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THE IMPACT OF HUMAN RELATIONSHIPS ON SEMI- CAPTIVE ASIAN ELEPHANT HEALTH AND WELFARE

Jennifer Crawley



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To my Nanabanana and Bapu

"One point in connection with elephants must never be lost sight of, namely, the importance of securing a maximum degree of permanency of the particular attendant of a particular animal. A keeper who is possessed even in a moderate degree of the qualifications already enumerated, and who has been with his elephant a sufficiently long time to have studied the peculiarities of his charge, has become invaluable to his employer. The knowledge in question can only be gained by experience and lengthened individual observation; it cannot be placed on record or otherwise handed down; hence any animal placed in the hands of a succession of keepers is subjected to treatment extremely calculated to prove prejudicial to its health, temper, and utility."

Evans 1910

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ABSTRACT

There are billions of animals living in close proximity to humans around the world from pets and livestock to laboratory, draught and zoo animals. The interactions these animals have with humans in captivity influence their physiology, behaviour, reproduction, growth, morbidity, and mortality. The Asian elephant (*Elephas maximus*) is an endangered species whose close history with humans has led to >25% of its total population living in captivity today, mostly in Asia and cared for by traditional handlers (mahouts). This thesis focuses on the relationship between mahouts and their elephants in the largest semi-captive population of elephants, the logging elephants of Myanmar. Most past studies have focused on mahout-elephant relationships from a human perspective whereas this thesis investigates their influence on elephants through measures of their physiology, immunology and behaviour. In **Chapter I**, I investigate current and past mahout handling systems of semi-captive elephants in Myanmar. I then assess the impact of this handling system on the elephants in **Chapter II**, exploring how mahout-elephant relationships and mahout experience influence elephant physiology and behaviour. **Chapters III-IV** focus on early mahout-elephant relationships, studying how calf traits historically were associated with mortality during taming ages (**Chapter III**) and monitoring calves for the first time during their traditional taming procedure (**Chapter IV**).

Whilst vast changes to the mahout profession have been reported in recent decades in many countries across Asia, Myanmar is often quoted as one of the last remaining reservoirs of traditional mahout knowledge and expertise. This thesis shows that there have also been recent changes to the mahout profession in Myanmar, with interviews of >20 experts and >200 current mahouts finding that mahouts today tend to be younger, less experienced and to change elephants more frequently than in the past. Less experienced mahouts may maintain good quality care however, with indicators of physiological stress from >150 elephants not dependent on mahout-elephant relationship lengths or past mahout experience. Yet both specific relationship lengths with mahouts and total mahout experience had important implications for other elephant physiological measures and elephant behaviour, and I discuss potential management adjustments to account for these effects.

Juvenile mortality is one of the main factors limiting population growth of these elephants. I show a >50% increase in mortality between age three and the taming age of four years, suggesting taming as an issue of both individual welfare and

population sustainability. Taming is highly criticized among welfare advocates and the media, yet it has never been empirically studied. I first investigate traits associated with historical taming-age mortality in >1900 calves, showing younger calves and those born to less experienced mothers to have higher mortality risk at taming ages. I also show recent improvements to taming practices, with the taming-age mortality of calves born after 2000 one third of those born in the 1970s. I next focus on the impact of taming today, collecting data from 41 calves undergoing traditional taming; I find evidence of acute stress, mostly over the first 10 days of the taming process, with one measure suggesting chronic stress lasting up to two months. I also emphasize that mahout safety should be at the forefront of decisions surrounding changes to taming methods.

I hope this will be the start of many empirical assessments of how both mahout interactions in general, and particularly the taming procedure, influence elephant welfare. This will bring much needed evidence to these areas of research to optimise the management of thousands of captive elephants across Asia and around the world. This thesis contributes to a growing area of research studying the impacts of human interaction on animal health and welfare applicable across a variety of contexts and species as more animals face anthropogenic impacts worldwide.

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TIIVISTELMÄ

Ihmisen kanssa elää lukemattomia eläinlajeja lemmikeistä ja karjasta koe-eläimiin, eläintarhaeläimiin sekä ihmisen kanssa työskenteleviin eläimiin. Vuorovaikutus ihmisen kanssa vaikuttaa eläinten fysiologiaan, käyttäytymiseen, lisääntymiseen, kasvuun, sairastuvuuteen ja kuolleisuuteen. Aasiannorsu (*Elephas maximus*) on uhanalainen laji, jonka historia ihmisen kanssa on johtanut siihen, että nykyisin yli neljäsosa norsukannasta elää osittaisessa vankeudessa Aasiassa perinteisten norsunhoitajien (engl. mahouts) huomassa. Tässä opinnäytetyössä tutkitaan Myanmarin metsätyönorsujen ja norsuja hoitavien ihmisten välisiä vuorovaikutussuhteita suurimmassa osin vankeudessa elävien norsujen populaatiossa. Useat aiemmat tutkimukset norsunhoitajien ja norsujen välisistä vuorovaikutussuhteista on tehty ihmisen näkökulmasta, mutta tässä opinnäytetyössä vuorovaikutuksen vaikutusta norsuihin tutkitaan eläimen kokeman fysiologisen stressin, immunologian ja käyttäytymisen kautta.

Luvussa I tutkin Myanmarissa aiemmin käytössä olleita ja nykyisin käytettäviä norsunkäsittelymenetelmiä. **Luvussa II** tarkastelen näiden menetelmien sekä norsujen ja niiden hoitajien välisten suhteiden vaikutusta eläinten fysiologiaan ja käyttäytymiseen. Seuraavissa luvuissa tutkin poikasajan varhaisten vuorovaikutussuhteiden ja yksilöiden ominaisuuksien vaikutusta poikasten kuolleisuuteen kesyttämisen aikana historiallisella aineistolla (**luku III**) sekä tutkin norsunpoikasten stressitasoja ja yleistä fysiologiaa perinteisten kesyttämistoimien aikana, ensimmäistä kertaa maailmanlaajuisesti (**luku IV**).

Norsunhoitajan ammatti on kokenut monia muutoksia viime vuosikymmeninä useissa Aasian maissa, mutta Myanmaria pidetään yhtenä viimeisistä jäljellä olevista perinteisen norsunhoitajien tuntemuksen ja osaamisen keskuksista. Tämä opinnäytetyö osoittaa, että myös Myanmarissa norsunhoitajan ammatti on viime aikoina muuttunut, sillä yli 20 asiantuntijan ja yli 200 nykyisen norsunhoitajan haastattelusta ilmenee, että norsunhoitajat ovat nykyään aiempaa nuorempia, heillä on vähemmän kokemusta ja he vaihtavat norsuja useammin kuin ennen. Vähemmän kokeneet norsunhoitajat voivat silti tarjota eläimille laadukasta hoitoa: yli 150 tutkitun norsun fysiologisen stressin mittarit eivät olleet yhteydessä hoitajasuhteen kestoon tai hoitajan aiempaan kokemukseen. Silti sekä hoitajasuhteen kestolla että hoitajan yleisellä kokemuksella oli merkittävä vaikutus muihin norsujen fysiologisiin mittareihin ja norsujen käyttäytymiseen. Saamaani tutkimustietoon

perustuen esitän suosituksia norsujen käsittelykäytäntöjen muokkaamiseksi, jotta nämä vaikutukset voidaan vastaisuudessa huomioida.

Nuorten norsujen kuolleisuus on yksi merkittävimmistä norsukantojen kasvua rajoittavista tekijöistä. Tutkimukseni osoittaa, että kuolleisuus on noussut yli 50% neljän vuoden kesytysissä normaaliin kuolleisuuteen verrattuna, mikä korostaa kesyttämistoimenpiteiden merkitystä paitsi yksilön hyvinvoinnin, myös norsupopulaation koon ylläpitämisen kannalta. Kesyttämistoimia arvostellaan ankarasti eläinsuojelun ja median toimesta, mutta niiden vaikutuksia ei ole aiemmin tutkittu empiirisesti. Selvitin aluksi historiallisen aineiston avulla yli 1900 norsunpoikasen piirteiden yhteyttä kesytysiän kuolleisuuteen. Aineisto osoittaa, että nuorilla poikasilla ja vähemmän kokeneille äideille syntyneillä poikasilla on muita suurempi riski kuolla nelivuotiaana kesytysissä. Viime aikoina kesytyskäytäntöihin on kuitenkin tehty parannuksia, joiden myötä vuoden 2000 jälkeen syntyneiden poikasten kuolleisuus kesytyksen yhteydessä on ollut enää kolmasosa verrattuna 1970-luvulla syntyneiden poikasten kokemaan kuolleisuuteen samassa iässä. Seuraavaksi tutkin kesyttämisen vaikutuksia nykypäivänä ottamalla tarkempiin tutkimuksiin 41 perinteisellä menetelmällä kesytettävää poikasta. Tutkimukseni osoitti merkkejä akuuttista stressireaktiosta lähinnä kesyttämisen ensimmäisten kymmenen päivän aikana, sekä yksi tutkittu fysiologinen mittari viittasi krooniseen stressiin, joka kesti jopa kaksi kuukautta. Eläinten kokeman stressin lisäksi tärkeää on myös norsunhoitajien turvallisuuden huomioiminen, kun kesyttämismenetelmiin suunnitellaan jatkossa muutoksia.

Toivon, että tämä työ edesauttaa jatkotutkimusten tekemistä norsujen ja niiden hoitajien välisistä vuorovaikutussuhteista yleisesti sekä erityisesti siitä, miten kesyttämismenettely vaikuttaa norsujen fysiologiaan ja hyvinvointiin. Tämä opinäytetyö tuottaa näistä aiemmin tutkimattomista aihealueista arvokasta tietoa, jonka avulla voidaan optimoida tuhansien osin vankeudessa pidettävien norsujen käsittelytapoja Aasiassa ja muualla maailmassa.

Tämä tutkimus luo pohjaa ihmisen kanssa vuorovaikutuksessa olevien eläinten terveyttä ja hyvinvointia koskeville tutkimuksille. Suosittelen tutkitun tiedon soveltamista eri yhteyksissä ja eri eläinlajien kohdalla, sillä ihmisen vaikutuksen piirissä eläviä eläinlajeja sekä eläimiä on yhä enenevässä määrin maailmanlaajuisesti.

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Abbreviations/Glossary

MTE	Myanma Timber Enterprise
NGO	Non-governmental Organisation
FGM	Faecal Glucocorticoid Metabolite
CK	Creatine Kinase
SC	Serum Cortisol
AIC	Akaike Information Criterion
K-foldIC	K-fold Information Criterion
H:L	Heterophil:Lymphocyte ratio
SQ	Supplementary Questionnaire
PCA	Principal Components Analysis
ML	Maximum Likelihood
KMO	Kaiser-Meyer-Olkin
HPA	Hypothalamus-Pituitary-Adrenal
Mahout	General term for traditional elephant handler (Burmese name: oozie)
Sin-Gaung	Burmese term for head mahout
Sin-Oke	Burmese term for regional head mahout
Phajaan	General term for elephant taming (literally “crush” in Thai)
Shaw Pike	Traditional song used in elephant training
Kunki	Well-trained adult elephant used to control calves during taming
Wan-U	Burmese term for traditional treatment of inflammation made from a plant

List of Original Publications/Manuscripts and Author Contributions

This dissertation is based on the following original publications/manuscripts, which are referred to in the text by their Roman numerals:

- I **Crawley, J A H**, Lahdenperä, M, Seltmann, M W, Htut, W, Aung, H H, Nyein U K, Lummaa, V. Investigating changes within the handling system of the largest semi-captive population of Asian elephants. *PLoS ONE*, 2019; **14**(1).
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- III **Crawley, J A H**, Lahdenperä, M, Min Oo, Z, Htut, W, Nandar, H, Lummaa, V. Taming age mortality in semi-captive Asian elephants. *Scientific Reports*, 2020; **10**:1889.
- IV **Crawley, J A H**, Nandar, H, Thi Zaw, H, Franco Dos Santos, D J, Seltmann, M W, Brown, J L, Goodsell, R M, Min Oo, Z, Htut, W, Nyein U K, Aung, H H, Lahdenperä, M, Lummaa, V. The first empirical assessment of physiological stress during taming of Asian elephant calves in Myanmar. *Submitted Manuscript*.

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LAB ANALYSES/SUPERVISION		JB, DJFDS,		JAHC, HN, HTZ, MWS, JLB,
STATISTICAL ANALYSES	JAHC, MWS	JAHC, OL	JAHC	JAHC, RMG
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WRITING: REVIEW AND EDITING	JAHC, VL, ML, MWS	JAHC, VL, ML, OL, JLB	JAHC, ML, VL, HN, ZMO	JAHC, JLB, VL, ML, DJFDS, HN

1 Introduction

1.1 Human-animal relationships

Animals have lived amongst humans for thousands of years. The majority of domestication arose in the Neolithic period ~12 000 years ago, but estimates of dog domestication stretch back as far as ~30 000 years ago (Germonpré *et al.*, 2009). Animal domestication occurred via a variety of pathways, ranging from anthropophilic commensal species adapting to human environments (e.g. dogs, cats), traditionally hunted prey species managed to maintain stocks (e.g. cattle, goats, sheep, pigs), to the directed use of particular species for specific purposes (Zeder, 2012). Animals that were domesticated for use in draught work often lack the docile, anthropophilic behaviour typical of the other domestication pathways. They also tend to be large, necessitating a greater degree of specialised handling expertise, e.g. horses, camelids and donkeys (Zeder, 2012). A group of these species requiring particularly specialised management are the “tame captives”, which are trained for a particular purpose but not fully domesticated as their reproduction is not managed (Driscoll *et al.*, 2009; Zeder, 2012). This thesis focuses on the relationship between humans and animals in one such species, the Asian elephant (*Elephas maximus*).

1.1.1 Human-elephant relationships

Humans have maintained a close working relationship with Asian elephants for millennia, yet have not truly domesticated them due to their body size, resource demands, longevity and slow life history which are incompatible with reproductive management (Sukumar, 2006; Driscoll *et al.*, 2009). Historically, training-age elephants would be captured when needed from a naturally reproducing wild population to save investment in juveniles not old enough to work (Trautmann, 2015). Emperor Akbar the Great maintained the first captive population in the 16th century as part of the Mughal empire in India (Hart and Sundar, 2000), however within this and subsequent captive populations, mating was not controlled by humans, and wild elephants frequently contributed to the gene-pool, removing the element of human selection associated with domestication (Lair, 1997). Despite this lack of full domestication, many countries across their range in South Asia have an

intimate relationship with elephants and 24-29% of the world's remaining endangered Asian elephants today (~16,000) live in captivity to some extent, mostly within their range countries in Asia (Sukumar, 2003; Leimgruber *et al.*, 2008; Jackson *et al.*, 2019; see **Figure 1** [Sukumar, 2006; Pebesma, 2018]). They are important both symbolically - in culture and religion - and logistically as draught animals, being an important part of the economy in some countries (Hart and Locke, 2007; Mar, 2007).

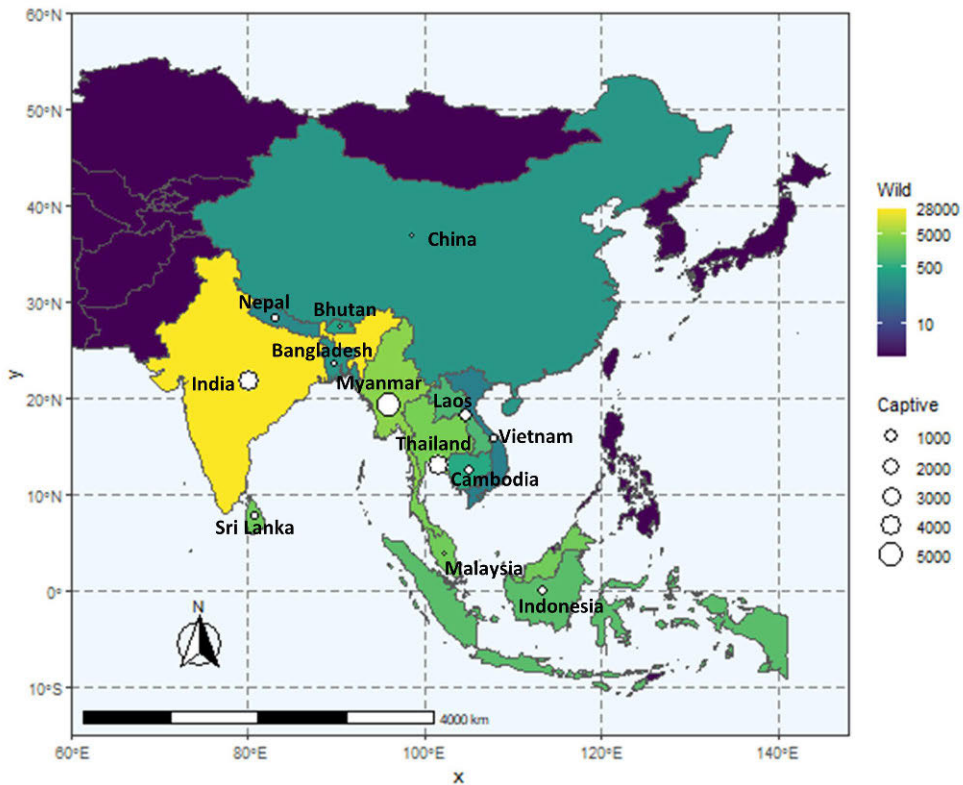


Figure 1. Population size estimates for Asian elephants in the wild and captivity (data from Sukumar, 2006) plotted using the *sf* package in R (Pebesma *et al.* 2018).

Rather than selecting for certain behavioural traits through domestication, elephant management has relied on expert knowledge of elephant handling practices accumulated over generations of elephant handlers. These elephant handlers are referred to herein with their widely used Hindi name, mahout. Many forest communities are specialised in this traditionally well-respected mahout profession (Mar, 2007; Locke, 2011). Studies over the last couple of decades however have found that the level of respect extended to mahouts from the prestige of caring for

elephants has decreased in India, Nepal, Laos and Thailand (Phuangkum *et al.*, 2005; Hart and Locke, 2007; Vanitha *et al.*, 2009; Suter *et al.*, 2013). Vanitha *et al.* (2009) found that the decline in respect, coupled with a lower salary, has led to a loss of traditional, expert mahouts from temples in India, which the authors linked to a higher incidence of elephant-related human mortality in temples compared to the forest department or privately owned elephants, which have retained more traditional handlers. Similar changes to the mahout profession had not been investigated in Myanmar prior to this thesis, home to the largest captive population of Asian elephants in the world and often quoted as one of the last remaining strongholds of traditional mahout knowledge and expertise (Lair, 1997; Sukumar, 2003). However, political shifts over the last decade have likely affected the lifestyles and attitudes of mahouts in Myanmar. The country opened up to the international community following the end of military rule in 2011 and technology became more widely available and affordable, substantially improving communication and access to remote areas. For example, there were significant rises in rural motorcycle use (ADB, 2016), and mobile phone access rose from less than 2% of the population in 2011 to almost 100% in 2016, after a 2000% drop in the price of a sim-card (Ling *et al.*, 2015; Handerson, 2016). These changes reflect an increase in urbanisation (Dobermann, 2016), which is likely to have affected the traditional mahout lifestyle, as well as influencing the availability and accessibility of alternative professions. This thesis aims to further our understanding of the mahout-elephant relationship specifically in Myanmar in the context of these changes, as well as more generally how mahout-elephant relationships influence elephant wellbeing, including during the most intense period of mahout-elephant interaction, the traditional taming procedure, a neglected area of research.

1.1.2 Human-animal relationships and welfare

The interactions that animals have with humans in captivity have been shown to impact indicators of welfare such as physiological stress and behaviour but also fundamental processes such as growth, reproduction, and health (Hemsworth, 2003). A review of the human-animal interactions literature found that almost 75% of studies have focussed on either companion or agricultural animals (Hosey and Melfi, 2014a), and only recently have studies expanded to include a wider range of human-animal relationships, such as those in zoos. Generally, studies of livestock often focussed on negative interactions associated with fear of humans (Hemsworth, 2003), whilst studies of interactions between humans and zoo/ companion animals often focussed on affiliative human-animal interactions (Ward and Melfi, 2013), though in companion animals this was often from the viewpoint of how interaction influence humans (Hosey and Melfi, 2014a). These differences are also reflected in

the terminology used in different disciplines, with authors' use of the terms human-animal- "relationships", "interactions", or "-bonds" denoting varying emphasis to the humans and animals involved and with more positive or negative connotations. In this thesis, I use the terms interaction and relationship interchangeably, and although I recognise that the relationship is dyadic and mutually affects both parties, I mostly study the outcomes from the perspective of the elephants.

Affiliative human-animal interactions can be influenced by the familiarity of the handler. For example, more time spent with familiar keepers was associated with lower faecal corticoid concentrations (a hormone involved in the stress response, see **Section 1.3**) in clouded leopards (*Neofelis nebulosa*, Wielebnowski *et al.*, 2002) and positive welfare indicators (increased grooming and playing behaviours/ decreased self-scratching and locomotion) in marmosets (*Callithrix jacchus*, Manciooco *et al.*, 2009). In addition to familiarity, we may expect a handler's past experience to impact the care they provide and their interactions; e.g. dogs (*Canis Lupus familiaris*) with more experienced owners showed less owner-directed aggression (Jagoe and Serpell, 1996; Kobelt *et al.*, 2003).

1.1.3 Human-elephant relationships and welfare

There have been many studies assessing elephant welfare in zoo environments (Clubb and Mason, 2002; Williams *et al.*, 2018), relating various aspects of their husbandry to measures of reproductive and stress-related endocrinology (Kumar *et al.*, 2014), health indicators (Edwards *et al.*, 2019), and behaviour (Clubb and Mason, 2002; Greco *et al.*, 2016). Following a pattern across many species (Carlstead, 2009), studies have begun to consider handler-elephant interactions in assessments of elephant welfare in zoos, finding strong relationships to be beneficial to both keepers and elephants (Carlstead *et al.*, 2019). However, assessments considering the mahout-elephant relationship in captive settings in Asia are rare, despite being applicable to >90% captive elephants and >20% of the entire remaining global Asian elephant population. It is interesting to note that although horses (*Equus caballus*) are recognised as companion animals in a review on this subject (Hosey and Melfi, 2014a), elephants are categorised as agricultural animals, which reflects a tendency to classify elephants as livestock across Asia, which has been criticised in respect to how their welfare is considered (Baker and Winkler, 2020; Schmidt-Burbach and Hartley-Backhouse, 2020) and may explain why elephants have been largely overlooked in this study area.

Most previous studies of mahout-elephant interactions have focussed on the relationship from an anthropological viewpoint, providing valuable insights into mahout livelihoods, traditions, knowledge and perspectives (Hart, 1994; Locke, 2011; Lainé, 2016; Mumby, 2019; Shell, 2019). These many studies appeared after

a call from Lair (1997) for more research and documentation of mahout traditions across Asia, and specifically the human-elephant relationship (Lainé, 2020). However, little research has focussed on the mahout-elephant relationship from the perspective of the elephant, and how different aspects of the relationship influence elephant health and welfare. A comprehensive set of studies assessing elephant welfare across US zoos and Thai tourism camps (see Bansiddhi et al., 2019; Brown et al., 2020 for reviews) covered numerous aspects of elephant management (e.g. housing, daily activities, diet, exercise, sex, age etc), and though they gave a general summary of mahout relationships (average relationship 3 years, $n=61$, Bansiddhi *et al.*, 2020), they did not examine the impacts of these relationships on elephants, instead highlighting it as a vital focus of future studies. A series of reports on state-owned elephants in India similarly reported detailed information on both specific mahout-elephant relationships and total mahout experience (e.g. average relationship 5 years, experience 15 years, $n=112$, Srinivasaiah et al., 2014; average relationship 8 years, experience 15 years, $n=306$, Varma et al., 2010), which are vital for documenting the current handling systems and informing comparisons to other populations, but few have assessed in detail how these relationships link to elephant welfare measures. Assessments by Srinivasaiah et al., (2014) suggested that specific mahout-elephant relationships may be more important than overall experience in elephant handling. Elephants were more cooperative, sociable and less aggressive towards their assistant mahouts than their main mahouts, who were more experienced but spent less time with them and elephants generally showed more “positive behaviours” towards mahouts scoring higher on a “keeper effort” measure.

1.2 Past studies of Training

Many animals that experience a lot of human interaction undergo some form of training, to control these interactions, ensure they are safe, and train certain behaviours. Studies of training practises have been carried out in some species, especially dogs (Fernandes *et al.*, 2017; De Castro *et al.*, 2020) and horses (Visser *et al.*, 2009; Olczak *et al.*, 2016). Although both can be considered companion animals, they are also used in working purposes (e.g. racing, police services, draught work, herding), and training is required in both contexts (Warren-Smith and McGreevy, 2007; Fernandes *et al.*, 2017). Most training depends on operant conditioning, in which a behaviour depends on a human-induced stimulus (Fernandes *et al.*, 2017). This can either be realised through encouraging a behaviour (reinforcement), or discouraging a behaviour (punishment), and these can be split further into either relying on adding a stimulus (positive), or removing a stimulus (negative), as demonstrated in **Figure 2**. Positive punishment and negative reinforcement are sometimes described as “aversive” training as they are based on the principle of

asserting dominance. The suitability and relevance of dominance-based techniques are debated in the context of dogs and can be criticised for their welfare impacts (Fernandes *et al.*, 2017).

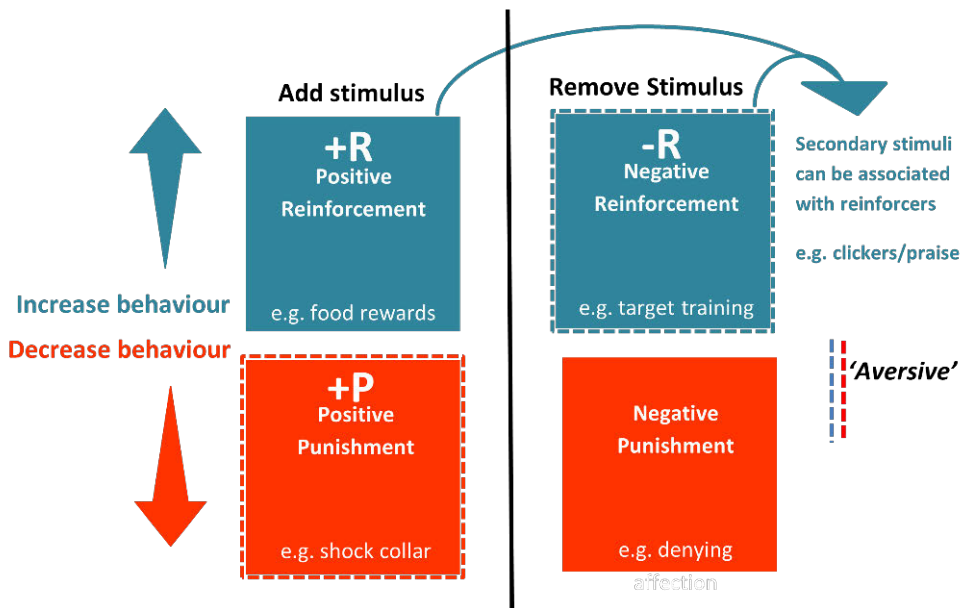


Figure 2. Schematic diagram of training or operant conditioning based on encouraging (reinforcement) or discouraging (punishment) behaviours by either adding or removing stimuli.

There is a general trend for dogs trained using aversive techniques to show more stress related behaviours (e.g. lip licking, yawning, panting, tongue flicking) and elevated cortisol (Fernandes *et al.*, 2017; De Castro *et al.*, 2020), and certain aversive-based tools such as shock collars, have been banned in some countries. However, this debate is ongoing, with the Companion Animal Welfare Council declaring there to be insufficient scientific evidence to draw policy decisions, and a recent review of the literature showing conflicting evidence and highlighting the fact that studies often rely on owner reports rather than objective measures to be a limitation (Fernandes *et al.*, 2017).

1.2.1 Elephant Training

Early human-elephant interactions have been particularly understudied, with very little empirical study of elephants undergoing training (Baker and Winkler, 2020; Schmidt-Burbach and Hartley-Backhouse, 2020). This is despite a lot of discussion of the welfare implications of traditional training methods, including a lot of

attention from international NGOs and the media. This was sparked following the circulation of a video taken during elephant taming in Thailand in 2002, leading to animal rights groups calling for tourists to boycott visiting elephant camps, and even Thailand as a whole (Cohen, 2015). Within this criticism however, all traditional elephant training is often homogenised into the general term “phajaan” without consideration of different techniques used and the relative impact of different training methods on calf welfare (Baker and Winkler, 2020). To my knowledge, though studies have described the different training techniques used across Asia (Nepal: [Locke, 2009, 2011], India: [Lainé, 2020], Thailand: [Schmidt-Burbach and Hartley-Backhouse, 2020], Myanmar: [Min Oo, 2010]), no studies have monitored calves during their taming or assessed the outcome of different training techniques on calf health or mortality, though many have highlighted the need for such studies (Brown *et al.*, 2008, 2020; Bansiddhi *et al.*, 2020).

Although training methods differ both within and between countries, the process often involves calves being separated from their mother and restrained by ropes, and undergoing close tactile training sessions with mahouts, generally consisting of a mixture of negative reinforcement and positive punishment, but also including positive reinforcement. A level of training is also incorporated into elephant husbandry in zoos to enable safe handling and veterinary intervention, and some training techniques are often considered to be enriching across animal species (Pomerantz and Terkel, 2009; Shepherdson *et al.*, 2013; Ward and Melfi, 2013) although there is little empirical evidence of this (Melfi, 2013). A transition to positive training or positive reinforcement techniques in Western institutions started in the late 20th Century (Desmond and Laule, 1994), along with increased adoption of protected contact environments (barriers separating elephants and humans). Positive training techniques attempt to remove pain from the process but often still use pressure as a negative reinforcement stimulus in combination with rewards, whereas full positive reinforcement training focuses solely on rewards (Schmidt-Burbach and Hartley-Backhouse, 2020).

Although positive training is also being considered in captive settings in Asia, it has mostly been employed on elephants already trained using traditional techniques (e.g. Fagen *et al.*, 2014), or those in protected contact. Advocates of positive training in free contact environments (no barrier between elephant and human) suggest it requires early exclusive human-elephant contact (at 1–3 years) and may not be possible for bull elephants (Lehnhardt and Galloway, 2008). Although it most likely benefits animal welfare, positive training is also likely to result in different behavioural outcomes and the safety of training exclusively with positive training in free contact management - which is used in most captive Asian elephant populations - is debated. For example, a study found slower responses and more refusal of commands in elephants when relying on positive reinforcement in protected contact

compared to those using negative reinforcement by an ankus in free contact (Wilson *et al.*, 2015). This may suggest elephants exercised more behavioural choice, associated with better wellbeing, but it also has important implications for the suitability of using positive reinforcement in high-risk free-contact environments, where elephants can pose a substantial danger to their mahouts (Radhakrishnan *et al.*, 2011; Bansiddhi *et al.*, 2020). Furthermore, a study comparing elephants managed in free vs protected contact found no differences in their overall or baseline cortisol concentrations, reinforcing the importance of relying on empirical assessments rather than simply assumptions (Proctor and Brown, 2015). There was a trial introduction of positive training in Nepal between 2006-2011 (Telkänranta, 2009; Varma and Ganguly, 2011), but there has been little public follow-up or data-driven evidence of the outcome of this, including to my knowledge, any measure of its impact on elephant behaviour and welfare or mahout safety.

1.3 Measures of Health and Welfare

In order to understand impacts of human interactions on elephant welfare, it is important to identify measures of elephant wellbeing. Many studies have assessed Asian elephant welfare, in both western institutions and across their range countries, particularly Thailand (e.g. Bansiddhi *et al.*, 2020; Brown *et al.*, 2020; Proctor & Brown, 2015; Vanitha *et al.*, 2010; Williams *et al.*, 2018; Mason and Veasey, 2010). There are many different ways to assess animal welfare, often through assessing either physiology, health parameters or behavioural indicators. Studies often use endocrinology to measure concentrations of glucocorticoid hormones secreted by the adrenal cortex in response to stressors. The stress response is a natural process, with acute stress priming the immune system to react to stressors, and even chronic stress (long-term activation of the hypothalamus-pituitary-adrenal [HPA] axis) arguably adaptive in some circumstances (e.g. in response to predation; Boonstra, 2013). However, both repeated acute stress and prolonged chronic stress can have detrimental impacts on bodily processes, such as the immune system, reproduction, growth, motor and sensory performance and brain function (Metz *et al.*, 2001; Conrad, 2010; Seltsmann *et al.*, 2017; Sarjan and Yajurvedi, 2018; Valenzuela *et al.*, 2020). See Box 1 for further definitions.

Box 1. Definitions of key concepts

Stress(or)	A threat to homeostasis or the body's physiological and psychological equilibrium. (Moberg & Mench, 2000; Mason & Veasey, 2010)
Wellbeing/	The overall state of an individual, "which encompasses both mental and physical
Welfare	health, engagement with the physical and social environment and the opportunity to exhibit control or choice." (Williams <i>et al.</i> 2018)
Acute stress	"A suite of physiological and behavioral changes that are thought to help
Response	an animal survive in the wild ... [to] temporarily suspend otherwise normal life-history functions, quickly counteract the impact of the stressor, and allow the animal to return to normal activities" (Dickens & Romero, 2013)
Chronic stress	"Occurs when the stress response system has been pushed beyond its
Response	normal capacity by either an altered intensity and/or persistence of stressors...normal activities become chronically disrupted and physiological systems begin to break down" (Dickens & Romero, 2013)

It is common to measure the concentration of glucocorticoid hormones circulating in the blood for tame animals, for whom blood collection can be achieved quickly and without inducing a stress response, to observe immediate changes (Proctor and Brown, 2015). Studies also often measure hormone metabolites in the faeces as a non-invasive measure which does not require manipulation or handling which can themselves cause stress (Stead *et al.*, 2000), and provides a cumulative measure of glucocorticoid hormones built up over the digestion process of up to a couple of days (Brown *et al.*, 2008). Other non-invasive measures such as infra-red thermography (Stewart *et al.*, 2005) and heart rate variability (von Borell *et al.*, 2007) are also becoming more widely measured. Monitoring the leukocyte (white blood cell) profile can be informative, both to recognise an immune response (increase in white blood cells), and as an indicator of chronic stress (elevated heterophil/neutrophil to lymphocyte ratio) (Fowler and Mikota, 2006; Davis *et al.*, 2008; Swan and Hickman, 2014; Selmann *et al.*, 2017). Creatine kinase is often used as an indicator of muscle damage, especially in livestock research in the context of, for example, pre-slaughter or transportation stress (Kannan *et al.*, 2000; Chulayo and Muchenje, 2013).

Physiological indicators are however always approximations and interpretation of results can be complex. For example, although acute stressors tend to be associated with increased glucocorticoid hormones, their response to chronic stress

can be less predictable (Miller *et al.*, 2007; Mason and Veasey, 2010; Dickens and Romero, 2013). Studies have found both increased and reduced HPA activity in chronically stressed individuals, with activity generally diminishing as time passes (possibly due to adrenal exhaustion or habituation) with varying effects on glucocorticoids throughout the day (Miller *et al.*, 2007). A review on the subject concluded that whilst it is valuable to monitor the glucocorticoid response in relation to chronic stress, the direction of change may not be important as much as the change itself: a reduced response may leave an individual unable to cope with the stressor, whilst a heightened response may endanger their long-term health and fitness (Dickens and Romero, 2013). One way to test whether lack of glucocorticoid response is due to adrenal exhaustion rather than lack of stressor would be to carry out an ACTH or CRH challenge, which involve injecting corticotropin releasing hormone or adrenocorticotropin to assess adrenal sensitivity and pituitary function respectively (Miller *et al.*, 2007; Brown *et al.*, 2008), an interesting development for future studies. Additionally, interpreting the health implications of leukocyte counts can be complex (Davis *et al.*, 2008) as both high and low counts can be indicative of a problem (low counts could reflect immunosuppression and high counts an immune response). For this reason, it is beneficial to understand a population's "normal range" to put changes into context, in this case I can refer to previously estimated reference intervals for this population (Franco dos Santos *et al.*, 2020). It is important to interpret findings with these limitations in mind and collect multiple complementary measures simultaneously for a wider and more reliable view of responses (Mason and Veasey, 2010).

In addition to physiological measures, it is also important to consider behavioural indicators of welfare, which can be indicated by abnormal behaviours such as stereotypies (Greco *et al.*, 2017), reduced exploratory and reproductive behaviours, reduced behavioural complexity, negative cognitive bias and increased aggression (Morgan and Tromborg, 2007; Mason and Veasey, 2010). In relation to human-animal interactions, studies often focus on negative interactions by assessing animals' fear of humans, especially common in studies of livestock or zoo animals, through avoidance or vigilance tests (Carlstead, 2009; Quadros *et al.*, 2014). It is also informative however to measure their cooperative behaviours, studied more in companion animals e.g. to understand owner-dog communication (Gibson *et al.*, 2014; Rehn and Keeling, 2016), which I do in this thesis to assess the mahout-elephant relationship.

This thesis employs a combination of measures as recommended for a comprehensive overview of elephant wellbeing (Wielebnowski, 2003; Brown *et al.*, 2008; Swan and Hickman, 2014), in line with the "one welfare" approach to animal welfare, a framework recognising the interconnection between animal welfare, human wellbeing and the environment (García Pinillos *et al.*, 2016).

1.4 Aims of the Thesis

This thesis aimed to further our understanding of mahout-elephant relationships in the largest (semi-)captive population of Asian elephants in the world. Little had been documented and quantified about the mahout system in Myanmar, or how mahout relationships in range countries impact their elephants in general prior to this thesis. The mahout handling system was previously assumed to resemble the ancient system described for centuries across Asia, with long-term mahout-elephant relationships and gradual apprentice-based teaching passed down generations. However, many aspects of this livelihood were likely to have been affected by recent socio-political and economic changes in Myanmar, as has been found elsewhere in Asia. In this thesis, I first present an overview of the handling system in Myanmar: outlining mahout ages, experience working with elephants, commitment and views surrounding elephant handling, as well the changes observed by those working in this system over recent decades (**Chapter I**). I then study these variables in the context of the elephants to assess how these mahout demographic factors influence the elephants in their care (**Chapter II**). I cover a suite of measures including indicators of physiological stress, immune function and behavioural responses to form a comprehensive view of how mahout-elephant relationship lengths and general mahout experience impact elephant welfare. I then focus on a particularly understudied and arguably the most intense period of mahout-elephant interaction, studying elephant calves during their taming when they are first introduced to human contact around the age of four years. Juvenile mortality is a major limitation for this population of endangered Asian elephants, with 25% of calves dying before the age of five years (Mar *et al.*, 2012), found to be one of the most important factors limiting their population growth (Jackson *et al.*, 2019). I revisit these past findings of a peak in mortality at the taming age of four years (Mar *et al.*, 2012), to understand whether certain calf traits are associated with higher mortality at these ages (**Chapter III**) and provide evidence-based recommendations for taming management. Finally, I extend this assessment of the effects of the taming procedure to collect data for the first time from calves during their traditional taming process (**Chapter IV**). I again investigate an extensive range of measures for a full overview of calf physiological stress, closely over the first 10 days of taming, and over the following six months to assess both the extent and duration of any negative impacts. This data is crucial to empirically inform this heated area of debate and will hopefully be the start of assessing different populations under varying management.

2 Materials and Methods

2.1 Study Population

This thesis investigates mahout-elephant relationships in the largest (semi)-captive population of Asian elephants in the world, the timber elephants of Myanmar. Myanmar is home to ~6000 captive elephants in total and around half of these are owned and managed by the governmental agency, the Myanma Timber Enterprise (MTE) (Hedges *et al.*, 2018); the elephants studied in this thesis. MTE elephants work dragging felled logs through the forest, but there are strict regulations in place in terms of their working hours (~4–8 hours/day, 5 days/week), the tonnage they drag (according to season and size/condition/age of elephant) and all elephants are rested during the hot season from March-May (Mar, 2007). Females are relieved of working duties from mid-pregnancy until their calves are ~18 months old and all individuals cease working at age 55 or when sick, but still receive the same care. Calves freely accompany their mothers and suckle at will, having limited contact with humans until the age of 4–5 years when they are separated from their mothers to be tamed (more information in **Section 2.2**). All elephants are paired with one mahout once tamed, who is responsible for their everyday care, such as bathing them each morning and monitoring their diet, condition, and behaviour for any abnormalities. Elephants are organised into camps of ~6 elephants, which are overseen by head mahouts (sin-gaungs), who do not have their own elephant but are generally more experienced and responsible for the group as a whole, and regions of elephants (~100) are overseen by the most experienced mahouts (sin-okes). The elephants are classed as semi-captive because they are released into the forest every afternoon/evening to freely forage, socialise and mate with conspecifics until the following morning (though sometimes with hobbled front legs) and therefore their diet and reproduction is not managed by humans. Each elephant has an individual ID number with a corresponding logbook detailing important demographic events such as their date and location of birth, their sex, information on their offspring, mother, and mahout, and when applicable date and cause of death; logbook data were analysed in **Chapters II & III**. The elephants are inspected at least once a month by specialist elephant veterinarians, and

important health information is also recorded, such as results from health checks, instances of injury or medication/vaccination.

2.2 Taming Procedure

Chapters III and IV focused on calves undergoing their traditional taming procedure which is generally considered aversive, with calves often resisting the rope restrictions and vocalising frequently. The taming procedure for captive-born MTE calves is always conducted in the cold season (beginning November-December), to reduce heat stress. Calves are usually tamed in the cold season of their fifth year, when they are 4–5 years old, but early taming can be granted for calves e.g. with weak younger siblings competing for milk or for calves whose behaviour or that of their mother is particularly difficult, if they are large enough (>5 feet 2 inches/ 157.5cm). The main initial taming procedure lasts around a month (though training continues over the following months and years) and is conducted in a special taming camp, located in a cool, well-shaded, level area of the forest with plenty of surrounding fodder, and a close running water source. There should ideally be no more than 4-5 calves tamed at one camp, although it depends on the number of calves born in an area- one camp observed in this thesis tamed 10 calves in one year but staggered it so there were six calves initially followed by four a week later. There should be a similar number of well-trained adult elephants, known as kunkis, in the camps as calves, who should be used to direct and control calves to limit direct mahout-calf contact. Each taming camp is overseen by at least one veterinarian and experienced sin-oke (and one location in this thesis had two specialist taming teachers). Prior to taming, the veterinarians treat each calf for parasites (particularly targeting nematodes and botflies) and provide a tetanus vaccination. Veterinarians monitor each calf daily, providing preventative care (e.g. daily vitamins and laxatives), any required medication (e.g. antibiotics for possible infection, fluids for dehydration, analgesic and anti-inflammatory drugs), and treatment for rope burns or wounds as needed (e.g. applied pressure and dressing with a combination of “wan-u” – a hot fermentation made from the bark of a common creeper in the forest (Min Oo, 2010, **Figure 3**), potassium permanganate, hydrogen peroxide, gentian violet, and “earth oil”) – see **Supplementary Information** for full list of medications.



Figure 3. Mahouts preparing “wan-u”, by **A)** soaking in hot water, **B)** softening it against solid surface, and **C)** using it to treat inflammation

Each calf is designated a cradle in the taming camp, which is constructed out of three large pillars of hard wood placed in a triangular formation with two posts at the front ~9 feet apart and the third ~11 feet from the centre of the front two (Min Oo, 2010; **Figure 4A**). Each post is securely embedded ~4 feet into the ground and extends ~9 feet above ground, forked at the top with extra posts lashed for support. The cradle structures usually face towards a sacred area at which offerings are left (**Figure 4B**), with “weaker” calves placed at the front so they cannot see the others, which is considered stressful. On the first day of taming, calves and their mothers are brought to the taming camp early in the morning, they are led to the cradles using food and injected with a 2% xylazine sedative, adjusted for their weight (0.002–0.007 ml/kg), usually around 2–4 ml per calf. Once the calves show clear signs of sedation (still, trunk/penis dropped) and are positioned in the middle of their cradle, mahouts begin tying the ropes, and the calf’s mother is led away, sometimes requiring a kunki elephant tied to her. Calves are restrained by ropes to their legs and shoulders and a strong breast band made from strands of the shaw tree (**Figure 4C**; (Mar, 2007)) around their chest, to prevent injuries to the mahouts and calves themselves. The mahouts apply pork fat onto any skin in contact with the ropes daily to minimise rope burn, ropes are adjusted and loosened as required, and sometimes covered in plastic to reduce friction (e.g. in **Figure 3C**).

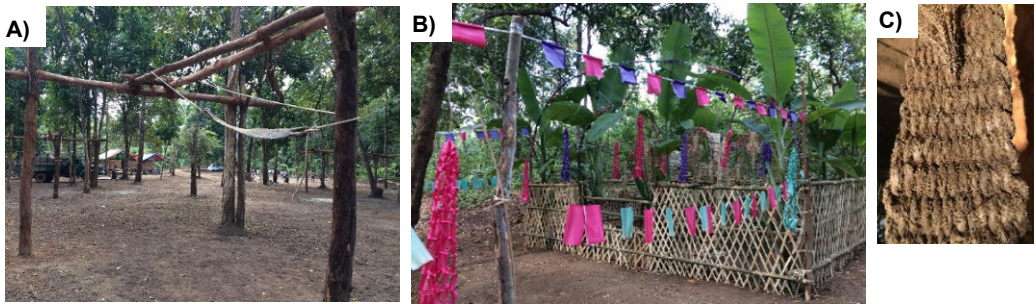


Figure 4. **A)** Cradle constructed from 3 main vertical posts and extra supporting beams across the top with the breast-band suspended across, **B)** shows the sacred area and **C)** shows the breastband close-up.

The ropes are loosened and removed over the coming days as it becomes safe to do so, leaving only a long rope to the back leg of the calf around day 10. Early each morning, veterinarians conduct health examinations and administer medication either via injections or orally in tamarind. Daily monitoring routinely involves taking body temperature as an indication of infection as well as observing feeding, urination and defecation habits and checking calf energy levels and mucous membrane colour. Blood and faecal samples are also collected, which were used in **Chapter IV** to analyse indicators of physiological stress (concentrations of blood glucose, creatine kinase, serum cortisol and faecal glucocorticoid, and differential white blood cell counts). Calves are taken for a walk daily tied to at least one kunki elephant (**Figure 5A&B**), for exercise and to drink from and bathe in a running water source. Mahouts collect fresh fodder from the surrounding forest which are constantly provided to the calves (e.g. bamboo leaves, paddy, broom grass) in addition to daily supplements (e.g. rice, tamarind with salt, banana stem, sugarcane) and water is offered frequently in a bucket, though many calves prefer to drink from a running water source.

Each calf has a designated team responsible for their taming, made up of ~10 mahouts, of whom ~3 are experienced sin-gaung and one is selected as the future mahout of the calf. The main training sessions (**Figure 5C**) consist of the group of mahouts surrounding the calf, singing a repetitive traditional *shaw pike* song whilst rubbing the calf all over, for repeated periods of ~45 minutes, punctuated by ~30 minutes of rest, which can last many hours and usually take place in the evenings and mornings when it is coolest. At first these sessions are to familiarise the calf with the mahouts' presence and touch, but gradually the calf is taught to accept a rider on their head, to accept opening and closing fetters around their front legs and to respond to commands important for their working life.

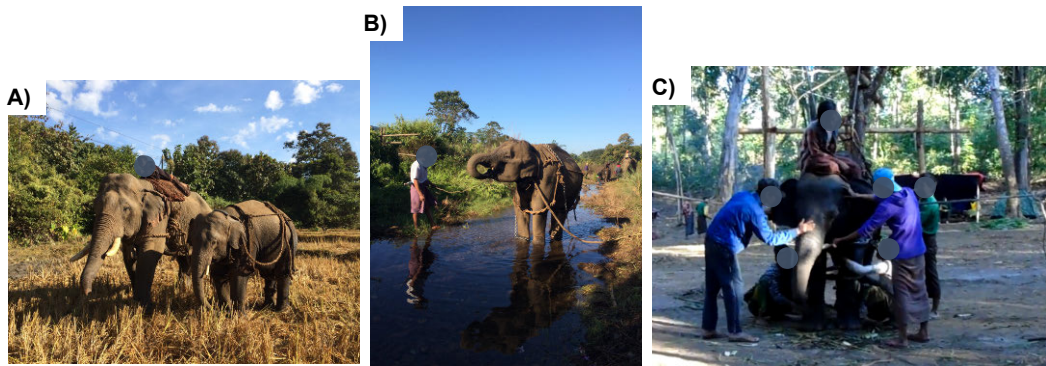


Figure 5 A) & B) Calf and kunki on their daily walk, pausing to A) feed, and B) drink and bathe and C) calf undergoing taming session.

2.3 Mahout Data Collection

I collected mahout information through interviews with mahouts and others involved in MTE elephant management (**Chapters I, II & IV**), and from information recorded in elephant logbooks (**Chapter II**). **Chapter I** investigated recent changes in the MTE mahout system through interviews with 23 experts with >10 years of experience working with MTE elephants (**SQ1**) as well as gathering information and perspectives from interviews with 210 current mahouts working in the MTE (**SQ2**), both based on semi-structured questionnaires. **Chapter II** linked mahout ages, relationship lengths with their elephants, and total experience collected from these interviews to elephant physiological health measures and behavioural indicators (n=87–136 observations of 65–81 individuals) as well as information on mahout changes gathered from elephant logbooks and from head mahouts. **Chapter IV** also included answers from interviews with 164 mahouts, based on a different questionnaire (**SQ3**). **SQ3** included some of the same questions as **SQ1**, but mostly investigated mahout attitudes towards various aspects of the taming procedure. All interviews included a mixture of multiple choice and open-ended questions and were conducted verbally in Burmese to aid less literate mahouts, allow questions to be explained further if necessary and to obtain consent.

2.4 Physiological Data Collection

Both **Chapters II** and **IV** investigated physiological measures deriving from either blood or faecal samples; see **Table 1** for an overview of measures and abbreviations. I collected both faecal and blood samples early in the morning to reduce diurnal variation, taking blood from an ear vein into a vacuette with either ethylenediaminetetraacetic acid (for TWBC/ H:L), or serum separator/clot activator

(for CK/ SC). I measured glucose immediately from fresh blood using an ACCU-Chek Aviva glucometer. I collected faecal samples as soon after defecation as possible and froze them before drying in a hot air oven to be analysed for FGM concentration at The Chiang Mai Diagnostics Laboratory, Thailand. FGM was determined using a double-antibody enzyme immunoassay relying on a polyclonal rabbit anti-corticosterone (CJM006) antibody (validated by Watson *et al.*, 2013). I obtained serum by centrifuging clot activator vacuettes for 20 minutes at 3400 rpm and sera were frozen until analysis. Sera were analysed for CK at the Crown laboratory in Yangon, Myanmar using an IDEXX VetTest analyser (IDEXX, USA) and for cortisol concentrations at the University of Turku, Finland using a species independent enzyme immunoassay (Arbor Assays) relying on a monoclonal mouse antibody (goat anti-mouse IgG). I counted TWBC counts manually under a microscope at x10 magnification from blood in a Neubauer haemocytometer after lysing the red blood cells with Turk's solution. To obtain H:L ratios, I identified 100 cells as either monocyte, heterophil, lymphocyte, basophil or eosinophil under a microscope at x40 magnification after staining blood smears with a Romanowski stain. Many of these methods are described in Franco dos Santos *et al.*, (2020). The number of overall samples collected, and individuals covered differed between the different physiological measures in **Chapter II** (n=307–1964 observations for 116–151 individuals), and **Chapter IV** (n=262–485 observations for 24–38 calves and n=41–266 observations for 11–16 kunkis), with specific sample sizes found in each chapter.

2.5 Behavioural Data Collection

Chapter IV assessed the elephants' cooperative behaviour in relation to mahout familiarity and experience measures. Simple behavioural tests were conducted in which mahouts asked elephants to cross an arena (8 x 3m) towards them. I quantified the elephants' behavioural responses by measuring an elephant's i) task success (binary: crossed/failed) and ii) response time (duration from command to entering the arena). These measures were then assessed in response to the familiarity of the calling mahout (binary: own mahout/ unknown mahout), the relationship length with the calling mahout (0 for unknown mahouts, range 0–7 years) and the calling mahout's total experience (categorised into 1:<24 months, 2= 24–38 months, 3= 39–119 months, 4=>120 months).

Table 1. Overview of physiological variables used in Chapters II & IV

Variable	Indication	Family	Code	Chapter	References
Total White Blood Cell Count	Immune Reaction		TWBC	II	(Fowler & Mikota, 2006; Seltmann <i>et al.</i> , 2017)
	High count suggests inflammation or infection	Gaussian			
Heterophil: Lymphocyte	Chronic Stress		H:L	II;IV	(Davis <i>et al.</i> , 2008; Swan & Hickman, 2014; Seltmann <i>et al.</i> , 2017, 2020)
	Low count suggests immunosuppression	Gaussian (log transformed)			
Glucose	Nutritional and Physiological Stress		Glucose	IV	(Kannan <i>et al.</i> , 2000; Murray <i>et al.</i> , 2003)
	Glucocorticoids in the blood can increase gluconeogenesis and inhibit glucose action outside of the liver	Gaussian			
Creatine Kinase	Muscle Damage		CK	II; IV	(Fowler & Mikota, 2006; Chulayo & Muchenje, 2013)
	The presence of creatine kinase in the blood plasma, outside of muscles indicates damage and rupture of muscles	II: Negative binomial IV: Gaussian (log transformed)			
Serum Cortisol	Physiological Stress		SC	IV	(Moberg & Mench, 2000; Morgan & Tromberg, 2007; Proctor & Brown, 2015)
	Glucocorticoid hormones secreted by the adrenal cortex often in response to acute stress can be directly measured in blood serum	Gaussian			
Faecal Glucocorticoid Metabolites	Physiological Stress		FGM	II; IV	(Stead <i>et al.</i> , 2000; Laws <i>et al.</i> , 2007; Brown <i>et al.</i> , 2019)
	Glucocorticoid hormones secreted by the adrenal cortex often in response to acute stress can be measured as metabolites in faeces, a non-invasive accumulated measure built up over ~24-48 hours of digestion	Gaussian (log transformed)			

2.6 Additional Data Collection

Although not included in the thesis chapters, I will briefly outline additional data collected during the taming procedure to give a more complete picture of the full project and an idea of future research directions. I recorded daily videos of each calf for four minutes during their early morning training sessions with their mahouts, with the aim to code these using an ethogram to identify certain behaviours (e.g. escape/stereotypic behaviours, affiliative behaviours, vocalisations etc.) to further assess their welfare. I collected additional physiological measures to assess their overall health over the same time period as the measures in **Chapter IV**, which includes blood pressure, TWBC, electrolytes (Na, K, Cl, Hb, Hct) and various blood chemistry parameters (BUN, creatinine, Ca, total protein, albumin, AST, ALKP, triglycerides) which will be analysed in collaboration with MTE veterinarians. Finally, I have measured indicators of oxidative stress, which I will combine with measures of mitochondrial density and telomere length to understand the impact of taming on allostatic load and biological ageing.

2.7 Statistical Analyses

This thesis employs a selection of statistical analyses carried out in R (R Core Team, 2020), covering Principal Components Analysis/PCA (**Chapter I**), generalised linear mixed-effects models using both Maximum Likelihood (ML) (**Chapters I, II & III**) and Bayesian frameworks (**Chapter II**), survival analysis (**Chapter III**), generalised additive models and posterior simulations (**Chapter IV**). Specific details can be found in the methods sections of each chapter. The family used in each model depended on the distribution of the response variable with some variables log-transformed (using the natural logarithm) before analysis (see **Table 1** for families used for physiological measures). Statistical significance was interpreted at the 95% level i.e. $p < 0.05$ in ML models. Continuous variables were scaled to aid model convergence, and mean centred if comparisons to zero were meaningless (e.g. mother's age in **Chapter III**).

2.7.1 Confounding variables

Specific details can be found in each chapter, but common variables accounted for included the season of measurement as a fixed effect (three factors, hot: March-May, monsoon: June-October, cold: November-February) as seasonal effects are known to be important in this population (Mumby *et al.*, 2013; Franco Dos Santos *et al.*, 2020). I also included important demographic information for focal elephants, such as their age (continuous, scaled) and sex (binary factor) as fixed effects, known to have important impacts on physiological measures (Franco dos Santos *et al.*, 2020).

I also included the location of elephant camps within Myanmar as a random intercept in **Chapter III** (eight level factor) and fixed effect in **Chapter IV** (binary factor) to account for spatial heterogeneity. Models in **Chapter III** contained additional maternal information known to have important impacts on offspring fitness in this population (Reichert *et al.*, 2019). These included fixed effects of mother's age at the birth of the focal calf (continuous, scaled, and mean centred), the calf's birth order (three level factor, 1: 1st born, 2: 2nd–3rd born, 3: 4th–11th born) and mother's origin (binary factor, captive: 0, wild: 1) in interaction with a continuous variable of the time since capture (log-transformed) at the time of the calf's birth, with captive born mothers coded as 0 to account for negative impacts of capture lessening over time (Lahdenperä *et al.*, 2018, 2019). I included a random intercept term for individual ID to account for pseudoreplication in models with repeated sampling of the same individuals. I tested for non-linear relationships if exploratory plots suggested they were appropriate and for interactions between variables where dependencies were hypothesised (e.g. a calf's FGM response to taming may depend on their sex). I also included variable-specific terms in models such as a random intercept for FGM measurement batch (eight level factor) in **Chapter II** to account for temporal measurement differences and fixed effects of days between collection and analysis of serum samples in CK models (continuous, scaled) in **Chapters II & IV** to account for storage time effects.

2.7.2 Model Selection

I assessed the distributional assumptions of fitted models, thereby evaluating their appropriateness, through residual diagnostics from the DHARMA package (Florian, 2020) and through the *gam.check* function for generalised additive models in **Chapter IV** (Wood, 2017). Additional analyses to account for heteroskedasticity in Model Cii) in **Chapter II** are shown in the additional supplementary information. I compared model predictive performance based on the Akaike Information Criterion (AIC) values in ML models and Kfold-Information Criterion (Kfold-IC) in Bayesian models. Specifically, I mostly used likelihood ratio tests (the *anova* function in R; Chambers and Hastie, 1992) to compare two hierarchically nested models to test whether adding complexity to the model (an extra term) improved model predictive performance, which I considered as a reduction in AIC of 2 or more, or a reduction in Kfold-IC with no overlap of SE's (Burnham and Anderson, 2004; Bürkner, 2017). When drawing conclusions, I retained non-significant terms if they had a strong biological hypothesis for inclusion, though I also compared conclusions when removing all non-significant terms. Test statistics of predictors in ML models were determined using likelihood ratio tests with the Chi-squared distribution as well as Tukey's post hoc tests for the significance of levels within a term, whilst the

importance of predictors in Bayesian models were assessed based on whether credible intervals encompassed zero. Prior to the PCA in **Chapter I**, I removed statements with >97% respondent agreement and allowed only statements which correlated >0.3 with at least one other statement, and never >0.9, suggesting there was no multi-collinearity (Budaev, 2010). I measured the sampling adequacy of the correlation matrix using the Kaiser-Meyer-Olkin (KMO) value and set the threshold for statements loading onto components at 0.5.

3 Results & Discussion

3.1 Current and past mahout demography in the MTE

Chapter I provided evidence of recent changes in the MTE mahout system in Myanmar over the past decade or more, with a significant majority of experts having perceived past mahouts to have been older (83%), more experienced (78%) and to have spent longer in the job (73%) than today's mahouts. Although similar changes have been reported in other countries across Asia, such as India, Laos, Thailand, and Sri Lanka, Myanmar has often been considered one of the last remaining areas of experienced traditional mahouts (Lair, 1997; Sukumar, 2003). Changes were pinpointed to have occurred in 2011/13, which correspond to important periods of social and political change in Myanmar (Parmar *et al.*, 2015). Interestingly, despite these changes to mahouts, a significant majority of experts (73%) thought elephant treatment had improved, explaining there were "more techniques/ training/ care" now, which may be more related to veterinary care and management rather than handling. I quantified the average (median) demography of current mahouts as 22 years old, with a total of 3 years of experience working with elephants in general, and only a one-year relationship with their current elephant, having worked with two elephants on average. My results suggest the traditional familial links to the profession are likely in decline, with <50% of mahouts having a family connection to elephant handling and less than a third of mahouts with children expecting them to become mahouts too, findings mirrored in other countries (Varma *et al.*, 2010b; Suter *et al.*, 2013; Srinivasaiyah *et al.*, 2014). Overall, these changes threaten the traditional mahout profession and its reliance on observation-based apprenticeship and training over many years for the passage of knowledge and expertise. The risk these changes could pose was demonstrated by a study by Vanitha *et al.* (2009), which found the loss of traditional mahouts to be coupled with more fatal accidents with temple elephants.

Chapter I also provided key mahout perspectives on various aspects of elephant management including elephant behaviour, job commitment and the taming procedure. Most handlers (70%) had been involved in the taming process, which contrasts to populations in other countries where there is lessening knowledge on

taming practises (Lair, 1997) and calves are sent away to be tamed (Schmidt-Burbach and Hartley-Backhouse, 2020). Most mahouts (60%) expressed pity for calves undergoing taming, with many (66%) showing support for training based on positive reinforcement, issues explored further in **Chapter IV**. I finally assessed mahout-elephant interactions through 17 questions which were reduced by PCA into the 5 components: “job appreciation”, “experience is necessary”, “human-elephant interaction”, “own knowledge” and “elephant relationship”. Investigations into variables associated with these components found that experienced mahouts agreed more with statements loading onto the “experience is necessary” component, suggesting they have an appreciation for the challenges and risks associated with working with elephants (Radhakrishnan *et al.*, 2011). Mahouts caring for bulls and younger elephants- both of which could be considered more difficult to manage- also agreed more that “experience is necessary”, suggesting that particular care should be taken to train mahouts in managing difficult behaviour, especially in younger elephants and during musth, in accordance with Srinivasaiah *et al.*, (2014). This could also help alleviate the fact that mahouts of difficult elephants scored lower on the “human-elephant interaction” component, consistent with findings from zoo elephants that keeper-elephant relationships are mutually beneficial (Carlstead *et al.*, 2019).

3.2 Influence of the mahout-elephant relationship on elephant physiology and behaviour

Chapter II investigated how the mahout-elephant relationship influences elephants in the MTE, finding both specific mahout-elephant relationship lengths and total mahout experience to influence elephant physiology and behaviour. Past studies into specific relationships from the perspectives of elephants have been rare, and mostly restricted to those kept in zoos (Carlstead *et al.*, 2019). My results suggested that neither an elephant’s FGM nor H:L, both indicators of physiological stress, were related to their relationship length with their mahout, their mahout’s age or their mahout’s prior experience of working with elephants. This could suggest that the mahout relationship is not a major driver of physiological stress in this population of elephants, or that the elephants are buffered from the negative impacts of inexperienced mahouts in some way. There may be enough mahout expertise remaining within the MTE to maintain adequate training for inexperienced mahouts to provide quality care. One of the few studies including a measure of mahout experience in an assessment of elephant welfare also found no link between mahout experience and elephant welfare, instead finding factors such as location, work type, shade and food availability to be the most important (Chatkupt *et al.*, 1999). The latter findings were in line with other studies (Vanitha *et al.*, 2010; Schmidt-Burbach

et al., 2015; Bansiddhi *et al.*, 2020; Brown *et al.*, 2020), though these did not directly assess the influence of mahout variables. On the other hand, I showed mahout relationship measures were associated with changes in other physiological measures, with elephants who had longer relationships with their mahouts showing lower levels of muscle damage (CK) once they reached the working age of 18 years. This likely reflects the importance of trust between elephants and their mahouts when working together, and how mahout understanding of their elephant's individual behaviours is built up over time working together. In apparent contrast to these findings, an elephant's CK, and to some extent their TWBC, logarithmically increased with longer mahout total experience, suggesting greater muscle damage and immune response in elephants of more experienced mahouts, though average TWBC counts remained within the reference intervals determined for this population (Franco dos Santos *et al.*, 2020). The increases in these measures with mahout experience is likely reflecting the fact that more experienced mahouts are generally involved in harder logging work which may be associated with muscle strain and inflammation. It is also possible that these measures are indicating mahout complacency over time, which has been suggested both from studies of zoo keepers and semi-captive elephants in India (Carlstead, 2009; Srinivasaiah *et al.*, 2014).

Finally, I found that elephants responded more to their own, familiar mahouts during behavioural tests, and faster to mahouts they had known for longer. These findings are in line with past studies interviewing mahouts who claimed it takes ~3 years to develop understanding and ~5 years to build trust with an elephant (Hart, 1994; Srinivasaiah *et al.*, 2014; Mumby, 2019). My results suggest the frequent mahout changes that are becoming more common across Asia could have important implications for elephant behavioural management, which has repercussions for mahout safety. Trends from zoos suggest animals are more likely to attack when cared for by many different keepers or new, unfamiliar keepers (Hosey and Melfi, 2014b), and temple elephants cared for by less experienced mahouts were found to be involved in more fatal accidents (Vanitha *et al.*, 2009). Such risks are relevant to the MTE population which has previously been estimated to lose 10-20 mahouts per year to accidents (Lair, 1997). It is also a concern for the mahouts of thousands of captive elephants across Asia, especially those who are mediating their interactions with unfamiliar humans such as the public and tourists in unpredictable environments with an estimated 200 mahouts lost in Thailand every year (Phongkum, 1995; Vanitha *et al.*, 2010; Radhakrishnan *et al.*, 2011).

3.3 Calf traits associated with taming age mortality

Chapter III focussed on one of the most intense periods of mahout-elephant interaction, the taming procedure, to assess whether certain calf traits were

associated with mortality during the taming ages of 4–5.5 years for 1,947 calves born in the MTE population between 1970–2013. I first showed that the calf mortality rate increased by >50% from age three to the taming age of four years for MTE calves, a peak not observed in wild African elephant calves (Moss *et al.*, 2011), suggesting it is likely linked to their management rather than their natural life history (e.g. weaning and dispersal from their mother). This reflects a similar pattern to previous studies of aversive events in elephants, with early maternal separation and inter-zoo transfers found to reduce survivorship in zoo elephants (Clubb *et al.*, 2008, 2009), and wild-capture to reduce survivorship in the MTE population (Lahdenperä *et al.*, 2018). I found that calves born earlier in the year, who were therefore older during the taming procedure which usually takes place in the November/December of a calf's fifth year, were less likely to die during taming ages. This was also true for calves born to more experienced mothers, in line with past findings both from the MTE population and others that first born calves have lower survival both at birth and later ages (Moss *et al.*, 2011; Mar *et al.*, 2012). Birth season has previously been shown to be associated with calf mortality in this population, seeing the highest survival in calves born between Dec–March, so the birth month effect found here may also reflect environmental influences (Mumby *et al.*, 2013). It could be that those calves born to more experienced mothers and those born earlier in the year may have a larger body size (both linked to being older and born in a preferable season), which often dictates mortality risk and may influence for example an individual's response to infectious diseases, exhaustion, parasite infection and gastro-intestinal issues, which together made up >50% of the taming age causes of death. Promisingly, the results showed that taming mortality dropped over the study period, with taming mortality rates of calves born after 2000 being a third of those born in the 1970's. This suggests that changes to calf management during taming in the MTE over recent decades have been beneficial, and I invite more studies into taming impacts in other elephant populations across the Asian elephant range to further our understanding of the impacts of different practices and optimise conditions, issues explored more in **Chapter IV**. It would be interesting for future research to investigate the optimum age of socialisation in elephant calves, to compare for example the relative benefits of gradual introduction to human contact from a young age vs. extended undisturbed mother-calf contact.

3.4 Monitoring calf physiological stress for the first time during the traditional taming procedure

Chapter IV sought to look closer at the taming procedure used in the MTE today, collecting data for the first time from calves undergoing traditional taming. I assessed measures both closely over the critical first 10 days and over the following six

months, to assess the extent and duration of effects. The traditional taming procedure has been widely criticised in the media and among welfare advocates, though it is often homogenised into general “phajaan” (Baker and Winkler, 2020) and there has been no empirical study of its impacts and the relative risks of different techniques in populations under varying management. Here I monitored five physiological measures (FGM, SC, glucose, CK, and H:L) for an extensive understanding of the physiological stress response, from 41 calves undergoing taming and 16 control adults in the same environment but not undergoing taming. I found that all five measures increased by at least 50% in the calves with most peaking around 2-3 days after taming began, and whilst increases were also observed in the control adults for some measures they were not to the same extent. Three of the calf measures stabilised after the first week (FGM, SC, glucose), but CK and H:L took one-two months to stabilise respectively, the latter of which could be an indication of chronic stress. The most extreme increases were seen in creatine kinase, which rose by >900%, likely reflecting muscle damage in calves struggling against ropes, though there were large differences between individuals. Increases of a comparable magnitude have been reported in the same measures in past studies of animals following transportation (Tarrant *et al.*, 1992; Marco *et al.*, 1997; Knowles, 1999; López-Olvera *et al.*, 2006), as well as in aversively trained dogs (Beerda *et al.*, 1998; Schalke *et al.*, 2007; De Castro *et al.*, 2020), though the durations of effects in these studies were mostly assessed in the short term (<24 hours). A past study of elephant FGM following transportation showed increases of greater magnitude, with effects lasting up to one month (Laws *et al.*, 2007; Millspaugh *et al.*, 2007).

I also gathered mahout perspectives on the taming procedure from those engaged in it, and when compared to answers given in **Chapter I** at another time of year, more mahouts expressed pity for the calves during taming (80% vs 60%), but fewer were confident of the suitability of positive reinforcement training for MTE elephants (41% vs 66%). This could be due to the experience and risks of taming being fresh in their minds, with many mahouts citing the danger posed by the calves as a counterargument to positive training methods, which can be substantial (at least one calf in this study had been involved in the death of a mahout). It is thus vitally important for future changes to the taming procedure to prioritise not only the welfare of elephant calves but also the safety of the mahouts involved both directly in the taming and working later in life with tamed elephants. This is especially relevant as elephants trained using positive reinforcement methods have been shown to be less responsive to commands than those trained using negative reinforcement (Wilson *et al.*, 2015).

4 Summary/Conclusions

This thesis can be loosely divided into two parts: **Chapters I & II** provide insight into mahout-elephant relationships in the MTE, how they have changed in recent years and how they influence the elephants' physiology and behaviour, whilst **Chapters III & IV** focused on the taming procedure in the MTE, highlighting calf traits associated with higher mortality during taming and providing the first empirical assessment of calves undergoing traditional taming.

Whilst changes to the mahout profession have been highlighted in other countries across Asia (Phuangkum *et al.*, 2005; Hart and Locke, 2007; Vanitha *et al.*, 2009; Suter *et al.*, 2013), Myanmar has often been assumed to retain a reservoir of traditional mahout knowledge and expertise (Lair, 1997; Sukumar, 2003). This thesis provided evidence to suggest that mahout experience and long-term relationships may be lessening at least in the Sagaing region of Myanmar (**Chapter I**), which is home to the largest population (~1000) of MTE elephants (Hedges *et al.*, 2018). It seems the MTE mahout system is able to maintain high quality care despite these changes, with **Chapter II** providing no evidence that two elephant physiological stress measures related to measures of mahout age, experience and specific relationship lengths. However, the mahout-elephant relationship did have important implications for other measures of elephant physiology and elephant behaviour, which should be considered and accounted for both in this population and others undergoing similar changes, with particular attention to mahouts of young elephants and bulls whose behaviour can be particularly difficult to manage (Hart and Sundar, 2000; Vanitha *et al.*, 2009).

High juvenile mortality has been shown to be one of the biggest limitations to the growth of the MTE elephant population (Jackson *et al.*, 2019), with 25% of calves dying before the age of five years (Mar *et al.*, 2012). **Chapter III** of this thesis showed that there was a mortality peak around the taming age of four years in the MTE population, with mortality increasing by >50%, which was not the case in a population of wild African elephants (Moss *et al.*, 2011), highlighting taming as a key focus of elephant management to optimise both individual welfare and population sustainability. This thesis took a vital step in this direction. I first identified young and first-born calves to be particularly at risk during the taming

procedure but provided evidence of improvements to taming practises in recent decades in the MTE, seen in higher calf survival (**Chapter III**). I also provided the first empirical evidence that calves experience acute stress in the first 10 days of taming, and potentially chronic stress lasting up to two months (**Chapter IV**), though further investigation of how taming influences the calves' psychological wellbeing, their overall health and their long-term fitness is needed. I argue that developments in future taming techniques must prioritise both calf welfare and mahout safety.

Both mahout-elephant interactions and the traditional taming procedure are often discussed as major issues in the literature of elephant welfare, but empirical assessments of their impacts on elephants are rare or non-existent. This thesis provides much needed evidence of how these factors can influence elephant welfare and shows how important it is for future studies to include these factors in welfare assessments of the thousands of captive Asian elephants under varying management conditions around the world. This also highlights a major issue not only in global elephant care but in captive animal management in general, which is a lack of data-driven decisions and dependence on *folklore husbandry* (Arbuckle, 2013; Hosey and Melfi, 2019). *Folklore husbandry* describes practices established without proper evaluation or simply because it has “always been done that way” which can also reflect a lack of integration of empirical findings into applied management (Arbuckle, 2013). Although it is particularly prevalent in elephant management due to the tendency to form emotional connections to these iconic animals and particular pressure from the public which can lead to a sometimes *elephantocentric* view (Wemmer and Christen, 2008; Lainé, 2018), it is true of the captive management of many species.

Even practices meaning to improve welfare, which have become widely applied, often lack empirical evidence to support their use, such as free contact handling for elephants (Proctor and Brown, 2015). Lack of assessment does not suggest practices are necessarily detrimental, but there should be more systematic assessment of both existing and introduced management practices to ensure decisions are evidence-based (Hosey and Melfi, 2019). The studies in this thesis provide a framework for conducting assessments of the impacts of human-animal interactions, covering indicators ranging from animal health, endocrinology, immunology and behaviour to inform management decisions which could be applied both in the context of elephant management and studying human-animal interactions in general. As human populations grow and expand, more and more species are forced into regular human contact and closeness, both in captivity and the wild, and it is crucial to understand the impact of such interactions in order to mitigate costs to animal health, reproduction and survival.

The most significant findings from this thesis are outlined below:

- The MTE elephant keeping system differs from assumed lifelong relationships and generational knowledge transfer (**Chapter I**)
- Mahout-elephant relationship lengths and mahout experience can have important implications for elephant physiology and behaviour (**Chapter II**)
- Calves born later in the year and to less experienced mothers historically had increased mortality at taming ages, whereas overall taming survival has tripled since the 1970s (**Chapter III**).
- Five measures of physiological stress monitored in calves during their taming increased by 50->900% in the first week of taming indicating acute stress, and one indicated potential evidence of chronic stress lasting one-two months (**Chapter IV**).

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I hope this helps to answer the recurring, ever-asked question of "How are the elephants?"

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Jennie Crawley

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