until a stable social structure is established [52]. Although the hierarchy was more stable in the new enclosure compared to the old one, it still fluctuated after the habituation period. The fluctuation in the social structure of habituated individuals was likely caused by their proximity in hierarchical positions. Indeed, while social structures usually consist of vertical hierarchies in both wild [32] and captive [19,53] giant tortoises (but see [19] for an exception), in our group, the two most dominant individuals had very close hierarchical ranks compared to the least dominant individual, in both the old and the new enclosures. Moreover, the identity of these more dominant individuals varied, and the hierarchy was inverted when the enclosure was changed: while one individual (Tortoise A) remained the second-most dominant across all enclosures, the historically most dominant individual became least dominant in the new enclosure (Tortoise C) [18]. This latter individual had arrived in the old enclosure at ZSL three years before the two others, but during the 2021 move under operant conditioning, it arrived last in the new enclosure. Because hierarchies often emerge in captivity in territorial species [54], and Galapagos tortoises have a long-term memory [55], the establishment of their hierarchy could originate from territoriality. The enclosure change could have re-set the hierarchy associated with the old enclosure, thus permitting the establishment of this new hierarchy based on the order of arrival of the tortoises. Clearly, Galapagos tortoises have a complex hierarchy and range of sociability [52], and much work is still needed to understand the dynamics of hierarchy establishment in both wild and captive Galapagos giant tortoises [32]. Our study was limited in time and only represented a snapshot of the effect of the enclosure change on our three individuals—it is possible that a more vertical hierarchy was established later.

4.3. Drivers of Aggressions and Audition in Giant Tortoises

Characterising the factors that lead interactions between individuals to escalate into fights is essential for adapting enclosure design and husbandry protocols to avoid injuries in captive animals. Although phenotypic traits such as shell coloration and age are associated with aggressive personalities in tortoises of various species [56,57], the individuals studied here were siblings, and thus had limited physical differences. Most of the factors we had hypothesised to influence the outcome of the interactions within our group of tortoises did not have a significant effect. Nevertheless, this lack of significance could simply result from our limited sample size, and the tendencies associated with each of the factors tested were consistent with our initial hypotheses.

In keeping with the growing evidence that reptiles have personalities [58,59], one pair of individuals (Tortoise A and Tortoise B) tended to have more agonistic interactions than pairs involving the third individual (Tortoise C). This latter tortoise was of lower hierarchical rank in the new enclosure, thus less likely to initiate fights and more likely to be submissive when being the recipient of an aggression. In contrast, the two other individuals shared close, dominant hierarchical ranks in the new enclosure: their increased likelihood of interactions escalating into fights may have resulted from a competition to become the most dominant.

Interactions occurring in close proximity to a resource, such as food, heat stations, water ponds or the mud wallow, tended to escalate more into fights, as suggested by Freeland et al. [18]. This likely results from "resource-guarding", i.e., agonistic behaviour to retain control of food or non-food items in the presence of another animal [60]. The number of resources was increased in the new enclosure compared to the old one, and each resource could largely accommodate all tortoises at the same time. Therefore, our results suggest that despite an excess of resources, tortoises tend to guard them aggressively from their conspecifics.

Interactions happening in the morning were less prone to escalate in a fight, in keeping with a study that reported thrice as many aggressive interactions in the afternoon in Galapagos tortoises [19]. Subtle management of circadian rhythms and of internal clock systems is essential for animal welfare in captivity [30] and might therefore be a new avenue for reducing aggressivity.

Noise generated by visitors is known to affect the behaviour of captive mammals [61], but no literature is available on its effect on reptiles to our knowledge. Here, we found that a higher noise volume significantly increased the likelihood of interactions escalating into fights. Indeed, loud ambient noise may reduce tortoise welfare, thus leading to aggression, or impair intra-specific communication channels otherwise used to mitigate interactions [62]. The effect of the number of visitors on the outcome of interactions was not significant, but this likely resulted solely from the low number of visitors allowed during the period of our study. Indeed, odds-ratios indicate that visitors had an effect of a similar magnitude to that of the noise volume on the outcome of the interactions. Visitors entering the enclosure had previously been shown to increase aggression in our tortoises [18] and even visitors remaining outside of enclosures are generally known to affect the behaviour of captive animals [26,63]. However, Freeland et al. [18] showed that the visitor effect was strongly tied to physical interaction with the animals, and that the presence of humans that did not engage in physical interaction with the animals was not nearly so associated with aggression.

5. Conclusions

This study represents a snapshot of the effects of an enclosure change on the behaviour of Galapagos tortoises. A longer-term evaluation of the efficiency of this strategy is needed, and more research should be conducted to identify the determinants of aggressiveness and of hierarchical structures in these threatened animals. However, even in this short timeframe, we found that the change in enclosure was successful in increasing the welfare of the tortoises and in reducing the occurrence of fights, and had important implications on the social dynamics of the group. Therefore, our results add to the evidence that enclosure modifications have the potential to improve individual welfare in reptiles, and provide useful insights for their management in captivity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jzbg3040037/s1, Figure S1: Design of the old (A) and new (B) enclosures.

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