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Subspecies identification with mtDNA and morphometrics in captive palm cockatoos, *Probosciger aterrimus*

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Abstract

The European breeding programme (EEP) for palm cockatoos *Probosciger aterrimus* has managed two subspecies, *P. a. goliath* and *P. a. aterrimus*, separately since it was found that only these two subspecies were distinct genetic lineages. Until recently a captive palm cockatoo was assigned to one or the other subspecies relying solely on morphology, *P. a. goliath* being reputedly larger than *P. a. aterrimus*. This study aimed at first to determine the subspecies of 78 captive palm cockatoos – mainly members of the EEP population – by sequencing a mitochondrial marker which had proved relevant for wild specimens. We also collected several anatomical measurements in order to compare the morphology with the molecular marker and to assess the presumed link between morphology and subspecies. Ten different haplotypes were found over 54 non-related samples, which could be arranged into two groups consistent with the subspecies *P. a. goliath* and *P. a. aterrimus*. Morphometric analyses revealed significant differences between the two subspecies, although there was some overlap between values for *P. a. goliath* and *P. a. aterrimus*. A stepwise discriminant analysis, which included one criterion for females and two criteria for males, allowed a correct assignation of 95% on average for our sample. These results allowed us to confirm that the captive population of palm cockatoos consists of two distinct genetic subgroups, which overall match with morphotypes. Therefore to preserve these two different conservation units we advise that *P. a. goliath* and *P. a. aterrimus* continue to be managed as two separate breeding populations. Morphology using a recommended set of measurements gives a fairly reliable insight into the subspecies identity for a newly introduced palm cockatoo, but testing mtDNA is highly recommended to confirm the correct subspecies determination.

Introduction

In modern zoology, including ornithology, the subspecies is the only recognised infraspecific taxonomic rank; it is the lowest rank included in the official nomenclature of the International Code of Zoological Nomenclature (ICZN 1999), which has established the standard trinomial nomenclature of genus–species–subspecies. The subspecies concept was originally based on differences in geographical distribution of phenotypic traits in populations within a species (Mayr 1942; Wilson and Brown 1953). This concept has, however, encountered numerous criticisms and remains controversial: in the biological species concept as defined by Mayr, subspecies, unless separated by physical boundaries, interbreed at their boundaries resulting in gene flow between them and blurring of morphological characters. However many species unquestionably consist of a set of populations represented by subspecies, so detractors have not criticised the concept of subspecies in itself but rather its improper application. Indeed taxonomists have often focused on mean differences for a character between populations regardless of the extent of overlap, thus ignoring the predictability issue (Patten and Unitt 2002). For that matter, a standard level for defining a subspecies has been largely established on the "75% rule", meaning that 75% of a population must lie outside 99% of the range of other populations for a given defining character or set of characters to be recognised as a subspecies (Patten and Unitt 2002; Mayr and Ashlock 1991).

Controversy further intensified with the development of genetic tools used to study infraspecific variations. So far molecular studies have relied mainly on mitochondrial DNA (mtDNA) markers, which trace back the history of the different populations forming a species (Joseph and Omland 2009). Phylogeographic studies now tend to use mtDNA together with several loci from nuclear DNA (nDNA) to take into account demographic processes such as gene flow (Zink and Barrowclough 2008; Backström et al. 2008; Joseph and Omland 2009). The subspecies definition now includes this molecular criterion: it is usually considered as a breeding population that occupies a distinct segment of the geographic range of its species

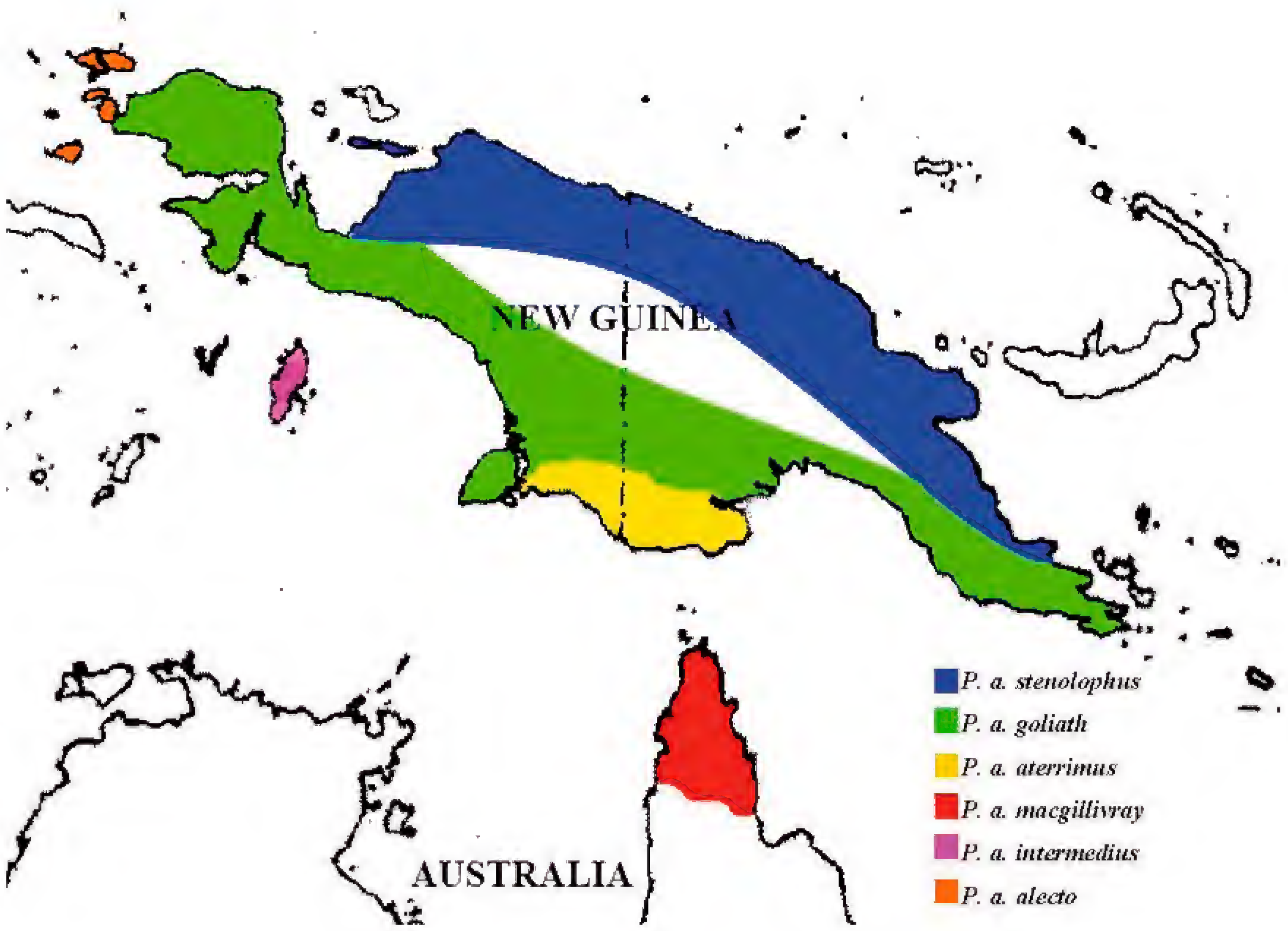

Figure 1. Approximate distribution of *Probosciger aterrimus* subspecies, as accepted before the research on the phylogeography of palm cockatoos by Murphy et al. (2007). Based on data in Murphy et al. 2007.

and that is measurably distinct in its phenotype, genotype or both. Ideally these multiple criteria would co-vary but several studies have revealed a lack of consistency between the traditionally recognised subspecies and the phylogenetic clusters identified using molecular methods. Subspecies traditionally recognised are rarely distinct phylogenetic units, but island subspecies are more likely to show monophyly compared to continental subspecies (Zink 2004; Phillimore and Owens 2006).

The palm cockatoo *Probosciger aterrimus* is a large black parrot and the only member of the tribe Microglossini. It is classified as Least Concern by IUCN and has ever been since 2004, though the population trend is known to be decreasing. Its range consists of New Guinea and surrounding islands and Cape York Peninsula in Australia. Traditional taxonomy recognised three subspecies: *P. a. aterrimus, P. a. goliath* and *P. a. stenolophus* (Coates 1985, Rowley 1997; Marchant and Higgins 1999; Taylor 2000). Other subspecies that have been proposed include *P. a. macgillivrayi*, *P. a. intermedius* and *P. a. alecto*, but these are reported to lack any clear morphological distinction (Rand and Gilliard 1967; Schodde and Mason 1997; Marchant and Higgins 1999). Figure 1 shows the approximate location of subspecies. *P. a. goliath* is reputedly larger than *P. a. aterrimus*, and *P. a. stenolophus* is described as the same size as or larger than *P. a. goliath* and to have narrower crest feathers (Rand and Gilliard 1967; Rowley 1997; Marchant and Higgins 1999, Taylor 2000), although no quantitative data on biometrics are available for any of the subspecies. More recent research on the phylogeography of palm cockatoos has revealed that, according to use of an mtDNA marker, only two east–west lineages could be distinguished (Murphy et al. 2007). Murphy et al. (2007) therefore proposed that only two subspecies should be recognised and given independent conservation status: *P. a. goliath* in the Vogelkop up to the Weyland Range and in the western islands, and *P. a. aterrimus* elsewhere.

Captive management of palm cockatoos has been supervised since 1988 in North America by a Species Survival Plan (SSP) (Taylor 2000) and since 1991 in Europe by a European Breeding Programme (EEP) (Bairrão Ruivo 2012). In both cases the captive population is believed to consist of both *P. a. goliath* and *P. a. aterrimus*, and both the SSP and EEP want to avoid the production of subspecific hybrids (Taylor 2000; Bairrão Ruivo 2012). In France, this management decision follows a ministerial decree enacted in 2004 by the Ministry of Ecology and Agriculture (Article 17 Arrêté du 25 mars 2004). Until the current study was undertaken for the EEP population, subspecies determination was based on weight and size only. The SSP generally consider birds weighing less than 800 g to be *P. a. aterrimus* and those weighing near to or more than 1000 g to be *P. a. goliath* (Taylor 2000), whereas the birds in the EEP were considered as *P. a. aterrimus* when weighing around 500–600 g and as *P. a. goliath* aat round 800–1000 g (Bairrão Ruivo, personal communication).

The use of an mtDNA marker (Murphy et al. 2007) represented a new opportunity to have the captive population of palm cockatoos studied genetically. The palm cockatoo EEP thus decided to support genetic research to investigate subspecific identification in the EEP population of palm cockatoos. It was acknowledged that some individuals may be hybrids between the two subspecies and that these could not be identified through maternally inherited mtDNA.

This research thus aimed first at assessing whether the same haplotypes as identified by Murphy could be found in the palm cockatoo EEP population. This possible genetic test would bring an additional criterion to help identify captive individuals as

belonging to one or the other currently recognised subspecies. The second aim was to assess the correlation between the genotype and the phenotype using several anatomical measurements and weight. These results would indicate if it was relevant to maintain breeding populations for the two presumably distinct groups. If so, each individual palm cockatoo within the breeding programme should be investigated and its subspecies updated according to genetic and morphological data.

Materials and methods

Sampling

In 2011, institutions holding palm cockatoos included in the EEP were sent an information note and a questionnaire to complete. It was indicated that all palm cockatoos in the EEP should be tested as to subspecies and those that were not tested could not have a breeding recommendation. The participants were requested to take a blood sample using the tubes provided, containing EDTA and thymol, to provide information on identification, suspected subspecies, sex and age, and to take body measurements according to illustrations provided (see “Morphometrics” below for more details). All the information and blood samples were to be sent back to us, and we stored the blood samples at around 4° C until processing them.

Genetic analysis

DNA extraction was performed with DNeasy Blood and Tissue kit (Qiagen®), following the indications for blood samples with nucleated blood cells.

Sex determination or confirmation was performed by PCR using primers 2550F and 2718R to amplify introns from the CHD-Z and CHD-W genes (Fridolfsson and Ellegren 1999).

A 280 base pair (bp) sequence in domain III of the control region – a non-coding area of the mtDNA – was amplified using primers designed by Murphy et al. (2007) (PCd3F#3: CGTTTGTTCGTGATCAACTCGTGTC; PCd3R#1: TGGTGGTAATCCATCTTAGCATC)

Each reaction contained 2 µl buffer (containing 25 µM $MgCl_2$), 1 µl DMSO, 0.8 µl of a 6.6 mM dNTP mix, 0.32 µl of a 10 pM/ml solution of each primer, 0.12 µL (0.6 units) of Quiagen Taq polymerase and sterile water up to 20 µl.

PCR thermal cycling for both consisted of an initial denaturing step of 5 min at 94° C, followed by repeated denaturing, annealing, and extension steps for 35 cycles of 40 s at 94° C, 40 s at 49° C, and 60 s at 72° C, with a final extension step of 5 min at 72° C.

Samples were then placed in a 2% agarose gel containing 10 µl ethidium bromide and electrophoresis was run in 0.5X TBE at 135 V for approximately 15 min. Gels were visualised under UV light and photographs were taken of all successful runs.

Female sex was assigned if both the CHD-Z and CHD-W bands were present, and male sex was assigned if a single CHD-Z band was present.

The amplification of the mtDNA marker was considered successful if a band appeared around 300 bp, comparing it to the 100 bp ladder obtained with the DNA molecular weight marker XIV.

Sequencing was performed on ABI 3730xl (96-capillary) following the manufacturer’s instructions and the sequences were then aligned in CodonCode Aligner 3.7.1. Analyses of sequences variability were performed with Mega 5.1.

Morphometrics

Eight body-size measurements were taken for each bird: body mass (±1 g), wing chord length (carpal joint to longest primary feather unflattened chord, ±1 mm), tarsometatarsus length (±0.5 mm), beak length, beak height and beak width (all ±0.5 mm), red cheek patch height and length (±0.5 mm). We gave no particular recommendation concerning the time when the measurements should be taken, so weight could correspond to an empty or full crop weight.

Statistical analyses were performed with Tanagra 1.4.42 Software (2003). Since no data on biometrics had been published for the wild population, the statistical analysis only concerns the samples collected for the purposes of this research.

A multivariate analysis of variance (MANOVA) was performed to evaluate overall differences between sexes and subspecies based on the genotype determined previously. Analysis of variance for each measurement was then performed separately for males and females, with a t-test assuming unequal variance between the two subspecies groups.

In order to test for clustering in groups based on morphology, regardless of the subspecies – either assumed or based on the mtDNA marker – a clustering analysis using a k-means algorithm was performed. This method enables later evaluation of the extent to which the subspecies and/or the sex subgroups match with the morphology subgroups.

Finally a forward step-wise general discriminant analysis (Williams 1983; Wilson et al. 2012) was performed separately for males and females to evaluate whether the morphology could be used to identify reliably an individual’s subspecies.

Results

Sampling

We collected 78 samples from 19 institutions, among which 60 – from 18 institutions – are part of the palm cockatoo EEP. Twelve birds from Jurong Bird Park in Singapore and six confiscated birds from the CITES Centre in Prague were also included. After determination by PCR, 45 males and 33 females were found. This population includes known close relatives (parents–offspring or siblings).

Genetic analysis

The amplified sequences were aligned and consisted of up to 286 bp. For further analysis, only the sequences from site 6 to site 221 – resulting in a 216-bp sequence – were taken into account in order to exclude from the analysis end sequences, which were of poor quality for some samples.

Of the 78 samples collected, 24 were either offspring of a female included in the study and/or siblings. These individuals obviously shared exactly the same sequence, and their sequences were therefore discarded to avoid biases in genetic distance analyses. The following statistics have thus been calculated with 54 *a priori* non-related samples.

Over the 216-bp defined sequence, 14 variable sites were identified, among which six sites were singletons – each of these six mutations was present in one sample only. Apart from these single nucleotide mutations, two insertion–deletion (indel) mutations were identified: a single nucleotide indel in one individual and the same 7-bp indel as found by Murphy et al. (2007) in 10 birds – and eight offspring. We found 10 different haplotypes, five of them showing the 7-bp indel (Table 1).

A network representation was drawn manually to show all 10 haplotypes according to the number of mutations that link them, thus representing genetic distances among them (Figure 2). They can be clustered into two groups, A and B, separated by seven mutational steps. Group B gathers samples with the 7-bp indel. Note that for the network’s construction we chose to consider each base substitution and each indel as one mutational event. Therefore the 7-bp indel here appears as one event, but should it result from seven independent events, the distance between groups A and B would be increased by six additional steps.

Table 1. Haplotype diversity within 54 palm cockatoos for a 216-bp sequence.

Haplo-type	Informative sites: 30	42	43	66	68	121	122	134	137	146	148	151	161	162	163	164	165	166	167	168	180	215	Number of individuals[1]
1	G	C	C	C	T	C	T	C	-	T	T	C	-	-	-	-	-	-	-	T	C	T	35
2	.	.	.	.	.	.	C	.	-	.	.	.	-	-	-	-	-	-	-	.	.	.	6
3	.	.	.	T	.	.	.	.	-	.	.	.	-	-	-	-	-	-	-	.	.	.	1
4	.	.	T	.	.	.	.	.	-	.	.	.	-	-	-	-	-	-	-	.	.	.	1
5	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-	-	-	-	-	.	.	.	1
6	A	.	.	.	.	.	.	.	-	C	.	T	A	T	C	A	C	C	T	C	T	C	2
7	A	.	.	.	.	T	.	.	-	C	.	T	A	T	C	A	C	C	T	C	T	C	5
8	A	.	.	.	.	T	.	.	-	C	C	T	A	T	C	A	C	C	T	C	T	C	1
9	A	.	.	.	.	T	.	T	-	C	.	T	A	T	C	A	C	C	T	C	T	C	1
10	A	T	.	.	C	T	.	.	-	C	.	T	A	T	C	A	C	C	T	C	T	C	1

[1]Haplotype 1 and Haplotype 8 were shared respectively by 16 and 8 other individuals, offspring or siblings.

In group A, two haplotypes were linked by only one mutational step and in group B two haplotypes were linked by one or two mutational steps. Nucleotide divergence was found to be 1.25% overall. It reached 3.65% between groups A and B and was 0.164% and 0.572% within each respective group.

According to this genetic analysis, we found 44 non-related birds plus 16 offspring or siblings – 33 males and 27 females – belonging to group A and 10 non-related birds plus 8 offspring – 12 males and 6 females – belonging to group B. All these results are summarised in Table 2.

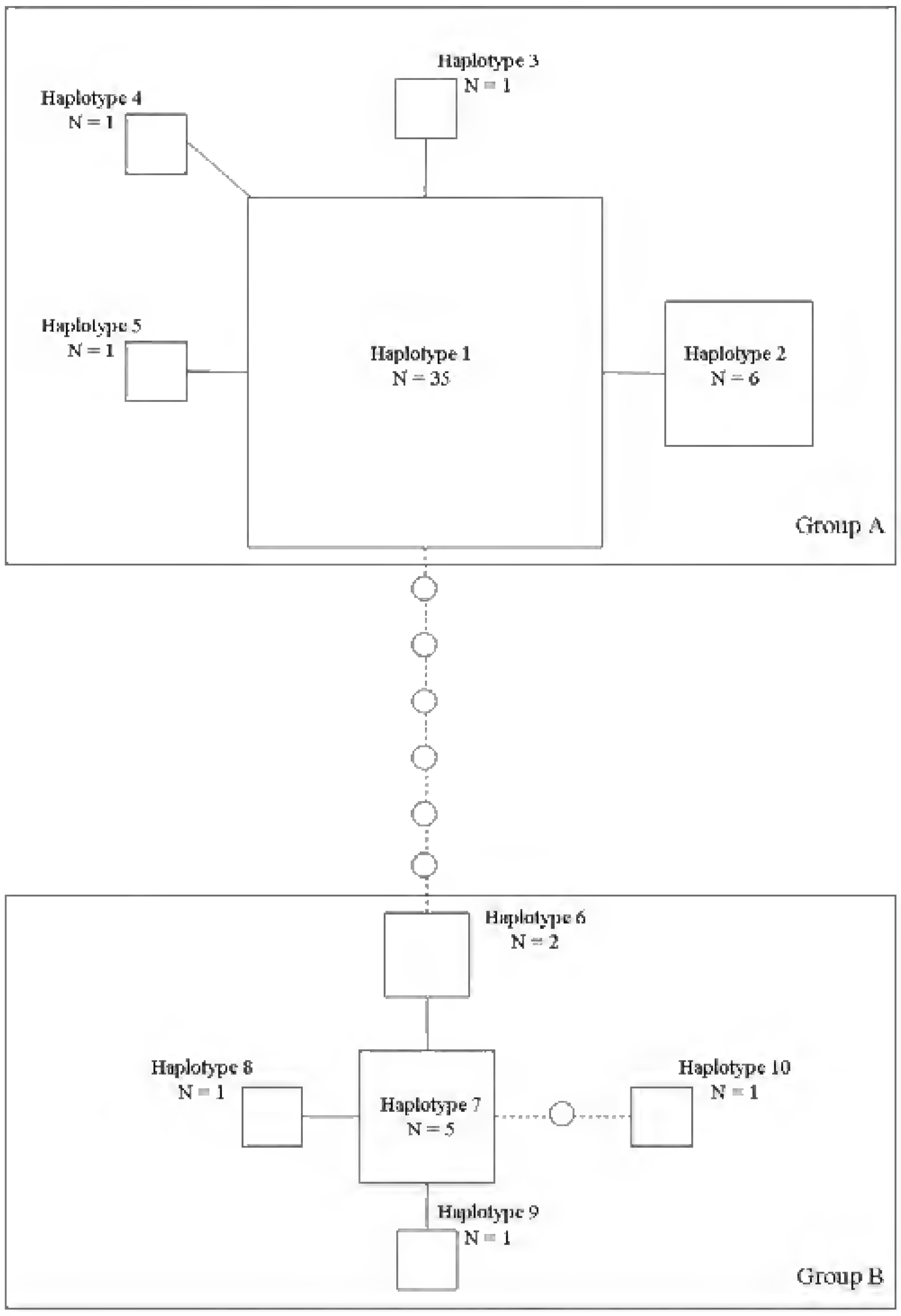

Figure 2. Network representation of the 54 non-related palm cockatoos in this study. Note: Boxes represent haplotypes and their size is proportional to sample size. Circles indicate missing haplotypes.

Referring to the findings of Murphy et al. (2007), groups A and B defined here correspond to clades 2.1 and 2.2, which were found to gather eastern – *P. a. aterrimus* – and western – *P. a. goliath* – birds respectively. Individuals belonging to either group A or B will therefore be referred to as *aterrimus* or *goliath* respectively in the remainder of this paper.

Morphometrics

All the birds were at least one year old – and up to 37 years old – at the time when measurements were taken, and were thus all considered fully-grown adults. We relied on growth curves recorded in the birds' husbandry notes at ZooParc de Beauval, which showed that hand-reared palm cockatoos reached adult size before one year. For the 69 individuals – 39 males and 30 females – for which all measurements were available, overall morphology differed between the sexes (Wilks' $\lambda = 0.24$, $F_{(8, 60)} = 24.28$, $p < 0.0001$). This result led us to compare each measurement and perform further discriminant analyses separately for males and females.

An outcome of the previous mtDNA analyses was that six of 69 individuals were found to have parents belonging to the two different genetic groups. Unless indicated otherwise, morphometric data for these six hybrids were discarded from further statistical analyses.

For the remaining 63 individuals – 17 *goliath* and 46 *aterrimus* – the overall morphology differed between subspecies (Wilks' $\lambda = 0.29$, $F_{(8, 54)} = 16.66$, $p < 0.0001$).

Table 2. Distribution of the 78 individuals in our sample[1] according to genetic results.

	Male	Female	Total
Group A	33 (26)	27 (18)	60 (44)
Group B	12 (11)	6 (5)	18 (16)
Total	45 (37)	33 (23)	78 (60)

[1]The numbers of individuals from the palm cockatoo EEP population are indicated in brackets.

Table 3. Body size (mm) and body mass (g) measurements.

	aterrimus[1]			*goliath*[1]			
	Mean	SD	Range	Mean	SD	Range	p-value[2]
Male							
Weight	793.5	108.1	593-1000	1056.7	97.8	790-1184	*<0.001*
Wing	339.1	19.3	277-369	384.5	13.5	353-405	*<0.001*
Tarso-metatarsus	35.9	3.8	24-41	41.9	2.1	38-45	*<0.001*
Beak length	87.1	7.9	60-108	95.1	5.9	85-106.5	*0.0019*
Beak height	41.3	2.7	34-45	47	4	42-53	*<0.001*
Beak width	23.7	3.2	18-36	28.1	3.1	19-32	*<0.001*
Red cheek patch height	61.4	6.3	40-71	65.7	7.5	56-81	0.11
Red cheek patch length	54.9	9.5	37-72	60.7	6.8	53-73.5	*0.047*
Female							
Weight	590.0	68.5	460-810	857.8	86.4	730-950	*0.0021*
Wing	321.9	18.8	260-355	360.8	19.0	325-380	*0.0093*
Tarso-metatarsus	33.5	4.1	24-39	38.0	3.0	33-42	*0.036*
Beak length	70.0	5.9	57-82	77.2	5.6	69-84	0.060
Beak height	35.4	2.4	29-41	41.4	3.2	37-46	*0.016*
Beak width	21.4	2.5	13-24	25.6	1.0	24-27	*<0.001*
Red cheek patch height	49.9	6.9	30-59	51.8	4.6	46-60	0.51
Red cheek patch length	49.3	11.9	28-83	50.6	4.5	46-59	0.70

[1]Sample sizes: *aterrimus* – 29 males except for weight (27) and red cheek patch length (28) and 22 females except for weight (20); *goliath* – 12 males and 5 females. [2]Significant comparisons (significance level set to 0.05) given in italics.

For males, all measurements except for the red cheek patch measures differed significantly with a strong p-value in *goliath* and *aterrimus* birds (Table 3). These measurements were approximately 9–19% higher – 33% for weight – on average for the *goliath* group. However, it should be noted that the ranges of recorded values overlap for all measurements between the *goliath* and *aterrimus* groups. This statement is also true for measurements among females. Only beak length and both the red cheek patch measurements failed to be significantly different in females. Wing length, tarsometatarsus length, beak height and width were significantly higher by 10–20% and weight by 45% on average for *goliath* female birds.

In the light of these results, red cheek patch measurements do not seem to be appropriate distinctive measurements. Several participants also reported these measurements as the most difficult ones to take and the most variable ones depending on the

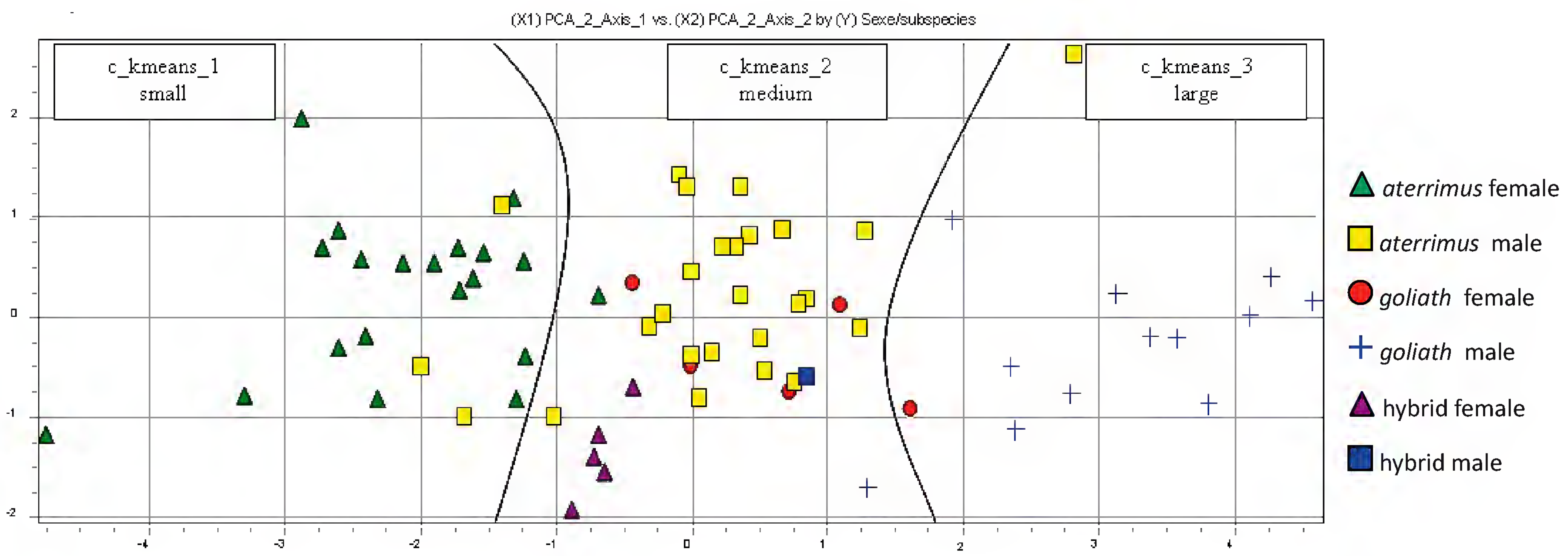

Figure 3. Principal Component Analysis (PCA) scatter plot representing the 69 palm cockatoos included in the k-means clustering.

Table 4. Contingency table displaying the distribution of the 69 palm cockatoos included in the k-means clustering among the three defined morphological clusters

	C_kmeans_1 "small"	C_kmeans_2 "medium"	C_kmeans_3 "large"	Total
aterrimus female	19	1	0	20
aterrimus male	3	22	1	26
goliath female	0	4	1	5
goliath male	0	1	11	12
hybrid female	0	5	0	5
hybrid male	0	1	0	1
Total	22	34	13	69

opening of the beak. These factors resulted in a lack of reliability for red cheek patch measurements, and so they were not used in further analyses.

The clustering analysis was performed using a k-means algorithm setting the number of clusters to three in order to get three different size groups referred to as small, medium and large. Our designation is arbitrary and does not reflect the actual size of the birds: all six measurements have equal importance in the determination of clusters. Since this statistical method does not depend on classification of individuals – neither for their subspecies or sex – all the palm cockatoos including hybrids were included in the analysis. A contingency table between the resulting morphological clusters and the "subspecies based on genotype + sex" group reveals a significant difference in frequencies (Table 4; $\chi^2 = 100$, $p < 0.0001$). It is noteworthy that there is no *goliath* individual, neither male nor female, in the first "small" group, and no *aterrimus* female in the third "large" group. Figure 3 shows the distribution of the 69 palm cockatoos included in the clustering analysis.

The final discriminant function obtained by a forward step-wise discriminant analysis selected two variables as significant measurements for distinguishing males between the subspecies: wing and tarso-metatarsus lengths. Assignation was correct for 26 out of 26 (100%) for *aterrimus* and 11 out of 12 (92%) for *goliath*. This model leads to the same error rates as when all six measurements were taken into account in the linear discriminant analysis. The unstandardised discriminant model derived from these two discriminating variables is as follows:

$$D = 0.058 \times \text{wing length (mm)} + 0.185 \times \text{tarso–metatarsus length (mm)} – 27.60$$

A male palm cockatoo was defined as belonging to the *goliath* subgroup if its measures led to a positive D, or to the *aterrimus* subgroup if D was negative; the overall error rate obtained by cross validation was 6.7%.

For females, only the weight was included in the final discriminant function. The percentage of correct assignation was lower, with 4 out of 5 (80%) *goliath* and 19 out of 20 (95%) *aterrimus* correctly identified. This model has again the same error rate as the discriminant analysis taking all six criteria into account. The unstandardised discriminant model derived from the variable weight is as follows:

$$D = 0.0133 \times \text{Weight (g)} – 8.53$$

A female palm cockatoo was defined as belonging to the *goliath* subgroup if its measures led to a positive D, or to the *aterrimus* subgroup if D was negative; the overall error rate obtained by cross validation was 10%.

Discussion

The two genetic clusters we found for captive palm cockatoos are consistent with the two clades identified by Murphy et al. (2007) from wild specimens. The nucleotide variability figures were also similar, although we found an average nucleotide divergence lower for each group. Since we excluded related samples – offspring and siblings – from the variability analysis, this observation cannot be explained by this bias. However, we cannot rule out a possible family relationship between founders of the captive population. The precise geographical origin of these palm cockatoos – which are of wild or unknown origin – is generally unknown; their distribution could therefore be less representative of the overall geographic range of palm cockatoos than the sample studied by Murphy et al. It may also be accounted for by lower sample sizes for both subspecies.

Mitochondrial DNA is maternally inherited and cannot therefore identify possible hybrids between *P. a. goliath* and *P. a. aterrimus* subspecies. For this particular aim, we tried simultaneously throughout this research to identify nuclear markers for subspecies. Because nuclear introns – non coding sequences – are known for their high sequence variation (Primmer et al. 2002), and thus more likely to show variability between the two subspecies, we investigated a dozen of them – autosomal or linked to the Z chromosome – for single nucleotide polymorphism (SNP). Unfortunately, no relevant SNP was found in the markers tested. Even though the sequenced introns overall represented 7060 bp, identification of relevant SNP for subspecies identification in palm cockatoos could be looked for in the future using restriction-site associated DNA (RAD) sequencing, which would enable much higher throughput genotyping (McCormack et al. 2013).

The morphometric analysis is most probably biased by a lack of consistency due to different people taking the measurements, and due to differences between institutions in diet and husbandry conditions that may affect body condition or other morphometric measurements such as beak length (which depends on how much it is worn down). Despite these biases, our morphometric analysis showed that there is a significant correlation between overall size – taking into account all six measurements – and the subspecies–sex category. Palm cockatoos carrying the *goliath* haplotype were found to have higher values for each measurement than those carrying the *aterrimus* haplotype. This finding is consistent with all previous morphological descriptions for *P. a. goliath* and *P. a. aterrimus*. However, we found that each morphological criterion measured here shows overlap between the two subgroups. Subspecies assignation relying on morphology cannot lead to a 100% correct classification. Predictive discriminant analysis using wing length and tarsometatarsus length remains satisfactory for males, since it enables a correct subspecies assignation with a 6.7% error rate, which is equal to the prediction obtained with six measurements. For females, the resulting equation is not as satisfactory since it only takes into account weight, which is a criterion dependent on other parameters – full vs empty crop, diet, activity, possible illness, reproductive status – and it shows a higher error rate of 10%. It would be interesting to have a larger sample size for females in order to calculate a more robust equation.

For 51 out of the 60 individuals included in the EEP, the marker sequence belonged to the group corresponding to the presumed subspecies. If we base this solely on genotyping, subspecies assignation was thus correct in 85% of the cases using a morphology overview – without precise measurement –

and of course based on the parents' origin when it was known. Among the remaining nine individuals, three had their subspecies determinations changed: two females from *P. a. goliath* to *P. a. aterrimus* and one male from *P. a. aterrimus* to *P. a. goliath*. These three individuals were of unknown or wild origin, without any geographical indication for the latter. Their previous subspecies determinations were based only on their size. We can assume that the two females were considered as rather large and the male as rather small to be respectively determined as *goliath* and *aterrimus*. However, in this study these two females are classified in cluster 1, "small", and the male in cluster 3, "large", which means that they have a morphotype corresponding to their subspecies. These three individuals indicate the lack of reliability of assigning a palm cockatoo to a subspecies only on visual inspection without precise measurements and without comparison with individuals belonging to both subspecies. Six individuals were determined to be hybrids: one was born from a female *goliath* and a male *aterrimus* and five were siblings and born from a female *aterrimus* and a male *goliath*. Interestingly all these hybrids – five females and one male – are assigned to group 2, "medium".

For captive breeding programmes, dealing with subspecies can be challenging. If breeding programme coordinators choose to manage subspecies separately, they create an artificial barrier that prevents gene flow for populations that may actually interbreed to a greater or lesser extent in the wild. In the particular case of palm cockatoos, little is known about the zone of hybridisation in New Guinea and the surroundings island: Murphy et al. (2007) mentioned the possibility of a hybrid population in the Weyland range. Nuclear markers are lacking to confirm this hypothesis. It has, however, been recommended that independent conservation status should be given to *P. a. aterrimus* and *P. a. goliath*, since they correspond to distinct east–west genetic lineages endemic to each area (Murphy et al. 2007). In terms of morphology, there are unfortunately no data available regarding geographical distribution *in situ* that could show a similar correlation as found in our sample.

From an ecological point of view, subspecies are populations with different phenotypes corresponding to adaptive traits to particular environments in terms of diet, habitat, population density, and competing species (Hamilton 1961; Grant 1968). Conservation efforts *in situ* aim at preserving these adaptive traits: subspecies are therefore considered as conservation units and should be maintained. Conservation breeding programmes in captivity should follow the same management directive, all the more when there is a clear discrimination between infraspecific populations as observed in this study. In the light of these results, we therefore conclude that captive breeding programmes for palm cockatoos should continue to manage separate breeding populations of *P. a. aterrimus* and *P. a. goliath*. The main difficulty for the future for the European Breeding Programme, which was confirmed by this research, is the very low number of representatives of *P. a. goliath*.

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Zoos as a context for reinforcing environmentally responsible behaviour: the dual challenges that zoo educators have set themselves

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Abstract

A strong focus for zoo education is inspiring visitors to care for the environment and this inevitably means reinforcing messages that relate to the adoption of sustainable lifestyles. Since lifestyle changes are likely to involve some level of personal cost or sacrifice, zoo educators are faced with the challenge of aligning this objective with the visitor expectation of a recreational day out to see the animals with the family. In order to evidence their effectiveness as education providers, zoos must also evaluate their educational activities and this represents a second challenge. The Learning Together intervention was devised specifically to address these two challenges as single parent families participated in zoo-based workshops with themes that related to environmentally responsible lifestyles. Results from focus groups in the zoo and semi-structured interviews in the community several weeks later showed that parents had gained a new understanding of the role of zoos and were motivated to make lifestyle changes that persisted over the lifetime of the project.

Introduction

The contextual framework that guides the direction of zoo education is based on the premise that modern zoos should inspire their visitors to care about the environment and instil a sense of personal responsibility for making behaviour changes that support sustainable lifestyles (WAZA 2005). A significant signpost for zoo education came from the Earth Summit in Rio in 1992, where zoos were identified as education providers for "Think Global, Act Local" – the framework that urged every citizen to take responsibility for making lifestyle changes to conserve the environment. More recently the World Association of Zoos and Aquariums pledged, on behalf of its membership, to support the Aichi Biodiversity Target 1 of the UN Decade on Biodiversity and commit to raising awareness of global issues and encourage zoo visitors to live sustainably (WAZA 2011).

In embracing this education role as a catalyst for change (Cachelin et al. 2009; Rabb and Saunders 2005; Hutchins and Smith 2003; Smith 1989), zoos have set themselves a challenge; in fact, two challenges. The first relates to how zoos attract their visitors and the effect this may have on visitors' mind-sets. The second relates to zoos providing evidence that they do indeed impact on their visitors in the way they purport to do.

To gain perspective on this first challenge, we must take into account, that unlike many museums and galleries that are publicly funded and offer free entry, zoos rely heavily on gate income to cover running costs and to fund their conservation activities. Attracting visitors in the competitive, leisure attractions' market is of prime importance. Public perception of zoos may be formed through pre-visit exposure to advertising (Falk, Moussouri and Coulson 1998; Moussouri 1997) that promotes fun, recreation and seeing the cute animals (typically mammals) that the media promote and the public find appealing (Moss and Esson 2010; Myers et al. 2003; Durham 1982; Resenbrink 1981; Bostock 1981). For many visitors the motivation to visit a zoo is not principally associated with education but recreation, particularly as a shared family experience (Hyson 2004; Pekarik 2004; Dierking et al. 2002; Turley 2001; Kellert and Dunlap 1989; Resenbrink 1981). Potentially worrying news about human impact on the environment may be in stark contrast to the family motivation for a recreational day out to see the animals (Esson and Moss 2013). Once in the zoo, visitors may not respond positively to having the agenda for their outing unsettled by challenges to their lifestyles. Contrary to facilitating a relaxing day out, raising awareness of pertinent environmental issues risks leaving visitors "in a state of anxiety about the problem and helplessness about the solution" (Sterling et al. 2007, p.44).

A number of studies in zoos, science centres, aquariums and museums have sought to shed light on the relationship between visitor self-identity and motivation to visit, compared with the impact this has on the enactment and outcome of the visit (Moussouri 1997; Packer 2006; Packer and Ballantyne 2005; Packer 2004; Packer and Ballantyne 2002; Briseño-Garzón et al. 2007; Falk et al. 2008). Broadly speaking these studies found a correlation between entry identity and motivation

to visit, and how the visit was enacted and later interpreted, including what was learned. As such, any disconnect between the anticipation of what the zoo visit promises and the reality of the educational experience once in the zoo, presents a real challenge to zoo educators who may be faced with reluctant learners and visitors who are not primed for learning. Encouragingly however, a small number of studies have demonstrated that motivation to visit zoos can include an educational focus. But is this just about the animals or is it about environmental behaviour change? For example, Morgan and Hodgkinson (1999) employed a quantitative approach and found that while recreation was the strongest motivation for a zoo visit, education was also seen as important, particularly for others in their group (namely children). Fraser (2009) describes an exploratory, qualitative study with families from a low-income background, reporting that parents saw the zoo as a valuable tool in promoting social relationships within the family and in encouraging moral development in their children, particularly related to altruism.

The second challenge for zoos is concerned with how to credibly measure impact. Zoos are asked to evidence that education that leads to behaviour change has taken place as a result of a zoo visit (Marino et al. 2010; Born Free Foundation 2011; Balmford et al. 2007; RSPCA 2006). There are inherent flaws associated with conducting research that seeks to evidence behaviour change since the research model usually relies on pledges made by the participants and not direct observations. Self-reports may be unreliable (Dierking et al. 2004) and even when it is possible to conduct a longitudinal study to witness behaviour change, the premise that the zoo could show causality for instigating that change is still fragile. Furthermore, knowledge gained as part of an educational intervention has often been conflated with corresponding changes in attitude and/or behaviour and behaviour –change researchers generally observe only limited correlations between knowledge and attitudes and behaviour (Heberlein 2012). The complexity of understanding behaviour change means that other factors can be influential. These include habit (behaviours that have become automatic), emotion (how someone might feel if they succeed or fail in performing a behaviour) and contextual factors (factors outside the control of the individual in question, such as access to information or financial restraints) (cf. Darnton 2008).

This paper describes the "Learning Together" project, a community and zoo-based educational intervention, for single parent families. A strong feature of the didactic teaching elements of the project was the delivery of specific action agendas – suggestions of how parents could alter their lifestyles to live more sustainably. The themes of the project workshops were closely aligned to Agenda 21 and Aichi Targets and were, to some extent, the generic environmental messages regularly disseminated by the broadcast media and government. Our aim was to provide a range of explicit prompts to encourage repetitive behaviours (McKenzie-Mohr 2012). Examples of themes are the effects of water pollution on wildlife and how to reduce this by choosing environmentally-friendly cleaning products, and the link between orang-utan survival, deforestation, our consumption of palm oil and value of checking food labelling before purchasing. We recognised that, for most people, infrequent zoo visits are unlikely to be life-changing events (Falk and Dierking 2000; Piper 1992) and we took the decision to contextualise the learning agenda to resonate with behaviours already promoted in the community (Adelman et al. 2000; Smith et al. 2008) in order to increase the likelihood of positively benefiting the environment. In positioning our learning agenda as a reinforcing agent, we borrowed from Prochaska and DiClemente (1986) whose model of behaviour change recognises that adhering to new behaviours over time requires maintenance in the form of positive reinforcement. This would suggest that the best opportunity zoos have to be successful in affecting behaviour change is to target their learning to resonate with behaviours already promoted in the community. Consequently our aims were to deliver a learning agenda that would reinforce positive environmental behaviours and to investigate if participants were tolerant of this context as part of a family zoo visit.

Materials and Methods

The Project

"Learning Together" was funded by the Big Lottery Family Learning programme and brought 263 single parents with 460 children into Chester Zoo. The project ran over two years and parents or guardians were recruited through a leading UK charity for single parents. The sampling criteria were single parent families with children over five years of age, living in areas of social and economic deprivation and attending Community or Children's Centres within the catchments of Chester Zoo. The data held by the charity were confidential and no record was kept of parent profiles or how many were approached or declined so it can be said that participants were self-selecting from within this demographic.

At the recruitment stage in the community centres, it was explained that parents and children would have planned educational activities and guided tours of the zoo. Participation in the project was free and an eight or nine week project cycle was devised to include a series of three, one-day visits to the zoo where we focused on flagship species to capture attention and introduce lifestyle and behaviour changes that would be considered convenient to adopt (McKenzie-Mohr 2012). Each visit had a specific theme. Visit one was called "Dangerous Beauty" and discussed how endangered animals are exploited for the illegal wildlife trade and how to avoid supporting this trade. Visit two, "Rainforests and us", focused on how we rely on rainforest products in our everyday lives and the ethical purchasing choices we can all make. Visit three, "Water and life", highlighted the precious nature of water as a resource for us all, and how we can conserve it.

Procedure

Focus groups were convened in the zoo, immediately post-experience, and semi-structured interviews were held in the community approximately six weeks later. By extending this time line we hoped that this afforded parents the opportunity to adopt rather than pledge lifestyle changes and that these would be affirmed at interview (Rennie and Johnston 2007). Some parents were lacking in confidence and exhibited low self-esteem, and some had not fully completed their formal education. We explained that participation in the study was not a prerequisite of joining "Learning Together" and informed consent was sought as part of an ethics protocol. All parents recruited to the project agreed to participate in the study. With parents who may have been lacking in confidence it was believed that the supportive setting of focus groups would encourage confidence and promote participation. A data collection approach that avoided the need for reading or providing written responses was thought to be less inhibiting for those parents who may have low levels of literacy and have had negative experiences of formal education. The additional advantage of using focus groups was that little effort was required of parents in terms of time and commitment.

A discussion framework for the focus groups was drawn up and this broadly reflected the aims of the intervention. Discussion typically commenced with opinions of zoos past and present and moved on to more specific impressions gained from "Learning Together". The framework was sufficiently loose to allow for the unexpected to emerge and the role of the focus group facilitator provided a light touch in order to keep the conversations broadly

on track, and for every voice to be heard. Since parents were from similar backgrounds, this created a powerful socialising agent which was helpful in accelerating discussion. The potential of context to bias data collection is acknowledged (Silverman, 2005), as is the possibility of willingness to please (Creswell 2003; Bryman 2001; Cohen and Manion 2007; Wragg 1984), though the alacrity with which unsolicited concerns relating to animal welfare in zoos were expressed would suggest parents felt sufficiently confident to openly express their opinions. The topic of animal welfare was an example of an unexpected topic emerging from lively focus group discussion. Focus groups were convened at the end of the third visit to the zoo and 100 parents participated in 18 focus groups. This sample represented 38% of the parents who initially agreed to participate in the project and 47% of those who attended all three zoo-based project days. There was a parent drop-out rate of 18% across the life of the project.

Discussion was recorded digitally and then transcribed. The process of coding the narrative from the transcriptions commenced as the focus groups convened in order to consider data analysis and to implement an iterative approach to data collection (Bryman 2004; Krueger and Casey 2000). As themes in the discussions began to emerge provisional descriptors for the codes were developed (Drever 2003). As transcripts were sequentially coded it became apparent over time that no new themes were emerging at which point no further focus groups were convened (Bryman, 2001). To evidence reliability, we adopted an inter-rated scoring approach to coding the transcripts; namely, the percentage agreement between two researchers scoring in isolation of one another. For this investigation, an inter-rater agreement of 91% was achieved and considered robust (Miles and Huberman 1994; Adelman et al. 2000).

As a delayed post-test component of the study, semi-structured interviews were conducted with individual parents in community centres, during their final group meetings. This allowed parents time to reflect on the zoo experience at the end point of the project (Bryman 2004). 40 parents who had previously participated in the focus groups volunteered to be interviewed. An interview framework was drawn up and interviews typically began with what parents had told family and friends about "Learning Together". We considered this relevant as participants in zoo education initiatives acting as message multipliers in the community is of benefit in reaching wider audiences (Domroese and Sterling 1999). Discussion then moved on to what parents had learned and acted upon as a result of the zoo visits. A relaxed style was adopted for interviews to encourage parents to respond in their own words (Drever 2003). The interview framework was not always followed in the same order and open-ended prompts were used to encourage expansion of answers. Conducting the interviews in community centres was viewed as a counter-balance of context to focus groups being held at the zoo.

Results

Focus groups

With the purpose of addressing the aims of the project we sought, as a priority, to capture those common conversational themes that dominated parent discussion. Four strong themes that spoke to the research questions emerged from the coding of the focus group data. These were: memories of past visits to zoos, present opinions of zoos, impressions formed from the three project days spent in the zoo and how behaviour changes can help conserve the environment. Parents were not directly asked about planned changes in behaviour. We found that in 16 of the 18 focus groups discussion moved to what parents had learned about human impact on the environment and how they could act to alleviate this.

Theme 1: Memories

The focus group discussion typically began with reminiscences of any previous zoo visits and what parents could recall. Family memories tended to be nostalgic in character as these recollections were childhood memories of spending time with their parents when they were children themselves.

> "Whenever I come back to the zoo I'm reliving my childhood. So it's all them memories of being a kid."

Parents seemed to place value on these zoo visits in terms of recollections of happy family life, broadly in line with the findings of Packer and Ballantyne (2005). In contrast, however, these happy memories were tinged with some concern and an historical association between zoos and issues of animal welfare was evidenced in the descriptive language of 11 of the 18 groups; for example, "*pens*", "*bars*", "*small and tiny cages*", "*cruel*" and "*bare concrete*".

Happy memories were associated with re-visiting zoos with their own children. This is consistent with the findings of Moussouri (1997), Packer and Ballantyne (2002) and Briseño-Garzón et al. (2007) who identified re-living past experiences as a motivation for making a visit. However, groups tended to discuss zoo visits with their own children in terms of a rather shallow, ill-planned experience, frequently using the word "just":

> "It was just a day out really, with the kids," and "It was a day out, full stop."

Parents associated the visit with seeing the animals, fun for the children and moving quickly from one exhibit to another without paying much attention. When probed about which animals they wanted to see responses typically centred around large mammals, for example: elephants, lions and tigers. Parents appeared to have had low expectations of the educational value of a zoo visit and consequently to have taken little in the way of learning from these visits in the past.

> [I saw the zoo] …"not as educational. I think we thought it was nice for the kids to see the animals."

Theme 2: Present opinions of zoos

Discussion flowed on to present opinion of zoos based on their "Learning Together" experiences and parents expressed a change of opinion when compared to past perceptions of zoos as attractions that "just" exhibited animals. They discussed the need for high standards of animal welfare, how they associated zoos with preserving endangered species and having a wider conservation role.

> "Now I'd say not only do you learn about those animals in the wild but you also learn about habitats and what can damage the environment."

There was a notable fluency in the discourse as groups demonstrated they had acquired the language of conservation; for example, "*endangered species*", "*extinction*" and "*habitat*". This provided some insight into the power of zoos to change opinion and suggested that "Learning Together" had been successful in broadening an understanding of the role of the modern zoo.

Theme 3: Impressions

Parents had a lot to say about their impressions of the "Learning Together" workshops. The understanding evidenced by the parents related to the content of all three workshops and not only from the same day that the focus groups were convened which

was encouraging, since it could be up to four weeks between their first and last zoo visit. The emotional appeal of the animals, especially the large mammals was evident.

> "Yes then we went to see the elephants, the oldest one in the zoo is 53. Then she [the educator] was saying something about its teeth. She'll have to have dentures! [Laughter] She said they can't live without their teeth."

> "She said, all the females in the herd whenever they have babies, she goes and looks after them. That made me cry."

> "That's a kind of relationship like we have isn't it?"

The discussion around time budgets and animal enrichment is an example of what appears to be depth of understanding of a zoo practice:

> "The animals have to search for their food. They're kept busy aren't they?"

Animal enrichment was a feature of one of the early zoo workshops. The degree of accuracy of what the parents discussed was impressive. There were very few instances where what was said was technically incorrect and occasionally where parents were unsure others in the group were quick to respond.

> [We learned] "where the animals come from and why they are that colour."

> "Especially the frogs - the poisonous ones and the copy-cat one."

> "And the apes put their tongue out to taste the wee to find out if the monkey's pregnant."

> "And the chimps we saw with the bums - the way the females get bigger because the male likes it like that." [Loud laughter]

Theme 4: Environmental

The mood of the focus groups often became rather collaborative when parents discussed the state of the environment and what they could do to help, often making verbal commitments as a group. Borrowing from Knapp (2000), a modified approach to transcript coding was adopted. Knapp distinguishes between three components of citizen development: gaining understanding, the ability to apply that understanding to different situations and learning to make lifestyle changes. The discussion that was first coded "Environmental" was scanned for the active verbs parents used to describe their levels of engagement and three groups of verbs emerged. These reflected degrees of action or intent:

"*Internaliser*": verbs that related to an inner understanding; for example: "realising", "taking in" and "wishing".

> "I'm starting to <u>care</u> more about the rainforest now because I don't want to see it getting destroyed."

"*Message Multiplier*": verbs that suggested parents felt sufficiently confident to communicate their understanding to others in different situations, for example: "passing on", "talking about" and "describing".

> "My brother - when we said you've gotta recycle. He said "why have we gotta do that?" With being here now you can see why you've gotta do it and its essential to do."

"*Eco Warrior*": verbs that represented a level of engagement where parents said they had made lifestyle changes, for example; "changing", "cutting down" and "refusing".

> "I'm not gonna buy bleach now when I go home. I'm forever doing that, just straight down the sink, clean the sink out. But now I think of the poor little frog now."

> "I've skinned about ten frogs in my life. The eco-friendly stuff, I didn't realise it smelled so nice, before when she [the zoo educator]passed them round; I thought well I'll get that then. Cos bleach reeks doesn't it."

> "I won't be using none of that no more"

> "I've done that [saved water] and I'm gonna get 'ECOVER' stuff 'cos it's the same price as the other stuff anyway."

The discussion above relates to the "Water and Us" workshop where the effects of damaging cleaning agents on amphibians were discussed. The discussion below was part of the topic about recycling and how wildlife can be injured by items like fishing line and plastic bags.

> "Before [the zoo workshops] you would think just stick it in the normal bin, throw it over the fence or whatever."

> "I'm recycling 'cos when I seen them pictures [turtle suffocated by plastic bag]. It's awful."

> "Yes definitely."

It may be naïve to expect parents to challenge one another's views on the need to protect the environment, so caution must be applied here in being overzealous in the desire to seek out positive opinions that evidenced the success of the project. What can be said to have been objectively witnessed was the degree to which parents adopted the Internaliser persona and appeared to be processing the information and giving the subject consideration by:

"...becoming more [aware]...of when you're buying things", and "...thinking about... the consequences."

Thinking about the environment may have been the easiest option since this required no action and we may have predicted that passive engagement would be most frequently referred to in discussions. Encouragingly, it seems that parents felt empowered to act as Message Multipliers and to advise others how to act. This show of confidence is a particularly rewarding finding from parents considered to be lacking in confidence at the start of the project.

> "Yes. If you see someone doing something you're gonna say "hey don't be doing that"

> "What you do affects other parts of the planet. You can educate other people about it."

"The next time someone throws away you can tell them to recycle……"

In terms of taking action themselves, parents appeared to be equally committed, discussing recycling, litter and purchasing decisions: *"There's a lot of stuff we could do without."* These were also strong themes in the workshops and, parents were often taken aback with how long it takes for some materials to degrade; that disposable nappies are not disposable and how indestructible cigarette filters and chewing gum are.

"You don't think a cigarette and all that would take that long [to degrade]".

Interviews

Those parents who agreed to be interviewed appeared confident in expressing their opinions and this is to be expected as they were a self-selecting group. When they were asked if they had passed on any information to friends or family, ten of the 40 interviewed could recall specific instances where they had done so, including offering advice.

"I've spoken about what we learned – cleaning products, bleach and things and what they do to the skin of a frog. I've been telling people about the plastic rings and animals getting stuck in them."

Parents talked confidently about what they had learned themselves. Our reliance on rainforest products, the need to recycle, to avoid dropping litter and polluting water, were the most widely discussed topics.

"It's so easy to buy and so hard to get rid of. I thought everything was disposable but it stays around. Rubbish doesn't go anywhere. You don't think about it when you go shopping."

It was encouraging that 37 of the 40 parents interviewed were able to explain, often in some detail, lifestyle changes they had made that they attributed to what they had learned from their zoo visits and actions were often linked to conserving wildlife.

"In shops I'm reluctant to accept carrier bags. I've bought several eco bags to use instead. I've changed to eco-friendly bleach and how it can cause harm to the environment and I've told other people about that. I say 'You've probably killed about 20 frogs using that stuff'."

"I never knew about water pollution and frogs or about nappies and how much water it takes to flush a toilet. Now we never leave the tap running. We turn it off."

Discussion

The findings from this research combine to reveal a complex and challenging landscape for modern zoos. "Learning Together" provided a lens through which parents could contrast their impressions of zoos from previous visits with that formed as a result of joining "Learning Together". We evidenced some success as far as it was possible to ascertain in the time frame of the project. Parents who previously viewed zoos as "just" popular places for family outings now recognised the role of zoos for education and in conserving endangered species. It is worth reflecting, however, on the point, that it was only after intense management of the zoo experience that the educational potential of zoos appeared to be realised.

The role of the zoo as a catalyst for behaviour change also met with some success, as parents affirmed their commitment to a range of environmental actions. We recognise that there are challenges associated with conducting research that seeks to evidence behaviour change and, even with the quasi-ethnographic and longitudinal research model that was applied in this study, the measurement of behaviour change relied on self-reports. Follow up interviews several weeks after the focus group discussions did indicate further commitment to lifestyle changes. The limitation of these findings is that behaviour change was affirmed by the participants and not directly observed. The literature is quite well-populated with zoo-based studies that deal with changes in visitor knowledge and/or attitudes (for example: Marseille et al. 2012; Randler et al. 2007; Lindemann-Matthies and Kamer 2006; Spotte and Clark 2004) but much less so with studies that have directly measured behaviour; in fact, we can locate only one zoo study (Swanagan 2000). This disparity would seem to reinforce the notion of behaviour change being an elusive variable to measure.

The strongly qualitative nature of this study granted us the opportunity to gain a rich understanding of the opinions and intentions of the parents, and we recognise that these qualitative data, along with the convenience sampling utilised, somewhat limits the generalizability of the findings too much beyond the study sample.

Future research might seek to pursue a quantitative approach based on the findings generated in this, more inductive study. It is also acknowledged that pre- and post-test focus groups may have yielded more robust data in relation to shifting opinions of the role of zoos, but this proved logistically impossible with this demographic. However, we are confident that within this, normally atypical, zoo visitor demographic, we have demonstrated that zoos and zoo education can achieve outcomes that are both beneficial to the environment and to wildlife conservation. Numbers directly participating in "Learning Together" may have been small, but we should consider the message multiplier effect (Domroese and Sterling 1999).

The focus on environmental action and empowering zoo visitors to engage in sustainable lifestyle behaviours emerges as a strong role for modern zoos. Zoos would appear to be in a good position to forge connections between local action and global environmental awareness, using the appeal of the live animal, particularly large-bodied mammal species, as a conduit (cf. Moss and Esson 2010). Zoos worldwide attract hundreds of millions of visitors and claim to have huge potential to educate such large audiences (Gusset and Dick 2011). Unlike many school visits, the majority of zoo visitors are free to interpret the zoo in their own way and the premise of allowing free choice zoo visitors to construct their own meaning from the visit (Falk and Dierking 2000) appears a rather precarious position for zoos to take in terms of meeting the challenges they have set themselves. The dilemma for zoo educators is to match message and delivery to the wider visitor audience and "Learning Together" provides some direction for content. The workshops deliberately focussed on some of the most popular species in the zoo, to build empathy and create an entry point for engagement. It is that same appeal that is most frequently used in zoo promotional materials to stimulate business so, to some extent, matching the expectation of the visit to the experience. The ability of parents to commit to behaviour changes may, in part, have been due to familiarity with the workshop themes. These reinforcing messages were selected to ensure we planned for success and they offered participants achievable goals and a choice of opportunities to act. If zoo education content can be thought of as reinforcement and linked to the plight of the most popular species in the zoo, then this is a direction that all zoo educators should consider worthwhile.

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Risk-based testing programme for *Mycobacterium bovis* following a clinical case in a zoological garden

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Abstract

Mycobacterium bovis is a strictly controlled disease. Outbreaks in zoos result in animal movement bans, disease investigation and euthanasia of infected animals. Both specific tuberculosis legislation and European Directive 92/65, often known as the "Balai" directive, require zoos to be free from tuberculosis in order to import and export animals. This paper describes the use of a risk based targeted testing programme for tuberculosis following a confirmed case of disease. This regime ensured a comprehensive but proportionate disease investigation developed through close co-operation with government veterinary officials, therefore limiting the impact of anaesthetic procedures and animal handling required to complete the necessary testing.

Introduction

Tuberculosis is a zoonotic disease caused by bacteria of the *Mycobacterium tuberculosis* complex, and tuberculosis infections caused by several species have been reported in zoological collections.

Mycobacterium tuberculosis is suspected to have been transmitted from infected human visitors on multiple occasions to a range of primates, antelope and tapirs (Michel et al.. 2003). Several reports of outbreaks of the newly identified *Mycobacterium pinnipedi* describe cross-species transmission, including to a human, originating from infected sealion reservoirs (Moser et al. 2008; Jurczynski et al. 2011).

Mycobacterium bovis is the cause of tuberculosis in cattle and has been reported in zoo felids, where it is thought to have been transmitted from infected meat (Helman et al. 1998; Thorel et al. 1998). Primates have also been infected on several occasions (Wilson et al. 1984; Thorel et al. 1998). There are surprisingly few accounts of this disease in antelope, deer and camelids in zoos, although it is known that these species are susceptible as it is seen in wild, ranched and farmed animals (Bengis 1999).

M. bovis is widespread in the United Kingdom and is subject to official disease control measures. Holders of susceptible animals are required to test stock for the disease and infected animals are culled. In the UK the badger (*Meles meles*) is an important reservoir of the disease (Corner et al. 2011).

Tuberculosis is of very high concern to zoological collections due to the potential public health risks, the potential loss of rare or endangered animals and the impacts of national disease control measures enforced on infected premises, which include a ban on imports or exports to the site and thus preventing participation in conservation breeding programmes.

In Europe, Directive 92/65 EC, known as the "Balai Directive", provides a basis for institutes such as zoos to become approved for use of a less stringent animal import procedure following implementation of a comprehensive programme of veterinary intervention, including disease surveillance, preventative medicine, post-mortem investigation and isolation procedures. This affords the zoo the benefits of revised animal health certification and reduced pre- and post-import disease testing, and avoids the need for officially supervised quarantine. In order to gain and retain "Balai-approved" status the zoo's animals must be free from mycobacterial infection.

This paper describes the implications of a case of *Mycobacterium bovis* infection in an animal held in a zoo approved under Directive EC 92/65, and how risk-based investigations and disease testing were utilised to allow disease control measures to be lifted and-approval under this legislation to be reinstated.

Background

A pair of llama *(Lama glama)* were tested using a comparative intra-dermal skin test in the axillary region, for routine disease surveillance purposes. The animals appeared healthy. They had been imported into the zoological collection nine months previously from a local private holder who had not had cases of

tuberculosis on their property. When re-examined 72 hours later, no reactions were seen and the animals were accepted as being free from *Mycobacterium bovis* infection.

One week after this test, the male animal was reported as being disorientated, weak and anorexic. On examination it was pyrexic and had an increased respiratory rate. Over the next 10 days the animal's condition developed, with ophisthotonus, paddling and other neurological signs. The animal was euthanased and the carcass submitted for pathological investigation. This demonstrated both gross and microscopic signs consistent with mycobacteriosis and samples were submitted for bacteriological culture. Infection with *Mycobacterium bovis* was confirmed.

Due to this finding, the zoo's Balai approved status was revoked and the zoo was placed under a Movement Prohibition Notice under the Tuberculosis (England) Order 2007. This resulted in the zoo being unable to move any animals susceptible to bovine tuberculosis until the premises were officially declared tuberculosis free by the official government veterinarians. A disease investigation was initiated to identify the source of infection, any transmission to other animals in the zoo and to facilitate eradication of the disease on the zoo site. This disease investigation was conducted under the direct supervision of government veterinary officials, who reviewed and approved the criteria used to determine the targeted testing programme. The diagnostic test regimes used in this official investigation are set out in UK law under the Tuberculosis (England Order) 2007. Any animals found positive for tuberculosis would be destroyed in order to return the zoo to its disease-free status.

Action

The concepts of the OIE Risk Analysis Framework (1994) were applied to this investigation using the risk question "What is the likelihood that *M. bovis* has been transmitted to other animals in the zoo?"

The standard risk terminology for this framework was used as shown in Table 1, and a risk pathway was produced (see Fig. 1).

Transmission of tuberculosis can be by a number of routes, including direct contact, short distance aerosol, contaminated fomites or reservoir species. The risk pathway was used to identify which animals could have been a source of the disease and which could possibly have been infected by the llama. The animal at highest risk was the female llama, as the only animal the male llama had been in direct contact with since his arrival at the zoo. There were no other animals sufficiently close for aerosol transmission.

The llamas were cared for by a team of keeping staff who could have inadvertently transmitted the disease on clothing, tools or other equipment, and therefore all susceptible species cared for by the same keepers were considered at higher risk of infection. It was unlikely that susceptible species cared for by other keeping teams would be at risk as all equipment was kept separate and only used within a specific house or area.

Table 1. Standard risk terminology.

Term	Definition
Likelihood	Probability; the state or fact of being likely
Likely	Probable; such as well might happen or be true; to be reasonably expected
Negligible	So rare that it does not merit to be considered
Very low	Very rare but cannot be excluded
Low	Rare but does occur
Medium	Occurs regularly
High	Occurs very often

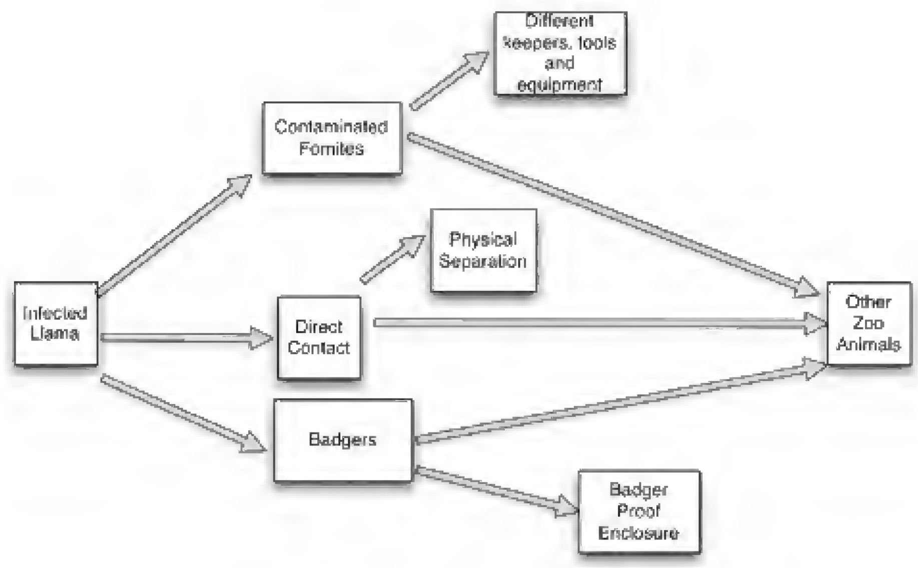

Figure 1. Risk pathway.

The final risk factor considered was wildlife reservoirs. The zoo does have a number of setts of the European badger on its site that in theory could be the source of the infection or transmit the disease to other zoo animals. Any dead badgers found on site were examined for evidence of disease and due to the lack of previous cases of *M. bovis* in the badgers in the zoo it is likely that the resident population was free of the disease.

The badger population on the zoo site is isolated from other populations, separated from them by major roads and heavily populated urban development. The nearest cattle and badger populations are significantly beyond the foraging or dispersal range of the species. This significantly reduces the risk of badgers being the source of the infection. However the badgers move around the zoo freely and can enter some enclosures and therefore could have become infected and could be a risk for onward transmission. As the badgers were rarely seen during daylight hours, those species that were housed indoors overnight or had enclosures that prevented access to the badgers were excluded from the investigations.

So in summary the animals that were of highest risk of being infected were the animal in direct contact with the infected llama, those susceptible animals cared for by the same keeping team as the llama and those susceptible animals that may have had contact with badgers.

This risk assessment determined which animals needed targeted testing for *M. bovis* to identify spread of the disease. As the risk of the female llama being infected was high and ante-mortem testing had already proved unreliable, it was decided to euthanase this animal so that definitive investigations could be undertaken. The animals cared for by the same keepers were Bactrian camels (*Camelus bactrianus*), guanaco (*Lama guanaco*), alpaca (*Lama pacos*), domestic sheep, domestic goats and domestic pigs. The camelids could also have had contact with the badgers. The only susceptible species other that could have had contact with the badgers was the group of reindeer (*Rangifer tarandus*), which was located closest to the llama exhibit.

All of the animals were tested using comparative intra-dermal skin tests, except for the Bactrian camels ,which were tested using lateral-flow rapid tests (RT) as described by Dean et al. (2009). These are the diagnostic tests that are required to be used by UK law in these species.

Government public health authorities were notified of the outbreak but due to the routine vaccination of humans in the UK, lack of reported clinical signs in the keepers, that this outbreak was due to *M. bovis* and that an official veterinary investigation was being undertaken the zoonotic disease risk was very low and no action other than biosecurity precautions needed to be taken.

Table 2. Risk assessment.

Source of TB	Justification	Risk Assessment before further testing	Risk Assessment after further testing
Direct contact with infected zoo animals	Only had contact with female llama	High to female llama, Very Low to all other stock.	Negligible – PME examination negative
Indirect contact through contaminated fomites	All other animals cared for using same tools and keepers etc.	Medium	Very Low – all in contact animals tested negative
Infection carried by badgers to other susceptible stock	No evidence of badgers infected with TB on site. Only reindeer and camelids have potential contact with badgers other than llamas.	Low	Very Low – all other stock tested negative

Consequences

The post-mortem examination and bacterial culture from the euthanased female llama did not demonstrate infection with *M. bovis*. The two camels had negative serological tests. All of the animals that were tested using comparative intra-dermal skin testing were negative except a single 16-week old reindeer calf and a domestic goat, which both had inconclusive tests. Therefore these animals needed to be retested at 120 days and 60 days respectively after the initial test. These animals were found to be negative on retesting using the same methodology.

This testing programme demonstrated that the risk of transmission of *M. bovis* from the original animal to others on the site was very low to negligible. The specific testing was supported by the zoo's ongoing programme of clinical examinations and routine post-mortem examination of all animals that die. On the very rare occasion a badger carcass is found, it is also examined for signs of disease.

As no other animals were found to be infected it was concluded that the source of the disease was the male llama and that it is likely he was latently infected when brought into the zoo. This is supported by the fact that the female llama was not found to be infected. This animal had been at the zoo for longer than the male and they had only been housed together.

As all the at-risk animals had tested negative and therefore the site was considered to be free *of M. bovis*, the Movement Prohibition Notice was lifted 326 days after the death of the male llama. Approval under Directive 92/65 was reinstated 331 days after the death of the male llama, allowing movements of susceptible animals into and out of the zoo to recommence.

This case study demonstrates the potential devastating impacts of infection with *M. bovis* both to the health and welfare of zoo animals but also the operation of the zoo as a conservation breeding centre. For almost a year this zoo was unable to import or export animals susceptible to *M. bovis*.

One of the many challenges of mitigating and investigating tuberculosis in zoos is the lack of validated tests. The "gold standard" of confirming an infection with organisms from the *M. tuberculosis* complex is the isolation of the bacteria. The intra-dermal skin test, which is the accepted method of detection in tuberculosis in domestic hookstock, primates and man, has not been standardised in many zoo mammals but is used widely (Kaandorp 1998; Sternberg et al. 2002). Use of the intra-dermal skin test raises several issues. It requires double handling for injection and then reading of the test, therefore requiring two anaesthetic procedures in many zoo animals. It detects only infection, not necessarily active disease, and it has a low sensitivity: animals with advanced disease can give anergic responses to tuberculin (Bengis, 1999,) The low specificity of this test can cause false-negative test results in animals other than domestic hoofstock and nonhuman primates (Bengis 1999; Kaandorp 1998). In some instances, even false-positive reactions can occur. Reindeer have been shown to regularly produce false positive reactions to the intra-dermal skin test (Palmer et al. 2006; Waters et al. 2005). These false positive results could lead to the unnecessary euthanasia of these animals if this species anomaly was not recognised. However, it has been determined that reindeer infected with *M.bovis* do reliably develop a robust response to the intra-dermal test and that false negatives are rare (Palmer et al. 2006).

As in this case, inconclusive tests can occur. This happens when there is a small reaction to the tuberculin but under the thresholds set for confirmation of a positive response (Sternberg et al. 2002). In this case the thresholds were set by the veterinary authorities, with a positive test being described as "an increase in skin thickness at the site of injection of PPD *M. bovis* by more than 2mm above the amount of increase at the site in injection with PPD *M. avium*". Inconclusive tests can be caused by incorrect injection of the antigens, incorrect reading of the test or abnormal immune reactions, for example in young animals with maternal antibodies. In these cases the animals are retested at a defined interval following the initial test.

The camelids have proved to be especially problematic to test reliably for tuberculosis. The intra-dermal skin test has been demonstrated to have a sensitivity of only 14% in llama. Twomey et al. (2010) identified seven animals out of a cohort of 70 that tested negative for tuberculosis by intra-dermal skin test, but were confirmed to have the disease by post-mortem examination within one to two months. Therefore at least some of them will have been infected with *M. bovis* at the time of the skin test.

In this case the male llama was definitely infected at the time of the initial routine skin test as he was examined by post-mortem that confirmed infection only 19 days later. Anergy to tuberculin when an infected animal fails to give a measurable hypersensitivity cutaneous response is a potential cause of a false-negative result but has never been proven in South American camelids (Twomey et al., 2010).

The lateral-flow rapid test VetTB STAT-PAK is a serological test that has been used widely in domestic and zoo animals (Lyashchenko et al., 2008). This test has the advantage that it only requires the collection of a single blood sample. In zoo animals it has been used where the risk of two anaesthetic procedures in a short period of time is considered to be high. In this case the RT was used on the Bacterian camels, which were not handled and therefore would have required anaesthetising for effective intra-dermal skin testing. The sensitivity and specificity of the rapid test in Bactrian camels is unknown, but during investigations of an *M. bovis* outbreak in a racing herd of dromedary camels, the rapid test accurately detected all three confirmed infected camels out of the 55 tested with no false positive results (Wernery et al., 2007).

Again South American camelids are the exception, Twomey et al. (2010) found a high rate of false positives with only 10 animals out of 54 testing positive to tuberculosis by the RT being confirmed to be infected with *M. bovis* at post-mortem examination. The RT is not specific to *M. bovis* and so the results could be caused by

infections with other mycobacterial agents, but no evidence of this was found. The most appropriate testing regime, which should have been used initially for this llama, is a combination of intra-dermal skin test and serological testing as described by Bezos et al. (2013). Preventative veterinary medicine best practice indicates that the llama should have been tested for tuberculosis prior to import as this outbreak and its consequences would have been prevented by the disease being detected at this stage (BIAZA 2008). However, this is not required by either tuberculosis legislation or EC Directive 92/65. Balai-approved premises are required to have a general disease surveillance programme and post-import isolation process in place, both of which were complied with. The zoo should review its standard disease prevention measures and surveillance programme in light of this outbreak.

With the difficulties of handling, restraint and potential anaesthetic procedures combined with the challenges of reliably testing the animals for *M. bovis*, a risk-based process for undertaking the testing regime was required. This attempted to avoid testing animals where the risk of infection was considered low and there was the possibility of negative impacts on the health and welfare of the animals through undertaking the procedure. These risks could be further mitigated by the choice of test, as in the case of the Bactrian camels.

By applying the risk-based criteria the number of animals that required testing was considerably reduced and the process eliminated many zoo species where interpretation of the test results is not standardised and therefore interpretation difficult. The risk-based procedure had the unforeseen advantage that the highest-risk species were primarily domestic species from the zoo's children's farm. Diagnosis of *M. bovis* in domestic species has been standardised and specific protocols and test interpretations have been defined.

In this case the zoo worked effectively with local environmental health officers to ensure that the potential risks to humans were being addressed appropriately, through biosecurity, disinfection, protective clothing and equipment, and access restrictions. This avoided any adverse impact on the zoo's visitors and allowed the zoo to operate during the disease incident.

Close cooperation, collaboration and communication with the government veterinary authorities was essential for the successful resolution of this disease incident. The control of *M. bovis* on infected premises is defined in legislation written primarily for domestic commercial farms and therefore its application in zoos and non-domestic animals can be problematic, primarily in interpretation of diagnostic testing. The risk-based testing regime was defined by the zoo and the veterinary authorities working in collaboration, following discussion, inspections of working practice and assessments of operating procedures to define transmission risks. This resulted in an evidence-based investigation which was proportional and effective. Further, by having defined testing regimes and defined test interpretations, even more prolonged restrictions on the zoo premises were avoided.

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Zoo BAPs: biodiversity action plans for conserving native wildlife in and around zoological gardens

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Abstract

Failure by the international community to meet Convention on Biological Diversity targets has heaped added pressure on national and local biodiversity action plans (BAPs). Zoological gardens are playing an increasing role in practical conservation of wild habitats, but zoos have rarely developed formal BAPs. Here we introduce the concept of zoo BAPs, i.e. strategic plans for the conservation of biodiversity in and around zoological gardens. We use the first formal zoo BAP, developed at Flamingo Land Theme Park and Zoo in the UK, to introduce a framework for the establishment, monitoring and re-evaluation of a Zoo BAP. Throughout the framework we emphasise stakeholder participation, particularly involving zoo staff and local government biodiversity representatives. Species and habitats must be selected that are locally relevant, and are either threatened or have value as indicators or flagships for conservation. Each species or habitat must have targets that can be measured, monitored, and then evaluated for annual revision of conservation actions. This kind of "adaptive management" should allow a flexible, evidence-based approach to conservation. Use of national and international frameworks for biodiversity assessment should also help zoos to become increasingly aligned with the international conservation community.

Introduction

The term "biodiversity", the variety of life, was popularised by the Convention on Biological Diversity (CBD; United Nations 1992). Biodiversity measures can be used to estimate the value and health of ecosystems (Teder et al. 2007) and to identify priority conservation regions (Myers et al. 2000). Following the CBD, national and local Biodiversity action plans (BAPs) were established across the world to help conserve biodiversity. However, nearly 40,000 species remain threatened with extinction worldwide (IUCN 2012). The most biologically rich ecosystems are also declining, e.g. 70% of coral reefs are either threatened or destroyed (Wilkinson 2004), 35% of mangrove forests have been destroyed in just 20 years (Millennium Ecosystem Assessment 2005), and 13 Mha of forest are being lost annually (FAO 2010). In light of the abject failure of the CBD to halt biodiversity loss by 2010 (e.g. Pollard et al., 2010), BAPs are more crucial than ever for achieving success.

Biodiversity conservation by zoos

In its revised CBD targets, the United Nations (UN) Strategic Plan for Biodiversity 2011–2020 advocates an increasing role for zoological gardens (United Nations 2010). Accordingly, modern zoos have become centres for biodiversity conservation, through in situ and ex situ conservation, and through public engagement (WAZA 2005). Of the species held in World Association of Zoos and Aquariums (WAZA) zoos, one in seven is threatened in the wild (Conde et al. 2011a). Of 34 species listed by the IUCN as extinct in the wild, 29 are actively bred in captivity, and 22 have been reintroduced to the wild (Gusset 2011). At least 13 out of 68 downgradings on the IUCN Red List have resulted from conservation breeding and release into the wild by zoos (Conde et al. 2011b).

However, conservation breeding, fund-raising and environmental education can be thought of as peripheral to conservation success (Fig. 1). As a result of a need for direct conservation action to complement captive efforts, 92% of British and Irish Association of Zoos and Aquariums (BIAZA) zoos supported field conservation during 2010 (Marshall and Deere 2011). Furthermore, WAZA member expenditure on field conservation has risen to nearly US$350 million a year, and is now the third highest non-governmental contributor to biodiversity conservation globally (Gusset and Dick 2011). The World Zoo and Aquarium Conservation Strategy further states that zoos should try to focus their conservation activities using local, national or regional BAPs, and/or similar recovery plans (WAZA 2005).

Most zoological gardens are found in temperate regions, and typically hold a low representation of native wildlife from the surrounding area in their collections (Conde et al. 2011a).

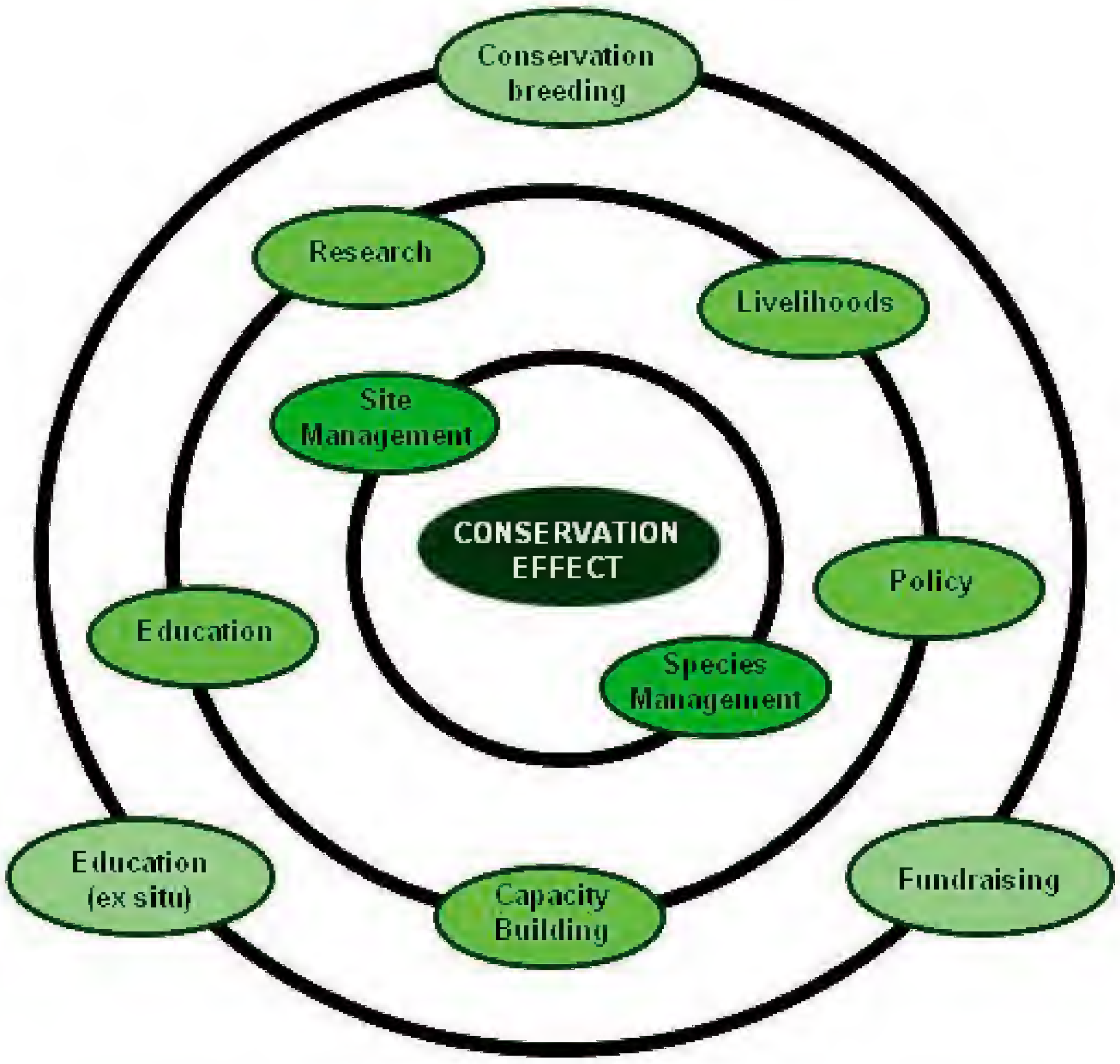

Figure 1. Schematic representation of the immediacy with which different types of conservation activity contribute towards conservation effect (adapted from Kapos et al. 2008). The ex situ activities of zoos contribute to conservation in a more indirect manner than other activities. Site and species management contribute directly as they deal with the actual conservation targets.

The UK is home to 100 zoos accredited by BIAZA (BIAZA 2012), which house hundreds of exotic species. However, the UK has also seen nearly 100 native species extinctions in just 100 years (UK Biodiversity Steering Group 1995). Accordingly, a growing number of zoos are contributing to native biodiversity conservation in their host countries (Marshall and Deere 2011). Activities include conservation breeding and reintroduction, community education programmes and habitat improvement. Quantitative data on the number of species found living wild in and around zoos are extremely limited, but the first in-depth survey of one Swiss city zoo found 3110 species in an area of only 13 hectares, including 31 species previously unknown from Switzerland, and 113 on the national Red List (Baur 2011). The potential for the broader zoo network as a metapopulation of biodiversity reserves may therefore be high.

Conservation planning

Structured and well-managed strategic plans are essential in order to achieve successful evidence-based conservation (Conservation Measures Partnership 2007). If measures of conservation success are not made, there is no evidence for the outcome of a management action. Furthermore, activities cannot then be fully explained to partners and stakeholders, and future management decisions become ill-informed. Accordingly, the United Nations has 97 operational indicators to monitor the progress of international biodiversity targets, within five strategic goals (UNEP 2012). However, conservation planning has traditionally suffered from a lack of indicators of success, with only anecdotal evidence as the major source of information for management actions (Pullin et al. 2004; Sutherland et al. 2004).

The aim of this paper is to give guidance for zoological gardens on the importance and procedures for the development of a "zoo BAP", a document used for planning the conservation of native wildlife in and around the grounds of individual zoological gardens. In achieving this aim we use the UK Biodiversity action plan (UKBAP; Maddock 2008) as an example of good practice for conservation planning, illustrating how this was used to develop the first zoo BAP.

Developing a BAP

BAPs and zoos

A biodiversity action plan (BAP) is a document that sets out targets for biodiversity conservation based on priority species and habitats. As a result of the CBD, over 170 countries have developed national biodiversity strategies and national BAPs (NBAPs; United Nations, http://www.cbd.int/2011-2020/). The UK was the first country to produce an NBAP in 1994 (Maddock 2008); however, conservation management actions can rarely operate at a national level, and hence at least 225 local biodiversity action plans (LBAPs) were then created in the UK to improve regional relevance. One of these, the Ryedale BAP, outlined a strategy for conservation for one North Yorkshire district (Ryedale Biodiversity Steering Group 2007), and was central to the implementation of the first zoo BAP.

There are few examples of zoo BAPs for planning the conservation of biodiversity in and around zoological gardens. The first formal zoo BAP was created for Flamingo Land Theme Park and Zoo (Hambly & Marshall 2011). The 150 ha acre site at Flamingo Land represented six habitats, including five of importance under the UKBAP (Maddock 2008), interspersed between a zoo, theme park and holiday village. In the year preceding publication of the first zoo BAP, the park attracted 1.3 million visitors (Mills 2011), and hence the aim was to conserve wildlife in full integration with human activities. In developing the first zoo BAP, a stepwise procedure was followed (Fig. 2), which we hope can form a blueprint for planning future zoo BAPs.

Consultations and assessment

The first stage of BAP development is an assessment of current knowledge regarding biodiversity and conservation management in the locality. In our experience, close collaboration with government LBAP representatives is particularly important for gaining advice on resources, contacts and priorities for BAP development and content. Involvement of local people increases the likelihood of success, and can be used as a form of environmental education (Elbroch et al. 2011). Accordingly, LBAP legislation emphasises that knowledge and opinions of both local

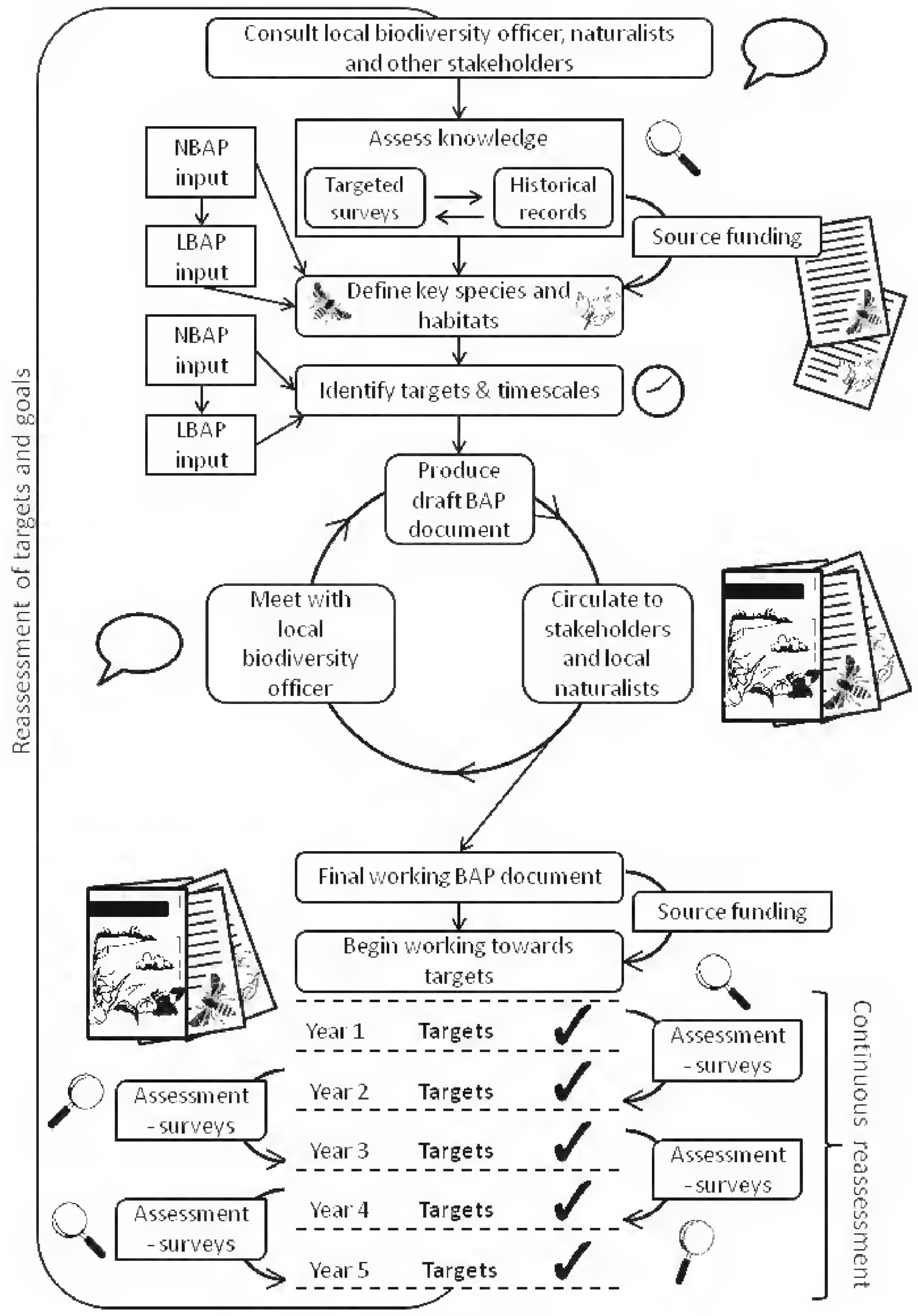

Figure 2. Recommended steps for creating and evaluating a five year Zoo Biodiversity Action Plan (Zoo BAP). NBAP = National BAP, LBAP = Local BAP.

people and conservation scientists should be included in BAPs (Harrison et al. 1998). Knowledge assessment would also therefore benefit from extensive stakeholder consultation, including zoo staff, local naturalists, conservation groups and land-owners. Discussion might include important features of biodiversity in the area, threats, timescales and identification of potential conflicts. In particular we found that long-standing members of staff were a valuable source for historical records of wildlife.

Alongside stakeholder consultation, pilot surveys are important to assess habitat types and the presence of LBAP species. Such surveys can benefit from partnerships with local communities, conservation/wildlife special interest groups, local naturalists and/or universities, thus providing high quality and yet low cost surveys (Harrison et al. 1998).

Defining key habitats and species

On completion of a full assessment of current knowledge, key habitats and species can be identified for priority conservation. These can be species and habitats currently on site, or species and habitats that may have existed in the past and could feasibly be restored. For instance, a water vole action plan may outline habitat improvements needed to encourage dispersal from surrounding waterways onto the Zoo BAP site (Hambly and Marshall 2011).

Species and habitats may be selected for a BAP based on a number of criteria. A good indicator species should be easily recognisable, easy to locate, occur in reasonable density, not highly mobile, and representative of ecosystem health (Caro and O'Doherty 1999; Hilty and Merenlender 2000). Selection of species and habitats based on an existing, locally relevant LBAP, is likely to provide the best linkage to national and international biodiversity targets. Using the Ryedale and UK BAPs, the first zoo BAP comprised species and habitats based on the following criteria (Hambly and Marshall 2011):

1. *National/local rarity:* Species/habitats that are nationally scarce, and/or species at risk of local extinction. This may also include species/habitats with localised distribution, e.g. common, but only in small areas due to specific habitat requirements.
2. *Nationally important populations, or populations in rapid decline:* Species that may be nationally common, but becoming rare elsewhere, and/or species that have suffered dramatic population reductions.
3. *Indicators of habitat quality:* Species that represent ecosystem health or threatened/regionally characteristic habitats. Indicator of habitat quality may even include relatively common species that represent ecological functions, e.g. restoring habitat connectivity, or providing microhabitats.
4. *Charismatic species:* Careful selection of attractive or well-known species may help the profile of biodiversity. These can be used in fund-raising or education, assuming their management will benefit ecosystem health.

Monitoring, verification and reporting

For successful conservation, measurements are needed to indicate progress towards targets (Sutherland et al. 2004). There have been a number of initiatives promoting the development of monitoring plans for gathering and using conservation data (e.g. Margoluis and Salafsky 1998; Salafsky and Margoluis 1999; Stolton et al. 2007). Management of the first zoo BAP has followed the "Open Standards for Conservation Practice", a now well-established procedure for planning conservation projects (Conservation Measures Partnership 2007). The CMP Open Standards uses a standardised terminology to help understanding of how project activities are intended to influence conservation targets. Zoo BAP managers might also benefit from determining targets for each species and habitat based on the relevant LBAP.

With measurement at the heart of conservation planning, the involvement of scientific expertise may be vital. The challenge here is to gather scientific information, but to report it in a way that is useful for practitioners. However, environmental scientists have a poor record of ensuring that their research has impact (Milner-Gulland et al. 2012) and conservation managers rarely read any scientific literature (Pullin et al. 2004). Consequently a BAP aims to bridge the research–implementation gap. The language used in a BAP is therefore intended to be neither simplistic nor specialist. A BAP also has a simple structure, for instance each habitat or species plan is typically written to stand alone.

A central database is also recommended for recording the progress towards conservation targets (Sutherland et al. 2004). One example is the UK Biodiversity Action Reporting System (BARS), a database of completed BAP conservation actions (BARS 2012). At the time of writing, the BARS database comprised records by 4339 conservation workers from 1311 organisations, hence providing a vital resource for assessing progress towards national targets. There is no zoo equivalent to BARS, but zoo native wildlife records can be sent to the BIAZA Native Species Working Group (http://www.biaza.org.uk/conservation/).

At the heart of all the various standards for conservation planning is the principle of "adaptive management", the cyclical process of assessment, reassessment and adaptation to experience and changing conditions (Conservation Measures Partnership

2007; Fig. 2). Stakeholder communication through workshops and circulation of documents remains crucial throughout the process to ensure expert input and partner satisfaction. Therefore, although the BAP document is fixed, the targets and strategies evolve according to monitoring outcomes. Finally, towards the end of any BAP term, plans must begin for a new document, starting with a repeat of the knowledge assessment process.

Conclusions

The emphasis here is the further progression of zoos from their original ex situ approach to biodiversity conservation, to ambassadors for field conservation. The concept of the zoo BAP aims to bring zoos another step further towards partnership with government, and the broader biodiversity conservation community. In contrast to larger scale BAPs, zoo BAPs are relatively easy to produce and provide targeted management guidelines. We see huge potential for the development of zoo BAPs for the promotion of zoos as miniature “biosphere reserves”, where people and nature co-exist sustainably. With over 700 million annual visitors, the world’s zoos provide an opportunity to conserve biodiversity in a human-dominated environment, while also promoting biodiversity conservation in a unique manner that is informal, educational and fun.

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Spontaneous recovery from reproductive failure in a hand-reared male western lowland gorilla (*Gorilla gorilla gorilla*)

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Abstract

An adult hand-reared male gorilla raised in bachelor groups was considered as not reproductive on the basis of his behavioural background, clinical history and the results of a physical examination (electroejaculation and ultrasound examination of the testes). He was kept for 3.5 years with a well-socialised adult female and, despite regular sexual activity, no reproductive success was achieved. However, this gorilla has since returned to fertility and within a few months of a second female joining the group, both females became pregnant, indicating that the male could no longer be deemed "infertile". Thus, male fertility evaluations must be interpreted carefully in relation to social structures in gorillas, and the establishment of a proper social group structure should be taken into consideration when establishing new gorilla breeding groups. The introduction of adequately socialised females to hand-reared males that have matured in bachelor groups may improve not only the social behaviour but also the reproductive capacity of such males.

Introduction

Success in the captive breeding of gorillas in European zoos has improved in the last decade and the population is increasing slowly (Abello et al. 2010). However, there is still a considerable number of non-breeding animals. Hand-reared males, raised in bachelor groups, are of particular concern as they are less successful as breeding animals (Abello et al. 2010, 2011). Nevertheless, there is little available information in the literature concerning ongoing monitoring of the reproductive success of these animals. The aim of the present report is to document the reversibility of infertility in a hand-reared male gorilla, highlighting that male fertility evaluations must be interpreted carefully in relation to social structure in gorillas.

Methods

A 16.5-yr-old male western lowland gorilla ("Mambie"), born in March 1991, was transferred on 14 December 2007 to Bioparc Valencia (Spain) as the breeding male for a new breeding group. The animal was hand reared and kept in a sibling group at his natal zoo, and later transferred to two different bachelor groups in two further zoos at the ages of eight and 11 years. Thus, he did not have any mating experience or the chance to observe mating in other conspecifics. On the same day an 8-yr-old mother-reared female gorilla ("Fossey") also arrived from a different zoo. This female had been parent reared in her natal group, was an adequately socialised animal, and should therefore have supposedly had a positive effect on the male by improving his social skills. The introduction of the gorillas proceeded without any difficulties within a few days. Both animals were considered sexually mature according to their age (De Vries and Glatstone 2005; Abello et al. 2010). Despite the limited social behaviour of the male, both animals were considered compatible and copulated regularly after their arrival, although no pregnancy was achieved. As previously mentioned, hand reared males raised in bachelor groups have particularly poor breeding results. Thus, on 4 June 2009, 1.5 years after the arrival of the male, a thorough physical examination of the male was carried out under general anaesthesia.

The ultrasonographic examination was performed with a GE Voluson 730 Pro equipment (General Electric Medical Systems, Kretzetchnik Gmbh co., 4871 Zipf, Austria) with a linear multi-frequency probe at 10 MHz for testicular examination. The bladder was emptied of urine and subsequently rinsed with

20 ml Human Tubal Fluid (HTF, LifeGlobal, Barcelona, Spain) in order to recover the retroejaculated sperm from the bladder. A rectal probe was inserted into the rectum and a series of low level electrical stimulations were delivered to the prostate gland and seminal vesicles via direct stimulations of the pelvic floor using a LifeSTIM™ System electroejaculator (Neurocontrol Seager, Ohio, Cleveland, USA). A total of eight stimuli of approximately five seconds were applied, starting at a voltage of five volts and increasing the voltage stepwise to a maximum of 25 volts and 600 milliamperes of current. The ejaculate was evaluated according to standard procedures as described for humans (WHO 2010).

Results

The 150 kg gorilla was found to be in excellent general condition. The ultrasonographic examination of both testicles showed diffuse microcalcifications throughout the testicular parenchyma (Figs 1 and 2). Testicular measurements (length x width) of the right and left testis were 37 x 16 mm and 39 x 17 mm respectively, within the published range obtained from reproductively successful gorillas (Schaffer et al. 1981). Approximately 0.3 ml of fluid was recovered during electroejaculation, which was then combined with the medium recovered by means of bladder catheterisation after electroejaculation. Only three sperm cells with progressive motility were found after an extensive search in multiple fields of centrifuged pellets. Thus, although the total ejaculate volume is not known, we may estimate the sperm concentration to be less than 0.1 x 10^6/ml. Hormone analysis included follicle-stimulating hormone (FSH; 11.95 IU/l), luteinising hormone (LH; 4.72 IU/l) and testosterone (4.2 ng/ml). An additional blood sample collected 6 months later during a minor procedure showed an FSH of 9.61 IU/l.

Based on the poor sperm quality, abnormal ultrasound and FSH findings and previous social history, a diagnosis of cryptozoospermia due to primary spermatogenic failure was made. Taking into consideration the elevated stress that invasive procedures cause on sensitive animals such as gorillas, further examinations were not carried out.

On 29 March 2011, 2 years and 3 months after the arrival of the first pair of gorillas, an additional 10-yr-old mother-reared female gorilla ("Ali") was added to the group. After a slow and progressive introduction process of two months, all three animals were finally housed together from 26 May 2011. Behavioural records collected on this day noted that the male displayed a complete silverback sequence towards the females, starting with soft clear hoots, then running bipedally sideways, throwing objects into the air and finally beating his chest. Both females retreated to a safe distance during the displays and were submissive towards the silverback, particularly the recently arrived female. A full silverback display towards other gorillas had never before been observed since the arrival of the male in Valencia. Ten months later, on 3 April 2012, the first female gorilla ("Fossey") gave birth to a normal baby. The average length of gestation for gorillas in captivity lasts 255 days (De Vries and Glatstone 2005), and fertilisation must therefore have taken place during July 2011, some five to nine weeks after the third gorilla was introduced. In fact, copulations were recorded on 18 and 25 July 2011. The second female was also fertilised by the male in February 2012.

Figure 1. Sagittal section of the right testicle showing "starry sky" appearance due to microcalcifications revealed by the ultrasound. Sagittal and transverse (not showed) sections of the gorilla's right testis revealed several microcalcifications throughout the testicular parenchyma. Volume = 4.7 cc; size = 37 x 16 mm.

Figure 2. Sagittal section of the left testicle showing similar but less severe appearance due to microcalcifications revealed by the ultrasound. Volume = 5.6 cc; size = 39 x 17 mm.

Discussion

The microcalcifications noted in the testicular parenchyma are described in human medicine as testicular microlithiasis (TM) (Yee at al. 2011). It is a relatively uncommon condition, and can be found in 0.6–9% of men referred for testicular ultrasound, although the true incidence in the general human population is unknown (Dohle et al. 2005). In a recent study, TM was diagnosed in 87 patients (6%) out of a total of 1439, and a significant co-ocurrence of TM, testicular cancer and infertility was noted (Yee at al., 2011). Its relationship with infertility is unclear, but probably relates to dysgenesis of the testis (Dohle et al. 2005; Yee at al. 2011). To our knowledge, TM has not yet been described in gorillas, and its significance in this species is unknown.

Standard semen characteristics alone are an indirect measure of sperm function and are not diagnostic of fertility (Spindler and Wildt 2010). However, although the minimum ejaculate characteristics required to produce a pregnancy in gorillas are unknown, this sample is far below the normality thresholds in gorillas and humans (Gould 1983; Sikaris et al. 2005), with an extremely low possibility of being able to achieve parenthood. Reference values for sperm concentration in gorillas range between 15 and 360 x 10^6 (Gould 1983). Testosterone values for mature gorillas ranged between 3.450 and 7.158 ng/ml (Gould 1983). Although we are not aware of reference data for pituitary hormones in gorillas, we used the reference intervals for FSH (1.3–8.4 IU/l) and LH (1.6–8.0 IU/l) published for humans (Sikaris et al. 2005), as gonadal and

pituitary hormone values for fertile adult male apes fall within the normal range for humans (Gould 1983). The hormones were considered within normal limits in this case, except for FSH, which remained elevated 6 months later. An elevated FSH value is an indication of damaged or dysfunctional spermatogenesis in both humans and gorillas (Gould 1983; Sikaris et al. 2005). Although the doses of anaesthetics applied were within the recommended range for this species (Sleeman 2007), it has also been noted that some anaesthetics might inhibit ejaculation (Spindler and Wildt 2010). Nevertheless, by the time of this examination, the male had been copulating regularly with the female for 1.5 years but no pregnancy was achieved.

The etiology of infertility is often obscure. In 40–60% of cases of infertile men, the only abnormality is found in the semen analysis and there is no relevant history or abnormality on physical examination and endocrine laboratory testing (Dohle et al. 2005). Also, spontaneous recoveries of fertility have been reported in couples with male infertility factor after discontinuation of assisted reproductive techniques (Osmanagaoglu et al. 2002).

Histological examinations performed on the testicles of male gorillas kept at Japanese zoos provided evidence for spermatogenesis in only 40% (n=10) of the samples analysed (Enomoto et al. 2004). In another study, spermatogenesis was evident in 100% (n=7) of the testicles of orangutans and 91% (n=11) of the testicles of chimpanzees also kept in Japanese zoos under similar conditions to those of the gorillas (Fujii-Hanamoto et al. 2011). Thus, spermatogenesis in captive gorillas appears to be more vulnerable in comparison with other great apes.

The total duration of spermatogenesis in most mammals lasts nearly 40 to 54 days (Hess and de Franca 2008). However, in great apes this process is longer, and lasts more than 70 days in humans (Hess and de Franca 2008), and 62.5±1.5 days in chimpanzees (Smithwick et al. 1996). To our knowledge, this process has not been studied in gorillas, but it is reasonable to expect a similar duration. Between the onset of the typical silverback behaviour and the estimated fertilisation date there is a period of around two months during which an entire new spermatogenic cycle may have been completed.

The remarkable gorilla display is a key communicatory signal in silverbacks. Agonistic displays by male western lowland gorillas in the wild are particularly intense in nature towards immigrant females, and are considered as "courtship aggression", which is an important part of the mating strategy of this species (Stokes 2004). Thus, it might be speculated that the addition of the second female to the gorilla pair provided sufficient social stimulation and finally triggered spermatogenesis. Spermatogenic arrest in gorillas may be corrected by alterations in their environment and management (Gould 1983). Also, social factors, such as the removal of a certain female, have been associated with the sudden decrease in the sperm quality of a proven breeder (Schaffer et al. 1981). In this particular case, the introduction of an additional female was the only factor changed, which finally resulted in two pregnancies within eight months with two different females after 3.5 years of reproductive failure, even though active copulatory activity with intromission was observed on a regular basis. This strongly suggests that his sperm quality must have improved sufficiently to have fathered offspring twice.

Spermatogenesis is a complex and species-specific biological process of cellular transformation, and is dependent upon numerous factors. In highly intelligent and social animals, such as great apes, social influences also play a major role. Western lowland gorillas in the wild live in cohesive, predominantly single-male groups with an average of 3.8 females per group (Stokes 2004). Keeping males of polygynous species such as gorillas in pairs may therefore have detrimental effects on their reproductive function, and this should be taken into consideration when establishing new gorilla breeding groups. The introduction of adequately socialised females to hand-reared gorilla males that have matured within bachelor groups may improve the social behaviour of the males, but may also have a stimulatory effect on their spermatogenesis and reproductive capacity.

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Evidence-based practice

Using student-centred research to evidence-base exhibition of reptiles and amphibians: three species-specific case studies

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Abstract

When zoo-housed animals have choice over aspects of their environment, and are able to exercise control over interactions with their surroundings, welfare can be improved and exhibits' value to the zoo increased. Reptiles and amphibians are not common subjects in enrichment studies yet their demanding captive requirements suggest a need for enclosure diversity and biologically sound enrichment programmes. As popular captive subjects, such animals are readily available for potential research projects that investigate behaviour, welfare and effects of enclosure design. Undergraduates on animal science courses that undertake a research-led dissertation or similar projects can collect data on such species that, if collected under a robust methodology, can be used to inform future husbandry decisions. This paper discusses three small-scale studies (on two reptile species and one amphibian species) that were designed to improve husbandry and welfare. The aim of the paper is to show that undergraduate projects, properly managed, can have a positive impact on overall day-to-day exhibition and management of these species. Results from these projects have shown that small changes to enclosure design can have a beneficial impact on activity patterns, and that overall enclosure design can help display the animals in a more interesting way to visitors. Potentially, the animal welfare benefits of enriched set-ups can be passed on to zoo visitors in the form of a more engaging, exciting and educationally relevant zoo experience.

Introduction

Reptiles and amphibians are some of the most commonly housed species in modern zoological collections and are kept by numerous private hobbyists globally. They also feature heavily in educational establishments teaching animal husbandry/management. Species-specific husbandry guidelines are lacking for many herptiles and recommendations for appropriate enrichment may be anecdotal. Our understanding of reptile and amphibian behaviour in captivity can also be limited, and enrichment projects that enhance the psychological welfare of reptiles and amphibians rarely appear in the peer-reviewed zoo literature. Improvements to the welfare of captive reptiles could be seen if behavioural repertoires are more closely examined and interpreted to enhance enclosures and support enrichment protocols. Students enrolled on degree programmes at establishments keeping such species are therefore an excellent resource that can be utilised to collect data to evidence-base husbandry regimes and enhance standards of care. Here we explain how such student-centred projects can have a positive impact on herptile exhibition and welfare, and are able to add to the scientific literature on reptile and amphibian husbandry requirements.

In many species it is known that provision of choice enables a higher welfare state to be reached (Bassett and Buchanan-Smith 2007; Nicol et al. 2009; Swaisgood 2007; Whitham and Wielebnowski 2013). Amphibians and reptiles both rely on environmental factors to regulate internal metabolism and hence require a thermally heterogeneous environment to move through daily to maintain homeostasis. When such heterogeneity is not provided, behavioural disturbances may result (Morgan and Tromborg 2007; Shah et al. 2003). As is the case in captive mammalian and avian species, reptiles have also been shown to develop stereotypic, abnormal behaviour patterns (Young 2003). Reduction and eventual eradication of such unwanted activities could be possible through increased stimulation from the environment in which the animal is housed. Both reptiles and amphibians have the capacity to benefit from well thought-out enrichment programmes that can be built in to their enclosures or be added to in a similar

fashion to that done with mammals and birds (Blake et al. 1998; Hawkins and Willlemsen 2004; Hayes et al. 1998).

There has been a growing call for more reptile enrichment to be undertaken and evaluated (Burghardt et al. 1996; Hernandez-Divers 2001) to ascertain the effects of behavioural enrichment on reptile activity, health and welfare. Husbandry regimes and enclosure design based around natural behaviour, termed "ethologically-informed" practices, are described by Greenberg (1995), yet the term "evidence-based husbandry" (Melfi 2009), coined for all captive animals, probably better sums up what is needed to further improve management of reptiles and amphibians in zoos. This would then ultimately enhance the message (and value) that such exhibits have to visitors. The design of zoo animal enclosures ultimately affects the public's perception of the occupants. Likewise, zoos strive to recreate naturalistic environments that promote "normal" behaviour patterns and give a positive image of the zoo as a whole (Robinson 1998). A positive visitor experience, (see Fernandez et al. 2009), can effectively champion the key aims of the modern zoo (conservation, education, research, entertainment). Such aims are better promoted when animals are housed in enclosures that mimic natural environments and engage visitors, as per Conway (1973)'s highly relevant, if now dated, article on displaying bullfrogs (*Lithobates catesbeianus*) effectively.

A dearth of research exists on the importance of such "good display" on the perception and educational or conservation relevance of reptiles and amphibians in zoos, as well as on the impact of such informed design on the occupants' behaviour. The common occurrence of popular "pet-style" species in educational facilities enables empirical research to be conducted in a situation that mirrors larger zoological collections, with the advantage of exhibits to be manipulated more quickly and set up in tandem to measure specific variables. Well managed undergraduate degree students that have access to these animals can be used to help increase the research output on such under-studied species.

This paper describes three experiments conducted by undergraduate students at Sparsholt College Hampshire (SCH), Winchester, UK. Students collected data on behaviour, visibility, activity levels and enclosure usage of two species of reptile (chuckwalla, *Sauromalus ater*, and corn snake, *Pantherophis guttatus*) and one species of amphibian (blue dart frog, *Dendrobates tinctorius azureus*) on display in the College's Animal Management Centre (AMC). The study species were chosen as they are representative of many such animals held in captivity and hence the results from these investigations would have far-reaching and relevant application to many other individuals. All students were registered on a BSc (honours) Animal Management degree and were working towards a dissertation project or involved in a personal development project to train and develop their research skills. Students were provided with standard ethograms and trained in the identification of specific behaviours and in the data collection procedures prior to each research project being implemented.

Case study 1: Chuckwalla enclosure design

Western chuckwallas are an arid-dwelling species that naturally occur in the deserts of south-western North America (Kwiatkowski and Sullivan 2002). Classified as "rock-dwelling" (saxicolous) lizards, chuckwallas actively avoid areas of habitat that are devoid of suitable rock structures for hiding and climbing (Goode et al. 2005). A chuckwalla's main defensive strategy is to expand lung capacity beyond normal volumes, thus inflating and wedging the lizard into a rock crevice or similar small space (Deban et al. 1994). This saxicolous lifestyle means that vivaria housing chuckwallas must enable the performance of an array of behaviours above the ground, on rockwork, in a similar manner to those documented in free-living animals.

Table 1. Ethogram of chuckwalla behaviour.

Behaviour	Description
Movement	The animal is walking, climbing, running around the exhibit and over furnishings.
Hidden	The animal is not fully on view and is using the furnishing of the enclosure to conceal itself.
Basking	The animal is flattened against a heat source or positioned under a radiant heat source. The animal is stationary.
General interaction	Social behaviour between the two chuckwallas of a non-threatening, non-aggressive nature.
Reproductive interaction	Head-bobbing, chasing, yawning and push-ups from male to female. Colour-flushing and orienting of body towards each other.
Other	Stereotypic movements against a glass barrier or stereotypic movement against a solid boundary.

Male chuckwallas are territorial (Alberts 1994), hence it is important to provide features of the vivarium that can be used as territorial markers. Familiarity with immediate environment is known to give a sense of security (Baumans and Van Loo 2013; Nikaido and Nakashima 2009) and hence is a means of decreasing stress in captive animals. The aims of this project were to provide two chuckwallas with the ability to improve overall behavioural repertoire by increasing spatial complexity of their enclosure (creating a micro-climate that would better resemble wild conditions) and to provide the chuckwallas with an outlet for key appetitive behaviours that may enhance reproductive potential.

Stress-related behaviours (in the form of repetitive interaction with a transparent boundary) have been noted in the past in the two subjects of this investigation. Re-evaluation and subsequent redesign of the enclosure has been undertaken to improve the likelihood of courtship display performance and to reduce the time spent stereotyping. By providing the two chuckwallas with a

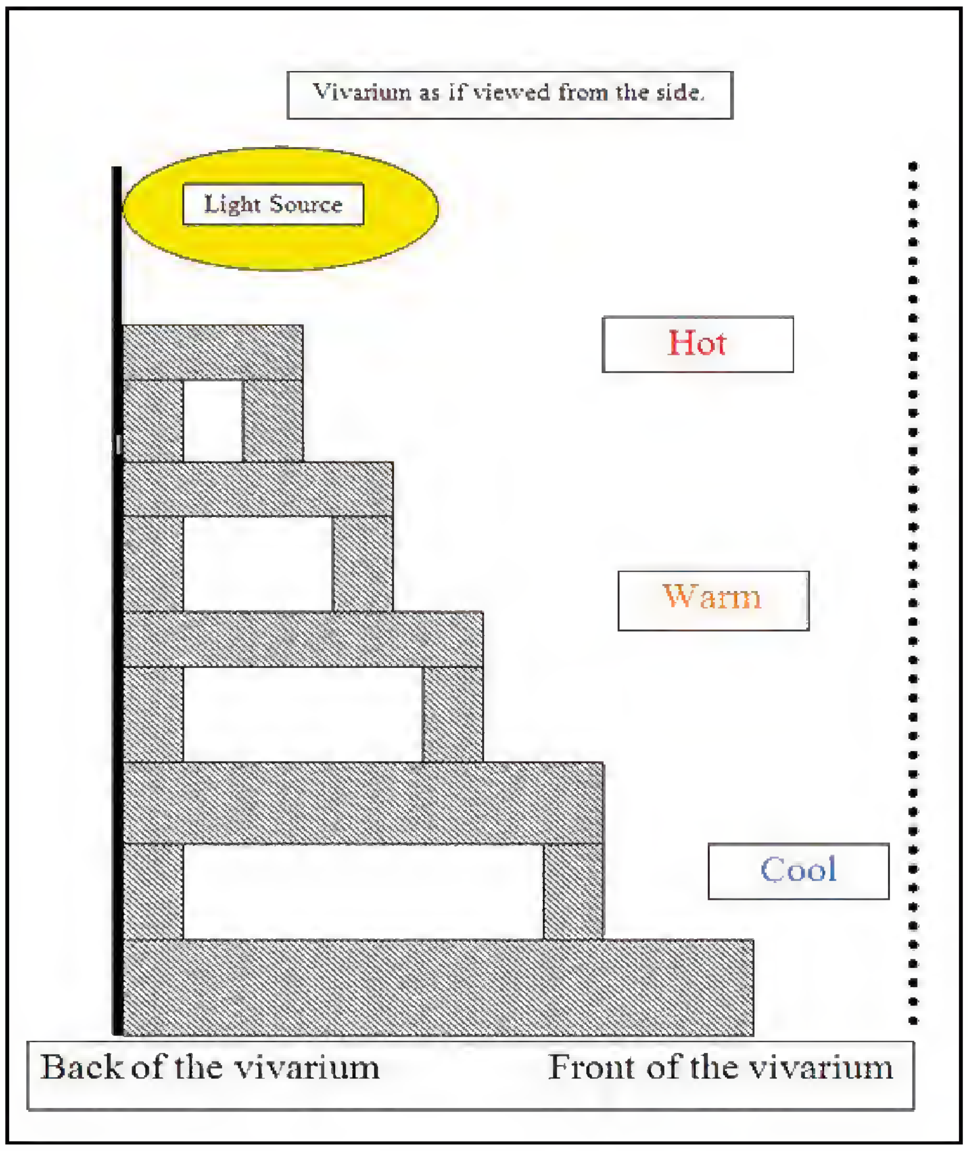

Figure 1. Schematic illustration of the "stack" built into the chuckwallas' enclosure.

Figure 2. Reduced number of hiding places (left) and new "stack" built for the chuckwallas to add spatial diversity, increased height and more hiding places into their exhibit (right).

range of basking areas as well as a gradual temperature gradient across the enclosure (via the construction of a basking stack out of blocks that enable the animals to move towards or away from heat; see Figure 1), it was hoped that an improved overall behavioural performance would be observed.

This project involved two adult western chuckwallas (one male, one female). To minimise the effect of extraneous variables, observations were conducted at the same time for each day of recording. An ethogram of chuckwalla behaviour was provided and explained to all data collectors (see Table 1). From October until December 2009, observations were conducted on the chuckwallas in their "normal" enclosure (as per College husbandry guidelines). A total of 1533 minutes of data were obtained and students worked in pairs to record state behaviours every minute for two (afternoon) 30-minute sessions, every Wednesday during term time using instantaneous scan sampling of focal individuals. The addition of new features to enrich the exhibit allowed for acclimatisation to environmental change before observations recommenced.

Comparison of before and after enrichment activity budgets, and use of the different features of the exhibit, was used to determine overall effects of enrichment (the increased "choice") on the activity levels, social expression and courtship displays of the two chuckwallas. During the entire experimental procedure,

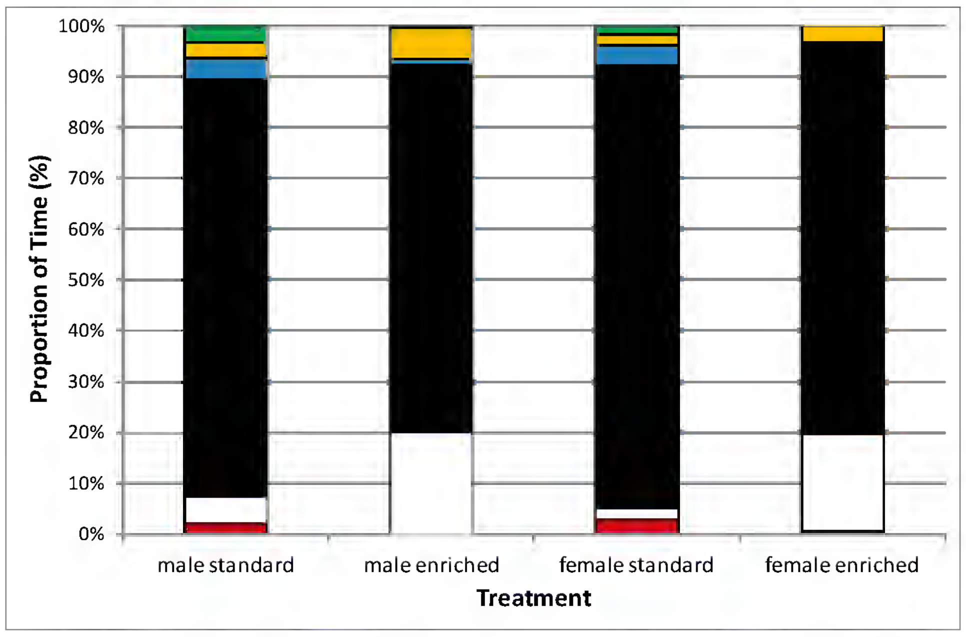

Figure 3. Activity budget for each chuckwalla (left two bars male; right two bars female) during the enriched and standard data collection periods. Behaviours shown are: red = movement; white = hidden; black = stationary/basking; blue = general interaction; orange = reproductive behaviour; green = other (e.g. stereotypy). Chuckwallas altered time spent hiding away and stationary when the basking stack was added in to the enclosure.

environmental conditions (lighting, heating and humidity) were maintained along the same parameters. At the time of the study, the chuckwallas' enclosure measured 1 m^3 in total volume. Figure 1 shows a schematic diagram of the alteration provided in the vivarium to encourage the chuckwallas to use a range of thermal conditions and hence display a more extensive range of behaviours. Figure 2 illustrates specific furnishings in the exhibits both before and after alteration of the enclosure. The "stack" was constructed of a brick and breeze block lattice, in a stepped formation across the back wall of the vivarium.

Data were analysed using Minitab 16 statistical software. As these data were non-parametric, one-factor and two-factor chi-squared tests were used to determine significant differences in behaviour patterns against enclosure style. Figure 3 outlines overall activity budgets for each chuckwalla.

Results showed that chuckwallas in the enriched environment significantly reduced the number of "general" interactions, i.e. those not associated with reproduction, (χ^2 = 7.58; df = 1; p = 0.006) but performed an increased frequency of reproductively-associated behaviours (from 9.5% to 17.5% of an average time budget) although this was not significant (see Figure 3). Whilst performance of stereotypical behaviours (interaction with a boundary) was low overall (only three minutes of "pacing" around a glass boundary), under enriched conditions no stereotypy was recorded. This suggests that the provision of the "stack" was important in altering the chuckwallas' ability to feel secure in their environment; this is perhaps supported by the result that both chuckwallas were hiding significantly more of the time when the "stack" was provided (χ^2 = 44.1; df = 1; p < 0.001). Whilst this may not make the animals a "better" exhibit from a display point-of-view, it highlights the fact that choice of escape area is important to psychological welfare. Careful positioning of hide spots would therefore enable chuckwallas to feel secure in their enclosure and be on view.

Figure 4 shows that under enriched conditions chuckwallas did use the extra height they were provided with and spent more time off the ground; this seems highly apparent in the male individual and provides scope for further investigation. It is possible that gender differences in resource use mean that male animals are more inclined to use height more frequently than female animals. A Pearson's chi-squared test for comparing the effect of enrichment on each individual's use of height shows a significant difference for both animals' behaviour between enriched and standard conditions (χ^2 = 389.9; df = 2; p < 0.001). Prieto and Ryan (1978) state that chuckwallas are normally found in multi-sex groups so it may be that a pair is an unnatural social structure to

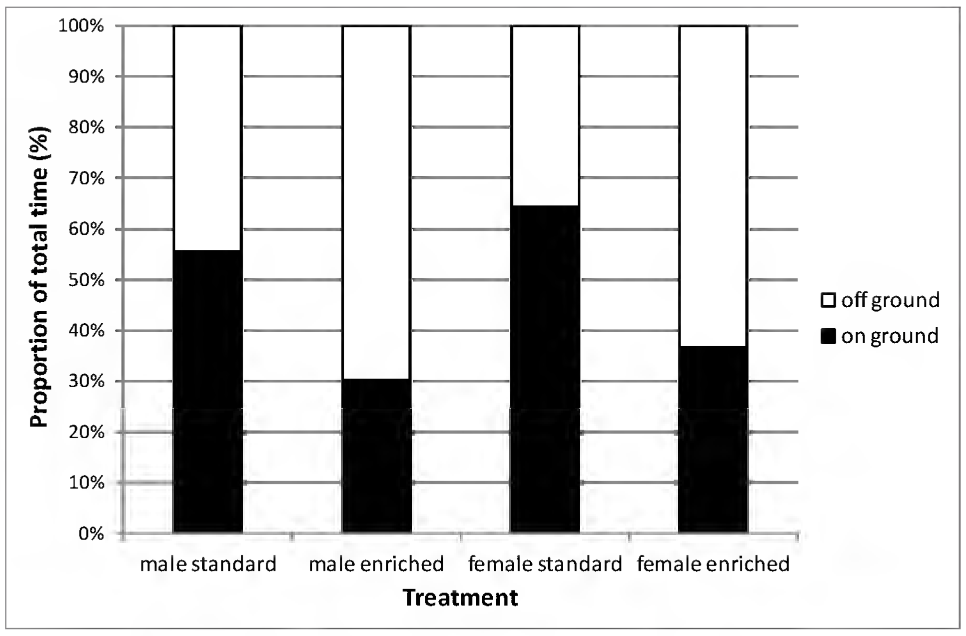

Figure 4. Differences in time spent on and off the ground under the two different enclosure treatments for each chuckwalla (male, left two bars; female, right two bars).

Table 2. Ethogram of corn snake behaviour.

Behaviour	Description
Hiding (terrestrial)	Majority of body concealed in substrate or furnishing on the ground.
Hiding (arboreal)	Majority of body concealed in vegetation.
Exposed(terrestrial)	Majority of body visible but stationary and respiring.
Exposed (arboreal)	Majority of body visible but stationary off the ground and respiring.
General locomotion and climbing	Movement: actively moving from one location to another. Actively climbing on furnishings of enclosure.

live in. Differences were noted in amount of time spent basking; this could be attributed to inconsistencies between observations whereby animals that were simply "exposed" or sat out in the open were deemed to be basking. Nevertheless, this is a particular aspect of the chuckwalla's behaviour that could be investigated further. Some arid-dwelling lizards are known to gather heat from rocks (thigmothermy) as well as radiant heat from the sun (heliothermy) (Belliure and Carrascal 2002); thus it is possible that differences in time basking/exposed could be attributed to use of the new rockwork as a means of regulating body temperature.

Case study 2: Behavioural enrichment of corn snakes

Corn snakes are often kept in flat, rather barren exhibits and numerous "pet literature" articles are available advising owners to maintain snakes in little more than plastic storage boxes (Jones 2000). Corn snakes are classed as rat snakes (Bartlett and Bartlett 2006), a group of snakes known to be highly exploratory (Almli and Burghardt 2006; Mullin and Cooper 1998), preferring a heterogeneous habitat of natural and man-made environments. These exploratory traits should be encouraged in captive enclosures. Casual observation of adult corn snakes at Sparsholt College indicated a preference for resting above ground, providing the foundation for this investigation. Research has shown that young corn snakes have a keen ability to learn from their surroundings (Holtzman et al. 1999), suggesting that behavioural patterns are modified via experience and indicating the importance of a stimulating environment to cognitive development. Almli and Burghardt (2006) noted that a related species, the black rat snake (*Elaphe obsoleta*), reacted in ways similar to those seen in mammalian species provided with enrichment, and that snakes in enriched exhibits are more behaviourally competent, further emphasising the importance of biologically relevant exhibits for captive reptiles and overall assessment of environmental enrichment.

This project involved behavioural observation of two populations of juvenile corn snakes housed in the Animal Management Centre at Sparsholt College Hampshire. The enriched population (two snakes) lived in a simulated planted enclosure, whereas the control group (two snakes) lived in a more basic style of accommodation (see Figure 5). Both populations were subject to similar environmental conditions (lighting, heating and humidity) and these variables were recorded throughout the study. Students worked in pairs and observed the snakes for two 30-minute periods every Wednesday in term-time from October 2008 until March 2009. Overall 540 minutes of behavioural data were recorded for each snake, using instantaneous scan sampling. The same

Figure 5. Furnishing of the enriched enclosure (left) and the pet-style, non-enriched, enclosure (right) for corn snakes used during the course of the study.

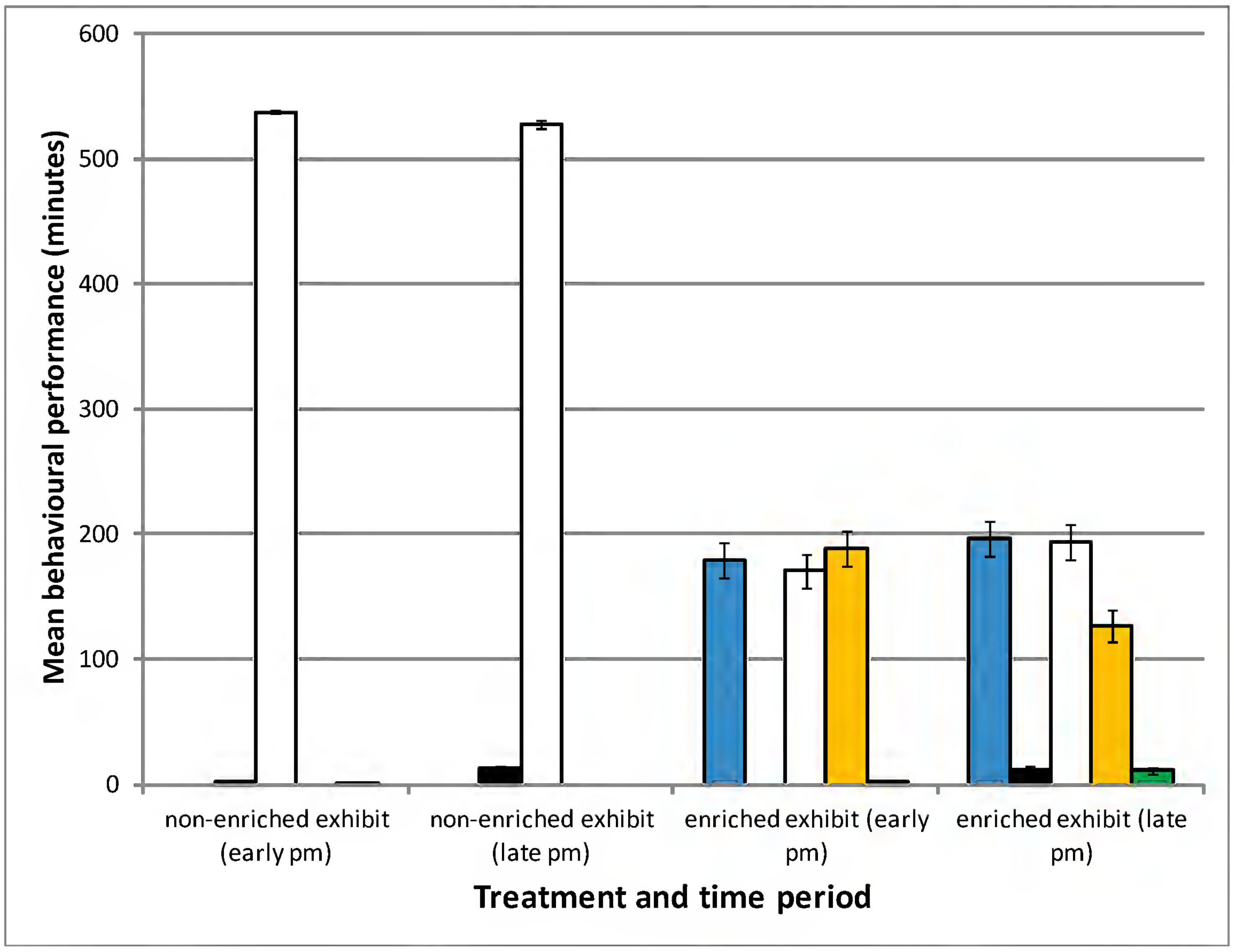

Figure 6. Mean activity budget (minutes) for each population of corn snakes within the two exhibits. Blue = exposed (arboreal); black = exposed (terrestrial); white = hiding (terrestrial); orange = hiding (arboreal); green = general movement (climbing, slithering).

time periods for observation were adhered to for each day of the project (1245 until 1315 and then 1515 until 1545). An ethogram (see Table 2) was produced to describe specific behaviours and activity patterns of note.

Data were analysed using Minitab 16 statistical software. All data were non-parametric and differences in behaviour, location and exhibit usage were evaluated using one- and two-factor chi-squared tests.

Snakes were provided with a complex environment, containing numerous branches for climbing and areas for off-ground resting increase the amount of time they are on view and are active (Figure 6). Comparison of the two populations highlighted the effect that extra furnishings have on the location of snakes in their enclosure; those in an enriched exhibit spent 31.7% of their time exposed and on view, whereas limited furnishings caused the snakes to be hidden for 98.6% of the total time observed. Snakes in the non-enriched exhibit, whilst provided with limited climbing opportunities, still did not use this element of their environment; yet an increase in availability of climbing "material" (as seen in the enriched enclosure) showed that the snakes spent 64.3% off the ground.

Using a one-factor chi-squared test, it was found that corn snakes spend significantly more of their time off the ground when given the opportunity ($\chi^2 = 88.4$; df = 1; $p < 0.001$) and using a Pearson's two-factor chi-squared test there is a strong significant relationship between enclosure type and whether the snake is on view or hidden ($\chi^2 = 443.6$; df = 1; $p < 0.001$). These trends are illustrated in Figure 7. Such results highlight the importance of a sound knowledge of natural activity patterns when designing reptile exhibits.

Snakes may feel more secure when provided with a planted, naturalistic environment and hence are on show more often, adding to their role as a zoological exhibit. Corn snake exhibits should, therefore, incorporate height as well as floor space and provide the snakes with useable space above ground level, giving opportunities for elevated locomotion, basking, resting and interaction. Hernandez-Divers (2001) calls for more behavioural enrichment to be performed with captive reptiles and in spite of the limited sample size and time available for study, these results clearly state that corn snake behaviour patterns are affected by the type of enclosure they are maintained in.

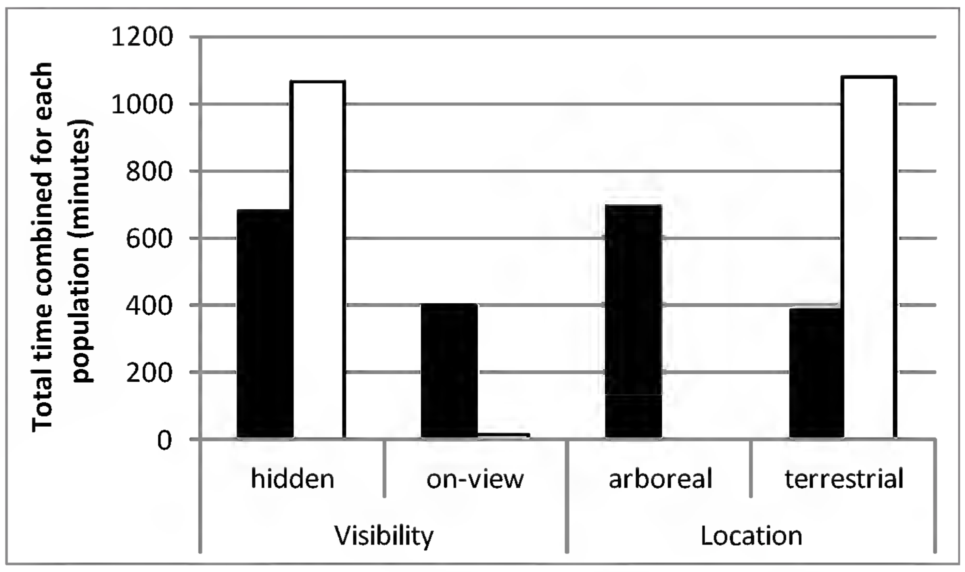

Figure 7. Relationships between style of the enclosure, location of the corn snakes and visibility to the observer. Black = enriched tank; white = non-enriched exhibit. Total time combined for both populations. Snakes were more likely to be on view and off the ground in an enriched environment.

Case study 3: Effect of exhibit design on poison dart frog activity

Amphibians are at risk of global extinction, and the importance of *ex situ* breeding programmes is a high profile topic (Griffiths and Pavajeau 2008; McGregor Reid and Zippel 2008). In order to promote zoo conservation the importance of public appreciation of species has been researched; findings show that "natural" or "unnatural" enclosure design can affect public perception of species within zoological collections. Zoos strongly promote their educational importance (Moss and Esson 2013), yet exhibits within zoos may not capture a visitor's interest and hence their message can be lost. Marcellini and Jenssen (1988) discuss the behaviour of visitors to a zoo reptile house and note that the public engage less with lizard and amphibian exhibits compared

Table 3. Ethogram of dart frog behaviour.

Behaviour	Description
Moving	A frog is hopping or crawling around its exhibit. Can include courtship wrestling.
Hunting	A frog is actively seeking out and searching for prey. Or pouncing and consuming prey.
Resting	A frog is stationary but completely visible.
Refuge use	A frog is partly visible or attempting to be fully concealed within foliage, furnishings or substrates in the enclosure.

to other species. The housing of many species of amphibian in relatively barren, "clinically sterile" exhibits because of heightened biosecurity could potentially detract from the exhibition potential of the frogs themselves. This study therefore explored the effects that enclosure design may have on the behavioural repertoire of amphibians held in the exhibit, and whether or not a clinical-style set-up altered the behaviour and activity level of the dart frogs in any way.

Two contrasting enclosures were created; one exhibit was representative of a naturalistic enclosure often seen at a larger zoo, and the exhibit was based upon a "clinical" style with non-natural furnishings and a bare substrate (see Figure 8). Due to constraints on the number of available animals, a sample of the same population of five frogs was used, first in one exhibit style and then in the other. Sixty hours of behavioural observation also took place on each frog population using a group scan sampling techniques every 5 minutes for 8 hours a day for 8 days in autumn 2011. An ethogram of behaviour (Table 3) was developed. Behavioural data were analysed using a one-factor chi-squared test (all using Minitab 16 statistical software).

The study found that enclosure design significantly affected the behavioural repertoire of blue poison dart frogs, with the naturalistic enclosure creating a more diverse activity range in the frogs (see Figure 9). The results of statistical analyses showed that the proportion of time spent active in the natural enclosure was significantly more than the time spent active in the non-natural enclosure ($\chi^2 = 147.91$; df = 1; $p < 0.01$). Likewise, frogs spent significantly more time resting in the clinical style set-up when compared to the naturalistic enclosure ($\chi^2 = 91.82$; df = 1; $p < 0.01$). Aspects of the three-dimensional, naturalistic enclosure were more likely to increase activity levels when compared to the unnatural enclosure, which was smaller and two-dimensional in design. This research can be used to understand how to increase the relevance of such exhibits (to visitors, to breeding potential, to positive animal welfare) in the zoo, as well as to highlight the importance of enclosure design in increasing the behavioural repertoire of poison dart frogs held in captivity.

Frogs still like to use refugia even if not provided in vast quantities; it is important to consider layout and planting of such exhibits so that poison dart frogs can feel secure enough to venture around the exhibit more often and use more of the available space. Potentially, frogs in the less natural set-up may have felt less inclined to move around their enclosure as they had less opportunity for retreating into refuge areas should they have felt the need to. Multiple refugia may also increase the range of microhabitats available to the frogs, thus providing increased environmental variables as well as hiding places, and hence enabling greater frog activity. The increased environmental diversity that the natural set-up provides enhances the activity patterns of the frogs themselves, suggesting they are more likely to be observed by visitors, and further increasing visitor engagement with the enclosure (and this species) in the zoo.

Discussion

All three case studies highlight that small-scale undergraduate projects can allow for evidence-based changes to enclosure design to be implemented that ultimately benefit the animals within the exhibit. Likewise, these studies highlight the positive responses that reptiles and amphibians give to an enriched set-up. Significant and beneficial differences in activity, space usage and, in some cases, visibility were noted in all three projects, demonstrating the importance of enriched housing for reptiles and amphibians. Building artificial cliff-faces for chuckwallas to choose where to thermoregulate, increasing the height that corn snakes can access, and recreating a small piece of rainforest to enhance a frog's chances of being more active are all small ways of improving the relevance of such animals within a captive setting. Developing an environment with added complexity can promote behaviours that make the animals more observable to zoo visitors and potentially, therefore, provide a better attraction overall.

Enrichment in the zoo can take many forms and does not necessarily have to be something added to the enclosure as a "toy" (Carlstead and Shepherdson 2000). Here, we have shown that the whole environment and overall layout of the enclosure can be enriching if this promotes a specific activity with a strong internal motivation (i.e. one that the animal is driven to perform). It is potentially easier to factor in an enriched enclosure

Figure 8. Furnishing of the naturalistic enclosure (left) and the clinical-style, non-naturalistic, enclosure (right) for blue poison dart frogs during the course of the study.

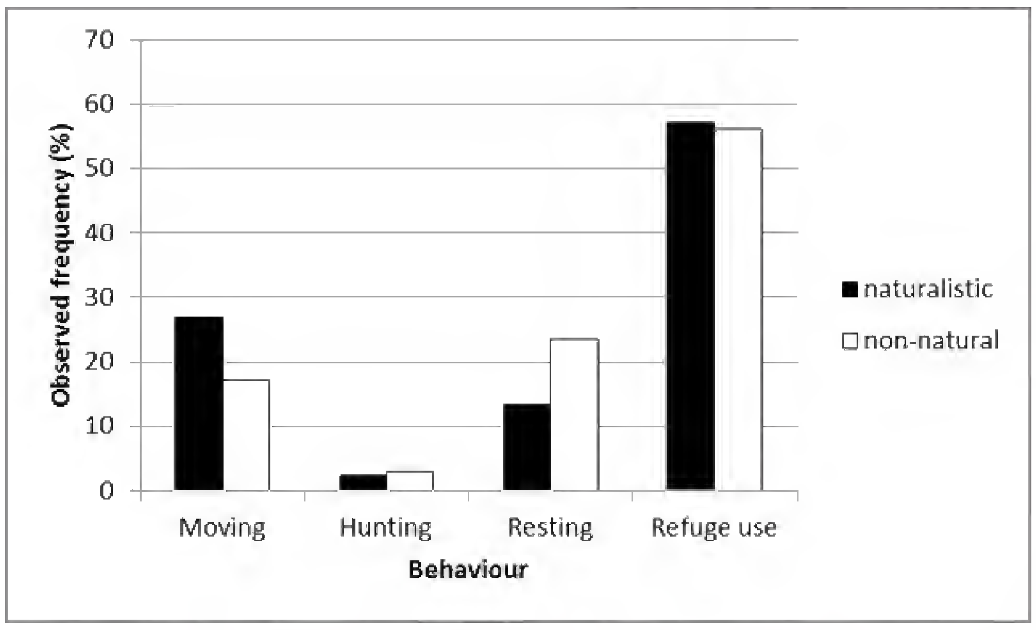

Figure 9. Activity pattern of the frogs when housed with a naturalistic (black bars) and non-natural, more clinical, enclosure (white bars).

layout, going back to the old principles of Greenberg (1995) and ethologically-informed schemes, from the inception of the exhibit rather than design a schedule that attempts to add enrichment in piecemeal afterwards. Lighting, heating, substrate composition and availability, water and planting are all key aspects of an enriched environment for reptiles and amphibians (Hawkins and Willlemsen 2004), and this research shows that provision of such factors in a manner akin to natural history enhances the behaviour and activity of a vivarium's inhabitants. Indeed, such a holistic approach to enrichment is championed by Mellen and Sevenich MacPhee (2001).

Ectotherms in general can be tricky subjects for which to gain public support and visitor interest. Reade and Waran (1996) noted that naturalness of zoo exhibits is the most important standard of husbandry noticed by zoo visitors, and that animals within "naturalistic" enclosures are more interesting, active and educational. Appropriate exhibition that engages the visitor's attention and provides the animals with a secure, relevant environment appears to be the best way to meet the needs of both stakeholders in this scenario. Current research suggests that evaluating the impact of species displayed in zoos on the education of visitors is multi-layered and that some taxa are viewed more favourably than others (Moss and Esson 2010). Zoos therefore need to be creative when integrating the exhibition of reptiles and amphibians into their long-term plans to ensure that such species are displayed in a fashion that engages the visitor and encourages natural behaviour.

Reptiles can be unappealing to zoo visitors, due to poor public image and ignorance of their natural history. Consequently, the provision of a spatially complex environment not only benefits the welfare of the animal, but can also further engage the viewing public, thus enhancing the zoo experience. The importance of this research to the development of reptile/amphibian-centred enrichment programmes is underpinned by Manrod et al. (2008), who state that whilst the welfare benefits of biologically relevant enrichment protocols for mammals and birds are well known, the benefits seen in reptiles post-enrichment suggest that this should become a standard procedure for husbandry practices in these species too. Results from the chuckwalla case study showed that animals budgeted their time differently when provided with an enclosure of increased temporal and spatial complexity. The stack provided for the lizards increased variation in thermal and lighting conditions, and it could be reasonable to assume that given the opportunity to regulate homeostatic mechanisms more precisely, the chuckwallas would then expend time (= energy) on diversifying specific behavioural displays. Dominance hierarchies, social flexibility and breeding success appear to be interlinked (Alberts 1994), and resource distribution can affect social stability. Consequently, spatially and temporally complex environments may help improve the behavioural repertoires of captive herptiles and lead to successful propagation. Provision of new spatial levels within enclosures may promote specific methods of social communication to enhance breeding success. Use of height by the corn snakes clearly shows that reptiles will utilise a range of three-dimensional spaces when given the opportunity to do so.

The research presented here was designed to show how these small manipulations to enclosure style can alter both animal behaviour and enclosure usage quickly and easily. Undergraduate projects can be beneficial to zoos and help advance the evidence basis of animal management. Directed research that is planned and explained to students, and thesis students under the supervision of a professional with expertise in the field, are useful ways that zoo research output can increase. The two groups of students (chuckwalla and snake projects) had all undertaken a second year-generic animal behaviour unit and had been trained in interobserver reliability (IOR), ethogram design and behavioural recording techniques. Data collection was practised with each group and behaviour of the animals observed *in situ* and explained to these students. Whilst a specific IOR was not generated for each cohort, we are confident in the credibility and validity of the results due to the extensive pre-experiment training that the students received.

Basic results from these case studies were presented, individually, at regional and international environmental enrichment conferences as well as at a British and Irish Association of Zoos and Aquariums (BIAZA) Research Symposium to demonstrate the efficacy of undergraduate research and the benefits of enrichment programmes for uncommonly chosen subjects (i.e. reptiles and amphibians). Increased collaboration between zoological collections and university departments, as described by Fernandez and Timberlake (2008), can provide more opportunities for the assessment and analysis of animal behaviour and visitor experiences if zoos are to move forward in their goal of adding science to all aspects of animal management, exhibit design and visitor education (Lawson, Ogden and Snyder 2008).

Conclusions

Overall, it can be concluded from these case studies that:

1. Small-scale undergraduate student projects can be used to alter husbandry for specific-species and evidence base management protocols for reptiles/amphibians housed in captivity.
2. Evidence from natural history can be implemented into enclosure layout in order to enrich the lives of the inhabitants within.
3. Spatially complex enclosures can enable a wider range of activities for reptiles and amphibians to perform.
4. Sociality between individuals that may be important for breeding activity can be promoted in an environment that provides for a fully-developed behavioural repertoire.
5. The style of the enclosure that reptiles and amphibians are displayed in increases activity and/or visibility and therefore may engage the observer for longer, thus increasing visitor engagement.

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