Elephant References: Stress and Cortisol
Elephant Care International Database
www.elephantcare.org
Accessed 2 Feb 2018

Ahlering, M. A., J. E. Maldonado, L. S. Eggert, R. C. Fleischer, D. Western and J. L. Brown (2013). "**Conservation outside protected areas and the effect of human-dominated landscapes on stress hormones in Savannah elephants**." Conserv Biol **27**(3): 569-575.

Biodiversity conservation strategies are increasingly focused on regions outside national protected areas, where animals face numerous anthropogenic threats and must coexist with human settlements, livestock, and agriculture. The effects of these potential threats are not always clear, but they could have profound implications for population viability. We used savannah elephants (Loxodonta africana) as a case study to assess the physiological stress associated with living in a human-livestock-dominated landscape. We collected samples over two 3-month periods in 2007 and 2008. We used fecal DNA to identify 96 individual elephants in a community conservation area (CCA) and measured fecal glucocorticoid metabolite (FGM) concentrations as a proxy for stress. The CCA is community Maasai land managed for livestock and wildlife. We compared the FGM concentrations from the CCA to FGM concentrations of 40 elephants in Amboseli National Park and 32 elephants in the Maasai Mara National Reserve, where human settlements and intense livestock grazing were absent. In the CCA, we found no significant individual differences in FGM concentrations among the elephants in 2007 ($p = 0.312$) or 2008 ($p = 0.412$) and no difference between years ($p = 0.616$). The elephants in the CCA had similar FGM concentrations to the Maasai Mara population, but Amboseli elephants had significantly lower FGM concentrations than those in either Maasai Mara or the CCA (Tukey pairwise test, $p < 0.001$), due primarily to females excreting significantly lower FGM relative to males ($p = 0.025$). In the CCA, there was no relation among female group size, average pairwise group relatedness, and average group FGM concentration. We found no clear evidence of chronic stress in elephants living on CCA communal land, which is encouraging for conservation strategies promoting the protection of animals living outside protected areas.

Alexander, R. M., G. M. O. Maloiy, B. Hunter, A. S. Jayes and J. Nturibi (1979). "**Mechanical stresses in fast locomotion of buffalo (*Syncerus caffer*) and elephant (*Loxodonta africana*)**." J. Zool. (Lond) **189**(2): 135-144.

Films of buffalo and elephant running, and detailed measurements on dissected legs, have been used to estimate the maximum stresses which occur in locomotion, in certain muscles, tendons and bones. These stresses are similar to stresses previously determined for some other, smaller mammals.

Ball, R. and O. Fad (2006). **Serum cortisols in captive Asian elephants (Elephas maximus) in different management systems at Busch Gardens Tampa Bay**. 2006 Proceedings American Association of Zoo Veterinarians.

Introduction:Cortisol is a widely accepted measure of stress in wild and captive animals. In the past, captive elephant management systems have been criticized as potential stress inducers. The analysis of fecal cortisols is non-invasive and has been used to give long term evalutions of

social and ecologic pressures in elephants and other species. Salivary cortisols have also been used as a minimally invasive technique to measure social stress in captive elephants. The herd of Asian elephants at Busch Gardens Tampa Bay (BGT) changed from a traditional contact managemnt (free ccontact, FC) to a protected contact (PC) system utilizing positive-reinforcement based operant conditioning in 2004. Serum cortisols were measured after the change and evaluated along wth banked samples from before. Long term sampling will be utilized to measure this transition but evaluating a single process will hopefully reflect the overall changes that can be expected with this change in management. While the individual variations are notable and other issues potentially confound the issue, it appears that this transition has lowered the serum cortisols in this herd. In addition to serum cortisol measurements, the actual process of collecting the samples appears to be less stressful behaviorally. Pathologic processes should not be discounted when considering cortisol levels in evalauting stress in captive elephants.

Methods and Materials: Six female Asian elephants (Studbook numbers 30, 32, 304, 34, 35, 3) had been managed in a free contact system for many years. Studbook number 304 was captive born and the others were wild born. Serum was collected intermittently during this management system to bank and for reproductive hormone analysis. The elephants were placed in lateral recumbency by the handlers and blood collected from the ear vein on the caudal aspect of the down ear. Reproductively sound animals were bled more frequently than the others. Serum was frozen at -80°C until analyzed. In August 2004, the first group of three animals was moved to the new barn and started the new positive-reinforcement, PC management system. Within 5 wk, all animals had been moved over. All animals had been trunkwashed and were culture negative for Mycobacterium tuberculosis and negative on the newly developed MultiAntigen Print ImmunoAssay (MAPIA) and lateral-flow technology (Rapid Test) developed to detected antigen to M. tuberculosis. As the caudal aspect of the ear was used for sampling, each elephant was asked to station in a static chute designed to allow training of voluntary ear-presentation for manipulation and blood collection. Handler safety and creating an effective learning environment for the elephants required training each to proceed to the chute solo and station there calmly. General desensitization techniques were applied as session durations were increased. Within the chute,individual elephants had significant room to maneuver. Since no physical restraint or sedation was utilized,animals were trained to cooperate fully and voluntarily allowing for blood sampling and other husbandry procedures. By May 2005, training for voluntary bloods draws was firmly established on all six animals. The first approximately 20 samples collected under this new system were matched against the samples collected in the previous system. Samples were selected against if the animal had an active problem or was on therapy for any reason. Several animals had undergone a drug trial and these samples were selected against as well. Serum was again stored in -80°C freezer until analyzed at Conservation and Research Center (CRC) Endocrine Research Laboratory, Smithsonian Institution, National Zoological Park, Front Royal, VA. T-tests were utilized to discern any statistically significant results in the mean serum cortisols collected from animals before and after the implementation of the new husbandry systems. Results were considered significant at alpha levels <0.05.

Results: The results and simple means of serum cortisols are listed in Table 1. Elephant No. 34 had essentially the same level of cortisol in both systems. Elephant No. 32 had a reduction in the mean cortisol level of approximately 32% (20.84 versus 14.28 ng/ml) from the FC to the PC

system. Elephant No. 304 had a similar reduction of 37% in the mean cortisol (22.59 versus 14.29 ng/ml). Statistical analyses results are reported here (means, standard deviations, t-test results).

Discussion: Serum was chosen over salivary and fecal sampling as a means to measure cortisol for several reasons. While fecal and salivary cortisol changes can reflect stresses within a reasonable period after the stressor (approximately 24 hr), serum cortisols is more likely to be reflective of the stressors closer to the moment of sampling. The methodology is straightforward and less subject to the hazards for sample storage. Timeliness of the sample result is also a benefit to serum sampling. Blood sampling is a required husbandry practice in all elephant holding facilities belonging to the American Zoo and Aquarium Association (AZA). While fecal cortisol samples may be useful to look at over a long term period to evaluate the transition from FC to PC, we choose to additionally look at how one specific task, blood collection, was affected by making this transition. Fecal cortisols have been used to measure stress in transportation and environmental stress in some species, but are not thought to be reflective of the stress in a diagnostic procedure itself. For this evaluation, the lag time period between the potential stressor (blood collection) and the means to measure the stressor are same. Elephants No. 304 and 32 both had significant reductions in the mean serum cortisol levels. Both are in good health and had no apparent inflammatory problems. The logical deduction here is that the sampling process itself is less stressful in the PC management than the FC management. Elephant 34 and 30 had essentially the same level of serum cortisol as measured by the mean in the different management systems. Elephant 34 has developed significant uterine leiomyomas during the time period measured. Elephant 30 has recently had clinical bouts of anterior enteritis and is suspected of having a dietary hypersensitivity to wheat. Even with these two pathologic processes, the serum cortisol did not rise. Elevations in cortisol are quite often explained as resulting from social, behavioral, or environmental causes and little attention is paid to inflammatory causes. Associations between infections and elevated cortisols have been noted in wild animals. It is reasonable to assume that if these two processes did not exist, these levels would indeed be lower. Based on the other two elephants, a reduction of approximately 30% could be expected. Overall it appears that collecting blood from the elephants at BGT in the PC system is less stressful that the FC system. As this is an example of how the routine husbandry and medical husbandry is now conducted, it can be expected that the overall net effect is going to be lowered stress in the elephants at BGT.
……

Ball, R. L. and O. Fad (2006). **Serum cortisols in captive Asian elephants (Elephas maximus) in different management systems at Busch Gardens Tampa Bay.** Proceedings International Elephant Conservation & Research Symposium.

Bechert, U. S. and S. Southern (2002). **Monitoring Environmental Stress in African Elephants (*Loxodonta africana*) through Molecular Analysis of Stress-Activated Proteins**. Proceedings American Association of Zoo Veterinarians.

Many disease outbreaks appear to be facilitated by increased stress due to overcrowding, and changing environmental conditions triggered by climate variability and human activities. Currently, the health of populations is typically assessed with the tools of population dynamics: estimations of trends in abundance, mortality, and reproductive rates. However, for populations that have long generation times, this approach is sometimes too slow to provide an early

warning about the impact of environmental stressors such as disease, pollution, and anthropogenic activities. We have developed new techniques for detecting chronic physiologic stress and disease in mammals, based on the molecular analysis of the expression patterns of multiple stress-activated proteins and genes. This approach represents a novel tool for health monitoring, and can provide an early warning of increased environmental stress and compromised health in elephants and other mammals. This paper describes a study in progress, in which the molecular analysis of stress is being used to explore correlations between stress level and information regarding population abundance, distribution, habitat needs, human-elephant interactions, and movements of elephants (*Loxodonta africana*) in the northern Botswana region. This technique will provide a more objective way to assess carrying capacity for African elephants, thus facilitating development of effective management plans for this species.

Boomershine, C. S. and B. S. Zwilling (2000). "**Stress and the pathogenesis of tuberculosis**." Clinical Microbiology Newsletter **22**(23): 177-182.

Brown, I. R. F. and P. T. White (1979). "**Serum electrolytes, lipids, and cortisol in the African elephant, *Loxodonta africana***." Comp. Biochem. Physiol. [A] **62**(4): 899-901.

1. Serum electrolytes were measured in the African elephant from the game parks of Uganda. The overall mean for sodium was 136.5 ± 5.6 mmol/l (n = 132) and for potassium 6.24 ± 0.86 mmol/l (n = 105). There was, however, some evidence for a seasonal variation in these values. 2. Serum lipid levels were considerably lower in the elephant than in man. The overall mean cholesterol was 1.58 ± 0.50 mmol/l (n = 84) and triglyceride 0.59 ± 0.29 mmol/l (n = 61). Some geographical variation was observed in the triglyceride results. 3. Serum cortisol levels were variable and ranged from 66 to 825 mmol/l in 33 elephants.

Brown, J. L., D. C. Kersey, E. W. Freeman and T. Wagener (2009). "**Assessment of diurnal urinary cortisol excretion in Asian and African elephants using different endocrine methods**." Zoo. Biol.

Longitudinal urine samples were collected from Asian and African elephants to assess sample processing and immunoassay techniques for monitoring adrenal activity. Temporal profiles of urinary cortisol measured by RIA and EIA, with and without dichloromethane extraction, were similar; all correlation coefficients were >0.90. However, based on regression analyses, cortisol immunoactivity in extracted samples was only 72-81% of that of unextracted values. Within assay technique, RIA values were only 74-81% of EIA values. Collection of 24-hr urine samples demonstrated a clear diurnal pattern of glucocorticoid excretion, with the lowest concentrations observed just before midnight and peak concentrations occurring around 0600-0800 hr. These results indicate that elephants fit the pattern of a diurnal species, and that glucocorticoid production is affected by a sleep-wake cycle similar to that described for other terrestrial mammals. Cortisol can be measured in both extracted and unextracted urine using RIA and EIA methodologies. However, unexplained differences in quantitative results suggest there may be sample matrix effects and that data generated using different techniques may not be directly comparable or interchangeable. Zoo Biol 28:1-10, 2009. (c) 2009 Wiley-Liss, Inc

Buss, I. O., J. A. Estes, L. E. Rasmussen and G. L. Smuts (1976). "**The role of stress and individual recognition in the function of the African elephant's temporal gland**." Mammalia **40**(3): 437-451.

Biochemical measurements were made from a sample of temporal gland secretion from each of five wild African elephant bulls (23 to 38 years of age) collected in Kruger National Park, South

Africa between November 1974 and April 1975. Total protein content was high (26-57 mg/ml), acid phosphatase ranged between 1.9 and 6.3 mM/h/mgm protein, and lactic dehydrogenase levels were undetectable. Total lipid content in the secretion averaged 80 mg% and ranged from 75 to 87 mg%. Triglycerides were just detectable, varying from 2 to 8 mg%, and phospholipids ranged from 9 to 11 mg% (ave. 10 mg%). Cholesterol content was surprisingly high, measuring 12, 19, 26, 36, and 70 mg% for five samples of secretion. Field observations indicated that stress triggers liberation of temporal gland secretion. Among 116 elephants collected in Uganda, secretory activity of their temporal glands was more frequent during dry (probably more stressful) than during wet seasons. Among 62 elephants driven by helicopter to roadways for collection in Kruger National Park, 23 driven relatively far and fast were in prominent musth; most of those driven slower and shorter distances showed no evidence of musth. The matriarchal leader of an elephant family near Lake Albert, Uganda developed very prominent temporal gland activity after an hour and 45 minutes of vigorously defending three of her family members. Chemical individuality of cholesterol levels in temporal glands of five adult bulls suggests a pheromone-producing function which serves for individual recognition by the African elephant. Direct observations of wild elephants also suggest that the temporal gland functions as a scent gland helping to recognize other members of the group or to find them.

Carter, S. (1989). **Occupational stress and elephant management**. Proc.Ann.Elephant Workshop 10.

Dathe, H. H., B. Kuckelkorn and D. Minnemann (1992). "**Salivary cortisol assessment for stress detection in the Asian elephant (Elephas maximus): A pilot study**." Zoo Biol **11**: 285-289.

Effects of introducing an unfamiliar female into an Asian elephant herd at Tierpark Berlin were monitored by means of salivary cortisol assessment. Saliva samples were obtained from a second female for comparative purposes. The period of familiarization was characterized by an enhanced cortisol level in both animals, with a maximum on the second day after joining. Cortisol returned to normal on the following day. Manipulations of the keepers caused a transitory increase on two other days. Possibilities for the use of this noninvasive method of stress monitoring in various management situations are indicated.

Dembiec, D. P., R. J. Snider and A. J. Zanella (2004). "**The effects of transport stress on tiger physiology and behavior.**" Zoo Biology **23**: 335-346.

Tigers are often transported for education, conservation, and zoo enhancement purposes, however the effect of transfer on them has not yet been documented. Our objective was to evaluate how transport affects the behavior and physiology of tigers, taking into account previous experience with the transport procedure. We simulated transport by relocating naive tigers in a small individual transfer cage. Two tigers had prior experience with the procedure, and three tigers were naive to it. After 30 min, each tiger was released back into their original enclosure. Physiological measurements were recorded for four of the naive tigers; these included respiration rate and immune-reactive fecal cortisol response using radioimmunoassay. We also recorded the behavior of all naive tigers before, during, and after transport. Our behavioral analysis included activity level, pacing behavior, time spent investigating, respiration rate, and ear position. Average respiration rates of all tigers increased from 56.1 breaths/min to 94.6 breaths/min during transport and to 132.3 breaths/min 10 min following release into their enclosures. Average immune-reactive cortisol concentrations peaked 3-6 days after transport at 239% above baseline and returned to baseline levels 9-12 days afterward. During their peak

time block, naýve tigers exhibited a higher average increase in cortisol levels (482% above baseline) than the experienced tigers (158% above baseline). The naýve tigers' average immune-reactive cortisol concentration remained elevated for a longer period (9-12 days) than the experienced tigers' (3-6 days). In both groups, behavioral responses ranged from active to inactive, however naýve tigers performed these repertoires with greater intensity by pacing faster and performing fewer state changes. Results suggest that prior exposure to elements of the transport procedure may lead to some level of habituation, thus reducing the effects of transportation stress.

Fanson, K. V., T. Keeley and B. G. Fanson (2014). "**Cyclic changes in cortisol across the estrous cycle in parous and nulliparous Asian elephants**." Endocr Connect **3**(2): 57-66.

In the context of reproduction, glucocorticoids (GCs) are generally considered to have negative effects. However, in well-studied model species, GCs fluctuate predictability across the estrous cycles, and short-term increases promote healthy ovarian function. Reproductive challenges have plagued captive elephant populations, which are not currently self-sustaining. Efforts to understand reproductive dysfunction in elephants have focused on the suppressive effects of cortisol, but the potential permissive or stimulatory effects of cortisol are unknown. In this study, we provide a detailed examination of cortisol patterns across the estrous cycle in Asian elephants (Elephas maximus). Time series analysis was used to analyze cortisol and progesterone data for a total of 73 cycles from eight females. We also compared cortisol profiles between females that successfully conceived and females that failed to conceive despite repeated mating attempts. Our results revealed that cortisol fluctuates predictably across the estrous cycle, with a peak during the second half of the follicular phase followed by low levels throughout the luteal phase. Furthermore, this pattern was significantly altered in nulliparous females; cortisol concentrations did not decline during the luteal phase to the same extent as in parous females. This study highlights the complexity of cortisol signaling and suggests future directions for understanding the role of cortisol in reproductive dysfunction.

Foley, C. A. H., S. Papageorge and S. K. Wasser (2001). "**Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants**." Conserv Biol **15**(4): 1134-1142.

Hattingh, J. and D. Petty (1992). "**Comparative physiological responses to stressors in animals**." Comparative Biochemistry and Physiology A-Comparative-Physiology **101**(1): 113-116.

The species-specific experimental response to stressors (SSERTS) analysis was applied to a number of species under varied short and long term conditions. The measure provides quantitative data relating to the physiological responses of animals when exposed to stressors and results are presented comparing these for different methods of immobilization, euthanasia, etc. at intra- and inter-species level. It is suggested that the SSERTS measure is of greater value for measuring the responses of animals to stressors than is the measurement of the concentration of single blood variables.

Hattingh, J. (1986). "**Physiological measurement of stress**." South African Journal of Science **82**(11): 612-614.

Jarjour, W. N., B. D. Jeffries, J. S. Davis, W. J. Welch, T. Mimura and J. B. Winfield (1991). "**Autoantibodies to human stress proteins**." Arthritis Rheum **34**(9): 1133-1138.

Unselected sera from patients with various rheumatic, inflammatory bowel, and autoimmune

skin diseases (n=268) were examined against human cell lysate by immunoblotting procedures, to determine the prevalence of autoantibodies to stress proteins (heat-shock proteins) hsp60 (homolog of *Escherichia coli* groEL and Mycobacterial 65K antigens), hsp73, and hsp90. Using standard, sensitive and specific assay conditions, IgG and IgM autoantibodies to these stress proteins were not demonstrable, or were detected infrequently, in sera from control subjects (n=36) and from patients with rheumatoid arthritis, Sjogren's syndrome, ankylosing spondylitis, Reiter's syndrome, systemic lupis erythematosus, and systemic sclerosis. Autoantibodies to hsp60 were relatively more common (>= 20% of sera) in patients with mixed connective tissue disease, polymyositis/dermatomyositis, psoriatic arthritis, inflammatory bowel disease, epidermolysis bullosa acquista, and bullous pemphigoid. Anti-hsp73 autoantibodies were detected in 20% or more of the sera from patients were Lyme disease and ulcerative colitis. Taken together, these data extend the spectrum of autoimmune and inflammatory diseases in which humoral anti-stress protein reactivity develops. However, the paucity of humoral autoreactivity to stress proteins in patients with systemic lupis erythematosus and rheumatoid arthritis argues against a direct role of anti-stress protein autoantibodies in the pathogenesis of these disorders.

Keay, J. M., J. Singh, M. C. Gaunt and T. Kaur (2006). "**Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review**." Journal of Zoo and Wildlife Medicine **37**(3): 234-244.

Kiley-Worthington, M. (1990). "**Are elephants in zoos and circuses distressed?**" Applied Animal Behaviour Science **26**(3): 299.

Kock, M. D. (1992). "**Use of hyaluronidase and increased etorphine (M99) doses to improve induction times and reduce capture-related stress in the chemical immobilization of the free-ranging black rhinoceros (*Diceros bicornis*) in Zimbabwe**." Journal of Zoo and Wildlife Medicine **23**(2): 181-188.

Kumar, V., V. Palugulla Reddy, A. Kokkiligadda, S. Shivaji and G. Umapathy (2014). "**Non-invasive assessment of reproductive status and stress in captive Asian elephants in three south Indian zoos**." Gen Comp Endocrinol **201**: 37-44.

Asian elephants in captivity need immediate attention to be bred so as to meet the increasing demand for captive elephants and to overcome the dependence on supplementing the captive stock with wild animals. Unfortunately, captive breeding programs across the globe have met with limited success and therefore more effort is needed to improve breeding in captivity. Endocrine profiling of reproductive hormones (progestagens and androgens) and the stress hormone (glucocorticoids) could facilitate better management and breeding strategies. In the present study, we investigated reproductive and stress physiology of 12 captive Asian elephants for 10-27 months using a non-invasive method based on steroid analysis of 1700 elephant dung samples. Most of the elephants were cycling regularly. Males during musth showed increased fecal androgen metabolite concentrations and exhibited a slight increase in fecal glucocorticoid metabolite levels. Elephants used in public festivals and processions showed significantly increased in faecal glucocorticoid metabolite levels. The results indicate that captive elephants require periodic health care, better husbandry practices and scientific management for sustainable captive population.

Menargues, A., V. Urios and M. Mauri (2008). "**Welfare assessment of captive Asian elephants (Elephas**

maximus) and Indian rhinoceros (Rhinoceros unicornis) using salivary cortisol measurement." Animal Welfare **17**(3): 305-312.

The measurement of salivary cortisol allows non-invasive assessment of welfare in captive animals. We utilised this technique to test the effect of zoo opening on six Asian elephants and two Indian rhinoceros at the Terra Natura Zoological Park, Alicante, Spain, during pre-opening, opening and post-opening periods. Salivary cortisol concentrations were found to be significantly higher during the opening period than during pre- and post-opening periods for both species. This method could prove a useful tool in monitoring the success of decisions taken to improve the welfare of captive animals.

Menargues, A., V. Urios, R. Liminana and M. Mauri (2012). "**Circadian rhythm of salivary cortisol in Asian elephants (Elephas maximus): a factor to consider during welfare assessment**." J Appl Anim Welf Sci **15**(4): 383-390.

Elevated glucocorticoid levels during an extended time period might be a stress indicator in nonhuman animals. Therefore, knowledge of the circadian pattern of cortisol secretion is very important to correctly interpret data obtained for welfare assessment of animals in captivity through salivary cortisol. In order to define the circadian rhythm of salivary cortisol secretion in the Asian elephant (Elephas maximus), morning and evening saliva samples of 3 Asian elephants were collected and analyzed by radioimmunoassay. Significantly higher salivary cortisol concentrations were found in the morning than in the evening in all individuals. These results show that salivary cortisol of Asian elephants follows a diurnal pattern of secretion, which could be taken into account when using this methodology to assess welfare in captive Asian elephants.

Menon, V., R. Sukumar and A. Kumar (1997). **A god in distress : threats of poaching and the ivory trade to the Asian elephant in India**. Bangalore, India**:** 1-91.

Mikota, S. K. (2009). Stress, Disease, and Tuberculosis in Elephants. **An Elephant in the Room**. D. L. Forthman, L. F. Kane, D. Hancocks and P. F. Waldau. North Grafton, Center for Animals and Public Policy, Cummings School of Veterinary Medicine, Tufts University**:** 74-84.

Mumby, H. S., K. U. Mar, A. D. Hayward, W. Htut, Y. Htut-Aung and V. Lummaa (2015). "**Elephants born in the high stress season have faster reproductive ageing**." Sci Rep **5**: 13946.

Senescent declines in reproduction and survival are found across the tree of life, but little is known of the factors causing individual variation in reproductive ageing rates. One contributor may be variation in early developmental conditions, but only a few studies quantify the effects of early environment on reproductive ageing and none concern comparably long-lived species to humans. We determine the effects of 'stressful' birth conditions on lifetime reproduction in a large semi-captive population of Asian elephants (Elephas maximus). We categorise birth month into stressful vs. not-stressful periods based on longitudinal measures of glucocorticoid metabolites in reproductive-aged females, which peak during heavy workload and the start of the monsoon in June-August. Females born in these months exhibit faster reproductive senescence in adulthood and have significantly reduced lifetime reproductive success than their counterparts born at other times of year. Improving developmental conditions could therefore delay reproductive ageing in species as long-lived as humans.

Mumby, H. S., K. U. Mar, C. Thitaram, A. Courtiol, P. Towiboon, Z. Min-Oo, Y. Htut-Aung, J. L. Brown and V. Lummaa (2015). "**Stress and body condition are associated with climate and demography in Asian**

elephants." Conserv Physiol **3**(1): cov030.

Establishing links between ecological variation, physiological markers of stress and demography is crucial for understanding how and why changes in environmental conditions affect population dynamics, and may also play a key role for conservation efforts of endangered species. However, detailed longitudinal studies of long-lived species are rarely available. We test how two markers of stress and body condition vary through the year and are associated with climatic conditions and large-scale mortality and fertility variation in the world's largest semi-captive population of Asian elephants employed in the timber industry in Myanmar. Glucocorticoid metabolites (used as a proxy for stress levels in 75 elephants) and body weight (used as a proxy for condition in 116 elephants) were monitored monthly across a typical monsoon cycle and compared with birth and death patterns of the entire elephant population over half a century (n = 2350). Our results show seasonal variation in both markers of stress and condition. In addition, this variation is correlated with population-level demographic variables. Weight is inversely correlated with population mortality rates 1 month later, and glucocorticoid metabolites are negatively associated with birth rates. Weight shows a highly positive correlation with rainfall 1 month earlier. Determining the factors associated with demography may be key to species conservation by providing information about the correlates of mortality and fertility patterns. The unsustainability of the studied captive population has meant that wild elephants have been captured and tamed for work. By elucidating the correlates of demography in captive elephants, our results offer management solutions that could reduce the pressure on the wild elephant population in Myanmar.

Oliveira, C. A., E. C. Felippe and M. O. Chelini (2008). "**Serum cortisol and progestin concentrations in pregnant and non-pregnant Asian elephants (Elephas maximus)**." Res Vet Sci **84**(3): 361-363.

Blood samples were collected during the estrous cycle (n=3), throughout gestation (n=3), and during the periparturient period (n=11) to assess serum concentrations of cortisol in pregnant and non-pregnant Asian elephants whose reproductive status was being monitored by serum progestin determination. While serum cortisol concentrations remained constant throughout gestation, progestin concentrations decreased significantly ($p<0.05$) in the second half of pregnancy, declining to undetectable levels by 3 days before calving. During the non-luteal phase of the estrous cycle serum progestins varied from undetectable levels to 100pg/ml (53+/-10.7pg/ml) then increased steadily during the luteal phase (322+/-207.5pg/ml). There were no significant differences between serum cortisol concentrations during the luteal and those of the non-luteal phase ($p>0.05$). The mean cortisol concentration during the estrous cycle was about twice that during pregnancy ($p>0.05$). No substantial changes in maternal cortisol were found during the course of pregnancy or the periparturient period.

Plotnik, J. M. and F. B. de Waal (2014). "**Asian elephants (Elephas maximus) reassure others in distress**." PeerJ **2**: e278.

Contact directed by uninvolved bystanders toward others in distress, often termed consolation, is uncommon in the animal kingdom, thus far only demonstrated in the great apes, canines, and corvids. Whereas the typical agonistic context of such contact is relatively rare within natural elephant families, other causes of distress may trigger similar, other-regarding responses. In a study carried out at an elephant camp in Thailand, we found that elephants affiliated significantly more with other individuals through directed, physical contact and vocal communication following a distress event than in control periods. In addition, bystanders affiliated with each other, and matched the behavior and emotional state of the first distressed

individual, suggesting emotional contagion. The initial distress responses were overwhelmingly directed toward ambiguous stimuli, thus making it difficult to determine if bystanders reacted to the distressed individual or showed a delayed response to the same stimulus. Nonetheless, the directionality of the contacts and their nature strongly suggest attention toward the emotional states of conspecifics. The elephants' behavior is therefore best classified with similar consolation responses by apes, possibly based on convergent evolution of empathic capacities.

Rubin, L. A. and G. A. Hawker (1993). "**Stress and the immune system: preliminary observations in rheumatoid arthritis using an in vivo marker of immune activity**." Arthritis Rheum **3**(2): 204-207.

Saikhun, J., N. Thongtip, K. Kornkaewrat, S. Mahasawangkul, T. Angkawanish, K. Boonprasert and A. Pinyopummin (2006). **Osmotic stress on motility and membrane integrity of Asian elephant spermatozoa analyzed by computer-assisted semen analysis.** Proceedings International Elephant Conservation & Research Symposium.

Schmid, J., M. Heistermann, U. Ganslosser and J. K. Hodges (2001). "**Introduction of foreign female Asian elephants (Elephas maximus) into an existing group: behavioural reactions and changes in cortisol levels**." Animal-Welfare **10**(4): 357-372.

The present study examined the extent to which the introduction of three female Asian elephants (aged 3, 11 and 27 years) into a group of 5 (1 male, 4 female) elephants at Munster zoo, Germany, affects the behaviour and urinary cortisol levels of the animals involved. At Munster, only the females were monitored , while the bull was mainly kept separate. Behavioural observations were carried out before transfer and during the six-month period following transfer. Urine samples were collected regularly from each elephant during the whole observation period. All the elephants showed behavioural changes to the process of introduction. The transferred animals increased their social behaviour after arrival in the foreign zoo. Two of them showed an increase in stereotypies and one a reduction in stereotypes. The elephants at Munster reacted with decreased frequencies of stereotypies and increased frequencies of social behaviour and manipulation/exploration behaviour. Six months after transfer, three of the four elephants at Munster and one of the three transferred elephants showed nearly the same behavioural activity pattern as before transfer. One female still showed elevated stereotypic behaviour. From the four elephants in which cortisol measurements could be reliably performed (two of the transferred elephants and two elephants at Munster), only one individual at Munster responded to the process of introduction with a short-term elevation in urinary cortisol levels. One elephant showed a negative correlation between locomotion and cortisol levels and one a positive correlation between stereotypies and cortisol levels. Taken together, the results suggest that transfer and introduction caused some stress responses in the elephants, but that stress was neither prolonged nor severe. Serious welfare problems may have been prevented through individual behavioural coping mechanisms and former experience with stressful situations.

Selye, H. (1956). Recent progress in stress research, with reference to tuberculosis. **Personality, stress, and tuberculosis**. P. J. Sparer. New York, Int. Univ. Press**:** 45-64.

Sikes, S. K. (1968). "**Habitat stress and arterial disease in elephants**." Oryx **9**(4): 286-292.

Elephant management in East African reserves and national parks has become one of the urgent conservation problems of today. In this study of the African savanna elephant, Dr. Sikes shows

that two diseases of the heart and arteries, found only in lowland elephants, were directly associated with the degeneration of the habitat when elephant numbers began to build up in the Tsavo National Park in Kenya and the Queen Elizabeth and Murchison Falls National Parks in Uganda. The two diseases thus appear to be natural factors tending to limit the elephant populations in these reserves, and she suggests four lessons to be drawn from this discovery by those concerned with elephant management in national parks.

Smith, T. (2004). **Zoo research guidelines: Monitoring stress in zoo animals.** London.

Southern, S. (2001). **Molecular analysis of stress-activated proteins and genes in dolphins and whales: a new technique for monitoring environmental stress**. Proc AAZV and AAAM Joint Conference.

In the past several decades, there has been a worldwide increase in marine diseases resulting in mass mortality among all major taxa and shifts in ecologic community structures in the oceans.1 Marine mammals have experienced a pandemic of morbilliviral infections and outbreaks of diseases caused by influenza viruses, fungi and algal toxins. Many of the disease outbreaks appear to have been facilitated by increased environmental stress burden in the global marine ecosystems due to changing environmental conditions triggered by climate variability and human activities. It is imperative to develop novel health-monitoring tools that could guide the management of marine ecosystems and facilitate the conservation of key species. Our research is focused on the molecular mechanisms underlying molecular stress response in humans and cetaceans exposed to

environmental stress and disease. We have developed new techniques for detecting the molecular signature of stress based on molecular analysis of stress-activated proteins and genes in field tissue specimens.2 The detection of molecular stress signature has been applied to evaluate the impact of tuna fishery on the spotted dolphins in the Eastern Tropical Pacific, the effects of coastal pollution on the beluga whales in the St. Lawrence River, and the idiopathic population decline of the North Atlantic right whale population.

Teixeira, C. P., C. Schetini de Azevedo, M. Mendl, C. F. Cipreste and R. J. Young (2007). "**Revisiting translocation and reintroduction programmes: the importance of considering stress**." Animal Behaviour **73**(1): 1-13.

It is widely known that the adverse effects of stress must be considered in animal conservation programmes. However, a full consideration of how and where stress occurs in animal conservation programmes has not been undertaken, especially in translocation and reintroduction programmes. The literature concerning these types of programmes shows high levels of mortality, despite researchers' consideration of the effects of stress. However, an analysis of the literature shows that many conservation biologists have only a superficial knowledge about stress. For example, most do not understand the importance of subclinical stress or the fact that the effect of successive stressors can be additive or accumulative. While most conservation biologists know that stress is bad for animal health, few have considered its adverse effects on cognitive abilities, which an animal needs to survive in the wild (e.g. memory). In this paper we conclude with suggestions for improving the efficiency of animal conservation programmes in terms of the number of animals surviving after reintroduction or translocation. The most important conclusion from this review of the literature is that there needs to be a greater interchange of information between animal welfare and animal conservation scientists.

Teubner, V. and S. Wells (1988). **Serum progesterone and cortisol levels in female Asian elephants (*Elephas maximus*)**.

Poster presented at Symposium on Vertebrate Models in Endocrinology. National Institutes of Health, Bethesda, MD. 1988. Abstract. In the past zoological gardens and parks have not been at the forefront of endocrine research. However, zoos and wild animal parks represent a relatively untapped source of vertebrates for use as endocrine models. The collaboration of academic institutions with zoo personnel on various projects will not only enhance the management of exotic species but will also add to the data base in endocrine research. This is of critical importance to the propagation of endanged species. The developement of radioimmunoassay techniques had made it possible to detect minute amounts of hormones in the blood or excreta of various species. This serves as a valuable tool in assessing the reproductive status of a given animal. In species that do not exhibit the classic behavioral signs of estrous, measurement of hormonal activity can be the only link to evaluating fertility. The female Asian elephant (Elephas maximus) has a covert estrous cycle that only an adult bull elephant can detect. Due to the problems associated with the maintenance of bull elephants in captivity the measurement of the hormonal activity of the female is a feasible alternative. The goal of our study is to measure sex hormones (i.e. progesterone,and estradiol) and cortisol levels in two adult, female Asian elephants housed at the Audubon Zoo and to determine their estrous cycles. Blood is sampled weekly from the ear vein of the each elephant. Whole blood is then centrifuged and serum is stored at -70C for hormone measurement via radioimmunoassay. Attempts to measure estradiol were unsuccessful as Asian elephants secrete a combination of estrone and estradiol. Therefore, we chose to limit our analysis to progesterone, as the indicator of ovulation and to the glucocorticoid cortisol. We determined that the elephants' estrous cycle has a duration of approximately 12 weeks with a 3.2 week follicular phase followed by a luteal phase of approximately 9 weeks. Elevation of serum progesterone of 50 pg/ml above baseline and remaining elevated for 2 weeks was used as an indicator of luteal activity. Cortisol levels (ug/dl) were also measured and expressed as a percentage. Serum cortisol was elevated either prior to a cycle or during a cycle. This suggests that cortisol may also be a useful indicator of fertility. The hormonal data from the Asian elephant indicate that this species has a predictable estrous cycle that can be readily and accurately assessed using radioimmunoassay. THe methodology used for this species may serve as a valuable research model to aid in captive breeding programs and to further our understanding of the endocrine systems of exotic species.

Vezina-Audette, R., C. Herry, P. Burns, M. Frasch, E. Chave and C. Theoret (2016). "**Heart rate variability in relation to stress in the Asian elephant (Elephas maximus)**." Can Vet J **57**(3): 289-292.

This study describes a safe, reliable, and accessible means to measure heart rate (HR) and HR variability (HRV) and evaluates the use of HRV as a physiological correlate of stress in the Asian elephant. A probabilistic model indicates that HRV measurements may adequately distinguish between stressed and non-stressed elephants.

Publisher: Abstract available from the publisher.
fre

Viijoen, J. J., A. Ganswindt, J. T. du Toit and W. R. Langbauer (2008). "**Translocation stress and faecal glucocorticoid metabolite levels in free-ranging African savanna elephants**." South African Journal of Wildlife Research **38**(2): 146-152.

There are local populations of African elephants (Loxodonta africana) which have increased to levels where they are implicated in altering vegetation types. The local reduction of elephant

numbers for wildlife management objectives can involve contraception, killing excess animals, or translocation to alternative habitats. The effects these management decisions can have on the physiological stress response of free-ranging African savanna elephants are still not fully understood. We examined the effect of translocation on faecal glucocorticoid metabolite levels of an African elephant family group, which was translocated within the Kruger National Park, South Africa. We found that translocation resulted in a significant increase in faecal glucocorticoid metabolite levels (up to 646 ng/g wet weight) compared to (1) pre-translocation levels in this group, (2) post-translocation levels in this group, and (3) levels measured in undisturbed 'control' groups in the area. However, the faecal glucocorticoid metabolite levels had returned to <100 ng/g by the time the translocated animals had navigated their way back to their previous home range, covering 300 km in 23 days.

Viljoen, J. J., A. Ganswindt, C. Reynecke, A. S. Stoeger and W. R. Langbauer (2015). "**Vocal stress associated with a translocation of a family herd of African elephants (Loxodonta africana) in the Kruger National Park, South Africa**." Bioacoustics-the International Journal of Animal Sound and Its Recording **24**(1): 1-12.

We used vocal indicators to examine the effect of a translocation of an African elephant family herd within the Kruger National Park. These animals were moved 300km from their home range, but returned unaided to this range within 23 days. We found that translocation resulted in a change in the mean fundamental frequency of low-frequency elephant vocalizations, known as rumbles. The rumbles increased significantly in pitch compared with pre-translocation levels during the 23 days the animals spent outside their normal home range. Mean fundamental frequency returned close to pre-translocation level by the time the animals had navigated their way back to their previous home range. Raised pitch is known to be an indicator of stress in humans and other animals. The observed acoustic results are consistent with a physiological measure of stress, faecal glucocorticoid metabolite levels, which were monitored from the same animals during the study and have already been reported elsewhere. To our knowledge, this is the first report of prolonged monitoring of vocal stress response in free-ranging animals. Measuring behavioural responses, such as vocalizations, may provide an objective non-invasive method for assessing stress. This could help in determining the effects that particular management actions might have on elephants.

Wilson, M. L., M. A. Bloomsmith, M. Crane and T. L. Maple (2001). **Behavior and serum cortisol concentrations of three captive African elephants (*Loxodonta africana*): preliminary results**. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Wilson, M. L., M. A. Bloomsmith and T. L. Maple (2004). "**Stereotypic swaying and serum cortisol concentrations in three captive African elephants (Loxodonta africana)**." Animal-Welfare **13**(1): 39-43.

The behaviour and serum cortisol concentrations of three captive female African elephants (Loxodonta africana) were studied to determine whether their stereotypic swaying was more prevalent before regularly scheduled events in the elephants' routine, and whether the elephants that exhibited more stereotyped swaying had lower mean serum cortisol concentrations. Behavioural data were collected during hour-long observations balanced across three periods, and during 15-min observations prior to the elephants being moved to different portions of their enclosure. Observational data were collected using instantaneous focal sampling of behaviours every 30 s. Serum cortisol measures were obtained through weekly

blood withdrawal from the elephants' ears. Of the three elephants, two exhibited stereotyped swaying, which accounted for a mean of 0.4% of the scans during the hour-long observations and a mean of 18% of the scans prior to the elephants being moved between different parts of the enclosure. Swaying was highly variable among the individual elephants during both categories of observations. Additionally, both elephants swayed more prior to moving in the afternoon than prior to moving in the morning. Analyses of serum cortisol concentrations indicated that each elephant had a different mean cortisol level, which did not clearly correspond with the expression of swaying. The findings indicate that a rigidly scheduled management event may elicit stereotyped swaying in the studied elephants. Future research should document the behavioural and physiological effects of an altered management routine to improve captive elephant welfare.

Wingfield, J. C. and R. M. Sapolsky (2003). "**Reproduction and resistance to stress: When and how.**" Journal of Neuroendocrinology **15**: 711-724.