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Cross-institutional assessment of stress responses in zoo animals using longitudinal monitoring of faecal corticoids and behaviour

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Abstract

Cross-institutional studies that combine non-invasive physiological measures of stress responses and the assessment of individual differences in behaviour and temperament have great potential as tools for assessing the well-being of zoo animals and for identifying key environmental stimuli relevant to well-being. In addition, such studies allow comparison of animals under a wide variety of conditions and enable researchers to obtain sufficiently large samples sizes for statistical data analyses. Faecal corticoid measurements, a method recently developed to monitor adrenal activity in wildlife and domestic species, can be obtained non-invasively as part of the normal husbandry routine. While basic techniques still need improvement, and interpretation of the acquired measures can be challenging, assessment of faecal corticoid concentrations can provide a useful indicator of stress responses under a variety of captive conditions. Here we report on three studies that illustrate this approach and the results that can be obtained. An on-going study reveals significant differences in the pattern of variability of faecal corticoid concentrations between polar bears that are reported by keepers to perform stereotypic behaviour and those that do not. In another study, faecal corticoid measures indicated that stress responses to certain extraneous noises might interfere with the breeding of Hawaiian honeycreepers in captivity. In a study of clouded leopards, higher faecal corticoid concentrations were measured when cats were kept on public display or near potential predators compared to individuals maintained off exhibit or in the absence of visible predators. The findings of an on-going experimental study suggest a causal relationship between the provision of additional hiding spaces and a decline in faecal corticoid concentrations in clouded leopards.

Keywords: *animal welfare, corticosteroid, housing and husbandry, stereotypies, stress, zoo*

Introduction

A new field of investigation (which may be) termed "zoo animal welfare research" is emerging in the area of zoo biology. This field of study aims to raise standards of animal care in zoos based on the systematic empirical analysis of animal–environment interactions. A key research area is the comparison of animal well-being under the various environmental and social conditions found in zoos to determine optimal animal management standards for a species. Zoo animal welfare research is multidisciplinary and cross-institutional. It involves the integration of several kinds of objective data, such as long-term behavioural observations, investigation of the causes of impaired health, reproductive function and abnormal or neurotic behaviour, and the assessment of behaviours considered 'normal' or appropriate for a given species (eg activity budgets, species-specific parental and social behaviour, exploratory and play behaviour, etc). Since these measures by themselves are frequently difficult to interpret in terms of an animal's state of well-being, additional independent measures of subjective emotional experience are now being employed. It is well known that threatening or challenging situations can induce ACTH (adrenocorticotropic hormone) release that, in turn, stimulates increased synthesis and secretion of glucocorticoids by the adrenal gland (Selye 1956; Mason 1968; Hennessy & Levine 1979; Hennessy *et al* 1979; Jones 1979).

A combination of non-invasive physiological measurements and individual behavioural assessments may provide an effective way of furthering our understanding of behavioural motivation and causation, as demonstrated in a number of recent behavioural studies within single institutions (eg Dathe *et al* 1992; Carlstead *et al* 1993; Mcleod *et al* 1996; Jurke *et al* 1997). Carlstead *et al* (1993) were able to show a reduction in both pacing and cortisol levels upon provision of concealment to leopard cats. Similarly, Jurke *et al* (1997) classified seven female cheetah into three categories of corticoid responsiveness (low, intermediate and high), and found that females in the high category were rated most 'nervous' and had compromised patterns of ovarian cycling. Capitanio *et al* (1999) found evidence that 'sociability' in macaques is related to immune parameters, since individuals that were more sociable showed a greater immune response and a more rapid decline in cortisol concentrations after inoculation with the simian immunodeficiency virus (SIV). Similarly, studies on farm animals have shown a link between social status and stress and immune responses to transportation (eg McGlone *et al* 1993). Several other studies have demonstrated that individual variation in stress responses is consistent and stable over time, and that a given stress response may remain characteristic of an individual over its lifetime (Pottinger 2000). For example, in domestic pigs an 'active' versus a 'passive' coping strategy to stress appears to be an individual characteristic (Schouten & Wiegant 1997, cited in Pottinger 2000). And in rainbow trout, consistent individual differences in responsiveness to stress are apparent for up to two years (Pottinger *et al* 1992).

A number of influential studies have demonstrated the power of cross-institutional behavioural research in revealing environmental effects on zoo animal behaviour, reproduction and well-being (see Mellen 1994 for a review). Cage size and topography have been shown to be significant factors associated with the performance of abnormal stereotypic behaviours in some zoo-housed bear species (Van Keulen-Kromhout 1978). A multivariate analysis of zoo husbandry survey data collected for the red panda revealed that large enclosures and the availability of several nest boxes were important factors in successful reproduction because red panda mothers need to be able to change the location of their pups frequently (Roberts 1989). In a multi-zoo gorilla study, Miller-Schroeder and Paterson (1989) found cage volume, complexity and the availability of privacy to be positively correlated with good maternal behaviour and reproductive success. In a zoo-based study of individual differences in the reproductive behaviour of small felid species, Mellen (1991) found that institutional differences in the quality of animal caretaking partially explained individual differences in reproductive success. Carlstead *et al* (1999a,b), in a cross-institutional comparison of 72 black rhinoceros, identified three statistically independent predictors of reproductive success: dominance behaviour of the female, enclosure area, and the absence of concrete walls around the enclosure. Their study also indicated that a high degree of exposure to zoo visitors along the enclosure perimeter might be a source of chronic stress for some individuals

A drawback of cross-institutional studies is the difficulty of standardising behavioural observations under such a wide variety of conditions. Therefore, we have relied on integrative assessments of behavioural distinctiveness, style or temperament made by animal keepers and researchers who have become familiar with individual animals over an extended period of time. Integrative behavioural measures may be predictive of an individual's reaction to environmental change and challenge, and can present a more comprehensive view of an individual's behaviour over time. They also allow us to combine behavioural information from a variety of institutions into a single database (see Gosling 2001 for a comprehensive review of measuring animal temperament and personality). There are two main sources of behavioural data for measuring temperament in animals: assessment by observers' ratings on scales with predefined scores (eg Stevenson-Hinde 1983) and assessment of responses to behavioural tests which directly measure an individual's response to an environmental change and/or challenge (Manteca & Deag 1993). Wielebnowski (1999) used keeper surveys and quantitative behavioural observations of responses to mirror presentations in a study of 44 adult cheetahs at five North American facilities. She found significant differences between non-breeders and breeders in the scores of 'tense-fearful' and 'readiness to explore new objects'. Cheetahs that had bred successfully also exhibited lower levels of fear-related behaviour and appeared more confident in their surroundings. To validate behavioural profiles based on keeper questionnaires for cheetah, maned wolf, black rhinoceros and great Indian hornbill, the Methods of Behavioural Assessment (MBA) project (Carlstead *et al* 1999a,b, 2000) used standardised tests of responses to novel objects and conspecific odours, and compared these with the ratings of keepers on temperament traits and frequencies of certain behaviours.

As an independent physiological measure, non-invasive monitoring of glucocorticoid concentrations is increasingly being applied to zoo animals through the sampling of urine, faeces and saliva. In particular, the measurement of corticoids in animal faeces not only offers the advantage of being almost entirely non-invasive, but also represents a pooled sample of corticoid output over a period of several hours or days, depending on the species (Whitten *et al* 1998; Wasser *et al* 2000; Möstl & Palme 2002). This can provide a highly useful longitudinal measure of adrenal output for detecting patterns potentially indicative of chronic stress. Monitoring stress responses for extended periods of time provides us with a window on the subjective emotional responses of individual animals in their particular environmental and social situations. By using a combination of physiological and behavioural measures *cross-institutionally*, we can begin to understand the particular challenges certain 'problem' species may face under captive conditions. This paper briefly reviews three on-going or recently completed studies of zoo animal welfare that employ this integrative approach.

Three case studies

1. Polar bear stereotypy

Concern has been expressed over the psychological health and housing of zoo animals since the 1950s (Hediger 1955). As one of the most easily recognised and possibly most prevalent abnormal behaviours seen in zoos, stereotypic behaviour has received considerable attention in the literature (eg Morris 1964; Boorer 1972; Dittrich 1984; Carlstead 1991; Marriner & Drickamer 1994; Carlstead 1998). Stereotypic behaviour is believed to be an indicator of reduced welfare because of its association with environments found by other measures to be 'stressful' or 'frustrating'. However, research to date has largely failed to reveal a simple relationship between welfare and the performance of stereotypic behaviour (Mason 1991; Mason & Latham

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2004, pp 57–69, this issue). It is becoming increasingly clear that stereotypies are a broad category of diverse behaviours with many different motivational underpinnings (Mason 1993; Rushen *et al* 1993). Stereotypies are also strongly individualistic in form and frequency, since not all individuals kept under the same conditions develop and perform these behaviours. It is clear that stereotypic behaviour cannot be understood outside the context of the individual animal's physiological and psychological responses to its environment.

As a group, bears are generally considered to be particularly prone to exhibiting stereotypic behaviour and, within this group, polar bears (*Ursus maritimus*) have been documented to be the most likely to show such behaviour (Richardson 1987; Ames 1993). Indeed, a 1997 survey of 16 North American zoos carried out by the American Zoo and Aquarium Association's (AZA) Bear Taxonomic Advisory Group (TAG) revealed that 43% of polar bears exhibited at least moderate amounts of stereotypic behaviour (K Carlstead 1997, personal communication). Considerable pressure has been placed on institutions in North America, the United Kingdom and other European countries by animal welfare interest groups either to improve the conditions that might be causing these problems or to cease keeping bears in captivity. Consequently, a number of small sample case studies have attempted to assess the prevalence of stereotypic behaviour in polar bears and its significance as an indicator of reduced welfare, and to test techniques for eradicating it (eg Wechsler 1991; Forthman *et al* 1992; Ames 1993). Carlstead and Seidensticker (1991) found a seasonal pattern in the stereotypy of an American black bear and hypothesised that the motivation to pace might be related to the reproductive cycle. A Lyles (1995, personal communication) has conducted studies at the Central Park Zoo, New York, that suggest a similar pattern in polar bears. Although these studies have produced some useful information and testable hypotheses, none of them has been conducted on a large enough scale with an adequate sample size for the results to be reliably generalised to the captive population as a whole. Research into the performance of stereotypic behaviour in bears, and particularly in polar bears, remains a high priority for zoos.

A major objective of this study, which is part of a larger ongoing project, is to investigate the relationship between stereotypic behaviour and stress hormones in polar bears. This is a step towards determining the role of stress in precipitating stereotypic behaviour and towards understanding the environmental factors that predispose polar bears to perform these behaviours. Answering these questions is an essential pre-requisite to the development and prioritisation of remedial protocols that minimise potential causes of chronic stress or frustration in bears.

Methods

This study involved 18 North American zoos and 48 subjects consisting of 22 males and 26 females ranging in age between 2.5 and 34.5 years. All of the bears had been living in their enclosures for at least one year. Enclosures were of a wide variety of designs and ages (ranging between 2 and 81 years), and varied in size (ranging between 139 and 836 m^2). Behavioural information about stereotypic behaviours displayed by the bears was collected by means of a questionnaire survey given to at least two animal keepers. The questionnaire asked if the bears displayed stereotypies (defined as 'an act or behaviour that is repeated in a stereotyped or invariant way with no apparent function') and if so, keepers were asked to describe the following characteristics of the stereotypy: the proportion of the day it occupied, the ease with which the bear could be distracted from it, the proportion of exhibit space used, and whether or not the stereotypy was normally a response to a particular stimulus. Nine of the 48 questionnaire responses were completed incorrectly and were subsequently excluded from the analysis involving keeper questionnaire data. Faecal samples were collected from each subject at the same time of day once every two weeks for a 12-month period and assayed for corticoids.

Results

A two-way ANOVA of all corticoid values (n = 26 per subject) revealed significant differences overall (n = 48, F_{47} = 2.74, P = 0.0001), with a stronger effect of zoo (n = 18, $F_{17,30}$ = 5.04, P = 0.0001) than individual (n = 48, $F_{17,30}$ = 1.44, P = 0.06). Further analysis of the behavioural and environmental data will be necessary to determine the significance of these findings with respect to potential institutional differences in stress levels.

Seven out of 39 (18%) bears were reported by their keepers as not displaying stereotypic behaviour. The bears that did display stereotypic behaviour were estimated to spend from less than 10% to 50–70% of their time engaged in this behaviour. The median percentage of time category spent engaged in stereotypic behaviour was reported as 10–30%. Using two sample t-tests, we compared six parameters of corticoid profiles between bears that did and did not display stereotypic behaviour and found no significant differences between mean (n = 39, t = 1.55, P = 0.13), median (n = 39, t = 0.30, P = 0.76) and minimum (n = 39, t = 0.52, P = 0.60) corticoid levels. However, there were significant differences between maximum corticoid levels (n = 39, t = 3.45, P = 0.001), variance (n = 39, t = 2.88, P = 0.06) and the coefficient of variation (n = 39, t = 3.43, P = 0.001) of corticoids. Bears that did not display stereotypic behaviour had higher peak corticoid levels and showed greater variation in corticoid levels than stereotypic bears. This result implies either that non-stereotypic polar bears are more reactive to particular environmental stimuli than stereotypic bears, or that they find their environments more acutely stressful. The latter explanation would support a coping function for stereotypic behaviour in these polar bears.

To summarise these findings, the corticoid profiles of stereotypic bears are different from those of non-stereotypic bears; this is consistent with the hypothesis that bears use stereotypic behaviour as a coping mechanism in stressful situations. However, it is clear that we need to know more to fully understand these results. We are now in the final stages of collecting detailed quantitative measures of the behaviour of these animals and others over a 12-month period.

2. Hawaiian honeycreeper stress responses

Very little research has been directed to the problem of how captive conditions might negatively impact the welfare and reproductive success of passerine birds, which generally do not breed well in captivity. A specific purpose of research at Honolulu Zoo and at five other zoos is to provide information to improve the success of captive propagation efforts for endangered endemic Hawaiian honeycreepers (*Drepanididae*). The propagation program focuses on the most common species as surrogates for more endangered species. In the wild, 'Apapane (*Himatione sanquinea*) and 'Amakihi (*Hemignathus virens*) live in mesic and wet native *ohia* forests where they feed mainly on nectar and insects. They defend breeding territories during an extended breeding season from about November until June (peak months are February to June). Since the captive breeding program began in 1989 at six zoos with between one and five pairs of birds each, only six pairs have produced chicks, most of which have not survived beyond fledging.

Our research is the first of its kind to identify the environmental factors in captive environments that potentially disrupt the reproductive efforts of passerine birds. The reproductive cycle of birds is responsive to a variety of seasonally predictable environmental cues that initiate gonadal development, control the onset of breeding and synchronise nesting and brood care efforts within a pair. These cues, when provided in a captive environment, are key to successful propagation. However, birds must also respond adaptively to unpredictable environmental perturbations (Wingfield *et al* 1997). For free-living wild birds these might include severe storms, increased predation pressure, social hierarchy in flocks, presence of competitors, low food availability, pollution or human disturbance (Silverin 1998; Wingfield *et al* 1998). Such environmental disturbances may cause an animal to shift to an 'Emergency Life History Stage' (Wingfield *et al* 1998), ie a 'stress response', consisting of facultative behavioural and physiological changes that allow the birds to avoid the deleterious consequences of long-term stress. Behavioural changes may include deactivation of territorial behaviour, suspension of social relationships, suppression of reproductive behaviours or seasonal migration, activation of refuge-seeking or escape from the source of disturbance, decreased or increased foraging activity, and/or settlement in alternative habitat (Wingfield 1994; Silverin 1997). In captivity, if birds are prevented from responding to environmental perturbations with these adaptive behavioural reactions, severe chronic stress may develop, resulting in immunosuppression, disruption of breeding efforts, neuronal cell death, protein loss and growth suppression (Wingfield 1994). Animal managers are well aware of this possibility when animals are subjected to various kinds of unusual disturbances, but there are no published, systematic investigations of the kinds of disturbance that birds respond to, or assessments of the severity of their responses.

This study asked two questions:

1) Which unpredictable noises and events in a zoo are associated with corticoid responses?

2) Do responses to these perturbations disrupt reproduction, as occurs in wild passerines?

Methods

Subjects were four 'Apapane (2 males and 2 females), all wild caught and housed at the Honolulu Zoo since 1990 and 1992 with the exception of one female that was wild caught in 1998, and six 'Amakihi (3 males and 3 females), all wild caught at the beginning of the study in December 1999 with the exception of one male that was wild caught in 1992. All birds were housed in pairs in cages 3.3 m × 3.3 m × 3.3 m inside an air-conditioned, single-wall plywood building that kept the cages mosquito-free. The cages were totally enclosed except for two bubble skylights in the ceiling of each cage. Behavioural observations were conducted via one-way glass in the cage doors.

Between February 2000 and July 2001, all fresh bird faeces were collected daily from each cage at approximately 0930h. Faecal samples were syringed from a large plastic bag suspended below a favourite perch. It was not possible to separate a pair of birds for individual faecal collection without causing undue stress, nor was it possible to determine the identity of the faecal samples. All samples were pooled into one tube for a given cage for one week. Faecal samples were frozen and later assayed for corticosterone at the Center for Conservation Biology at the University of Washington.

Throughout the 18-month study period, animal keepers and researchers noted daily if there were any unusual or unpredictable noises or events in or around the Hawaiian bird facility. These were classified into five categories of potential disturbance. On the day after a disturbance, faecal samples were collected as usual and stored in separate tubes. Faeces were not always present in every cage. The five categories of disturbance and the range of faecal samples collected per pair of birds were: 1) concerts on summer evenings between 1800–1900h (n = 6–19); 2) people going into the cages to service air-conditioning, to change lights or to catch the birds (n = 6–10); 3) people working on the roof of the building (n = 2); 4) social stimuli presented to the birds (call playbacks or mirror presentations) (n = 10–17); and 5) machine noises outside the building, such as helicopters, tractors, leaf blowers, a portable generator and chain saws, and construction noises from jackhammers and bulldozers, which caused vibrations to the building (n = 4–9). For comparison, single-day faecal samples were also collected on a few 'normal' days when nothing unusual happened (n = 4–8).

Continuous behavioural observations of each individual bird (n = 4 'Apapane and n = 6 'Amakihi) were made for 5 min per day in random order at some time between 1000h and 1500h, between two and five times per week. If a disturbance occurred on a particular day, all birds were observed for 5 min the following day. All behaviours are expressed as frequencies per 5 min observation period for a

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total of 432 observations for the two ‘Apapane pairs and 865 observations for the three ‘Amakihi pairs. In this paper results are reported only for behavioural elements that were combined into the categories ‘perch hopping or flying’, ‘foraging’, ‘nest-building’ and ‘courtship’.

Results

The study period encompassed parts of two breeding seasons and two non-breeding (moult) seasons. None of the pairs nested in either breeding season, but singing, courtship and the gathering of nesting material were observed. ‘Amakihi showed very low frequencies of these reproductive behaviours during their first season in captivity (January to June 2000), but more during the second season (December to June 2001).

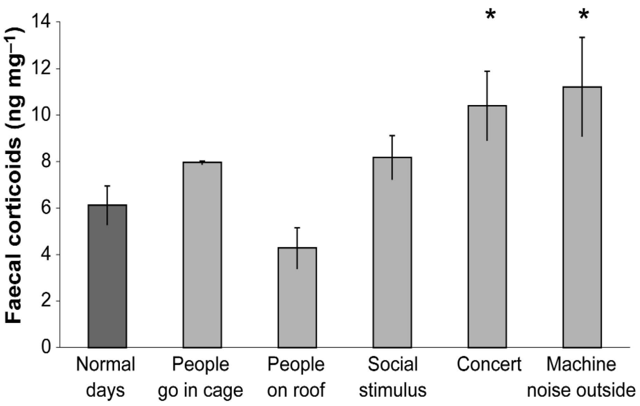
1. Which unpredictable noises and events in a zoo are associated with corticoid responses?

Faecal corticoid concentrations were averaged for each pair of birds (n = 5, species not distinguished) on the days after each type of disturbance and on days when nothing unusual happened (n = 4–8 samples per pair). A one-way ANOVA indicated that there were significant overall differences between disturbance categories ($F_{5,29}$ = 4.29, P = 0.006; see Figure 1). *Post hoc* Dunnett’s one-tailed t-tests were used to determine if corticoid concentrations associated with any disturbance category were significantly higher than on normal days. Figure 1 shows that faecal corticoids were significantly higher on days after concerts and after outside machinery noises than on normal days (t = 2.36, minimum significant difference = 4.18, difference between concert and normal = 4.27 and between outside noise and normal = 5.09, P < 0.05). Therefore, Hawaiian honeycreepers tend to respond with increased glucocorticoid secretion after unpredictable and unusual machinery noises and concerts outside their building, and this response lasts for at least one day after the event.

2. Do responses to unexpected environmental perturbations disrupt reproductive behaviour, as occurs in wild passerines?

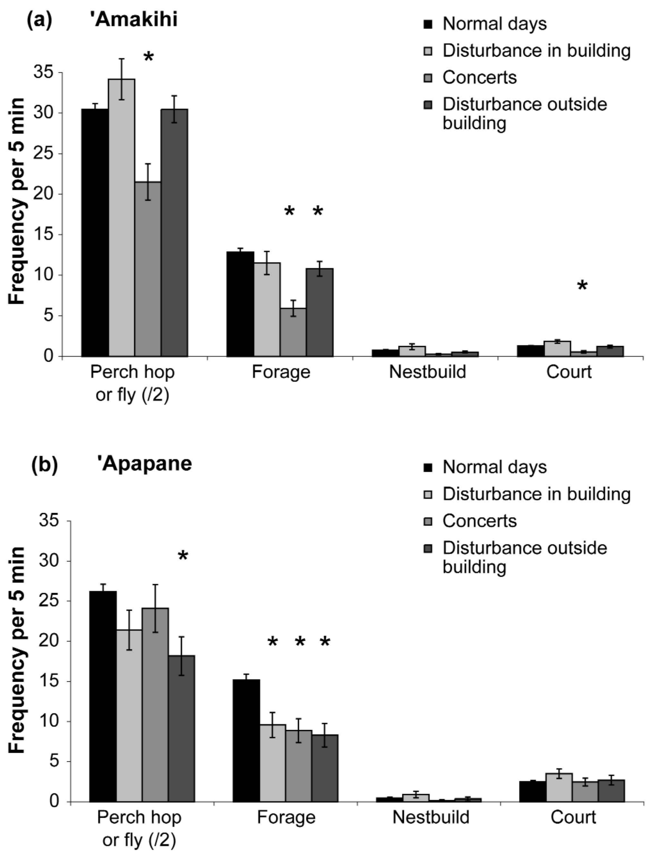
Behavioural frequencies of individual birds on normal days when nothing unusual happened (n = 170) were compared to: 1) days after people went into the cage for ‘unusual’ reasons or people were working on the roof (n = 26); 2) days after evening concerts (n = 22); and 3) days after outside machinery noises and construction noises (n = 37). Two-way ANOVAs, with disturbance category and individual bird as independent variables, were performed for each of four behaviours for each species separately. Figure 2 presents the average behavioural frequencies and the results of *post hoc* Dunnett’s one-tailed t-tests that were used to test whether behavioural frequencies on days following each category of disturbance were significantly lower than on normal days for a) ‘Amakihi, and b) ‘Apapane. For ‘Amakihi, all four behaviours had significant overall effects, and effects of disturbance and bird (‘perch hopping or flying’: $F_{3,5}$ = 8.42, P < 0.0001; ‘foraging’: $F_{3,5}$ = 17.99, P < 0.0001; ‘nest-building’: $F_{3,5}$ = 9.12, P < 0.0001; and

Figure 1



Average faecal corticoid concentrations (mean ± SE) of honeycreepers (‘Amakihi and ‘Apapane) on the day after different types of disturbance. Normal (control) days are those when nothing unusual occurred. Asterisks indicate significantly higher corticoid concentrations (P < 0.05) compared to normal days.

Figure 2



Behavioural frequencies measured on normal (control) days, and on the day after: 1) disturbance inside the building; 2) evening concerts outside the building; and 3) machinery and construction noises outside the building, for two species of honeycreeper (a) ‘Amakihi (n = 6), and (b) ‘Apapane (n = 4). Asterisks indicate significantly lower behavioural frequencies (P < 0.05) compared to normal days.

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'courtship': $F_{3,5} = 7.78$, $P < 0.0001$). *Post hoc* comparisons ($df = 799$, $t = 2.11$, $P < 0.05$) indicated that 'perch hopping or flying', 'foraging' and 'courtship' were all significantly reduced after concerts (Figure 2a). 'Foraging' was also significantly reduced after outside machinery and construction noises. For 'Apapane, only 'perch hopping or flying' ($F_{3,3} = 7.71$, $P < 0.0001$) and 'foraging' ($F_{3,3} = 7.89$, $P < 0.0001$) were significant overall and for the effects of disturbance and bird. *Post hoc* comparisons indicated that 'perch hopping or flying' was significantly reduced after outside machinery and construction noises, and that 'foraging' was significantly reduced after all three types of disturbance (Figure 2b).

The conclusion is that environmental disturbances caused by machine noises and concerts outside the building have species-specific effects on honeycreeper corticoid responses and behaviour. These types of disturbance increase faecal corticoid levels and decrease activity levels (hopping or flying) and foraging on the day after they occur. Additionally, 'Amakihi exhibit decreased courtship activity on the day after evening concerts. Therefore, for at least one of these species, environmental disturbances that elicit corticosteroid responses do indeed have disruptive, suppressive effects on some reproductive behaviours. The data indicate that other types of disturbance probably have a variety of behavioural effects as well.

This study gives a view into some of the causes of behavioural variation and disruption of reproduction in Hawaiian honeycreepers. It shows that captive passerines respond to environmental disturbances in a similar way as described for some wild passerine species, and it indicates how this might be a problem for captive breeding. It also raises many new questions about facility design and location in relation to non-localisable, persistent noises such as those made by machinery or concerts.

3. Adrenal activity in clouded leopards in response to enclosure modifications: a preliminary analysis

Clouded leopards (*Neofelis nebulosa*) in captivity are known to show a variety of behavioural problems, for example, fur-plucking, tail-biting, excessive pacing or hiding, and severe mate incompatibility (Yamada & Durrant 1989; Law & Tatner 1998; Kitchener 1999). These problems indicate poor animal well-being and severely hinder successful *ex situ* population management. Managers of clouded leopard facilities have frequently asserted that this species may be particularly susceptible to stress and that the specific needs of this species in captivity have to be identified to remedy these problems (N Fletchall 2003, personal communication). The relatively recent development of non-invasive faecal glucocorticoid monitoring now permits us to investigate whether the observed behavioural problems of clouded leopards may indeed be related to chronic captivity-stress (for reviews of the methodology see eg Wasser *et al* 2000; Möstl & Palme 2002). Using this hormone monitoring technique we first validated a faecal corticoid assay for clouded leopards using an ACTH challenge test, and then assessed baseline adrenal activity (ie corticoid concentrations) combined with various behavioural and husbandry variables in 65% (74 individuals) of the North American clouded leopard population (Wielebnowski *et al* 2002). Results from this multi-institutional study, carried out at 12 North American zoos, indicated that some of the observed behavioural problems might indeed be associated with increased adrenal activity. Keepers' assessments of 'stressed' versus 'calm' individuals during initial surveys appeared to be supported by behavioural and physiological data. Average faecal corticoid concentrations were positively correlated with the occurrence of self-injuring behaviours as well as with the frequency of pacing and hiding (Wielebnowski *et al* 2002). Furthermore, analysis of various husbandry factors showed that available enclosure height (ie height available to the cat by using climbing structures), available keeper time (ie time per day keepers spent not only feeding and cleaning, but also observing and providing enrichment), the visibility of potential predators (ie other large carnivores), and whether or not the animals were on public display, were significantly associated with faecal corticoid concentration. For example, cats that were housed in enclosures of greater height exhibited lower faecal corticoid concentrations. Similarly, available keeper time was negatively correlated with corticoid concentrations, indicating that the more time keepers were able to spend caring for the cats, the lower were the cats' faecal corticoid concentrations. Clouded leopards on public display or with visual exposure to predators showed significantly higher faecal corticoid concentrations than individuals housed off display (ie off exhibit) or without such 'predator exposure' (Wielebnowski *et al* 2002). Since multiple regression and other correlational data analyses can only infer associations, and not ascertain causality, it was decided to conduct a follow-up study to systematically test some of the variables associated with a rise in glucocorticoid concentration.

Two experimental set-ups were used to test for a potential cause–effect relationship between changes in enclosure design and concurrent measures of adrenal activity and behaviour. After an initial baseline period of data collection, hiding spaces were added in experiment 1 and climbing structures were added in experiment 2. This experimental follow-up study is currently on-going.

Methods

Twelve adult clouded leopards (6 males and 6 females) housed at four North American zoos (Buffalo Zoological Gardens, Cincinnati Zoo, Memphis Zoo and Point Defiance Zoo) are currently part of this study. Diet and other husbandry factors have been unaltered while one of two husbandry variables (available enclosure height or predator visibility/public display) has been changed for a period of between two and three months. Changes in enclosure height

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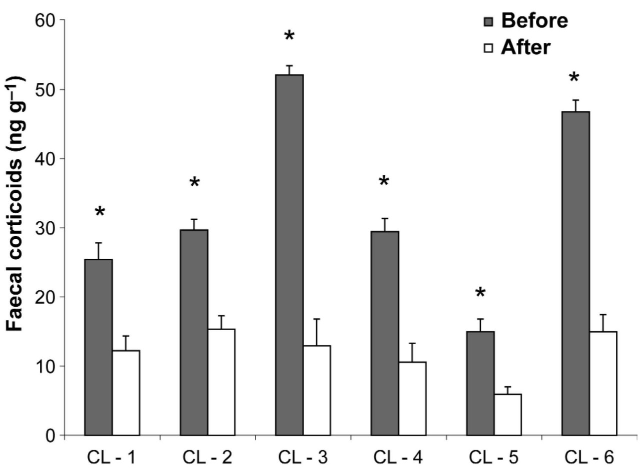
were achieved through the addition of climbing structures to allow the animals to access higher areas of the enclosure. Changes in predator visibility and public display were made indirectly by adding additional hiding spaces to allow animals complete seclusion from view. Each study animal was exposed to only one of the two treatments because of the logistics of implementing both changes consecutively at each of the facilities. The increase in climbing structures was implemented in the enclosures of four of the study animals (2 males and 2 females), while increases in the number of hiding spaces were made in the enclosures of the remaining eight animals (4 males and 4 females). Each animal served as its own control by monitoring all cats for at least two months prior to any enclosure change, and for between two and three months after the change had been made. Faecal samples were collected as close to daily as possible and quantitative behavioural observations were obtained between two and three times per week by one trained observer at each institution. While all data have already been obtained for eight of the 12 cats at this point, data are still being collected on the four remaining animals. Of the eight cats for which data analyses have been completed, six (2 males and 4 females) were exposed to a change in the available hiding space in their enclosure, whereas only two (1 male and 1 female) were exposed to a change in the amount and height of climbing structures. Thus, all data presented in the results section are still preliminary. The reported sample sizes show the number of faecal samples collected before and after the changes were made (n = before, after). Some faecal samples were not analysed because they were either too small or were of unusual consistency (mostly hair or diarrhoea) when samples were collected after a 'fast' day (ie a day when the animals were not fed). For some individuals this resulted in somewhat less than daily samples being available for statistical analysis. A non-parametric Mann-Whitney Rank-Sum statistic (t-test statistic, SigmaStat V2.03) was used to test for differences in faecal corticoid concentrations for each animal before and after the implementation of enclosure changes.

Results

Preliminary analysis of faecal corticoid data indicated that the addition of hiding spaces caused a significant decline in faecal corticoid concentration in all of the six clouded leopards (CLs) examined so far (CL1: n = 32, 37, $P < 0.001$, $t = 1532$; CL2: n = 81, 84, $P < 0.001$, $t = 8235$; CL3: n = 36, 66, $P < 0.001$, $t = 2953$; CL4: n = 74, 88, $P < 0.001$, t = 8011; CL5: n = 73, 85, $P < 0.001$, $t = 7534$; CL6: n = 42, 68, $P < 0.001$, $t = 2841$; see Figure 3). A decline in faecal corticoid concentration was also observed in the other two animals in response to the addition of climbing structures to increase available cage height. However, this decline was not statistically significant (CL7: n = 79, 65, $P = 0.189$, $t = 4385$; CL8: n = 69, 72, $P = 0.241$, $t = 5332$; see Figure 4).

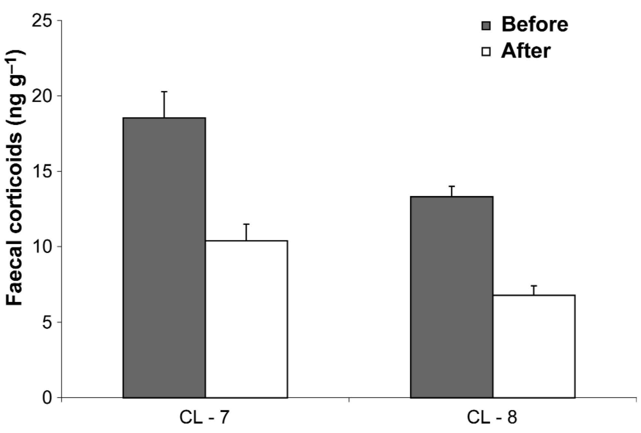
Overall these preliminary data analyses appear to indicate that modifications of enclosure design may indeed help to reduce chronically elevated glucocorticoid concentrations that are generally associated with distress in mammals.

Figure 3



Average faecal corticoid concentrations (mean ± SE) measured in six clouded leopards before and after additional hiding spaces were provided in their enclosures. Asterisks indicate significant differences ($P < 0.001$) between corticoid concentrations measured before and after the manipulation.

Figure 4



Average faecal corticoid concentrations (mean ± SE) measured in two clouded leopards before and after additional climbing structures were provided in their enclosures to increase available cage height.

General conclusion and animal welfare implications

Multi-institutional zoo studies, using measures such as non-invasive monitoring of physiological stress responses in combination with the assessment of individual differences in behaviour and temperament and/or detailed longitudinal behavioural observations, have great potential as tools for improving zoo animal well-being. These tools can be used at two levels: 1) at the population level to help define quantitatively what 'optimal' environments for a given species

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should be, and 2) at the individual level to help develop and establish new tools for assessing individual animal well-being. Such tools may help to facilitate the adoption of new management practices for individual animals, particularly with regard to environmental enrichment, behavioural training and husbandry recommendations for captive breeding.

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