Marwell Wildlife

UNIVERSITY OF Southampton

Behaviour and condition of individual sand lizards (Lacerta agilis) as predictors of long-term reintroduction success

A technical report prepared from an MRes Wildlife Conservation project, Marwell Wildlife & University of Southampton

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ABSTRACT

Anthropogenic activities are threatening global biodiversity and resulting in the need for conservation interventions. Comprehensive assessment of these interventions is often hindered by a lack of understanding of the focal species' behaviour. This study used sand lizards (*Lacerta agilis*) as a model species to investigate behavioural variation within a single population and determine the requirements for reintroduction success and population stability. This was achieved by profiling individual behaviour, social dynamics and fitness through behavioural observations and measurements of morphological traits and locomotor performance.

The study found marked behavioural variation within the captive population, indicating that individuals within the group consistently differed in their behaviour. Contrary to predictions, morphological traits had minimal influence on the behaviour and locomotor performance of sand lizards. Therefore, individual success and survival cannot be attributed to phenotypic variance in physical characteristics alone, but rather due to a complex interaction between an individual's personality, genetics and the environmental context. Maintenance of behavioural variation within captive and wild populations is vital to reintroduction success. Behavioural variation ensures population resilience and persistence through the 'portfolio' and 'insurance' effects that increase a population's adaptability to changing environments.

Overall, this study highlights the importance of integrating behavioural ecology with conservation biology when conducting and assessing conservation interventions. Further studies are required to assess the long-term consequences of personality in sand lizards to expand current knowledge of the drivers of individual reintroduction success.

***Key words**: Behavioural variation; animal personality; social dynamics; individual fitness; conservation biology; behavioural ecology; species reintroductions*

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Sand lizard (*Lacerta agilis*)

Figure i Juvenile climbing on some vegetation at Marwell Zoo, Hampshire
(Source: Author's own)

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ABBREVIATIONS

SVL – Snout-to-vent length
BCS – Body condition score
GLZM.NB – Negative binomial generalised linear model
MAM – Minimum adequate model
PCA – Principle components analysis
PC – Principle component (results derived from PCA)
DI – Dominance index

Total word count = 10,606

1. INTRODUCTION

1.1. Biodiversity conservation

Recent calculations indicate that biodiversity has fallen below ‘safe levels’ across more than half of the world’s land (Newbold *et al*., 2016). Declines of species populations across all taxa threatens the delicate balances within ecosystems, disrupting vital ecosystem processes and services (Díaz *et al*., 2006; Şekercioğlu *et al*., 2004). According to the largest assessment to date, nearly one-fifth of all reptile species are threatened with extinction (Cox and Temple, 2009). This stark assessment highlights the need for conservation interventions for the often-overlooked reptile species (Böhm *et al*., 2013). According to Burns *et al*., (2016), anthropogenic activities including habitat alteration and climate change are central to global biodiversity declines. Conservation biology aims to address biodiversity loss by evaluating the impact of human activities and developing practical solutions worldwide (Gerber, 2011). These interventions can include conservation initiatives such as captive breeding, species reintroductions and habitat management (Soorae, 2011).

1.2. Species reintroductions

Species reintroductions offer a promising conservation tool to mitigate the population declines seen across many taxa (Soorae, 2016). Reintroduction is a type of translocation whereby organisms are released to an area within their indigenous range, from which they have disappeared (IUCN and SSC, 2013). Evaluation of previous or ongoing programmes is essential for the adaptive management and continuous improvement of procedures (Sutherland *et al*., 2004). However, these assessments are often problematic and require long-term commitment and a detailed knowledge of species life history (Canessa *et al*., 2016). The diversity of single families can extend over taxonomy, ecology, behaviour and reproductive mode, creating a need for species-specific research when conducting and evaluating conservation interventions (Vitt and Caldwell, 2014). Santos *et al*., (2009) suggests that understanding the focal species’ behaviour is imperative to ensuring the greatest possible success of reintroduction initiatives.

1.3. Behavioural ecology

Behavioural ecology combines the study of animal behaviour with evolutionary biology, population ecology and physiology in order to study the fitness consequences of behaviour and gain an understanding of the evolutionary basis behind behaviour (Birkhead and Monaghan, 2010).

1.3.1. Behavioural syndromes

Individuals within a species or population often consistently differ in their behaviour. Correlated behaviours are defined as 'behavioural syndromes' and form an animal's personality (Sih *et al.*, 2004; Bell, 2007). Behavioural syndromes are heritable and incur life history consequences; making them subject to evolutionary processes and trade-offs (Smith and Blumstein, 2008; Sih *et al.*, 2010). For example, individuals who are generally more aggressive may succeed in situations where aggression is favoured, but be maladapted to situations where low aggression is favoured (Sih *et al.*, 2004). Likewise, the perceived benefits of monopolising ecological resources through dominance and boldness come at the cost of increased injury and predation risk (Taylor and Lattanzio, 2016).

A consistent personality indicates an individual has reduced capacity for behavioural plasticity in response to immediate circumstances. This reduced plasticity explains the preservation of between-individual variation alongside consistent individual responses in a variable environment, accounting for individual deviations from optimal behaviour (Sih *et al.*, 2012; Bergmüller, 2010). The 'ecological approach' to behavioural syndrome research seeks to provide explanations for individual variation in fitness related traits by identifying correlated behaviours (Bell, 2007). Behavioural syndrome research provides an integrative approach to studying animal behaviour by taking the causes and effects of individual variation in behaviour into account (Bergmüller, 2010).

1.3.2. Individual variation in reptile behaviour and social dynamics

To date, the understanding of how individual variation in behaviour effects fitness traits in reptiles is limited to a few studies and anecdotal evidence. Individual variation in behaviour can alter the outcomes of social interactions and subsequently the social dynamics within a group. For instance, differing social dominance can result in competitive interactions whereby only the most dominant individuals are successful in defending high quality ecological resources and mates (Carpenter, 1995; Taylor and Lattanzio, 2016).

House *et al.*, (1980) observed wild sand lizards (*Lacerta agilis*) and common lizards (*Zootoca vivipara*) in temporary outdoor enclosures. The study found higher social interference between sand lizard conspecifics resulting in fewer sand lizards being able to utilise optimal basking areas at any one time. Whilst the study allowed an acclimatisation period to captive conditions and anecdotally found no ill effects, this was never quantifiably assessed. The study also failed to include individual age in analysis, a limitation as behaviour has since been found to alter between life stages (Fearnley, 2009). Nevertheless, the results of the study by House *et al.*, (1980) provide some evidence for the role of social dynamics and individual behaviour in determining an individual's fitness and ultimately individual survival and population sustainability. Further exploration of the mechanisms behind individual fitness would benefit conservation initiatives worldwide.

1.3.3. Personality and conservation

There is a growing appreciation for the potential conservation benefits of integrating behavioural ecology and conservation biology. Sutherland (1998) recognised the importance of behavioural studies in 20 areas of conservation biology, including dispersal in fragmented populations, release schemes and captive breeding.

Personality can affect evolutionary and ecological processes. For example, personality-dependent dispersal can enable the successful colonisation of a species, affecting the population's persistence post-reintroduction (Michelangeli *et al.*, 2017). The variation of behavioural tendencies between dispersers and non-dispersers of a reintroduced population would influence the composition of sub-populations across the reintroduction site and subsequently affect colonisation success (Michelangeli *et al.*, 2017). In their study, Michelangeli *et al.*, (2017) found that dispersal tendency in the delicate skink (*Lampropholis delicata*) was associated with aggression, thought to be due to aggressive individuals forcing subordinates into hiding and thus restricting their ability to disperse. Dispersal in common lizards (*Zootoca vivipara*) is influenced by population density, with social individuals dispersing when population densities are low (Cote and Clobert, 2007).

Similarly, although empirical studies are scarce, populations harbouring multiple different behavioural types are expected to be less vulnerable to environmental change (Wolf and Weissing, 2012). This resilience and persistence is explained by the 'portfolio' and ‘insurance’ effects. In other words, behaviourally diverse populations are more likely to harbour a variety of personality types adapted to cope with a novel environment, leading to less extreme responses and population persistence (Schindler *et al.*, 2010; Wolf and Weissing, 2012). On an individual level, behaviour modulates interactions with conspecifics and the environment and thus plays an essential role in individual survival and reproductive success (Schlaepfer *et al.*, 2010). Conservation interventions, such as species reintroductions are therefore more likely to be successful if the target species or population’s behavioural ecology is considered (Schlaepfer *et al.*, 2010).

1.4. Individual fitness as potential indicators of reintroduction success

At present, reptile captive breeding and release programmes are managed on the assumption that bigger juveniles are fitter and therefore more likely to survive post-release. Offspring quality is commonly assessed by fitness proxies of size, growth and locomotor performance (Chapple *et al.*, 2004; Warner and Shine, 2007; Connolly and Cree, 2008; Hare *et al.*, 2012). The evidence behind this assumption in Squamates is limited and context dependent, whereby larger body size increases chance of survival in some scenarios and not others (Warner and Shine, 2007).

Locomotor performance is an important fitness proxy measure in lizards as it has been shown to affect foraging efficiency, predator avoidance and mating ability (Vanhooydonck and Van Damme, 2003; Husak *et al.*, 2007; Ekner-Grzyb *et al.*, 2013). Ekner-Grzyb *et al.*, (2013) found no significant effect of snout-vent-length on speed in their study of 142 individual sand lizards, supporting the notion of a complex relationship between size and fitness. The effect of other morphological traits on locomotor performance has not yet been investigated.

A five-year field study by Olsson, (1993) showed that natural near-equal sex ratios of sand lizards creates contest competition for females, with males exhibiting ritualistic displays that often escalate into physical aggression. Subsequent investigations found that larger males had more beneficial social interactions and higher rates of reproductive success (Olsson and Shine, 1996). In contrast, Metcalfe and Monaghan, (2001) state that high early growth rates increase an individual’s susceptibility to nutritional stress in a variety of taxa. Correspondingly, Olsson and Shine, (2002) assessed the long-term effects of manipulating growth rates of neonatal Tasmanian snow skinks (*Niveoscincus microlepidotus*) and concluded that high growth rates had a negative impact on longevity over a four year period. These studies provide evidence of the need for husbandry practices to enable appropriate development, in order to exploit the perceived benefits of larger body size without succumbing to the negative consequences of high growth rates.

1.5. The sand lizard (*Lacerta agilis*)

The sand lizard is a conservation priority in the UK due to declining numbers owing to habitat loss and fragmentation (Langham, 2008). Whilst classified as least concern on the IUCN Red List due to their widespread distribution across Europe and northwest Asia (Figure 1), the sand lizard is regionally rare within parts of its range (Agasyan *et al*., 2010; Andres *et al*., 2014).

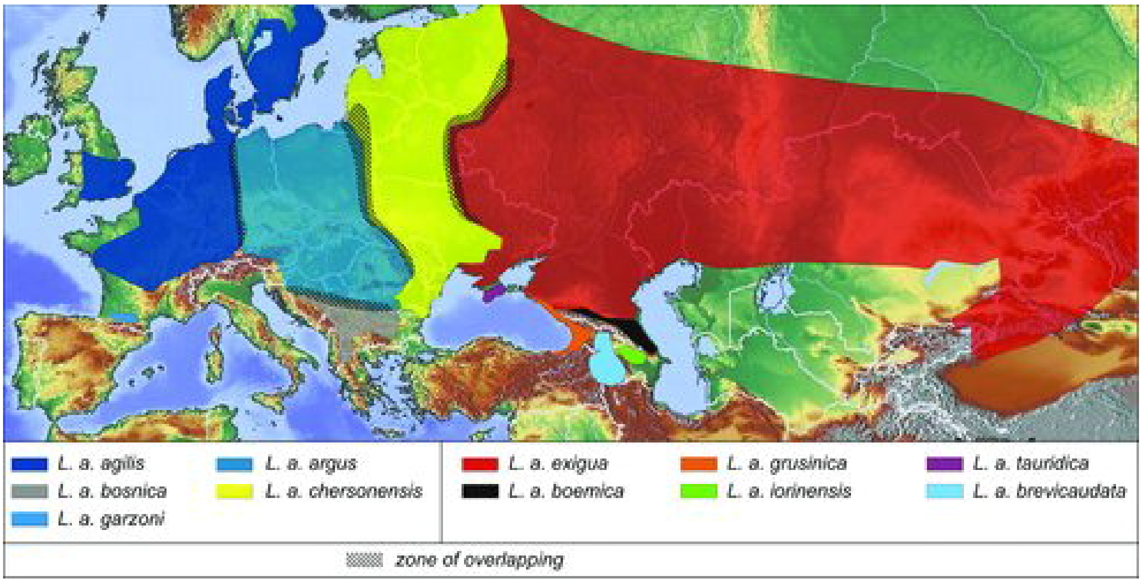

Figure 1 Distribution of sand lizard subspecies across Europe and northwest Asia (Andres *et al*., 2014)

The sand lizard is one of Britain's rarest reptiles, with populations limited to a few fragmented heathland and coastal dune systems in southern and north western regions, as shown in Figure 2 (Agasyan *et al*., 2010; National Biodiversity Network, 2012). Habitat loss and fragmentation has isolated the British populations, creating three identified 'races', the Wealdon (Surrey) race, the Dorset race and the Sefton (Merseyside) race (Langham, 2008). The relatively small home ranges of 41 to 1,396 m^2 for males and 51 to 600 m^2 for females restricts movement between fragmented patches and populations. Dispersal is therefore dependent on human-mediated connectivity (Nicholson, 1980; Woodfine *et al*., 2017). Sand lizards are a European Protected Species, protected under Schedule 5 of the Wildlife and

Countryside Act (1981) and schedule 2 of the Conservation of Habitats and Species Regulations (2010) (Langham, 2008; Fearnley, 2009; Moulton *et al*., 2011).

Figure 2 Distribution of sand lizards across Britain (National Biodiversity Network, 2012)

Sand lizards are the smallest oviparous, egg laying, species within the green lizard genus *Lacerta*. There is marked sexual dimorphism of body proportions and colour, with males on average having larger, broader heads and longer, thicker tails, whilst females have longer body lengths. Males also have vivid green flanks, which become more prominent in the mating season between April and May as shown in Figure 3 (Blanke and Fearnley, 2015).

Figure 3 Female (left) and male (right) sand lizard at the captive breeding facility at Marwell Zoo (Source: Author's own)

Due to the rapid decline in numbers within Britain, the remaining populations form the focus of specific conservation efforts, backed by European and domestic legislation. A captive-breeding and reintroduction effort headed by the British Herpetological Society and the Amphibian and Reptile Conservation Trust was established in the late 1960s. This project, coupled with habitat management, aims to re-establish viable populations, which persist within the historical UK range (Woodfine *et al*., 2017). During recent surveys, there have been records of sand lizards at 26 of the 27 release sites, indicating a positive outcome from the initiative (Woodfine *et al*., 2017). However, the inherent effects of small population size, including inbreeding and genetic drift, persist (Allendorf and Luikart, 2007; Frankham *et al*., 2010; Russell, 2013; Woodfine *et al*., 2017). In a genotypic study of UK sand lizard populations, Russell (2013) found variation in the genetic diversity of the three isolated races. While the diversity of the Dorset populations compared favourably to European populations, the diversity of Surrey and Merseyside populations was significantly lower. Genetic augmentation of the populations is therefore required in order to ensure long-term success of the initiative (Russell, 2013; Woodfine *et al*., 2017).

Additionally, further research and continuous monitoring is crucial in evaluating the projects effectiveness (Moulton *et al*., 2011; Russell, 2013). A systematic review uncovered a lack of detailed behavioural knowledge for the sand lizard (Fearnley, 2009; Ljungström *et al.*, 2015). This is thought to be partly due to the cryptic nature and seasonal behavioural fluctuations of sand lizards making field observations difficult (Fearnley, 2009). Assessing the phenotypic correlations between behavioural, morphological and fitness-related traits will enable stricter assessment of the current husbandry practices and aid in defining the determinants of individual reintroduction success.

2. AIMS AND OBJECTIVES

The research project was conceived to investigate the behavioural and fitness requirements for sand lizard reintroduction success and population stability. The work addresses the current knowledge gap in sand lizard behaviour.

The project **aims** and the corresponding *objectives* are as follows:

1. **Evaluate the effects of temporal, weather and individual-related factors to provide ecological context to sand lizard behaviour**
   a) *Use statistical tests to identify the main temporal, weather and morphological traits that affect sand lizard behaviour*
2. **Profile captive individuals to assess individual variation in behaviour and fitness as predictors for reintroduction success prior to release**
   a) *Compare time budgets and conduct a principle components analysis (PCA) to visualise behavioural variation within the captive population*
   b) *Evaluate the effect of social dynamics on the behaviour of individuals within the captive population*
   c) *Using locomotor performance as a fitness proxy, establish whether there is a direct correlation between an individual's morphometric measurements and their fitness*
3. **Inform conservation management by enabling a comprehensive assessment of the current reintroduction practices**
   a) *Assess the captive population using the acquired knowledge of the correlations between behavioural, morphological and fitness-related traits*
   b) *Evaluate the effects of the current management practices on the long-term success of the reintroduction*
   c) *Provide management recommendations to further increase the stability of the species*

3. MATERIALS AND METHODS

This study used sand lizards as a model species to investigate the requirements of reintroduction success and more broadly, population stability. This was achieved by profiling individual behaviour, social dynamics and fitness through behavioural observations and measurements of morphological traits and locomotor performance. Behavioural data collection took place five days a week, between April and June 2017. All research was conducted in accordance with Southampton University animal ethics guidelines.

3.1. Study area

The study monitored a captive population of sand lizards permanently held at Marwell Zoo in Hampshire. Adults are housed in a south-facing outdoor vivarium measuring 60 m^2 (Figure 4 and Figure 5). A 60 cm high acrylic perimeter fence prevents lizard escapes and the vivarium sits within a large mesh fruit cage, successfully inhibiting access by avian and rodent predators. The area is off-access to the public, minimising human disturbance (Fearnley, 2009). Whilst within the estimated home range size (Nicholson, 1980), the researcher acknowledges that the stocking density is unlikely to be reflective of naturally occurring population densities.

The vivarium mimics southern England's natural habitat and is planted with typical lowland heathland vegetation, *H2 Calluna vulgaris - Ulex minor*, as classified by the British National Vegetation Classification (NVC) (Rodwell, 1995; Fearnley, 2009). Routine enclosure maintenance removed unwanted vegetation and maintained bare sand areas to encourage oviposition. A variety of naturally occurring invertebrate species provided a source of prey items and the lizards' diet was supplemented with black field crickets (*Gryllus bimaculatus*) according to a seasonal feeding schedule.

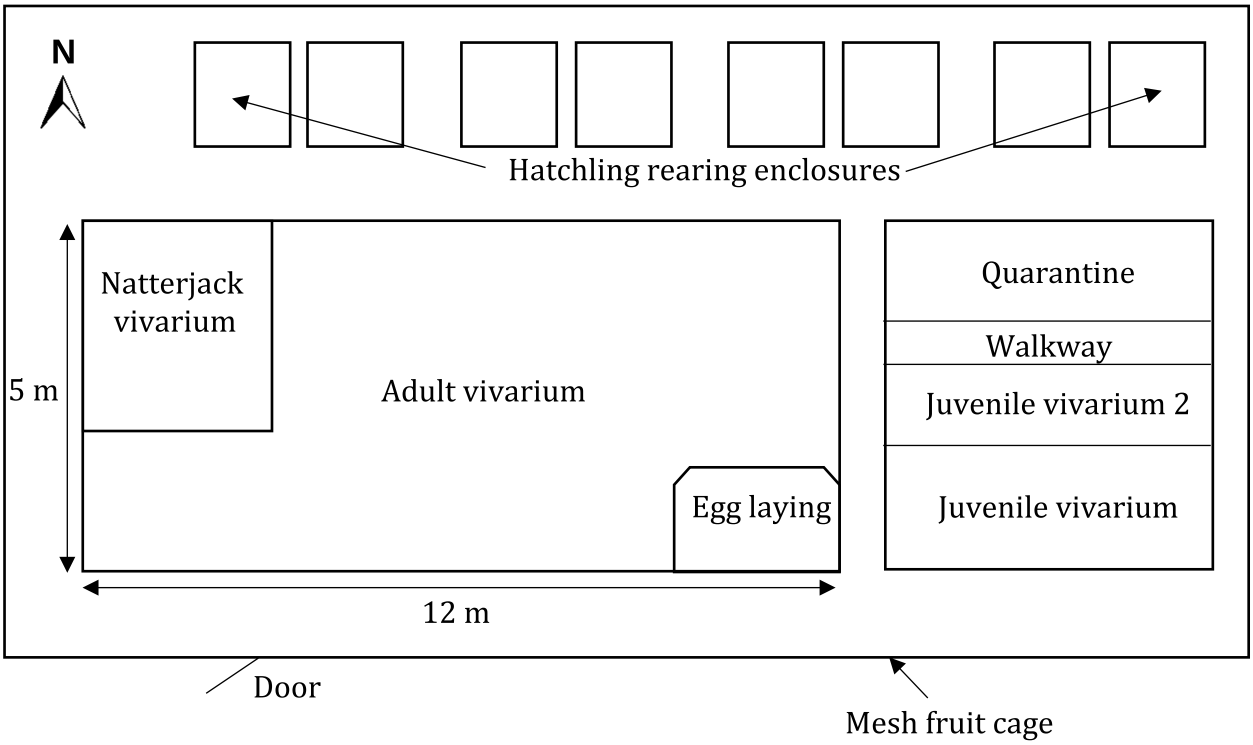

Figure 4 Design and dimensions of the vivarium at Marwell Zoo (Source: Author's own)

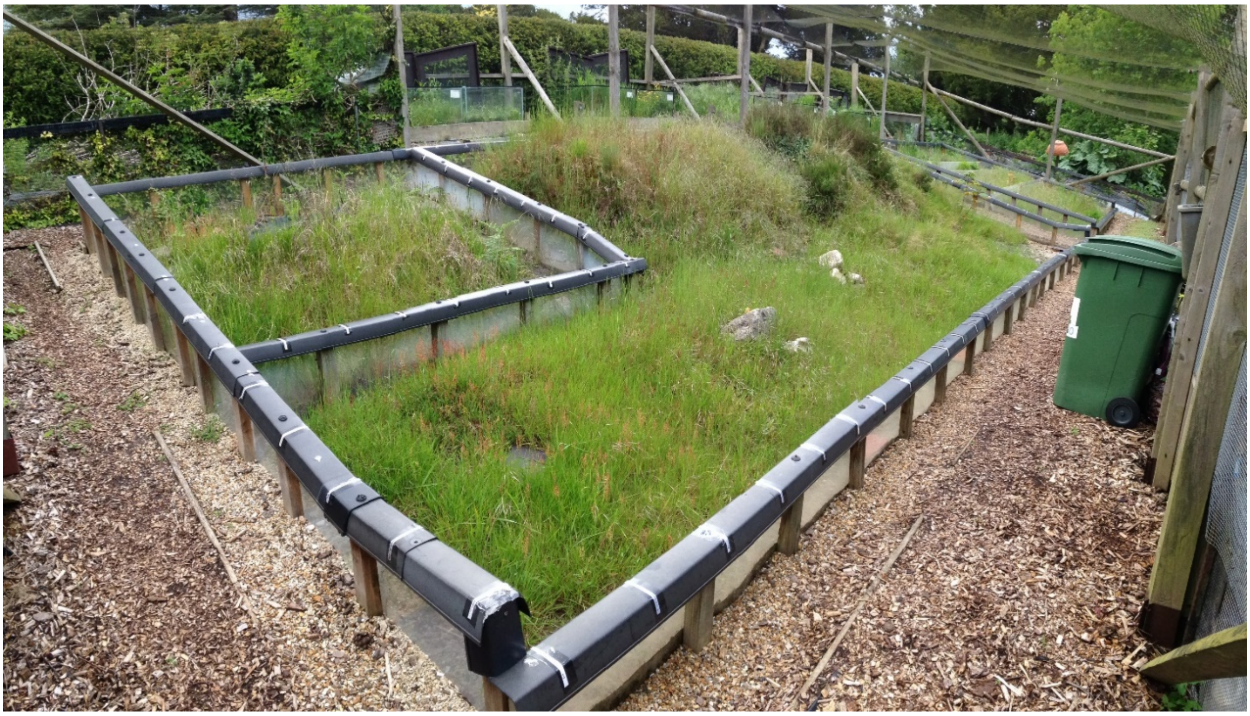

Figure 5 Adult sand lizard vivarium at Marwell Zoo (Source: Author's own)

3.2. Study population

The study observed 16 adult sand lizards, nine males and seven females, with an estimated average age of three to four years. The population is housed as part of the captive breeding and reintroduction project and consists of both captive-bred and wild-caught individuals (Fearnley, 2009). Eggs are excavated, artificially incubated and hatchlings reared outdoors prior to their release. The population is essentially closed, with limited recruitment through population augmentation and losses arising solely from mortality (Fearnley, 2009).

3.3. Individual identification

Each individual was allocated an identification number prior to the study. Unique dorsal patterning and characteristics, such as missing tail tips or scarring, enabled the researcher to distinguish between individuals during observations. Pentax Papilio II 8.5 x 21 close focus binoculars aided in the visual identification of individuals throughout the study.

3.4. Assessment of sand lizard behaviour

3.4.1. Ethogram

An ethogram, a complete behavioural repertoire, was created using information obtained during two preliminary continuous observations conducted subsequent to lizard emergence. The complete ethogram used in the main study is shown in Appendix A.

3.4.2. Behavioural observations

Behavioural observations were conducted in 30-minute sessions, using a cyclical instantaneous scan sampling technique. The observer walked anti-clockwise around the perimeter of the enclosure from the bottom left-hand corner every five minutes. Individuals were identified and their behavioural code was recorded. This technique was the most suitable due to the high level of behavioural detail required for each individual. The method also enabled more categories of behavioural data and time distribution of behavioural states to be recorded per individual than other techniques including focal sampling; which would not have been suitable due to the cryptic nature of the species (Altmann, 1974; Martin and Bateson, 2007).

Care was taken to ensure a consistent walking speed around the enclosure and a maximum of 10 seconds, timed on a stopwatch, was spent observing a single individual in order to minimise any potential bias. The five-minute interval period provided a balance between obtaining an accurate representation of behaviour and practicality of technique. The interval period also ensured each observation was statistically independent, allowing for analysis as separate data points (Martin and Bateson, 2007).

Each data collection day was split into three time blocks: 1000-1130, 1130-1300 and 1430-1600 hours. Within each time block, two half-hour observations were conducted, totalling six observations per day (Table 1). Observations had no fixed start time within their given time blocks, allowing for some flexibility whilst maintaining a reasonable level of structure. Breaks of a minimum of 10 minutes between each observation reduced the effects of observer fatigue and ensured each observation was independent. Table 2 illustrates a typical schedule for a data collection day. The pilot study and previous research (Fearnley, 2009) found that lizards returned to their burrows and remained hidden during periods of heavy rainfall; therefore, no observations occurred during such periods.

Table 1 Time division of behavioural observations

Time block	**BST (GMT+1)**	**Observations**
1	1000-1130	1 and 2
2	1130-1300	3 and 4
3	1430-1600	5 and 6

Table 2 Typical schedule for a data collection day

Time	**Activity**
1000-1030	Observation 1
1030-1045	Break
1045-1115	Observation 2
1115-1130	Break
1130-1200	Observation 3
1200-1215	Break
1215-1245	Observation 4
1245-1430	Lunch/other
1430-1500	Observation 5
1500-1515	Break
1515-1545	Observation 6

A single researcher conducted all observations to provide consistency across the data collection. Researcher effect on behaviour was minimised by the observer remaining outside the vivarium perimeter during observations and the pilot study provided a period of acclimatisation prior to the main study. Enclosure maintenance and provision of supplementary food and water occurred outside of the observation time blocks in order to avoid potential confounding effects.

3.5. Pilot study

A two-week pilot study was conducted in March prior to the main study. Throughout this period, the methodology and ethogram were assessed and adapted as required. The pilot study was a vital stage, enabling the researcher to develop skills in identifying individuals and distinguishing between behaviours under time constraints. Additionally, the pilot study provided an important period of acclimatisation for the study animals to the observer's presence, thus minimising any subsequent effect on behaviour.

3.6. Morphological traits

Adult morphological traits were measured once at the beginning of the study period in attempt to reduce disruption during breeding season. A warm, sunny day was chosen to increase the likelihood of capturing all individuals on a single day.

Individuals were captured by hand in the early morning, placed in separate ventilated plastic boxes and lined up along the perimeter fence. The early start ensured individual were at their most sluggish and easiest to capture. Individuals were identified and measurements recorded before the lizards were returned to their box ready for locomotor testing.

Measurements taken are shown in Figure 6 and were as follows:

1. Snout-to-vent length (SVL);
2. Tail length (TL) - from vent to tip of the tail;
3. Head width (HW) - at the widest point;
4. Head length (HL) - from the parietal scale to the snout;
5. Left hind-leg tibia length (TIB);
6. Body weight;
7. Body condition score (BCS).

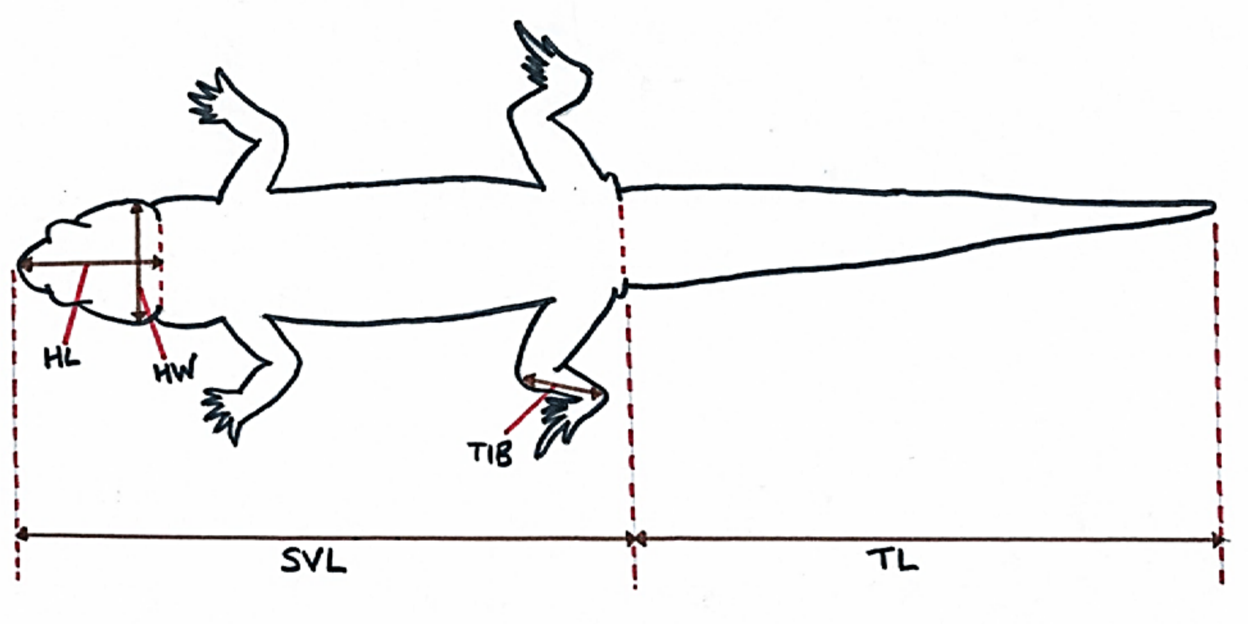

Figure 6 Morphometric measurements (Source: Author's own)

Measurements 1-5 were recorded to the nearest millimetre using callipers whilst holding the individual as straight as possible to ensure an accurate measurement (Figure 7). Body weight was measured to the nearest gram using digital scales. Individuals were weighed in their boxes and the weight of the box subtracted from the measurement. Individual BCS was allocated through visual inspection using the five-point scale as detailed in Table 3.

Figure 7 Body morphometric measurement procedure (Wilkie, M. 2017)

Table 3 Descriptions of body condition scores (BCS)

BCS	Description
1	Poor body condition, no fat deposits visible anywhere on the body, prominent visible vertebral column, multiple (5+) severe visible imperfections (scarring, missing digits, tail autonomy, missing scales…)
2	Poor body condition, minimal fat deposits, visible vertebral column, several imperfections (3-4)
3	Average condition, vertebral column visible but not prominent, some visible imperfections (≤ 2)
4	Good body weight, plentiful fat deposits, vertebral column not easily seen, minor scarring
5	Prime body condition, plentiful fat deposits, abdomen is slightly rounded, vertebral column not easily seen, no visible imperfections

3.7. Locomotor tests

Locomotor performance was measured as in studies by in Vanhooydonck and Van Damme (2003) and Ekner-Grzyb *et al.*, (2013). Locomotor tests were conducted following morphometric measurements to avoid unnecessary additional handling and reduce disruption.

A meter-long 'racetrack' (plastic patio drainage channel) was placed in a flat area of the adult vivarium and lined with compacted sand sourced from the vivarium to reduce confounding effects of novel stimuli. A digital video camera was positioned above the racetrack using a tripod as shown in Figure 8. The video camera was set to continuously record over the testing period. Individuals were released from the right end of the racetrack one at a time. Running was stimulated by moving a long-handled duster along the racetrack at a consistent speed. The same researchers conducted all tests to avoid experimenter effects.

Figure 8 Set up for locomotor testing (Source: Author's own)

Footage was downloaded onto a computer, converted into frames using VLC Media Player. These frames were analysed in AxioVision 4.9.1, a specialist software designed for analysing microscope images (Zeiss, 2009). Using the images as reference, a meter length scaling was created and used to annotate the runway into 0.1 m long segments. The number of frames for the lizard to move between segments was counted. The lizard was said to have entered a segment as soon as the tip of the nose had passed the annotated mark (Figure 9). The fastest speed of each lizard across a 0.2 m segment was calculated based on the frame rate of 25 frames per second.

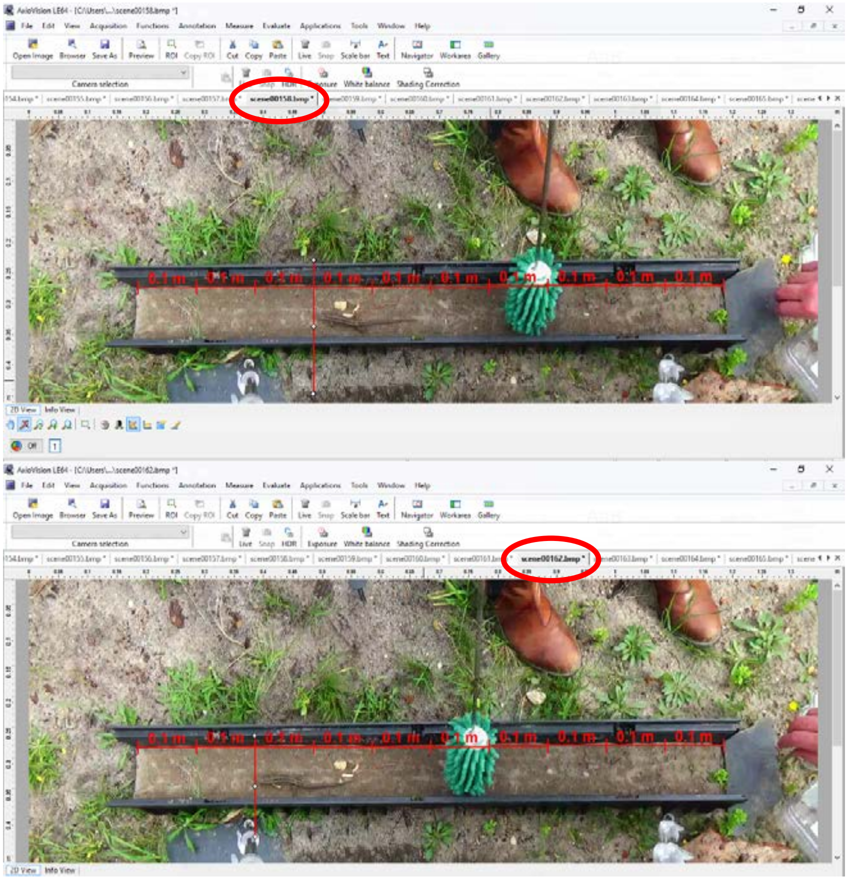

Figure 9 Screenshots from AxioVision computer software, detailing how locomotor performance was calculated based on video frames

3.8. Weather

An Oregon Scientific WMR200 static weather station located on the perimeter of the enclosure was set to record weather variables every minute. Variables recorded and used in analysis were temperature; relative humidity; wind speed; UV index and atmospheric pressure. Lux was measured via a portable lux-meter located within the enclosure. Weather data was averaged over each half hour observation period so that all variables had equal levels.

4. ANALYSIS

All statistical analysis was performed in R 3.3.3 (R Core Team, 2017) and significance assessed at the critical $p < 0.05$ level.

4.1. Temporal, weather and individual-related variables that influence general sand lizard behaviour

As expected with counts, preliminary exploration of the behavioural data through diagnostic plots revealed that the data was not normally distributed. Plotting the observed and expected residuals from various distribution families determined that the data best fitted a negative binomial distribution (see active behaviour example in Figure A1, Appendix b).

Additional visual checks of Rootograms were conducted using the "countreg" R package (REF) following recommendations in Kleiber and Zeileis (2016) (see active behaviour example in Figure A2, Appendix B). Behavioural data was analysed using negative binomial generalised linear models (GLZM.NB), allowing for mixed effects. Behavioural categories were analysed separately.

Table 4 describes the temporal, weather and individual-related variables included in all first full models. Individual ID was included as a random effect in all models to account for repeated measures. Lux, temperature and SVL were scaled. Non-significant terms were removed using backwards stepwise regression until only significant variables remained in the final 'minimal adequate model' (MAM). Goodness of fit tests and diagnostic plots were performed for each MAM using the DHARMa package for mixed models (Hartig, 2017) (see active behaviour example in Figure A3, Appendix B).

Table 4 Description of the variables included in the full statistical models prior to stepwise regression

Variable category	Variable	Units
Temporal	Month (F)	April, May and June
	Time block (F)	As in Table 1, listed as 1, 2 and 3
Weather	Temperature (N)	Degrees Celsius
	Lux (N)	Kilolux
	UV (I)	UV Index Scale
	Wind (N)	Meters per second
	Atmospheric pressure (N)	Millibars
	Relative humidity (N)	Percentage
Individual	Sex (F)	Male or female
	Age (F)	Adult or sub-adult
	SVL (N)	Millimetres
	Weight (N)	Grams
	Head length (N)	Millimetres
	Head width (N)	Millimetres
	Tail length (N)	Millimetres
	BCS (F)	Score ranging from 1 to 5.
	DI (N)	Index ranging from -1.00 to +1.00

(F) Factor, (N) numeric, (I) integer

4.2. Individual variation in behaviour and condition

4.2.1. Behavioural syndromes

In order to visualise individual variation in behaviour, PCA (principle components analysis) was used to reduce the data to manageable components. Interpretable loadings were decided at the minimum cut-off point of 0.3, as suggested by Budaev (2010). Individuals were then plotted against these components to produce a graph visualising behavioural variation within the population using the "factoextra" R package (Kassambara and Mundt, 2015). The PCA component that explained the most variance was then regressed with month, an explanatory variable from the GLZM.NB, using linear regression in order to test for changes across the observation period. Diagnostic plots were checked for model fit.

4.2.2. Dominance hierarchy

Behavioural data was used to create dominance matrices per month, where columns represented an individual's dominant interactions with each conspecific and rows equated to their submissive interactions. Each column and row was summed to give the total number of dominant and submissive interactions per individual. The pilot study and previous literature (Fearnley, 2009) found that females are more sociable and rarely engage in aggressive behaviours. Therefore, females were excluded from the final matrices and dominance calculations in order to avoid disproportionate calculations from limited results.

Using the totalled figures from the matrices, *Dominance Indices (*DI*)* for each individual were calculated using the following equation, adapted from Langbein and Puppe (2004):

$$\mathrm{DI} = \frac{dominant - submissive\ interactions}{dominant + submissive\ interactions}$$

The resulting DI are on an index ranging from -1.00, consistently submissive, to +1.00, consistently dominant (Langbein and Puppe, 2004).

4.2.3. Locomotor performance

General linear models (GLM) were built to test the effects of age, sex and morphological traits on mean lizard speed. All variables and two-way interaction terms were included in the first model which was subsequently simplified using backwards stepwise regression. Pearson's chi-squared test and diagnostic plots were used to assess model fit.

5. RESULTS

5.1. Temporal, weather and individual-related variables that influence general sand lizard behaviour

Objective *– 1.a) "Use statistical tests to identify the main temporal, weather and morphological traits that affect sand lizard behaviour".*

Initial analysis of the behavioural data highlighted several significant interactions between behaviour and the temporal, weather and individual-related variables. Variables had differing effects, affecting none, some or all behaviour categories.

5.1.1. Temporal variables

The group's time allocation towards each behavioural category varied across the three months and time of day, as shown in Table 5 and Figure 10. The large standard error bars on Figure 10 indicate high between-individual variation. This individual variation in behaviour is explored further in subsequent sections.

On average, the group was most visible during May ($n = 16$, $z = -2.78$, $p < 0.05$). Mating and courtship behaviours decreased over the months, in response to the end of peak breeding season ($n = 16$, $z = -4.54$ and -6.65 respectively, $p < 0.001$). Correspondingly, fewer active, aggressive and submissive behaviours were observed in June ($n = 16$, $z = -3.33$, -3.53 and -5.19 respectively, $p < 0.001$). Lone sedentary, vigilant and basking behaviours increased significantly throughout the months ($n = 16$, $z = 2.75$, 2.96 and 10.17 respectively, $p < 0.001$). Social basking was most frequent during May ($n = 16$, $z = 6.29$, $p < 0.001$), whereas social sedentary behaviours were most prominent in April and rarely observed in May and June ($n = 16$, $z = -5.96$, $p < 0.001$).

Table 5 Temporal variable results from the MAM GLZM.NB for each behavioural category (*n* = 16)

	TEMPORAL VARIABLE											
Behavioural	**MAY**†			**JUNE**†			**LATE AM**‡			**MID PM**‡		
category	**Est.**	**SE**	***z – value***	**Est.**	**SE**	***z – value***	**Est.**	**SE**	***z – value***	**Est.**	**SE**	***z – value***
Hiding	-0.33	0.12	-2.78 **	-1.36	0.17	-7.81 ***	-0.27	0.13	-2.03 *	-0.27	0.13	-2.01 *
Active				-0.27	0.08	-3.33 ***				-0.26	0.07	-3.61 ***
Vigilant	0.49	0.17	2.93 **	0.55	0.19	2.96 **						
Sedentary				0.27	0.10	2.75 **						
Social Sedentary	-1.33	0.23	-5.77 ***	-1.75	0.29	-5.96 ***						
Basking	0.53	0.07	7.39 ***	0.82	0.08	10.17 ***	0.17	0.07	2.42 *			
Social basking	0.96	0.15	6.39 ***				0.33	0.15	2.25 *			
Courtship	-0.28	0.14	-2.06 *	-1.26	0.19	-6.65 ***						
Mating				-1.09	0.24	-4.54 ***	-0.49	0.20	-2.43 *	-0.82	0.22	-3.83 ***
Aggressive	0.31	0.15	2.09 *	-0.73	0.21	-3.53 ***				-0.36	0.17	-2.16 8
Submissive				-0.98	0.19	-5.19 ***	-0.30	0.14	-2.15 *	-0.61	0.15	-4.04 ***

Est. = estimate, SE = standard error

† Month (April) was the reference category

‡ Time block (Early AM) was the reference category

'*' significant at $p < 0.05$ '**' significant at $p < 0.01$ '***' significant at $p < 0.001$

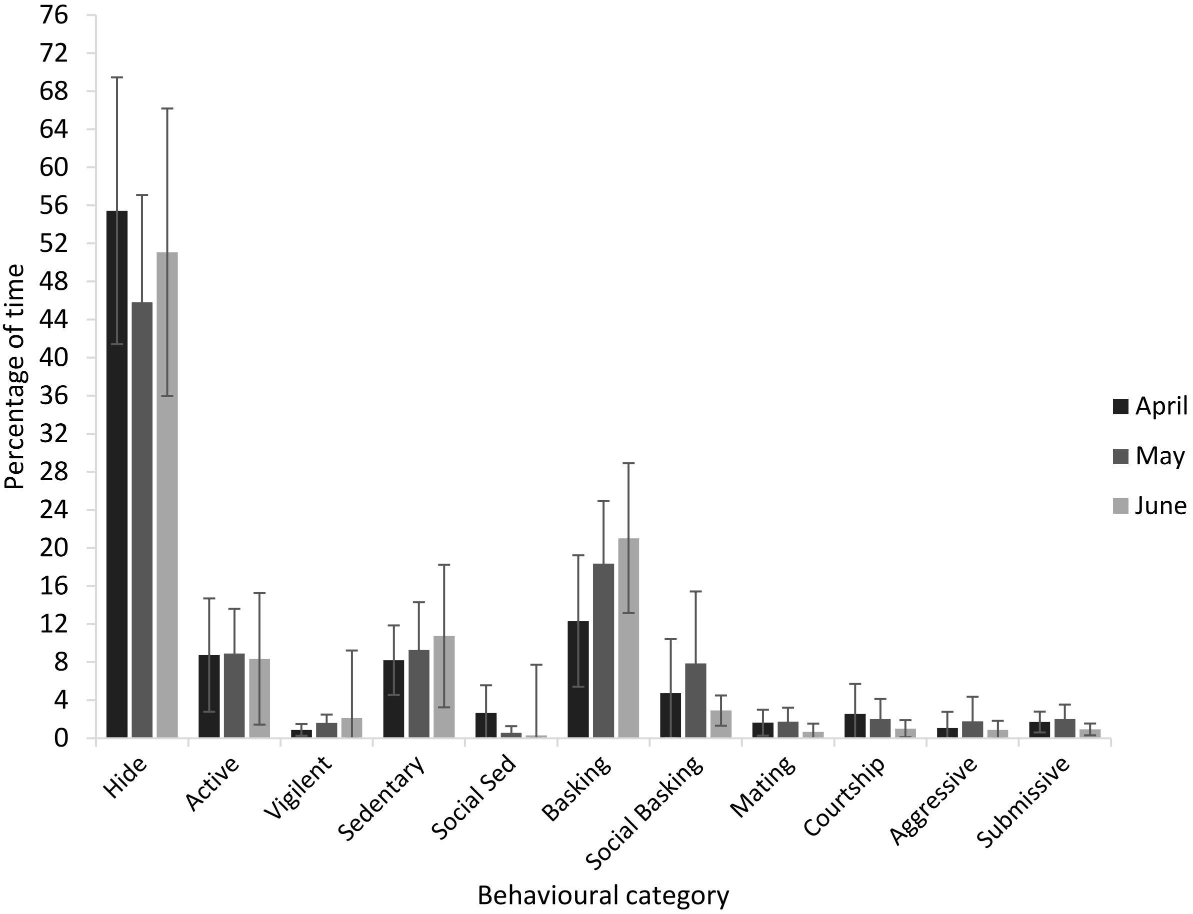

Figure 10 Group mean percentage of time spent performing behaviours in each behavioural category across the three months, including standard error bars

Time of day also affected the frequency at which behaviours were observed. Fewer hierarchy contest behaviours (aggressive and submissive) were observed during mid-afternoon than in the morning ($n = 16, z = -2.16, p < 0.05$ and $n = 16, z = -4.04, p < 0.001$ respectively). This corresponds with the timing of mating and active behaviours which also decreased in the afternoon ($n = 16, z = -3.83$ and $z = -3.61$ respectively, $p < 0.001$). Individuals remained hidden for longer during the afternoon than the earlier periods ($n = 16, z = -2.01, p < 0.05$). Social basking behaviours were most frequent during the late-morning ($n = 16, z = 2.25, p < 0.05$). The group's time allocation to each behavioural category over the different periods is shown in Figure 11.

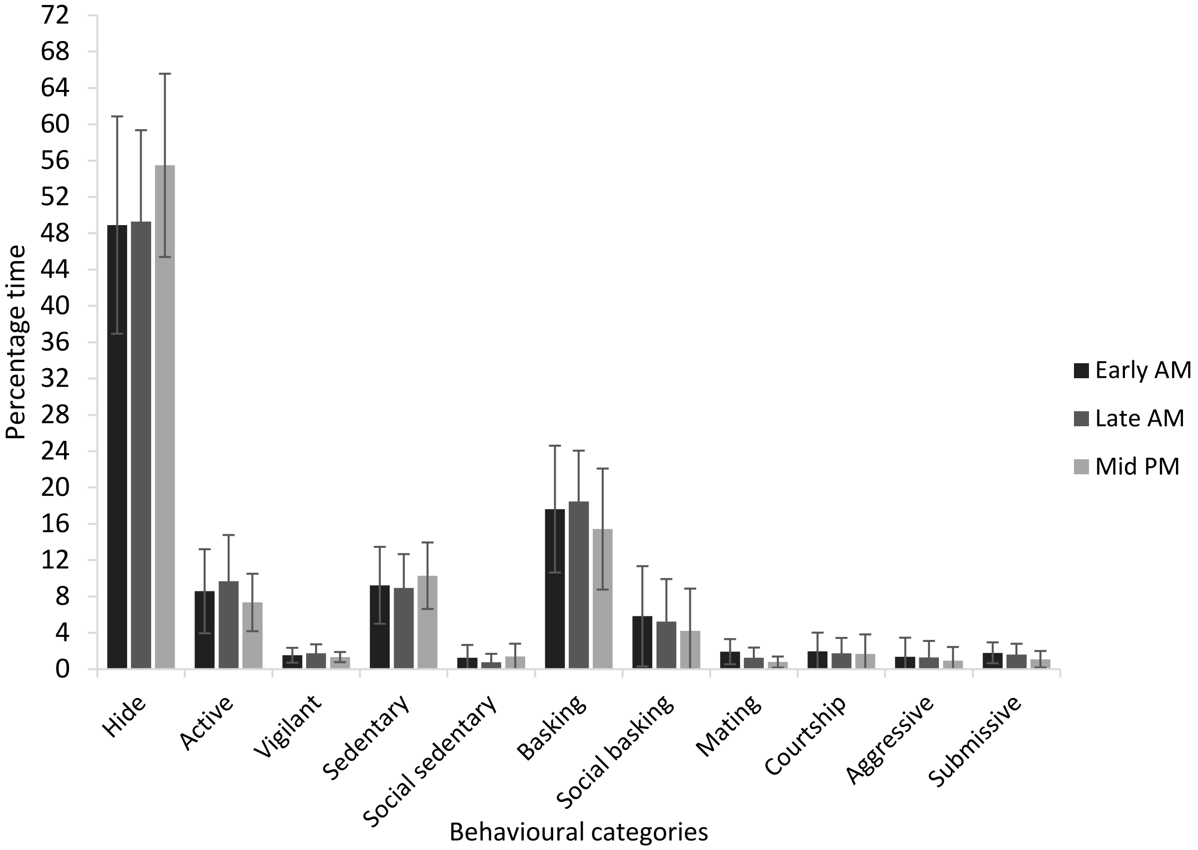

Figure 11 Group mean percentage of time spent performing behaviours in each behavioural category across the three time blocks, including standard error bars

5.1.2. Weather variables

There was no significant effect of relative humidity, wind or barometric pressure on any behavioural category and so these were excluded from all of the final models. The interaction between UV and lux was the only variable interaction to remain in any of the final models. Temperature, UV and lux affected the majority of the behavioural categories studied (Table 6). UV on its own had no effect on dominance hierarchy behaviours (aggressive or submissive). Social sedentary was the only behavioural category not to be affected by any weather variable.

Table 6 Weather variable results from the MAM GLZM.NB for each behavioural category (*n = 16)*

Behavioural category	WEATHER VARIABLE											
	TEMPERATURE			**LUX**			**UV**			**LUX:UV**		
	Est.	**SE**	***z – value***	**Est.**	**SE**	***z – value***	**Est.**	**SE**	***z – value***	**Est.**	**SE**	***z – value***
Hiding	0.24	0.07	3.57 ***	0.38	0.11	3.45 ***	0.10	0.05	2.18 *	0.13	0.04	3.59 ***
Active	0.14	0.02	6.62 ***	0.59	0.06	10.40 ***	0.15	0.02	6.62 ***	0.10	0.02	6.03 ***
Vigilant	0.24	0l07	3.69 ***	0.65	0.12	5.31 ***	0.14	0.04	3.00 **	0.11	0.03	3.15 **
Sedentary				0.26	0.08	3.10 **				0.18	0.03	6.50 ***
Social Sedentary												
Basking	-0.10	0.03	-3.24 **	0.14	0.06	2.29 *	-0.05	0.02	-2.23 *	-0.13	0.02	-6.20 ***
Social basking	-0.49	0.08	-6.39 ***	0.37	0.15	2.46 *	-0.20	0.05	-3.78 ***	-0.41	0.06	-6.94 ***
Courtship	0.22	0.07	2.92 **	0.41	0.13	3.21 **	0.11	0.05	2.13 *	0.13	0.04	3.29 **
Mating				0.52	0.18	2.85 **				0.14	0.06	2.46 *
Aggressive	0.25	0.08	3.28 **	0.51	0.13	3.96 ***				0.12	0.04	2.88 **
Submissive	0.27	0.07	3.83 ***	0.28	0.12	2.41 *						

Est. = estimate, SE = standard error
‘*’ significant at $p < 0.05$ ‘**’ significant at $p < 0.01$ ‘***’ significant at $p < 0.001$

5.1.3. Individual-related variables

Individual's age and sex had the most significant influence on sand lizard behaviour, affecting multiple behavioural categories (Table 7). Sub-adults interacted with conspecifics less than adult individuals (social basking $z = -5.16$, $p < 0.001$, social sedentary $z = -3.53$, $p < 0.001$, aggression $z = -3.49$, $p < 0.001$). Sub-adults were more sedentary than adults were but remained hidden for less of the observation period ($z = 4.31$ and $z = -5.37$ respectively, $p < 0.001$). As expected, males competed in more aggressive interactions and were more active and vigilant when compared to females ($z = 4.928$, 6.85 and 3.87 respectively, $p < 0.001$). Perhaps as a direct consequence of the increased activity, males were less sedentary and were observed social basking less than females were ($z = -3.97$ and -2.17 respectively, $p < 0.05$).

Individuals were hidden for an average 52.2% of the total observation period. Males on average remained hidden significantly less than females (mean ± SD, 46.5% ± 9.58 and 57.3% ± 7.39 respectively, $z = -2.88$, $p < 0.05$). There was marked sexual dimorphism of behaviour, with sex having a significant effect on all but two behavioural categories (Table 7, Figure 12).

Snout-vent-length (SVL) was the only morphological variable to have a significant effect on the performance likelihood of any of the behavioural categories. Larger individuals displayed significantly less submissive behaviours than smaller individuals did ($z = -2.43$, $p < 0.05$), but SVL did not affect any other behaviour category in the study.

Table 7 Individual-related variable results from the MAM GLZM.NB for each behavioural category (*n = 16)*

	INDIVIDUAL-RELATED VARIABLE								
	AGE †			**SEX ‡**			**SVL**		
Behavioural category	**Est.**	**SE**	***z – value***	**Est.**	**SE**	***z – value***	**Est.**	**SE**	***z – value***
Hiding	-1.99	0.37	-5.37 ***	0.93	0.32	2.88 **			
Active				1.14	0.17	6.85 ***			
Vigilant				0.60	0.16	3.87 ***			
Sedentary	0.71	0.16	4.31 ***	-0.61	0.16	-3.97 ***			
Social Sedentary	-1.47	0.41	-3.53 ***	-0.79	0.31	-2.59 **			
Basking				0.67	0.10	6.75 ***			
Social basking	-2.95	0.57	-5.16 ***	-0.96	0.44	-2.17 *			
Courtship	-1.98	0.37	-5.36 ***	0.91	0.32	2.86 **			
Mating	-1.93	0.31	-6.18 ***						
Aggressive	-1.64	0.47	-3.49 ***	2.21	0.45	4.93 ***			
Submissive							-0.65	0.27	-2.43 *

Est. = estimate, SE = standard error
† Figures refer to sub-adults. Age (adult) was the reference category
‡ Figures refer to males. Sex (female) was the reference category
'*' significant at $p < 0.05$ '**' significant at $p < 0.01$ '***' significant at $p < 0.001$

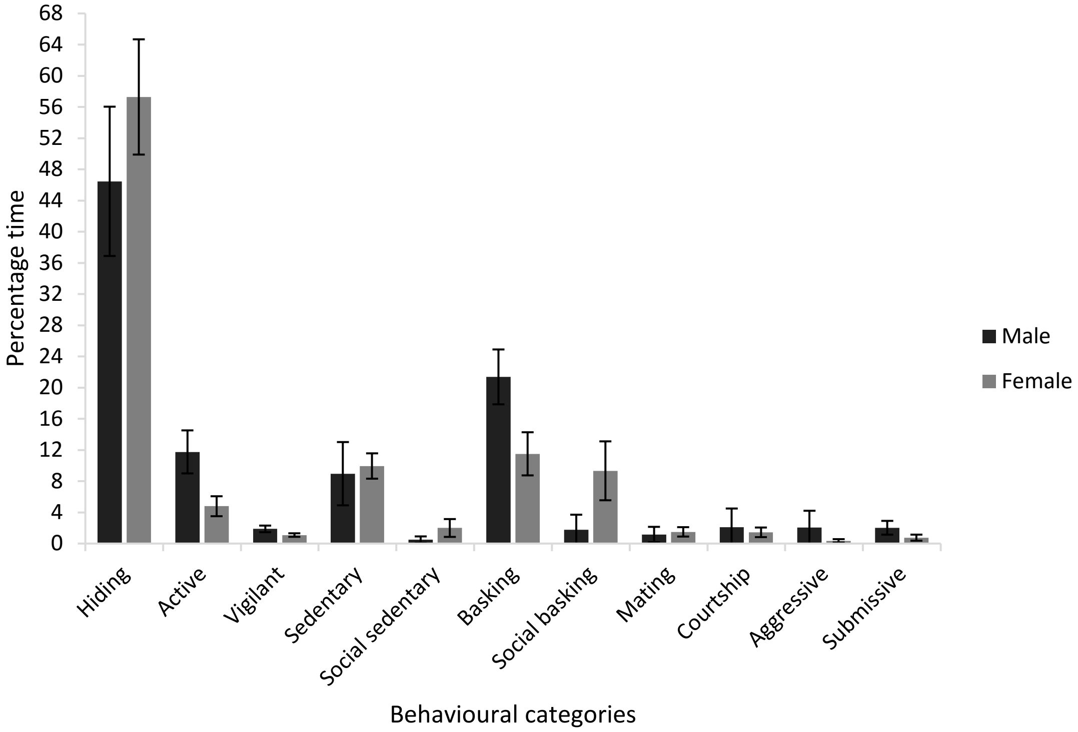

Figure 12 Sex differences in mean time allocation to behavioural categories including standard error bars

5.2. Individual variation in behaviour and condition

5.2.1. Variation in individual's behaviour time allocation

Objective – 2.a) *"Compare individual behavioural time budgets and conduct a principle components analysis (PCA) to visualise behavioural variation within the captive population".*

The results indicate high levels of individual variation in time allocated to each behavioural category, as detailed in Table 8 and visualised in Figure 13. The 'hide' behavioural category contained the most individual variation (Table 8). Individual number 2, who ranked most dominant (see section 5.2.3.), was hidden for less than 25% of the total observation period, whilst individual number 10 was hidden for almost 70%. This individual variation is displayed in Figure 14.

There are similarities in the time allocation of certain individuals, indicating that those individuals have similar 'personality types'. For instance, individuals 11, 12 and 13 have comparable percentage time allocation on all behavioural categories except sedentary and social basking. Equally, individuals 6, 7 and 8 spend an almost equal amount of time performing active, vigilant, sedentary and basking behaviours.

Table 8 Minimum and maximum individual percentage time allocation to each behavioural category across the total observation period.

	Behavioural time allocation (%)		
Behavioural category	**Minimum**	**Maximum**	**Range**
Hiding	23.93 (2)	68.68 (10)	44.75
Active	3.24 (11)	14.52 (8)	11.28
Vigilant	0.69 (16)	2.70 (2)	2.01
Sedentary	3.87 (5)	15.58 (1)	11.71
Social sedentary	0.47 (1)	4.15 (15)	3.68
Basking	8.26 (12)	27.39 (2)	19.13
Social basking	0.00 (1)	13.57 (11)	13.57
Courtship	0.08 (1)	2.65 (12)	2.57
Mating	0.14 (6)	8.02 (2)	7.88
Aggressive	0.00 (1)	7.47 (2)	7.47
Submissive	0.07 (2)	2.97 (4)	2.90

Corresponding individual ID shown in brackets

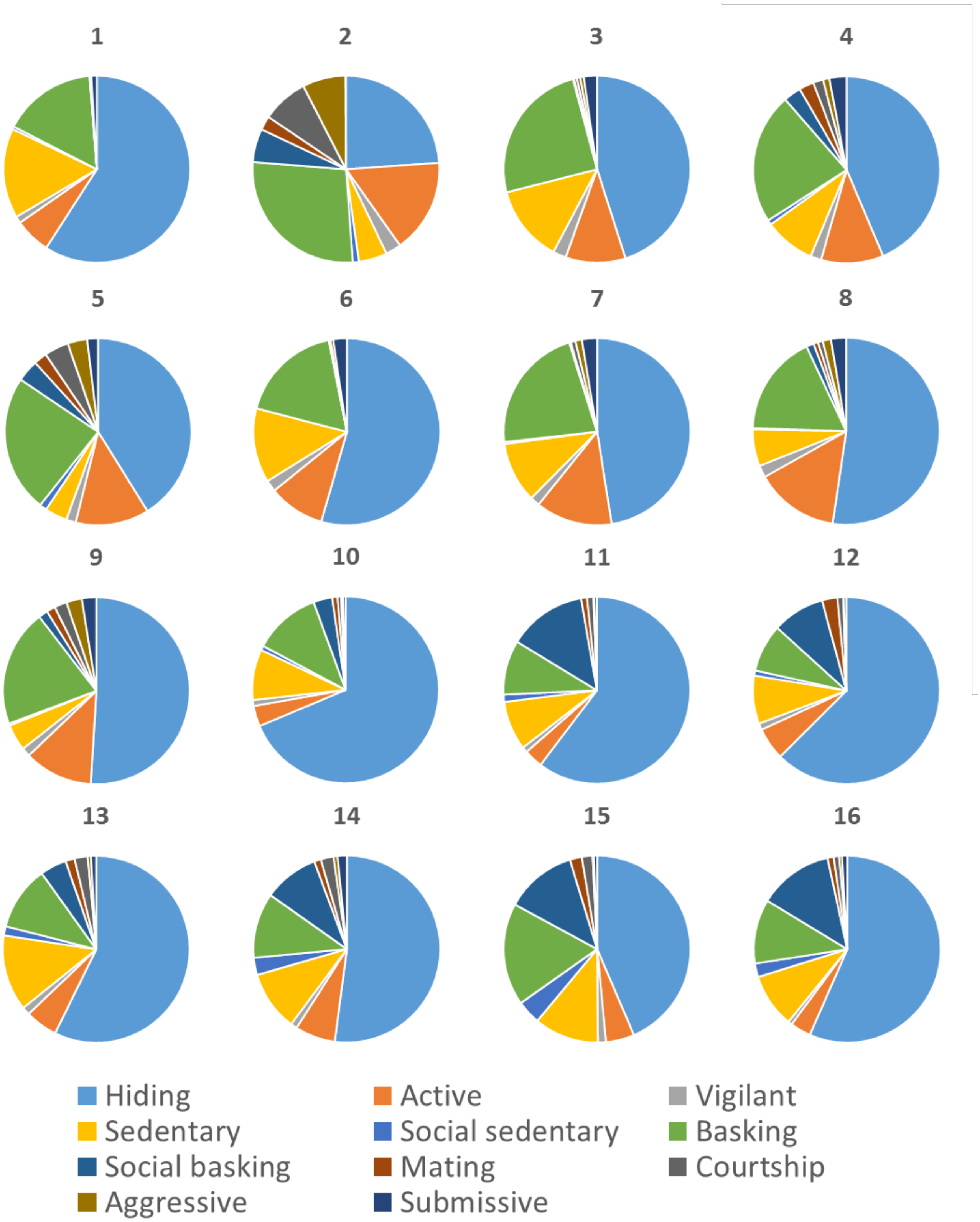

Figure 13 Each individual’s time allocation to the behavioural categories across the total observation period

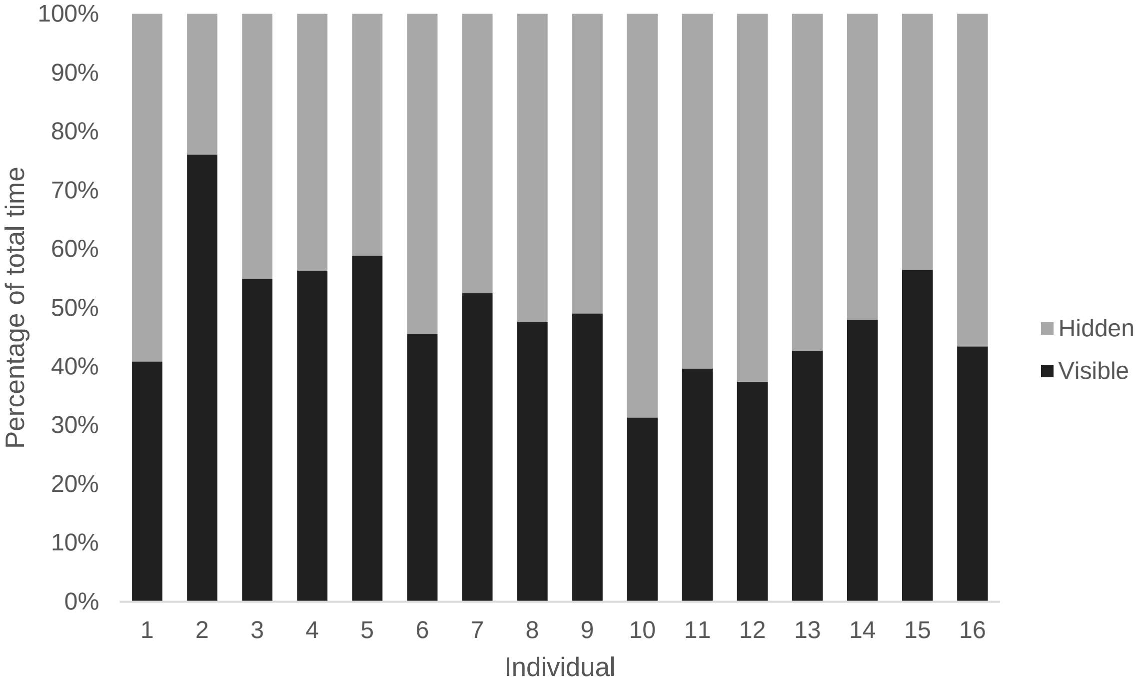

Figure 14 Percentage of time individuals were visible and hidden across the entire observation period

5.2.2. Behavioural syndromes and behavioural variation

Objective – 2.b) *"Compare individual behavioural time budgets and conduct a principle components analysis (PCA) to visualise behavioural variation within the captive population".*

The PCA produced 11 components, including all behavioural categories as variables in the analysis. The interpretable loading values for the first five components, that explained 59% of the variation in the data, can be found in Table 9. Each component had a different behavioural emphasis, which demonstrates the behavioural variation within the data. The positive and negative interpretable loadings of each component can be found in Table 10 PC1 explained the most variance within the data and was positively loaded on hiding behaviours, whilst PC2 loaded positively on active, vigilant and aggressive behaviours.

Table 9 First five components derived from PCA for the entire observation period on all of the behavioural categories in order of proportion of variance they explain.

	PCA components				
Behavioural category	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Hide	**0.73**	0.16	-0.01	-0.06	-0.02
Active	-0.27	**0.54**	-0.02	0.17	0.22
Vigilant	-0.18	**0.30**	0.22	0.17	**0.37**
Sedentary	-0.18	**-0.37**	0.07	**0.75**	-0.04
Social Sedentary	-0.08	-0.24	**-0.44**	0.15	-0.02
Basking	**-0.44**	-0.14	**0.46**	**-0.45**	-0.30
Social Bask	-0.15	**-0.42**	-0.21	-0.28	**0.53**
Aggressive	-0.20	**0.36**	-0.28	-0.09	0.21
Submissive	-0.14	0.27	0.02	0.19	-0.39
Courtship	-0.18	0.07	**-0.53**	-0.16	-0.09
Mating	-0.10	0.06	**-0.37**	-0.04	**-0.49**
Proportion of variance explained	0.17	0.12	0.11	0.10	0.09

Interpretable loadings are shown in **bold***, set at the 0.3 minimum cut off* point

Table 10 Behavioural categories identified as positive and negative interpretable loadings for each component of the total observation period PCA

PC	**Positive interpretable loadings**	**Negative interpretable loadings**
1	Hiding	Basking
2	Active, aggressive, vigilant	Social basking, sedentary
3	Basking	Courtship, social sedentary, mating
4	Sedentary	Basking
5	Social basking, vigilant	Mating, basking
6	Aggressive, courtship	Social basking, submissive, mating
7	Social sedentary, submissive	Mating
8	Vigilant, social sedentary, mating	Submissive
9	Aggressive, social sedentary	Courtship, vigilant
10	N/A	Active, aggressive, submissive, vigilant
11	Hiding, basking, sedentary, social basking	N/A

When combined, PC1 and PC2 explained approximately 29% of the total variance within the data. Spatial representation of the data in relation to these two components revealed between-individual variation. Figure 15 visualises this variation by different shaped and positioned ellipses that correspond to each individual and their behavioural emphasis towards the components. There was minimal variation in the shapes of the female ellipses (numbers 10 to 16, represented by the purple tones) which are all located at a near-horizontal angle with slight emphasis on PC1, the ‘hiding’ component. There was more between-male variation (numbers 1 to 9, represented by the orange-green tones) but on average male ellipses showed emphasis on the ‘active and aggressive’ PC2 and were located at an almost 45° angle to the female ellipses. This is indicative of marked sexual dimorphism in behaviour. Individual number 2 had the most distinct ellipses with strong emphasis on PC2 indicating a rare personality type.

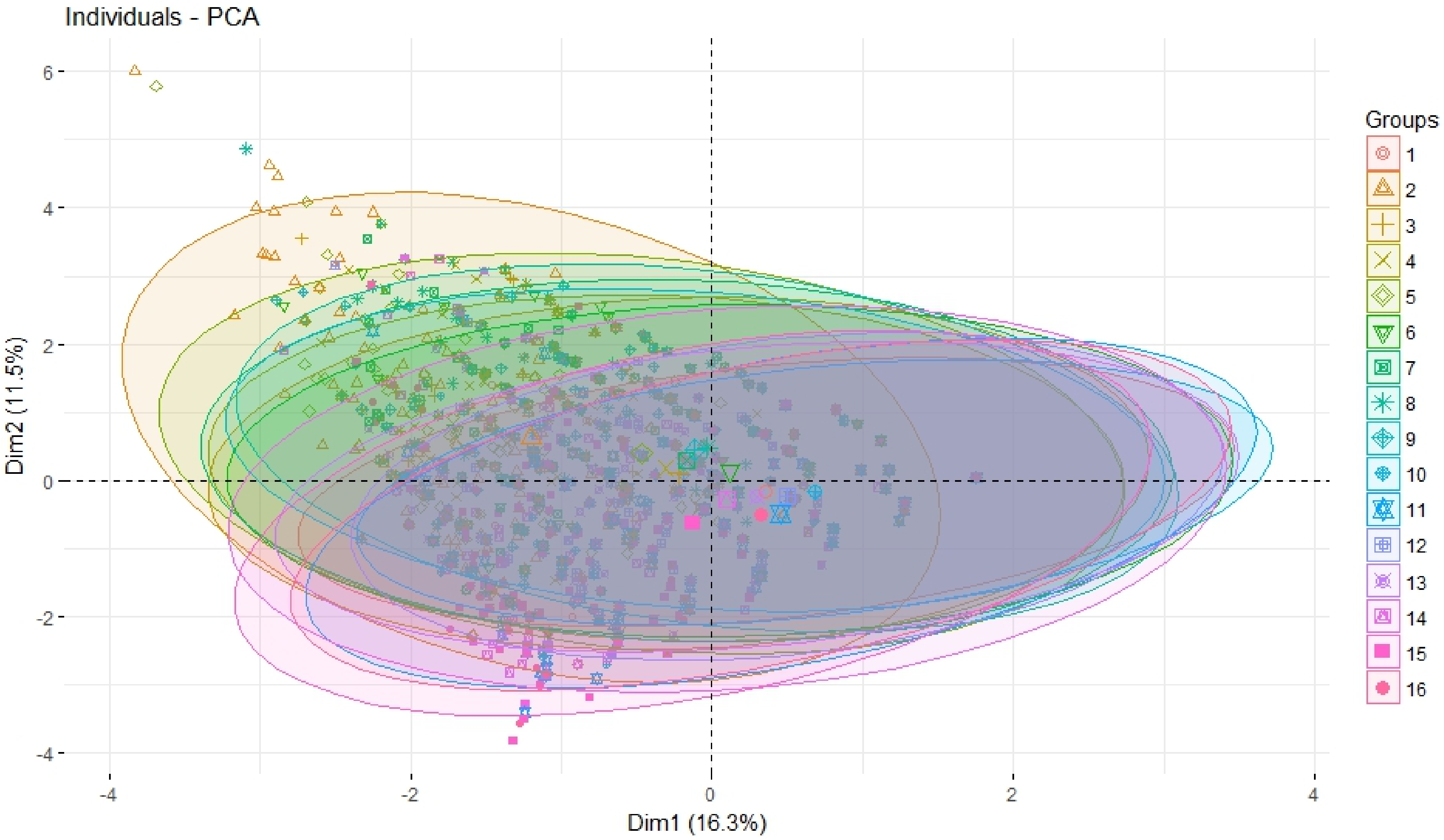

Figure 15 Spatial representation of data points on PC1 and PC2 from the PCA analysis using data from the entire observation period. Ellipses represent the positioning of each individuals data points, colour coded as on the right

Regression analysis found a significant effect of month on PC1 ($t = -2.77, p < 0.05$). Further PCA was then conducted on the data from each month independently. There were differences in the interpretable loadings for the first three components across the three months, as shown in Table 11. Spatial representation in Figures 16, 17 and 18 revealed that individual variation decreased throughout the months, with all individual ellipses, with the exception of individual 'number 2', converging in June.

Table 11 First three components derived from independent PCA on all of the behavioural categories for each month, in order of proportion of variance they explain.

Behavioural category	**PCA components**								
	April			**May**			**June**		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Hide	**0.70**	0.21	0.08	**0.74**	0.12	-0.06	**0.73**	0.13	-0.07
Active	**-0.31**	**0.49**	-0.07	-0.23	**0.50**	-0.14	-0.27	**0.58**	0.00
Vigilant	-0.21	**0.30**	-0.09	-0.11	**0.32**	0.14	-0.20	**0.38**	0.00
Sedentary	-0.16	**-0.44**	-0.13	-0.13	-0.27	0.25	-0.23	-0.17	**0.79**
Social sedentary	-0.14	**-0.35**	**0.43**	-0.08	-0.27	**-0.34**	-0.06	-0.02	0.29
Basking	**-0.37**	-0.04	**-0.50**	**-0.44**	-0.05	**0.47**	**-0.49**	**-0.30**	**-0.49**
Social basking	-0.14	**-0.39**	-0.03	-0.20	**-0.55**	-0.24	-0.09	**-0.37**	-0.07
Courtship	-0.22	0.02	**0.60**	-0.23	0.08	**-0.47**	-0.12	-0.05	-0.10
Mating	-0.13	0.00	0.15	-0.14	0.06	**-0.45**	0.01	-0.06	-0.10
Aggressive	-0.29	**0.31**	**0.37**	-0.19	**0.31**	-0.25	-0.13	**0.46**	-0.13
Submissive	-0.12	0.23	-0.09	-0.16	0.28	0.14	-0.15	0.18	0.13
Proportion of variance explained	0.17	0.12	0.10	0.16	0.11	0.11	0.16	0.12	0.11

Interpretable loadings are shown in **bold***, set at the 0.3 minimum cut off point*

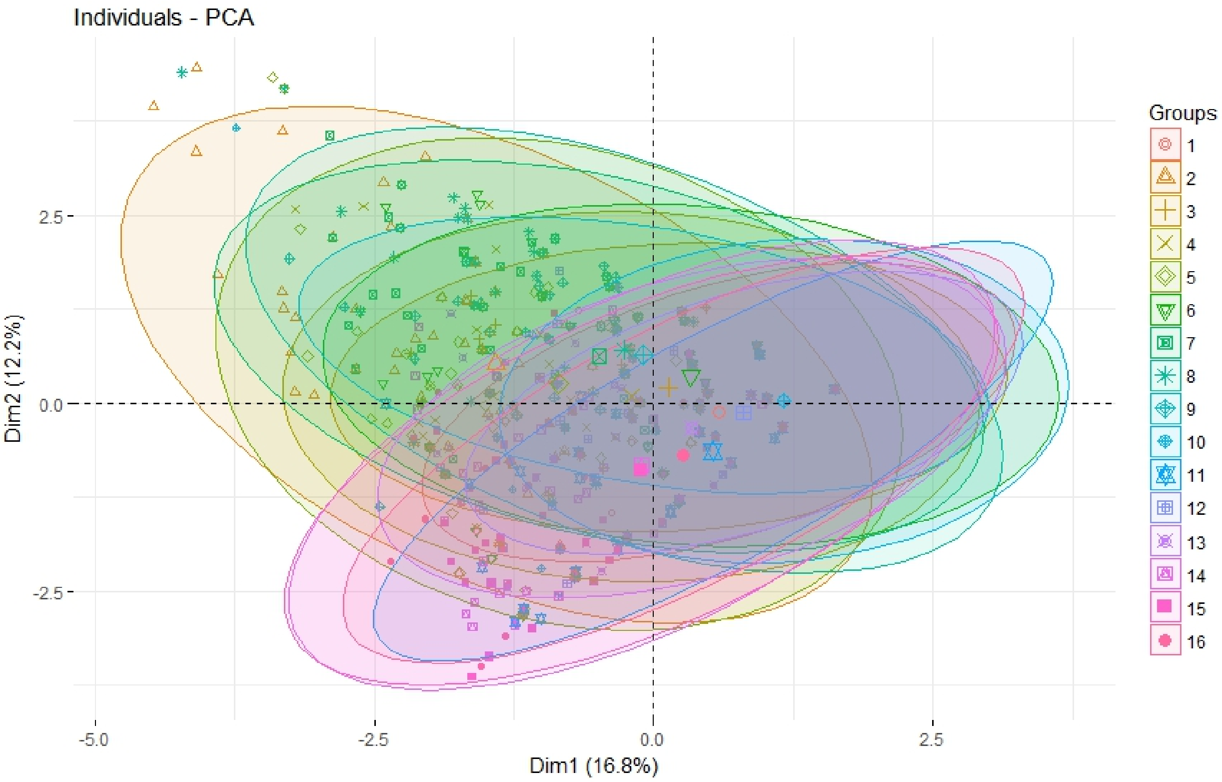

Figure 16 Spatial representation of data points on PC1 and PC2 from the PCA analysis using data from April. Ellipses represent the positioning of each individuals data points, colour coded as on the right

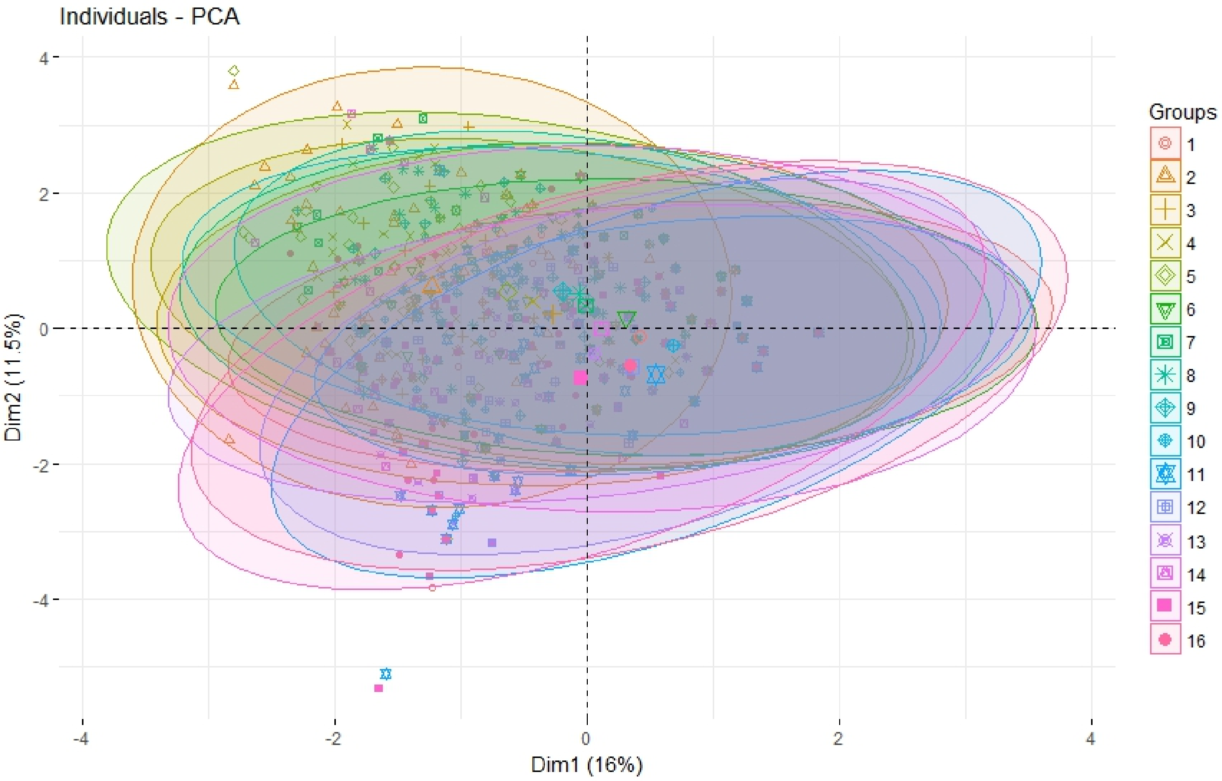

Figure 17 Spatial representation of data points on PC1 and PC2 from the PCA analysis using data from May. Ellipses represent the positioning of each individuals data points, colour coded as on the right

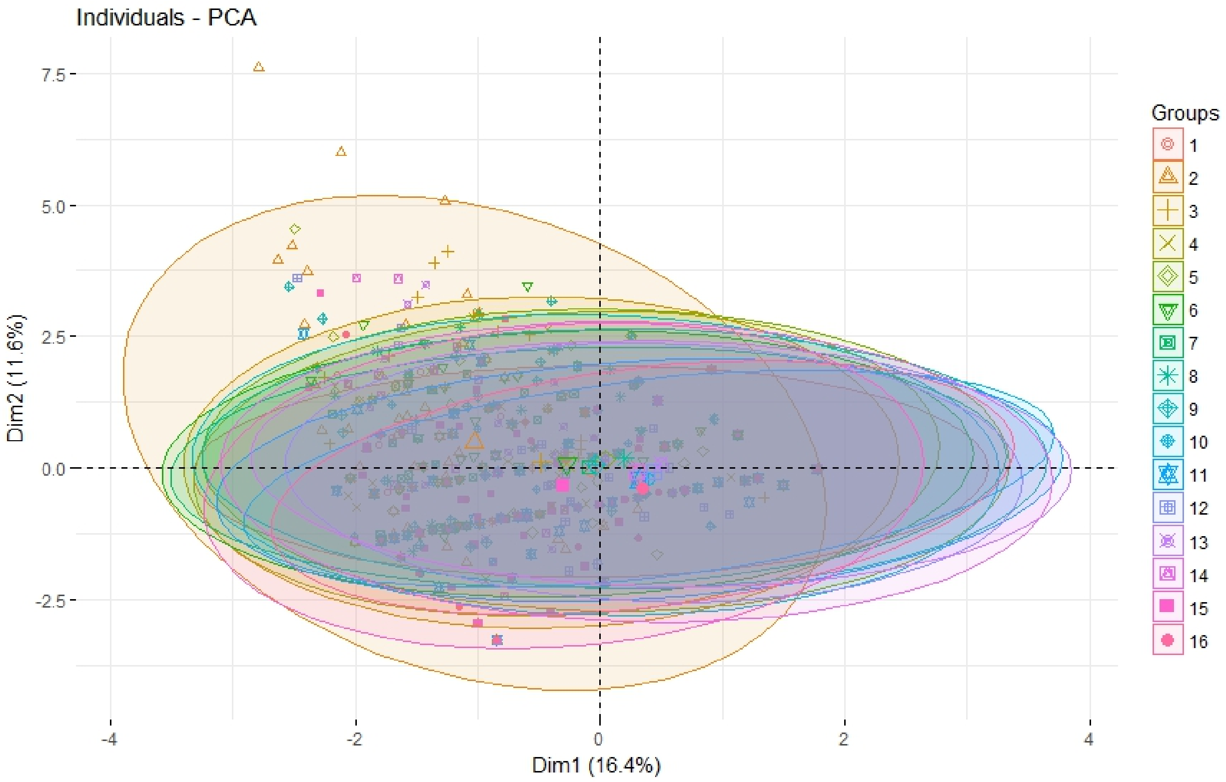

Figure 18 Spatial representation of data points on PC1 and PC2 from the PCA analysis using data from June. Ellipses represent the positioning of each individuals data points, colour coded as on the right

5.2.3. Social dynamics and the dominance hierarchy

Objective – 2.b) *"Evaluate the effect of social dynamics on the behaviour of individuals within the captive population".*

The male dominance matrices for the three months separately and combined are shown in Figure 19.

		DOMINANT									Total
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
SUBMISSIVE	**1**		0	0	1	0	0	1	0	0	2
	2	0		0	0	0	0	0	0	1	1
	3	0	4		0	2	0	1	1	2	10
	4	0	6	0		7	0	0	0	1	14
	5	0	8	0	0		0	1	0	0	9
	6	0	2	0	0	1		4	2	4	13
	7	0	2	1	1	5	0		0	2	11
	8	0	7	0	1	2	0	3		0	13
	9	0	10	0	1	5	0	2	2		20
Total		0	39	1	4	22	0	12	5	10	

		DOMINANT									Total
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
SUBMISSIVE	**1**		3	0	1	1	1	0	0	0	6
	2	0		0	1	0	0	0	0	0	1
	3	0	7		1	2	1	0	2	4	17
	4	0	11	0		8	0	0	1	1	21
	5	0	12	0	0		0	0	0	2	14
	6	0	2	1	0	0		1	3	4	11
	7	0	7		2	1	2		4	5	21
	8	0	8	1	2	4	0	2		3	20
	9	0	10	0	0	6	4	1	1		22
Total		0	60	2	7	22	8	4	11	19	

3 - JUNE

		DOMINANT									Total
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
SUBMISSIVE	**1**		1	0	0	0	0	0	2	1	4
	2	0		0	0	0	0	0	0	0	0
	3	0	5		0	1	0	0	0	1	7
	4	0	6	0		6	0	0	0	1	13
	5	0	4	2	0		0	0	0	0	6
	6	0	5	0	2	1		0	0	2	10
	7	0	3	0	3	1	0		0	1	8
	8	0	3	0	0	1	1	0		1	6
	9	0	1	0	2	1	1	0	1		6
Total		0	28	2	7	11	2	0	3	7	

TOTAL OBSERVATION PERIOD

		DOMINANT									Total
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
SUBMISSIVE	**1**		4	0	2	1	1	1	2	1	12
	2	0		0	1	0	0	0	0	1	2
	3	0	16		1	5	1	1	3	7	34
	4	0	23	0		21	0	0	1	3	48
	5	0	24	2	0		0	1	0	2	29
	6	0	9	1	2	2		5	5	10	34
	7	0	12	1	6	7	2		4	8	40
	8	0	18	1	3	7	1	5		4	39
	9	0	21	0	3	12	5	3	4		48
Total		0	127	5	18	55	10	16	19	36	

Figure 19 Male dominance matrices for April, May and June and the total observation period

The results of the DI calculations revealed that the top ranking individuals (2 and 5) and the lowest ranking individual (9) maintained their positions in the social hierarchy throughout the observation period. The middle ranking individual's DI and ranking fluctuated, with number 6 having the largest changes across the months. Across the total period, only two individuals (2 and 5) had a positive DI. Individual 'number 1' had a DI of -1.00 throughout the entire observation period, meaning he was consistently submissive, whereas individual 'number 2' had a minimum DI of +0.95 making him almost consistently dominant over all conspecifics. This dominant individual repeatedly displaced other individuals, defended a large territory and successfully mate-guarded a number of females. The presence of this individual within the captive breeding group therefore restricted the mating and dominance behaviour of other individuals who attempted to avoid costly competitive interactions. The largest difference in DI was between the top two ranked individuals, with a difference of 0.66 between them. The full list of DI calculations, rank and rank change from the previous month is shown in Table 12.

Table 12 Calculations of individual Dominance Index (DI), social hierarchy ranking and rank change for each month

	April		**May**		**June**		**Total period**	
ID	**DI**	**Rank**	**DI**	**Rank (change)**	**DI**	**Rank (change)**	**DI**	**Rank**
1	- 1.00	9	- 1.00	9 (=)	- 1.00	9 (=)	- 1.00	9
2	+0.95	1	+0.97	1 (=)	+1.00	1 (=)	+0.97	1
3	- 0.82	7	- 0.79	8 (⇩ 1)	- 0.56	6 (⇧2)	- 0.74	8
4	- 0.56	6	- 0.50	6 (=)	- 0.30	4 (⇩2)	- 0.45	6
5	+0.42	2	+0.22	2 (=)	+0.29	2 (=)	+0.31	2
6	- 1.00	9	- 0.16	4 (⇧ 5)	- 0.67	7 (⇩3)	- 0.55	7
7	+0.04	3	- 0.68	7 (⇩ 4)	- 1.00	9 (⇩2)	- 0.43	5
8	- 0.44	5	- 0.29	5 (=)	- 0.33	5 (=)	- 0.34	4
9	- 0.33	4	- 0.07	3 (⇧ 1)	+0.08	3 (=)	- 0.14	3

= Individual's hierarchy rank stayed the same
⇩ Individual moved down the hierarchy
⇧ Individual moved up the hierarchy

5.2.4. Locomotor performance, a fitness proxy

Objective – 2.c) *"Using locomotor performance as a fitness proxy, establish whether there is a direct correlation between an individual's morphometric measurements and their fitness".*

Locomotor performance was analysed for all 16 adult lizards, nine males and seven females. Body weight had a positive effect on the speed of sand lizards ($t = 2.563, p < 0.05$) (Table 13 and Figure 20). There was a significant difference ($t = 2.270, p < 0.05$) in the mean speed (m/s) between males and females (mean ± SD; 0.73 ± 0.26 and 0.58 ± 0.16, respectively). There were no significant effects of age, SVL, autonomy, BCS, head width or head length (Table 13).

Table 13 Statistical output from a general linear model examining the effects of age, sex, weight, SVL, autonomy, BCS, head width and head length on speed of the sand lizard (*Lacerta agilis*)

Fixed effect tests			
Source	**Estimate**	***t - value***	***p -value***
Age†	-0.022534	-0.131	0.8990
Sex‡	0.463474	2.270	0.0494*
Weight	0.063957	2.563	0.0305*
SVL	0.007089	0.331	0.7479
Head width	-0.032474	-0.519	0.6160
Head length	-0.058154	-0.949	0.3672
Autonomy	0.094229	0.710	0.4959
BCS	-0.143412	0.127055	0.2854

** Significant at the critical p < 0.05 level*

† Figures refer to sub-adults. Age (adult) was the reference category

‡ Figures refer to males. Sex (female) was the reference category

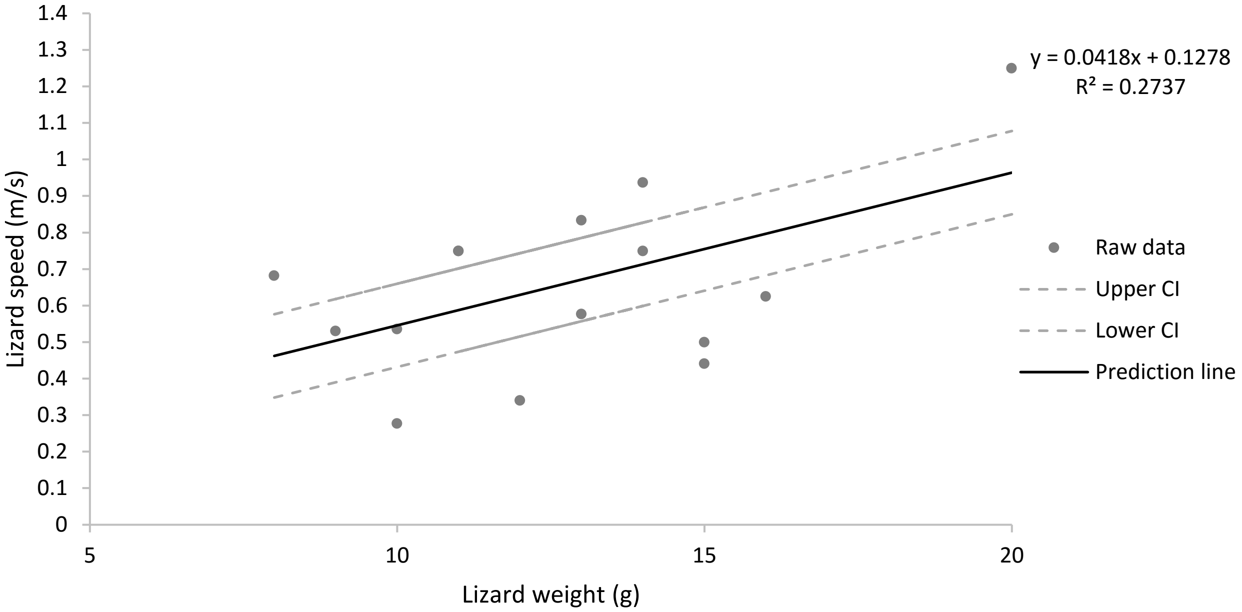

Figure 20 Relationship between lizard speed (m/s) and lizard weight (g)

6. DISCUSSION

This study aimed to use sand lizards as a model species to investigate the requirements for reintroduction success and more broadly, population stability. The study comprised of several sections which when combined provide a comprehensive understanding of sand lizard behaviour.

6.1. The effect of temporal and weather variables on sand lizard behaviour

Aim 1 *"Evaluate the effects of temporal, weather and individual-related factors to provide ecological context to sand lizard behaviour"*

Phenotypic traits, including behavioural traits, are a result of complex interactions between an individual's genes and their environment (Ghalambor *et al*., 2010). Familiarity of a species' ecology and the extraneous variables that influence behaviour is therefore imperative when looking at individual-level behavioural variation as it facilitates a deeper level of discussion and thus more meaningful conclusions (Santos *et al*., 2009; Schlaepfer *et al*., 2010). This study is understood to be the first to analyse the effect of these variables on specific behavioural categories in the sand lizard, with previous literature focusing on detectability and general activity levels.

The activity and detection probability of male and female sand lizards have been shown to vary with reproductive stage (Amat *et al*., 2003; Fearnley, 2009). The strong effect of month on all behavioural categories in this study could therefore be a result of the observation period straddling peak breeding season, during which there is high competition for mating (Fearnley, 2009). At the tail end of the breeding season in June, there were noticeably fewer hierarchical and mating behaviours observed, with a shift in behavioural focus towards basking and vigilant behaviours. This shift is most likely in attempt to regain body condition in preparation for the coming hibernation (NCC, 1983; Fearnley, 2009; Blanke and Fearnley, 2015). Additionally, for females, this increase in basking could be in response to egg development. Plentiful UVB light is required to synthesise vitamin D3 and the hormone calcitriol, which is essential for calcium regulation and thus necessary for egg development (Fearnley, 2009; Baines *et al*., 2016).

In the present study, temperature, lux and UV were strongly associated with behavioural changes. This indicates that not only do these variables influence sand lizard presence and general activity levels (Amat et al., 2003; Fearnley, 2009), but they also influence the specific behaviours being performed. Fluctuating environmental conditions due to climate change could therefore have life history consequences that requires further investigation.

As an ectothermic species, sand lizards actively maintain their optimal body temperature and organ function through basking (Fearnley, 2009; Baines *et al*., 2016). Keeping with findings by Fearnley (2009), lizards were less visible during periods of high temperature, lux and UV indices. Open basking is a potentially costly behaviour due to increased vulnerability to predators. Therefore, when possible during high temperatures, lizards will choose to cryptic bask via small rays of UVB penetrating through the vegetation and may not be detected (Fearnley, 2009).

6.2. Behavioural syndromes and behavioural variation

<u>Aim 2</u> *"Profile captive individuals to assess **individual variation in behaviour** and fitness as predictors for reintroduction success prior to release"*

In all PCA tests, there were vast contrasts in the interpretable loadings between the first two components that explained almost a third of the variation in the data. PC1 was consistently loaded on hiding behaviours with fewer basking behaviours, whereas, PC2 was loaded on active, vigilant and aggressive behaviours with fewer social behaviours. These contrasting interpretable loadings are indicative of different behavioural syndromes or personalities within the data set. When all individuals were plotted against these two components from the total observation period, there was variation in the elliptical shapes produced and marked sexual dimorphism. Each individual's behaviour therefore had a different emphasis towards the two behavioural syndromes, for instance, some individual's behaviour was consistently more active and aggressive. This is also shown by the differences in the time budgets of each individual and the dominance index calculations.

Having identified personality differences within the captive population, it is important to consider the potential evolutionary implications and consequences for the long-term viability of the population following reintroduction. Personality can effect evolutionary and ecological processes; including dispersal, foraging and mate choice (Brooks and Griffith, 2010; Hamilton, 2010; Michelangeli *et al*., 2017). The differences in personality between the individual sand lizards could have implications for dispersal following reintroduction as in Michelangeli *et al*., (2017) who found personality-dependent dispersal in the delicate skink (*Lampropholis delicata*) and the common lizard (*Zootoca vivipara*).

Maintenance of personality variation within the population is necessary for a self-prospering population due to the life history consequences of behavioural syndromes. Individuals of varying personality types will respond differently to a changing environment and therefore be well suited to certain contexts and maladapted to others (Sih *et al*., 2010). As with poor genetic diversity, poor behavioural diversity can be detrimental to the long-term resilience of a population through reduced adaptability (Sih *et al*., 2010). The heritability of behavioural syndromes highlights a further need to ensure varied parentage in the release population of a species reintroduction.

The convergence of individual ellipses in the PCA graphs across the months is indicative that variation in individual behaviour is condition dependent, mediated by an interaction with ecological context (Schlaepfer *et al*., 2010). This is supported by the findings in section 6.1. that showed sand lizard behaviour is modulated by temporal and weather variables. In the captive scenario, personality predominantly influenced male social hierarchy and mating behaviours, whilst female personalities were less varied.

6.3. Social dynamics and the dominance hierarchy

Aim 2 "*Profile captive individuals to assess* ***individual variation in behaviour*** *and fitness as predictors for reintroduction success prior to release*"

Only two individuals had a positive DI for the entire observation period, indicating that consistent aggression is limited within the population. The presence of a consistently dominant individual within the captive breeding group could be altering the behaviour of the surrounding individuals (McGlothlin *et al.*, 2010; Santostefano *et al*., 2016; Michelangeli *et al*., 2017). The responses to competition by the nonaggressive individuals in the present study was not consistent, with individuals altering their strategy based on context. In some scenarios, these individuals would choose to rival their competitor through aggression and in other scenarios they would flee, avoiding the costly competitive interactions. This is highlighted by the fluctuating rankings of the remaining individuals, with one exception who was consistently submissive, fleeing from all potentially negative interactions.

In contrast to predictions and findings by Olsson and Shine, (1996), morphological traits had minimal influence on the behaviour of the sand lizards in this study. Instead, the results of this study were consistent with the general conclusions from the meta-analysis of fitness consequences of personality by Smith and Blumstein (2008). The bolder, most dominant individuals spent more time mating, demonstrating personality dependent mate choice. Whilst in the captive context this personality type is proving advantageous, studies on a number of species including great tits (*Parus major*) and black field crickets (*Teleogryllus commodus*) have stressed that these bold individuals tend to incur a survival cost (Dingemanse *et al.*, 2004; Shackleton *et al.*, 2005). This trade-off in fitness consequences across contexts once again stresses the need for maintenance of personality variation within a population.

Not only did the dominant males have increased mating frequencies, they also limited the mating opportunities for the other males, by defending large territories, successfully mate guarding a number of females and repeatedly displacing conspecifics. This suppression in the ability of other males to reproduce due to over-dominance of a single male will result in limited paternity of the hatchlings used in the reintroduction. Due to the heritability of behavioural syndromes (Sih *et al.*, 2010), limited genetic diversity could additionally result in reduced behavioural variation within the population. This limited behavioural variation could restrict the ability of the population to adapt to a changing environment and thus be detrimental to the species' persistence.

6.4. Locomotor performance, a fitness proxy

Aim 2 *"Profile captive individuals to assess* ***individual variation*** *in behaviour and* ***fitness*** *as predictors for reintroduction success prior to release"*

Locomotor performance is an important fitness proxy in lizards due to its role in predator avoidance and hunting (Vanhooydonck and Van Damme, 2003; Ekner-Grzyb *et al.*, 2013). Body weight positively influenced sand lizard speed, perhaps a result of greater muscle mass. Additional research into body constituents is required to support this hypothesis. As previously mentioned, fast early growth rates can lead to various bone abnormalities and nutritional stress (Metcalfe and Monaghan, 2001; Olsson and Shine, 2002). When assessing the indicators of an individual's fitness it is therefore advised that more emphasis be placed on body condition as opposed to size.

In contrast to the study by Ekner-Grzyb *et al.*, (2013) tail length and autonomy had no effect on lizard speed. This indicates that the tail may not play a significant role in the horizontal locomotion of the sand lizard, as it does for other species (Medger, 2008). However, this inconsistency in findings may be due to the small sample size in the current study.

6.5. Management recommendations

Aim 3 *"Inform conservation management by enabling a comprehensive assessment of the current reintroduction practices"*

The outdoor vivarium mimics the sand lizard's natural habitat and enables the lizards to experience the same discrete changes in weather and circadian rhythm as their wild counterparts. This naturalistic enclosure provides opportunities for the lizards to perform appropriate behaviours in response to environmental conditions as shown by the findings of section 5.1.2. The successful hibernation, breeding and rearing of hatchlings over the past two decades is also testament to the current husbandry practices (Woodfine *et al.*, 2017). Nevertheless, the research findings have led to a few management recommendations. These recommendations, if applied, would help ensure high standards of animal welfare and the long-term viability of reintroduction populations.

Firstly, it is important to acknowledge the potential effects of personalities within the sand lizard population. Continuing to profile and monitor the captive and wild individual's genetic and behavioural diversity will identify any need for behavioural augmentation. Maintaining behavioural variation will ensure the population's capabilities of adapting to a changing environment and thus improve the long-term species stability (Sih *et al.*, 2010). The overly dominant males monopolised the mating opportunities in the captive group, at a potential cost to the subsequent generation's genetic and behavioural diversity. Removal of the most dominant male every couple of years and supplementing the breeding stock with individuals from other collections would help to provide the necessary genetic and behavioural augmentation of the population (Woodfine *et al.*, 2017). Ensuring biosecurity between collections would be of upmost importance to limit any disease transfer (Reiss and Woods, 2011).

Furthermore, emphasis should be placed on husbandry practices that maintain body condition and appropriate bone development for optimal locomotor performance. Whilst black crickets provide an adequate, easily accessible food source, it would be beneficial to introduce a variety of other adequately gut-loaded live food species. This would help to provide all the essential amino acids and appropriate calcium: phosphorus ratio for safe growth and maintenance of body condition (Courteney-Smith, 2014). The area surrounding the enclosure could also be planted with a variety of heathland plants to further increase the diversity and abundance of natural prey items available to the lizards (Courteney-Smith, 2014).

7. LIMITATIONS

As the study monitored a closed captive population, the sample size was relatively small (*n = 16*). This is not unusual in behavioural ecology where it is often difficult to obtain large sample sizes due to practical and ethical constraints (Nakagawa, 2004). Nevertheless, whilst the small sample size enabled the detailed examination of individual behaviour, care should be taken when interpreting the results due to limited statistical power.

As this project was part of a Master's degree the time available for data collection was inherently limited. Previous studies have concluded that sand lizard behaviour is seasonal and varies with reproductive stage; with males more active prior to mating and females most active post-mating (Amat *et al*., 2003; Fearnley, 2009). The study attempted to combat this limitation by ensuring the observation period covered the entire breeding season from April to June. Longer-term studies would be more representative of the phenology of the species and potentially capture more of the discrete changes in behaviour across the season.

There is a risk that the study subject's behaviour is not generalisable to wild populations due to potential adaptation to captivity. Although empirical testing of the intricacies of adaptation to captivity is required, the naturalistic enclosure design provided opportunities for the individuals to perform natural behaviours as observed in wild populations (Blanke and Fearnley, 2015). As the overarching aim focused on assessing behavioural variation within a single population of sand lizards, their origin was of lower importance. Furthermore, studying the captive population enabled multiple behavioural categories to be examined and attributed to each individual instantaneously. Obtaining this level of behavioural detail would not have been feasible in a wild population under the time constraints.

There were additional limitations of the locomotor performance testing. Firstly, due to ethical restrictions no repeats were conducted. Coupled with the small sample size, this greatly reduced the statistical power making it difficult to draw firm conclusions from the data. Secondly, whilst attempts to standardise body temperature and motivational state were made through holding individuals in identical boxes for an equal amount of time prior to testing, this was not quantifiably assessed. Although a previous study by Ekner-Grzyb *et al*., (2013) found no effect of cloacal temperature on speed, this potential limitation should still be acknowledged.

8. CONCLUSION

The study successfully met the set objectives; expanding the current knowledge of sand lizard behaviour. This research will assist conservation practitioners in monitoring existing sand lizard populations and ensuring the long-term species persistence.

Marked behavioural variation within the captive population indicates that individuals within the group consistently differ in their behaviour. Within the captive population, a consistently dominant male restricted the behaviour of the conspecifics and monopolised the mating opportunities. This behavioural restriction could negatively impact the long-term reintroduction success due to reduced genetic and behavioural diversity within the subsequent generations. Maintenance of behavioural variation within captive and wild populations is vital to population stability, increasing population resilience and persistence through the 'portfolio' and 'insurance' effects (Schindler *et al.*, 2010; Wolf and Weissing, 2012). Contrary to predictions, morphological traits had minimal influence on the behaviour and locomotor performance of sand lizards. Therefore, varying individual 'success' cannot be solely attributed to phenotypic variance in physical characteristics but rather a complex interaction between an individual's genetics, personality and the environmental context.

Overall, this study highlights the importance of understanding animal personalities and offers further evidence to challenge the concept that animals work on instinct alone. Behavioural ecology and conservation biology should not be seen as distinct sciences and researchers should collaborate to enhance the understanding of the drivers of individual success and provide the best possible chances of conservation success.

9. FURTHER STUDY

This research has expanded current knowledge of individual behaviour of the sand lizard, to further inform conservation management and ensure long-term stability of the species. However, as with any research project it asks as many questions as it answers.

Repeating the current study on wild and reintroduced populations would further enhance the knowledge of individual behaviour of the sand lizard. Determining the behavioural variation within the reintroduced population will prove useful to assess the current management practices. Additionally, further knowledge of wild behaviour would help to determine the level of adaptation to captivity within the Marwell population.

To date, identifying personality types within the species requires high survey effort due to the amount of behavioural data required and the species' cryptic nature. Testing the accuracy of using behavioural assay tests, such as response to novel stimuli, or fitness proxies, such as number of copulations, to determine an individual's personality type would be beneficial to the programme.

Long-term monitoring of the fitness consequences of personality in sand lizards would enable a more comprehensive assessment of the reintroduction programme and expand current knowledge of the drivers of individual success. Long-term studies could also test the use of fitness proxies, such as locomotor performance, as predictors of reintroduction success.

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APPENDICES

Appendix A

Table A1 Ethogram used in behavioural observations, including code, full description and behavioural category to which the behaviour was assigned for statistical testing

Behaviour (code)	Description	Category
Aggressive mating (AM)	Male has seized the female in his jaws, forcing coitus.	Mating
Basking (BI)	Lying flat on stomach	Basking
Basking together (BT)	Lying flat on stomach in close proximity to another individual (< 30cm)	Social basking
Bite (B)	Biting another individual and not immediately releasing	Aggressive
Burrow (BU)	Digging in the substrate	Active
Chase (CH)	Chasing another individual	Aggressive
Courtship (CO)	Male slowly approaching female, jittery movements, nipping females flanks	Courtship
Escape (E)	Attempting to run or jump up the vivarium walls	Active
Feed (F)	Successfully eating a whole item of food completely	Active
Flee (FL)	Running away from another individual	Submissive
Female acceptance (FA)	Female lying flat on the ground, turning hind legs towards the sky and waggling them	Courtship
Test hole (TH)	Females digging in substrate to test for egg laying sites	Active
Food-bite (FB)	Biting an item of food	Active
Food-shake (FS)	Biting an item of food and shaking it with rapid head movements	Active
Groom (GR)	Lizard grooms itself	Active
Lying (LY)	Individual lying with >50% body under cover	Sedentary
Lying together (LYT)	Lying with >50% of body under cover, in close proximity to another individual (< 30cm)	Social sedentary
Mate guarding (MG)	Male following female and basking next to her	Courtship
Mating (M)	Coitus - male usually seizes the female, at the base of her tale or mid-way along the body.	Mating
Nip (N)	Biting another individual and releasing immediately	Aggressive
Not visible (NV)	Individual is not visible	Hide
Oviposition (OVI)	Female laying eggs in the substrate - hole will be backfilled	Active
Scurry (SC)	Quick, urgent movement around the enclosure	Active
Stalk (ST)	Very slow movement around the enclosure	Active
Stop-start (SS)	Stop and start locomotion, vigilant, head held up	Vigilant
Tongue flick (TF)	Fast flicking of the tongue whilst staying still, to search for prey (Cooper, 1997)	Active
Wriggle (W)	Bodily movements whilst staying in the same spot	Active

Appendix B

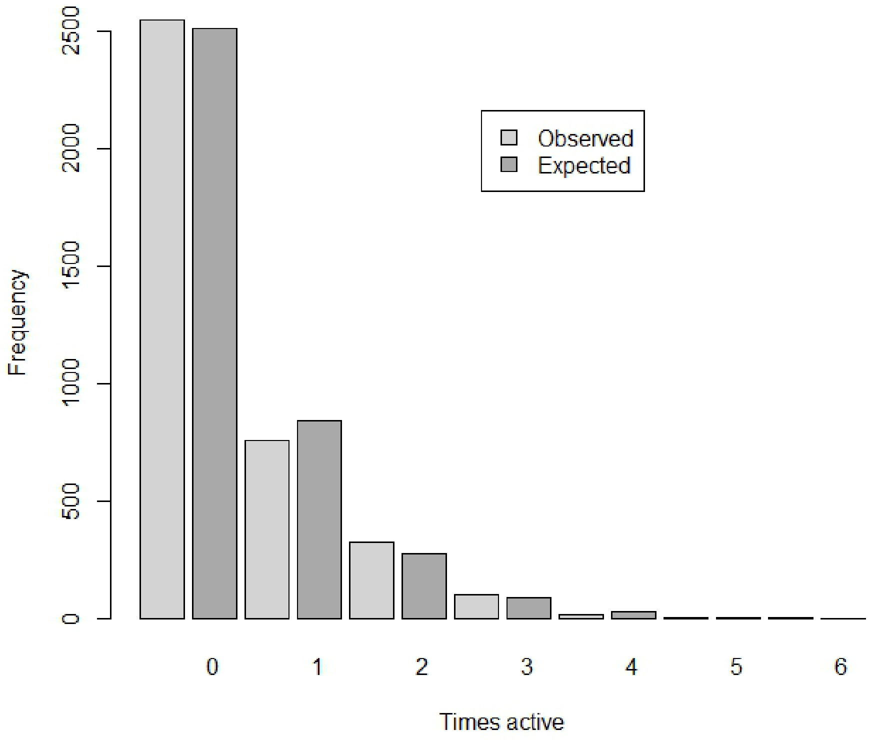

Figure B1 Observed and expected residuals of active behaviours, plotted to a negative binomial distribution family

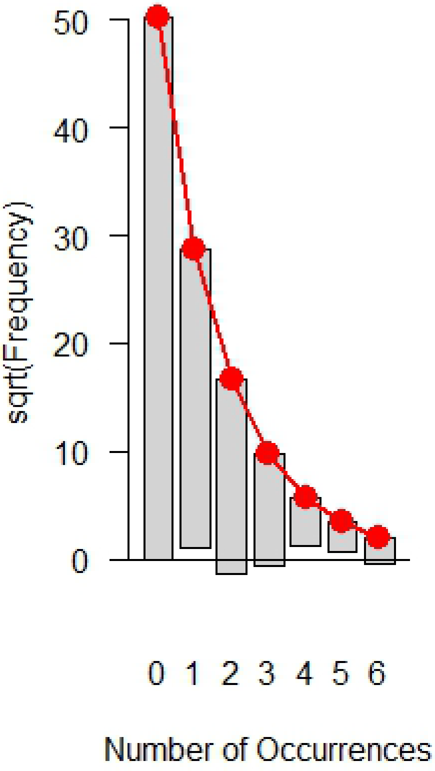

Figure B2 Rootogram of active behaviour data with a negative binomial distribution

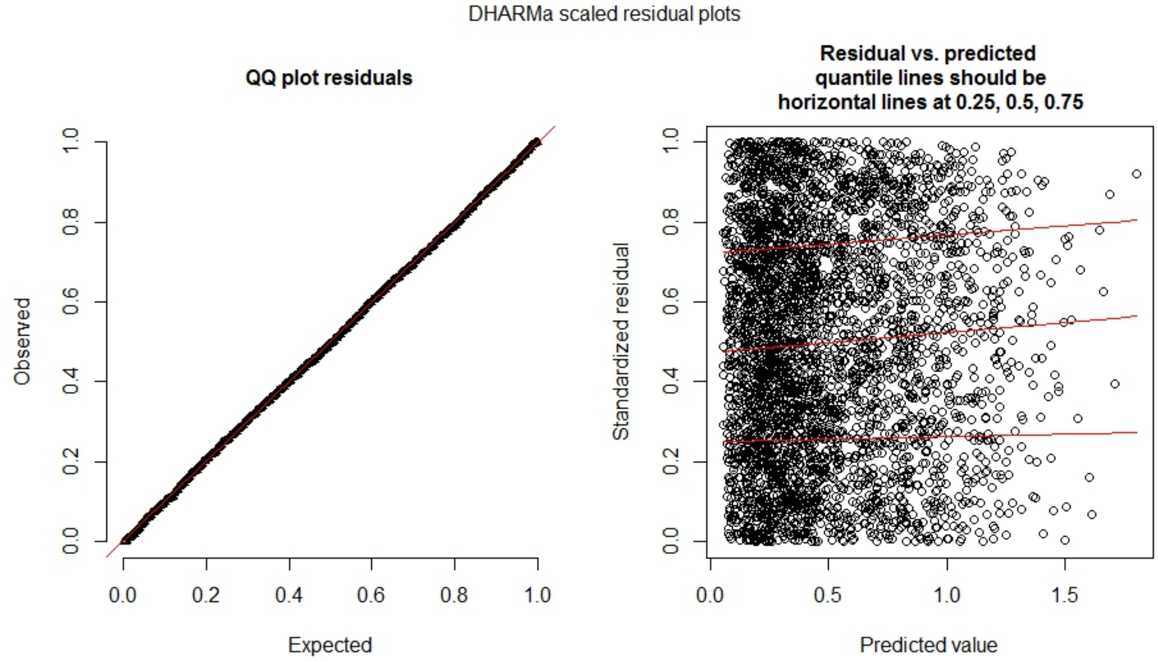

Figure B3 DHARMa diagnostic plots for active behaviour MAM