Effects of captivity and implications for *ex situ* conservation: with special reference to the red panda (*Ailurus fulgens)*

Submitted by Kristen Rebecca Jule to the University of Exeter as a thesis for the degree
of Doctor of Philosophy in Animal Behaviour, May 2008.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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ABSTRACT

This PhD thesis aims to improve the *ex situ* conservation of threatened and endangered species by investigating the effects of captivity and improving the methodology of current conservation techniques. The use of reintroduction as a tool for the purpose of conserving species is becoming increasingly popular. Since many wild populations are declining, captive-bred stock are frequently used to restore or supplement wild populations. Evidence suggests that captive-bred animals are less successful than their wild counterparts, but this has not been recently reviewed and there is limited research into investigating what aspects of captivity may be affecting success. Here, I conduct a review of carnivore reintroductions for projects carried out post 1990, which shows that captive-born animals are less likely to survive a release into the wild than their wild-caught translocated counterparts. A case study species, the endangered red panda (*Ailurus fulgens*), is used to investigate how a species involved in captive breeding for conservation responds to life in captivity. Results from analyses of lifetime reproductive success (and related variables) showed that both adaptation to captivity and inbreeding depression are occurring in the global captive red panda population. An investigation into behavioural adaptation to captivity was less revealing, although only generations three to seven from the wild were observed. The effects of captive environment and husbandry regime were also investigated and revealed that the size of the useable area and amount of human contact were among the factors influencing the behaviours of red pandas. How these findings contribute to a greater understanding of effects of captivity is discussed. The use of selection criteria based on temperament was also investigated in order to improve the likelihood of survivorship upon release into the wild. This method needs to be tested in practice, but based on the selection criteria used, there was evidence that unsuitability for release was positively predicted by generation time in captivity. Implications for the future use of captive red pandas in efforts to conserve the species in the wild are discussed, as well as how these findings can be utilised for other species involved in conservation efforts.

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STATEMENT OF THE CANDIDATE'S CONTRIBUTION TO CO-AUTHORED PAPERS

There are five studies included in this thesis, three of which have been written up as manuscripts for publication. As detailed below, the substantial contribution to the co-authored papers presented in this thesis was made by the candidate. However, while the candidate is fully responsible for the work presented in this thesis, where the first person is used it is in the plural (i.e., 'we' rather than 'I') as in the original peer-reviewed articles, to reflect the collaborative efforts guiding the research process. All chapters that have been written up as manuscripts are presented in the format requested by the respective journal; since each manuscript is meant to stand alone, some information may be redundant.

Paper 1: Chapter 2

Jule, K. R., Leaver, L.A., & Lea, S.E.G. (2008). Effects of captive experience on reintroduction survival in carnivores: A review and analysis. *Biological Conservation 141*(2), 355-363.

The first manuscript, presented in this thesis as Chapter 2, was submitted as a paper to the journal *Biological Conservation* and was accepted in November 2007. The data were collected and analysed by the candidate, with statistical advice from Prof. Stephen Lea. The paper was written with supervisory support from Dr. Lisa Leaver and Prof. Stephen Lea.

Paper 2: Chapter 3

Jule, K. R., Lea, S.E.G., & Leaver, L. A. (in prep.). Using a behaviour discover curve to predict optimal observation: captive red pandas (*Ailurus fulgens*) as a case study.

The second manuscript, presented in Chapter 3, was submitted the journal *Animal Behaviour* but was not deemed novel enough. It is currently in preparation for submission to *Conservation Biology*. The candidate designed the methodology of data collection as well as collected and analysed the data. Prof. Stephen Lea contributed to the model equation and the theoretical application of the model. The paper was written with supervisory support from Dr. Lisa Leaver and Prof. Stephen Lea.

Paper 3: Chapter 6

Jule, K. R., Lea, S.E.G., & Leaver, L. A. (under review). Examining the use of behavioural assessment in captive-born animals to identify temperament traits associated with reintroduction success. *Animal Conservation.*

The third manuscript, presented in Chapter 6, was submitted to the journal *Animal Conservation* in April 2008. The candidate designed the questionnaire, collected the data, analysed the data and wrote the paper with supervisory support from Dr. Lisa Leaver and Prof. Stephen Lea.

STATEMENT OF THE SUPERVISORS' CONTRIBUTION TO CO-AUTHORED PAPERS

As outlined in the candidate's statement above, the substantial contribution to the co-authored papers presented in this thesis was made by the candidate. This includes the review of the literature presented in each paper, study design, statistical analyses and interpretation of the data, together with the write-up for publication. The supervisors contributed to the papers by advising on statistical analyses and interpretational issues, relevant literature, and writing style. Moreover, the theoretical framing of the empirical work in this thesis and the arrangement of the papers is a product of a concerted discussion of the thesis content between the candidate and supervisors.

Dr. Lisa A. Leaver (first supervisor)

Prof. Stephen E. G. Lea (second supervisor)

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Chapter 1

Using red pandas as a case study to investigate the effects of captivity and their implications for *ex situ* conservation

Overview of thesis structure and hypotheses:

Introduction to thesis

This PhD thesis aims to improve *ex situ* conservation by providing a greater understanding of the effects of captivity as well as proposing improved practical and applied methodological techniques. I begin by introducing the objectives and relevant questions of the following chapters. A schematic representation of how the chapters contribute to our understanding of the effects of captivity and improved *ex situ* conservation is presented in Figure 1.1.

The literature review in this introductory chapter is broken into two sections. In Part 1, I introduce some of the history leading to the formation of reintroduction guidelines and review the use of captive-born animals in reintroductions; I then address some of the key factors relating to the successes and/or failures of reintroductions in order to provide a greater understanding of reintroduction as a conservation technique. In Part 2 of this introductory chapter, I review the literature on my selected species of study, which is the red panda (*Ailurus fulgens*). I conclude the chapter by reiterating the thesis aims and the rationale for the case study selection.

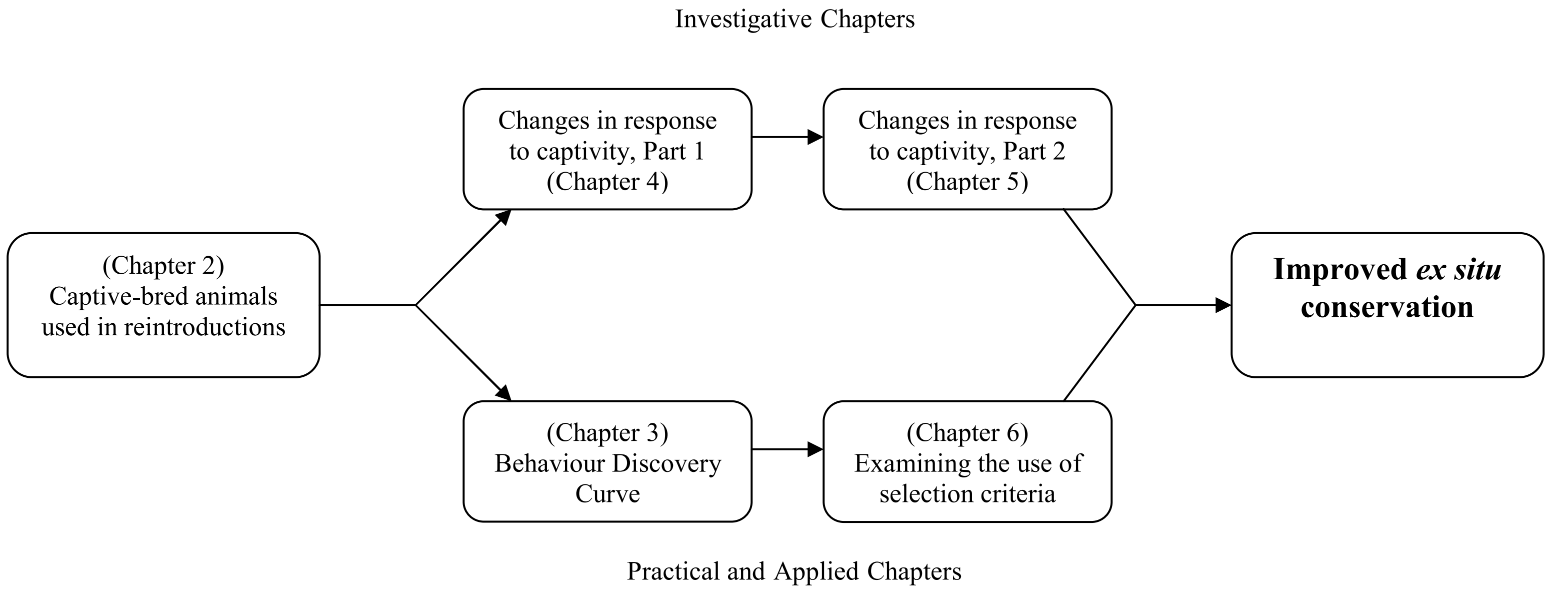

Figure 1.1. Schematic representation of how the chapters contribute to the overall aims laid out for this thesis

Brief outline of thesis

Since previous literature suggests that captive-born animals do not fare as well as wild-caught animals when released into the wild, the first aim of this thesis was to identify differences in survival between wild-caught and captive-born animals (focusing on carnivores) used in reintroductions; I explore this in Chapter 2. I next wanted to formalise a method for determining optimal observation time of animals both in the wild and in captivity. Behavioural observations are vital to furthering our knowledge of species' ecology. Having a method that formalises the length of behavioural observation time (coined Behaviour Discovery Curve in this thesis) is practical for both reducing disturbance to the animals and limiting costs to the researcher. The methods devised to determine the rate of behaviour 'discovery' are presented in Chapter 3 using captive red pandas (*Ailurus fulgens*) as a case study.

In addition to improving methodological techniques, the importance of investigating the effects of captivity is critical to improving *ex situ* conservation. As the release of captive-born animals in reintroductions becomes an increasingly popular trend, efforts need to focus on improving the success of these ventures. Adaptation to captivity, inbreeding depression, habituation towards humans and the physical aspects of the captive environment are all factors which contribute to how a species responds to life in captivity. These aspects will be investigated in Chapters 4 and 5.

Methods to improve the likelihood of survival of captive-born animals in reintroductions are also vital to conservation efforts. Using temperament characteristics as selection criteria for an individual's suitability for release into the wild is one potential method for improving survival success in released captive-born animals. Chapter 6 investigates this method for a captive population of red pandas housed in the UK.

Literature review: Reintroduction and the red panda (Ailurus fulgens)

Part 1.

Reintroduction as a tool for *ex situ* conservation

Brief Introduction and Background

Humans have a long history of translocating animals around the planet, whether through intention or not. Over the past half a century, there has been a marked increase in reintroduction and translocation projects of animals, primarily for the purpose of replenishing native game species (Griffith, Scott, Carpenter, & Reed, 1989). More recently, translocation for the purpose of re-establishing endangered animals into their native habitats has become increasingly popular (MacKinnon & MacKinnon, 1991; Stuart, 1991). The idea of reintroducing animals to their native habitats is not as new as one might suspect. In fact, reintroductions of capercaillie (*Tetrao urogallus*) have been recorded in Scotland as early as 1837, nearly 100 years after their extinction in the UK.

Reintroduction is considered by some as a tool with the potential to save many species from extinction (Kleiman, 1989; MacKinnon & MacKinnon, 1991; Sarrazin & Barbault, 1996; Seal, 1991; Stuart 1991; Tear, Scott, Hayward, & Griffith, 1993). However, reviews have found that translocations of native game species have been more successful (81-86%) than translocations and reintroductions of endangered species for conservation purposes (44-53%) (Griffith et al., 1989; Wolf, Griffith, Reed, & Temple, 1996). This suggests that the use of translocations and reintroductions as tools for the conservation of endangered animals needs to be further improved upon in order to increase success rates.

Formation of reintroduction guidelines

Despite the large number of translocations/re-introductions of animals, reintroduction success is relatively low (from 17% to 56%) (Fischer & Lindenmayer, 2000; Wolf et al., 1996). The first formal guidelines were set by the World Conservation Union (otherwise referred to as the International Union for the Conservation of Nature and Natural Resources or IUCN) when they released their Position Statement on the Translocation of Living Organisms in September 1987.

Following this statement, Devra Kleiman (1989) and Mark Stanley Price (1989), both of whom closely associated with well known reintroduction projects, published more detailed guidelines. They compiled criteria based on their experiences, Kleiman with the golden lion tamarin (*Leontopithecus rosalia*) project at Pocos das Antas in Brazil and Stanley Price with the Arabian oryx (*Oryx leucoryx*) in Oman.

The next large step in creating guidelines for reintroductions was a symposium entitled 'Re-introducing Endangered Mammals to the Wild' which was held by the Zoological Society of London in 1989 and the proceedings published in 1991 (Chivers, 1991; Gipps, 1991; Kleiman, Beck, Dietz, & Dietz, 1991; Stanley Price, 1991). This symposium paved the way for stricter guidelines that were set forth by the IUCN's Species Survival Commission (SSC) Re-introduction Specialist Group (RSG) and approved at the 41st Meeting of the IUCN Council in May 1995 and published in 1998 as the formal (but not all inclusive) IUCN Guidelines for Re-introductions. A comprehensive list of the reintroduction guidelines set forth by the IUCN (1998) can be seen in Table 1.1. Important points particularly relevant to this thesis are highlighted in bold.

The following are definitions of terms used by the IUCN's 1998 Guidelines (p.6): A ***re-introduction*** is an attempt to establish a species in an area which was once a part of its historical range, but from which it has been extirpated or become extinct.

A ***re-establishment*** refers to a reintroduction that has been successful. A ***conservation or benign introduction*** is an attempt to establish a species, for the purpose of conservation, outside its recorded distribution but within an appropriate habitat and eco-geographical area. This is only encouraged when there is no remaining area left within a species' normal historic range. A ***translocation*** is a deliberate and mediated movement of wild individuals or populations from one part of their range to another (this is not always done for conservation purposes, i.e. the goal may be restocking game species). A ***supplementation or re-inforcement*** is when individuals are added to an existing population of conspecifics.

The IUCN Guidelines for Re-introductions (1998) set the following aims and objectives for a re-introduction (p.6):

Aims:

1) To establish a viable, free-ranging population in the wild, of a species, sub-species, or race, which has become globally or locally extinct or extirpated in the wild.
2) Re-introduction should be within the species' former natural habitat and range and should require minimal long-term management.

Objectives:

1) To enhance the long-term survival of a species
2) To re-establish a keystone species (in the ecological or cultural sense) in an ecosystem.
3) To maintain and/or restore natural biodiversity.
4) To provide long-term economic benefits to the local and/or national economy.
5) To promote conservation awareness.
6) Any combination of the above.

Table 1.1 Summary of the IUCN Guidelines for Re-introductions (1998).

- I. Multi-disciplinary approach
  - a. projects should include the combined resources of
    - i) Government Natural Resource Management Agencies
    - ii) NGO’s (Non-governmental organizations)
    - iii) Funding bodies
    - iv) Universities
    - v) Veterinary Institutions
    - vi) Zoological/Botanical gardens
- II. Pre-project planning activities
  - a. Biological
    - i) Feasibility study and background research
    - ii) Review of previous re-introductions
    - iii) Choice of release site and type (e.g. within historic range?)
    - iv) Evaluation of re-introduction site (e.g. habitat sustainability)
    - v) Availability of suitable release stock
      - a. Preference for wild stock, but only if wild population sustain genetic loss?
      - b. Captive stock must be genetically managed
      - c. Minimal disease risk
    - vi) Release of captive stock (p.9)
      - a. ”Most species of mammals and birds rely heavily on individual experience and learning as juveniles for their survival; they should be given the opportunity to acquire the necessary information to enable survival in the wild, through training in their captive environment; ***a captive bred individual’s probability of survival should approximate that of a wild counterpart***.”
      - b. “Care should be taken to ensure that potentially dangerous captive bred animals (such as large carnivores or primates) are not so confident in the presence of humans that they might be a danger to local inhabitants and/or their livestock.”
- III. Socio-economic and Legal requirements
  - a. Long-term financial and political support
  - b. Assess impact to humans
  - c. Assessment of local attitudes to ensure long-term protection (in particular, if cause of decline was human factors)
  - d. Proper planning for potential risk of life and/or property
- IV. Planning, preparation, and release stages
  - a. Secure approval from all bodies, e.g. governments, land owners, funding bodies
  - b. Identification of short- and long-term success indicators
  - c. Design of pre- and post-monitoring programmes (and possible intervention policies)
  - d. Health and genetic screening of stock (including vaccinations if necessary)
  - e. Development of transport plans and release strategy
  - f. Development of conservation education and public relations
  - g. ***Of paramount concern throughout all stages is the welfare of animals for release***
- V. Post-release activities
  - a. Post release monitoring of all (or a sample) individuals (via tagging, informants, etc…)
  - b. Demographic, ecological, and behavioural studies of released stock *must* be undertaken
  - c. Study of long-term adaptation
  - d. Collection/Investigation of mortalities
  - e. Interventions (e.g. supplemental feeding, removal of animals)
  - f. Decisions for revision or discontinuation
  - g. Habitat protection/restoration
  - h. Continued public relations and education
  - i. Evaluation of success techniques
  - j. Regular publications in scientific/popular literature

Source of animals used in reintroductions

This thesis focuses on *ex situ* conservation, principally, the viability of using captive born animals in reintroductions. 'Source' refers to whether animals were wild-caught from a sustainable wild population existing elsewhere or obtained from captive breeding stocks. In most cases of trans-locating game species, the stock comes from a stable wild population. However, frequently the reason behind trans-locating and reintroducing animals for conservation purposes is due to a declining wild population. Thus, founder stock for conservation translocations and reintroductions are increasingly being sourced from captive populations (Ebenhard, 1995). There are many risks involved when reintroducing captive-born animals, however the main concern of conservationists is the loss of behaviours and traits associated with "wild fitness" (Gipps, 1991; Lindburg, 1994; Rabin, 2003). The implications of this decrease in wild fitness on captive-born animals will be discussed further in Chapter 2 and alluded to in the remaining chapters.

The Universities Federation for Animal Welfare (UFAW) has written guidelines specific to the welfare of reintroduced captive-bred animals to the wild (1992). The guidelines highlight the need for ensuring that "… [captive bred animals'] chances of survival should be equivalent to those of wild bred animals of similar age, sex, and status in the natural habitat" (p.1). The guidelines recognise captive-bred animals' lack of development in skills associated with survival and therefore stress the importance of preparing the animals for survival under natural conditions, including training, development of behaviour and/or skills, and appropriate selection criteria (such as age, sex, character, social group and health) to ensure that the most suitable animals are likely to be released (UFAW 1992). The American Association of Zoos and Aquaria (AZA) have also written guidelines for the reintroduction of animals born or held in captivity (1992). The main aims of the UFAW (1992), AZA (1992) and IUCN (1998) guidelines are to facilitate successful reintroductions while considering the well-being and welfare of the animals released. The Wildlife and Countryside Act of 1981 also prohibits releasing animals in circumstances that may inflict unnecessary suffering (DEFRA 1981).

Based on the considerations addressed above, some species conservation groups do not support releasing captive bred animals as a conservation technique. The European Association for Aquatic Mammals (EAAM) released an official statement of their position on the release of long-term captive cetaceans, maintaining that there is lack of evidence to suggest release is a viable method for conservation, and further stating that in the few cases where the outcome was known, most animals did not fare well. They conclude their statement by adding that "the success of reintroducing cetaceans to the wild will depend upon resources, methodologies, and techniques which still need to be tested" (EAAM 2004). The arguments that the EAAM present against the use of captive bred animals in reintroducing are viable concerns and only emphasise the need for further investigation into the effects of captivity and its implications on conservation efforts.

However, other researchers, such as Theodorou & Couvet (2004), suggest that using captive animals for release programmes can be beneficial when certain criteria are met: namely that i) the length of the captive breeding programme does not exceed a reasonable time frame (e.g. 20 generations) ii) introduction of captive born individuals should be kept at low levels (e.g. 1 or 2 individuals per generation), so that captive-born animals are only used as supplemental genetic stock and iii) the size of the captive population is reasonably large (relatively low inbreeding depression).

The main limitation in Theodorou and Couvet's (2004) recommendations is that they only consider the genetic consequences of captivity but do not consider captive animals' loss of appropriate behaviours associated with success in the wild. Learned behavioural (e.g. social interactions and hunting) traits can be lost more rapidly than genetic diversity in captivity and the further generation from the wild, the greater the loss of behaviours associated with wild fitness and survival (May, 1991; Rabin, 2003; Wallace, 2004). Traits associated with 'wild fitness' have been found to be negatively correlated to traits associated with adaptation to captivity (Gilligan & Frankham, 2003). Research carried out on the common fruit fly, *Drosophila melanogaster*, demonstrated that increases of reproductive fitness associated with adaptation to a captive environment corresponded directly with a significant reduction in reproductive fitness under conditions representing the 'wild' environment (Woodoworth et al., 2002). Based on this assumption,the terms 'adaptation to captivity' and 'captive fitness' will be used interchangeably throughout this thesis to denote the change in both behaviour and genetics as animals become better suited to life in captivity. Chapters 4 and 5 of this thesis will investigate how one species, the red panda, responds to life in captivity.

Pre-release training and experience have become increasingly popular in preparing captive-born animals for life in the wild. Techniques include providing naturalistic enclosures, training predator avoidance and providing hunting opportunities (Box, 1991; McLean, Lundie-Jenkins & Jarman, 1996; Biggins, Vargas, Godbey & Anderson, 1999; McLean, Holzer & Studholme, 1999; Griffin, Blumstein & Evans, 2000; Beck, Castro, Stoinski & Ballou, 2002). Along with a loss of wild-type behaviours, captive-born animals are also likely to have an increased habituation towards humans;

therefore, pre-release techniques have also been developed to help dishabituate animals from humans (Soorae & Stanley Price, 1997).

General review of reintroductions for conservation

Many well known reintroductions that have been carried out for the purpose of conservation, such as the California condor(*Gymnogyps californianus*), the black-footed ferret (*Mustela nigripes*), the golden lion tamarin (*Leontopithecus rosalia*), the Arabian oryx (*Oryx leucoryx*), and the red wolf (*Canis rufus*). It is from these successes and failures that we can learn how to improve future reintroduction efforts. For the purpose of this review there are far too many reintroduction projects to discuss individually (Kenyon, 1995); therefore, I will focus on previously published reviews of reintroduction projects.

There have been several reviews and assessments of reintroduction projects (Beck, 1995; Beck, Rapaport, Stanley Price, & Wilson 1994; Breitenmoser, Breitenmoser-Wursten, Carbyn, & Funk, 2001; Fischer & Lindenmayer, 2000; Griffith et al., 1989; Reading & Clark, 1997; Stanley Price, 1991; Wolf et al., 1996) but one large problem that remains consistent in all the reviews is a universal definition for success. Many researchers have attempted to define reintroduction success (Fischer & Lindenmayer, 2000; Kleiman et al., 1991; Kleiman et al., 2000; Seddon, 1999; Stanley Price, 1991), however, there are several difficulties with setting minimum success criteria.

Assessing reintroduction projects as a success or failure is problematic and can potentially be misleading. For example, self-sustainability does not necessarily imply long-term success of a population. Wolf and colleagues (1996) found that a portion of reintroduction projects classified as successful in 1987 had declining populations five years later. Classifications of project successes also have other dangers since they can lead to assumptions that there is an end-point after which new releases or continued monitoring of projects may no longer be required. Therefore, a reintroduction can only be considered as successful at a specific point in time. As a case in point, the re-introduction of the Arabian oryx has been considered to be one of the flagship re-introduction success stories (Stanley Price, 1989). However, continued poaching in the following decade left the population no longer viable (Seddon, 1999). There have been several subsequent supplementations to the population and this highlights the importance of setting initial criteria to determine the viability of a reintroduction project, since a “successful” reintroduction depends largely on solving why the population declined in the first place.

Aspects affecting success of reintroduction programmes

Previous reviews have highlighted several factors that contribute to the success and/or failure of a re-introduction project. For initial planning and preparation of a project, clarifying the *organisational aspects* is critical; this includes ensuring proper multi-organisational cooperation, which often ranges from local to national governments and non-profit organisations (Kleiman & Mallinson, 1998; Reading & Clark, 1997; Reading, Clark, & Griffith, 1997). Stanley Price (1991) specifically recommends that reintroduction programmes should be incorporated into national and international conservation strategies and they should incorporate a multi-disciplinary and scientific approach.

Procurement of secure long-term funding (Fischer & Lindenmayer, 2000; Lindburg, 1992; Soorae & Stanley Price, 1997) is also an important factor affecting success. The costs of many projects range into the thousands, even millions of dollars. Kleiman and colleagues (1991) reported estimated costs of approximately $22,000 per surviving individual in the golden lion tamarin (*Leontopithecus rosalia*) reintroduction programme. Between 1989 and 1991, it is estimated that the U.S. federal and state agencies spent $31,300,000 on the bald eagle (*Haliaeetus leucocephalus*), $13,600,000 on the Florida panther (*Puma concolor coryi)*, and $12,600,000 on the brown bear (*Ursus arctos*) (Soorae & Stanley Price 1997); the reintroduction of wolves (*Canis lupus*) in Yellowstone National Park and in central Idaho was estimated in 1996 to have cost $6,700,000 (Fischer & Lindenmayer, 2000). Due to the high costs associated with reintroduction projects, securing long-term funding is essential in ensuring that the project can be carried out in full, including appropriate post-release monitoring.

The *valuational aspects* surrounding re-introductions are also important, and in addition to long-term funding support, there should be a large amount of community support and the project should make efforts to educate the public (Kleiman et al., 1994; Reading & Clark, 1997; Wolf et al., 1996; Yalden, 1993). Human related incidents are the highest reported causes of death in animals involved in reintroductions; human intervention includes shooting, poisoning, capturing, and poaching (Soorae & Stanley Price, 1997; Woodroffe, Ginsberg & Macdonald, 1997). For carnivore species, many of these acts are done in retaliation for the loss of livestock and/or competitive resources. This again stresses the need for solving the cause of initial decline, which in most endangered carnivore species results almost exclusively from conflicts and/or competition with humans (Linnell, Swenson & Anderson, 2001). This in turn emphasises the futility of reintroducing animals when the surrounding community is not in support of the project. Extensive efforts should be made to reduce the risk of a poor investment (namely, failure of the re-introduction project), but also to reduce the risk of poor welfare (Beck, 1995) and/or fatalities of the released animals.

A key factor in attempting a successful re-introduction is identifying the reason for initial decline. As mentioned previously, a species' decline is often due to conflicts with humans and the reason for initial extirpation must be removed before a reintroduction can be successful. Loss of suitable habitat is often also a large factor in the cause of initial decline. The IUCN reintroduction guidelines (1998) state that the specific purpose of a reintroduction programme is to reintroduce animals back into their historic range, but in many cases, a species' initial decline is due to loss of habitat. In these cases, habitat restoration is the only way that animals can be reintroduced back into their historic range. In cases where habitat restoration is not possible (i.e. because of human development) in a declining population, then a benign introduction of a species may be considered outside of the animals' historic range. In either case, habitat suitability needs to be carefully considered prior to release, as well as the long-term effects of reintroducing or introducing a species to a particular area.

Studies on conservation efforts suggest that habitat destruction and fragmentation are the greatest threats to biodiversity (Bright, 2000), and that habitat characteristics were the most important factors affecting the reintroduction success of mustelids (White, McClean & Woodroffe, 2003). Characteristics within habitat suitability include: habitat management, viable self-sustaining prey and/or food densities, awareness of the impact of predation and/or foraging on the habitat, limited or no competitor species, suitable amount of area available for breeding sites, and sufficient amount of cover for animals that require refuge (Breitenmoser et al., 2001; Griffith et al., 1989; MacKinnon & MacKinnon, 1991; Miller, Ralls, Reading, Scott, & Estes, 1999; Reading & Clark, 1997; Wolf et al., 1996).

Release protocols

There are several aspects regarding the release of animals in a reintroduction or translocation. Releases can be either "hard" (immediate release, with little or no provisioning) or "soft" (acclimatisation period, sometimes with prolonged provisioning) (Kleiman 1989). It is generally believed that "soft" releases are better for animals, in particular for captive-born animals, and can improve reintroduction success (Bright & Morris, 1994; Letty, Marchandeau, Clobert & Aubineau, 2000). Soft releases often include additional aspects of pre- and post- release methods; these include behavioural training, preventive health care, socialisation, acclimatisation and provisioning (Beck et al., 1994; Reading & Clark, 1997). The extent to which these are carried out are often determined by assessment of costs, feasibility, and relative contribution to survivorship. Behaviour assessments, pre-release training, health screening and disease management will all be discussed in further detail shortly.

Another aspect of release protocol that has been investigated in relation to re-introduction success is the timing of release. Seasonal timing of releases has been found to have implications for successful breeding and/or survival (Bright & Morris, 1994). The survival and success of reintroduced animals depends heavily upon the environmental and social conditions the animal must confront, such as food availability, weather conditions and temperatures, as well as the breeding season of the existing wild population. For example, for trans-located black (*Ursus americanus*) and brown (*Ursus* *arctos*) bears, the season of release had a direct correlation on the success of the reintroduction; winter releases resulted in greater survival and reduced post-release movements (Clark, Huber & Servheen 2002) primarily due to the reduced likelihood that an animal would return to its home territory during the hibernation season.

The number of animals needed for release to sustain a viable population is a much studied, highly debated topic (Foose, 1991; Franklin & Frankham, 1998; May, 1991; Reading & Clark, 1997; Soule, 1987; Stanley Price, 1991). From an initial reintroduction success perspective, previous reviews have found that projects releasing fewer animals have a higher risk of failure than projects with larger numbers (Breitenmoser et al., 2001; Fischer & Lindenmayer, 2000). However, a more long-term concern regarding the number of animals released is the genetic considerations of a minimum viable population (MVP). When previously large populations quickly decline, they are susceptible to decreased viability and fecundity caused by inbreeding depression (May, 1991).

Inbreeding depression is of particular concern when a species becomes endangered and loses genetic variability. In captivity (which can be considered an isolated system) loss of genetic variability can continue for several generations. The danger of inbreeding depression has highlighted the need to determine a minimum viable population size (MVP) which, if it fell any lower, would result in the loss of genetic variability and would therefore likely imperil a population’s long-term survival. Although many prefer to ascribe a population size of 500 as an MVP, and the IUCN guidelines recommends this as a population size in their definition of a minimum self-sustaining population – viable population size actually depends on a variety of factors such as habitat type and mating and breeding strategies (Foose, 1991; Franklin & Frankham, 1998). Not only is the maintenance of individual populations important, but also the genetic management of existing meta-populations, which are collections of distinct sub-populations (Foose, 1991). Meta-populations, managed appropriately, can ensure sufficient genetic variability of a species. In effect, maintenance of genetic diversity within a meta-population and sub-populations is how captive breeding should be managed and this will be discussed further in Chapter 4.

Hence, the number of animals that should be released in a reintroduction project depends upon the need for genetic variability, the species-specific reproductive output and the purpose of the reintroduction (i.e. whether it is to establish a new population or to supplement an existing wild population). In any case, genetic considerations need to be addressed in order to reduce the risk of inbreeding depression, population decline and irreversible genetic loss.

Animal selection considerations

There are factors aside from source that have been found to the overall success of a reintroduction project. As mentioned previously, genetic representation must be considered and the degree in which the animal is inbred can directly affect its selection in a reintroduction project (Earnhardt, 1999), as well as its generational length of time in captivity.

Another consideration in animal selection is the gender of the animal (Kleiman, 1996; Letty et al. 2000; Moehrenschlager & Macdonald, 2003). Depending upon certain environmental or social situations, females or males tend to fare better or have a higher likelihood of reproducing. In many cases, females can integrate better into an existing population (Kleiman, 1996); however, males tend to travel greater distances and can establish larger areas (Letty et al., 2000). Moehrenschlager & Macdonald (2003) found that female swift foxes (*Vulpes velox*) were less likely to survive than males and therefore recommended that a greater proportion of females should be released in future translocations.

The influence of age at time of release has also been investigated (Kleiman,1996; Robert, Sarrazin, Couvet & Legendre, 2004). There is some contradictory evidence on the success of releasing adults versus juveniles. Robert et al. (2004) found that adults were more likely to survive than juveniles, but that juveniles were more likely to breed and are therefore important in boosting the genetic viability of future generations.

The health of the animals is also important in reintroductions (Bradshaw & Bateson, 2000; Bush, 1994; Cunningham, 1996; Woodford & Rossiter, 1994), both of the reintroduced animals as well as the existing wild population (in the case of a supplementation). Diseases can be carried by released animals, and have the potential to wipe out existing populations. Captive-born animals are particularly affected by infectious diseases and pathogens (Beck et al., 1994; May, 1991; Lafferty & Gerber, 2002), and although preventive health care (e.g. inoculations) can help protect founder stocks (Beck et al., 1994; Bush, 1994), second and third generation released animals are still susceptible to diseases in the wild that they may not be immune to.

Success upon release into the wild has also been attributed to temperament . The survival of captive-born released swift foxes (*Vulpes velox*) was found to be negatively correlated with boldness towards the captive environment (Bremner-Harrison et al., 2004). Appropriate selection of animals based on certain behavioural traits has the potential to greatly improve both *ex situ* conservation efforts on the whole, as well as individual project success (Mathews et al., 2005; McDougall, Reale, Sol & Reader, 2006). A method developed to select animals suitable for reintroduction is presented in Chapter 6.

In order to better evaluate the factors contributing to the successes and/or failures of reintroduction projects, more post-monitoring needs to be carried out on these projects. There are limitations in current projects on the length of time animals have been monitored after release, and there is also a lack of data on individual successes and failures. Many review papers also stress the difficulties in obtaining data on reintroductions and a difficult obstacle facing the reviews of reintroductions is publication bias (Fischer & Lindenmayer, 2000). It is much more difficult to find information on failed reintroduction projects, and reviews on reintroductions are therefore inherently biased by the published information that is available.

Taxonomic bias in reintroduction and in selection of species for re-introduction

Conservation societies often use flagship species, such as giant pandas (*Ailuropoda malanoleuca*), to promote conservation causes. Flagship species are often chosen for their visual appeal, i.e. they are often mammals, and typically large. Preferential species selection can also be seen in reintroduction projects, such that mammals, and to a lesser extent birds, are over-represented (Seddon et al. 2005). Despite their even greater endangered status, fish, amphibians, reptiles, invertebrates and plants comprise a much smaller ratio of reintroduction projects. Within mammals, two orders are particularly over-represented in reintroduction projects, artiodactylids (e.g. ungulates) and carnivores (Seddon, Soorae, & Launay, 2005).

This taxonomic bias can also been seen in the animals involved in endangered species breeding programmes in zoological parks. Mammals comprise 75% of the European Association of Zoos and Aquariums (EAZA) European Endangered Species Program (EEP), birds 20% and the remaining 5% consists of invertebrates and reptiles (EAZA, 2008).

Criteria for reintroduction

There are other factors besides public appeal that contribute to selection of species for reintroduction. With the formation of guidelines, criteria have also been designed to determine the suitability of a species for reintroduction. Extensive pre-planning is required for reintroduction projects and it is considered unethical to release captive bred animals simply because there may be a surplus in captivity (Kleiman, 1996; Lindburg, 1991). The welfare of the released animals must be of large import (Beck 1995; Bradshaw & Bateson 2000), as must be the success of the project.

Based on the guidelines set by the IUCN (1998), certain criteria must be met when considering a species for reintroduction (Kleiman, Stanley Price, & Beck, 1994). The following table (Table 1.2) assesses the feasibility of reintroducing giant pandas (*Ailuropoda malanoleuca*). At that point in time, a reintroduction project for pandas was not recommended, since a number of criteria were not met, such as insufficient founder stock, limited knowledge of the species' biology, and most importantly, the causes for initial decline had not been resolved. So, in spite of the panda's endangered status, the species was not a good candidate for reintroduction in 1994. The conclusion of this case study also has implications for the management of pandas in captivity. Due to relatively poor breeding success in captivity, continued removal of animals from the wild for breeding management/genetic variability was not recommended. Instead, *in situ* efforts such as habitat protection, which is understood to be the least expensive and most effective way of saving endangered species, should be targeted for this species in priority over *ex situ* efforts such as reintroduction and captive breeding. This is an example of why reintroductions or *ex situ* efforts should not be the only methods employed for conservation. Reintroductions have high criteria for acceptability and relatively low success rates. Considering the time, effort, and considerable funding required, reintroduction projects should be thoroughly reviewed before progression, in relation to alternative methods of conservation.

Table 1.2. Do appropriate conditions exist for the re-introduction of giant pandas (*Ailuropoda melanoleuca*) ? (scale 5 = best)

Condition of species	
1. Need to augment wild population	Yes
2. Available stock	No
3. No jeopardy to wild population	?
Environmental conditions	
4. Causes of decline removed	No
5. Sufficient protected habitat	No
6. Unsaturated habitat	No
Biopolitical conditions	
7. No negative impact for locals	No?
8. Community support exists	2
9. GOs/NGOs supportive/involved	Yes?
10. Conformity with all laws and regulations	?
Biological and other resources	
11. Re-introduction technology known/in development	1
12. Knowledge of species biology	2.5
13. Sufficient resources exist for programme	No
Recommended re-introduction/translocation	No

Table adapted from (Kleiman et al., 1994)

Part 2.

The red panda (*Ailurus fulgens*)

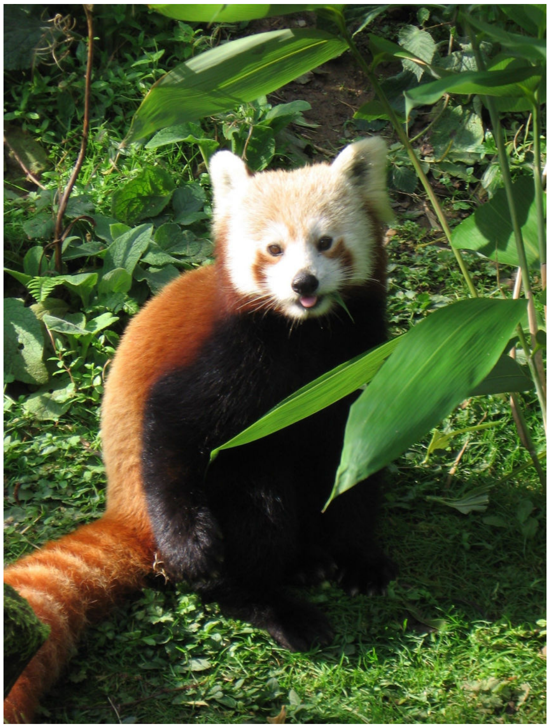

Photo by K. R. Jule

History

The first known written record of the red panda occurs in a 13th-century Chou dynasty scroll (Roberts, 1992). Red pandas were not recognised as a species in Europe and the western world until 1821 when English Major-General Thomas Hardwicke, identified the species in Nepal and brought a specimen back to London. There were several local names associated with the animal including “Wha”, which is a written description of the sound of its loud call, and “poonya” which has since been anglicised to “panda”. The French naturalist Frederic Cuvier gave the panda its official scientific name, *Ailurus fulgens* (which translates to brilliant coloured cat), in 1825.

For nearly half a century, *Ailurus fulgens* was the only known panda to Europe and the west, until a large black and white bear-like animal with a diet similar to that of the red panda was discovered in China in 1869. This animal was dubbed the "giant panda" and given the species name *Ailuropoda melanoleuca* to represent its believed relatedness to the red panda (Morris & Morris, 1968). *Ailurus fulgens* became the "lesser" panda, a name which has since become unpopular due to its inferior reference, so the *Ailurus* is now more commonly referred to as the “red panda”. The phylogenetic relatedness between the giant and the red panda is still controversial today, and the taxonomic and phylogenetic classification of the red panda is still highly debated (see section on genetics and classification and Figure 1.2 for discussion).

Since the discovery of the giant panda, research focus has shifted to the giant panda and there has been very little further study on the ecology of the red panda until relatively recently; and it is only in recent decades that the conservation value of red pandas has been recognised (Hunter, 1991). However, the conservation of both giant and red pandas has come under debate by advocates who suggest that these two species are ‘evolutionary failures’ due to their relatively low breeding rates and the combination of their low energy diets and lack of specialised digestive system (see Gittleman 1994 for a review).

Species Information

Red pandas are placed in the order Carnivora, the Superfamily Canoidea, the Family Ailuridae (although this is under some debate) and the genus *Ailurus*. Red pandas are placed in the order Carnivora due to their dentition, skull and jaw formation, and brain size relative to body size. All mammals in the order Carnivora are believed to be descended from a common ancestor from the Paleocene era thought to be carnivorous, although not all mammals currently within the order Carnivora are strict carnivores. For future reference, the term carnivore is used for red pandas to denote their taxonomic and phylogenetic placement rather than in reference to their diet. Pandas and their related ancestors are often referred to as herbivorous carnivores.

Historically, four species were identified after their initial discovery up to 1902- *Ailurus fulgens*, *Ailurus ochraceus*, *Ailurus refulgens* and *Ailurus styani*. However, it is likely that *ochraceus* and *refulgens* may have been synonymous and taxonomic evidence has since led to the classification of only one recognised species in the genus, *Ailurus fulgens*. There are now two recognised subspecies within this species, *Ailurus fulgens fulgens* and *Ailurus fulgens styani*. The former refers to animals found on the western slopes of the Himalayas (India, Nepal, Burma, Bhutan and Tibet) and the latter to animals found on the eastern slopes of the Himalayas (China's Sichuan and Yunnan provinces) (see Figure 1.2).

Genetics and Classification

The red panda is considered to be a living fossil; fossil remains of related ancestors, such as the Parailurus and the Simocyon – herbivorous carnivores – have been found across the earth from Western Europe, Russia and Japan (Wang, 1997; Sasagawa et al., 2003; Peigne et al., 2005; Sotnikova, 2008). There has been extensive research using mitochondrial DNA and RNA sequencing to uncover the phylogenetic placement of this species and its relation to fossil remains (Su et al., 2001; Liang et al., 2007; Zhang et al., 2008).

There are several phylogenetic hypotheses regarding the placement of red pandas including placement with ursids (Vrana et al., 1994), procyonids (Slattery & O'Brien, 1995; Wang, 1997), mustelids or in the Superfamily musteloidea (Flynn et al., 2005), or as a sister taxon to giant pandas (refer to Figure 1.1). However, the strongest evidence is that red pandas belong to their own distinct family (Ailuridae), which is placed nearer mustelids and procyonids than ursids (Ledja & Arnason, 1996; Bininda-Emonds et al., 2000).

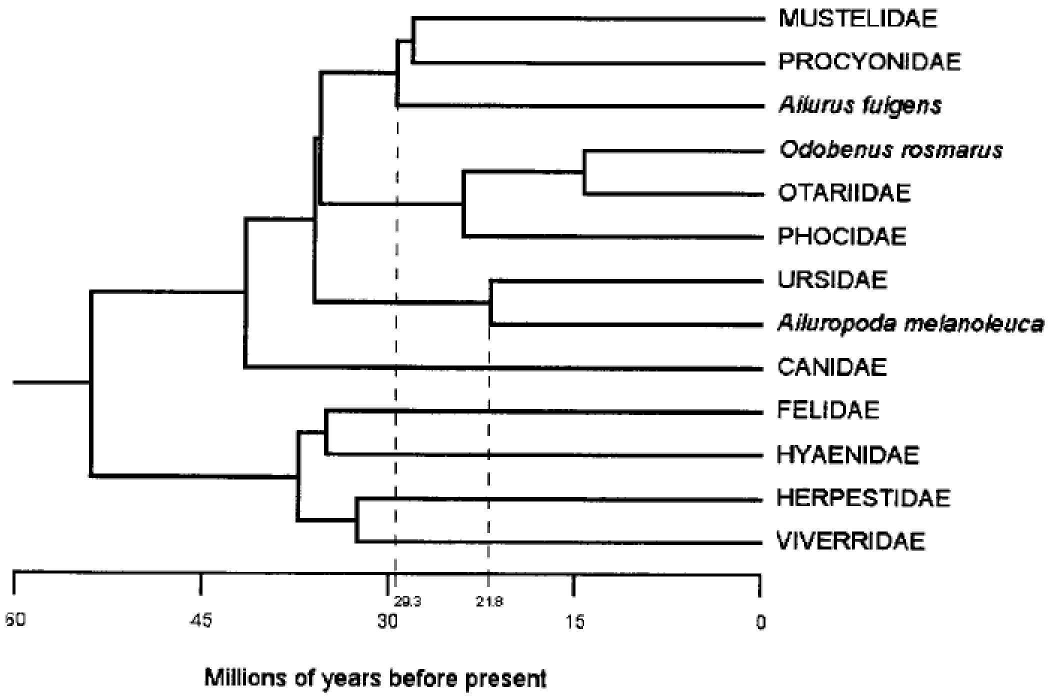

Figure 1.2. A phylogenetic tree for the higher groups of carnivores, figure taken from Bininda-Emonds (2000).

Energetic costs of an herbivorous diet

Red pandas are one of five known species of obligate bamboo eaters, although their diet has also been known to include a small portion of fruits, berries and mushrooms (Yonzon & Hunter, 1989). A bamboo diet appeared to have evolved far back in the ancestral history of the panda. Red panda-like fossils have been found from the Miocene (25 to 5 million years ago) and Pliocene (5 to 2 million years ago) eras with the physical dentition and skull shape necessary for the attachment of sufficiently powerful chewing muscles (Roberts, 1992).

Pandas have the short, relatively simple digestive tracts consistent with other mammalians placed in the order Carnivora, hence they cannot digest cellulose and are inefficient at processing an herbivorous diet of bamboo – approximately only 25% of the potential energy in bamboo is extracted (Gittleman 1988; Gittleman 1989; Roberts, 1992; Wei et al., 1999a; Wei et al., 1999b). There has been research to suggest that red pandas have evolved a very low metabolic rate so as to expend as little energy as possible (McNab, 1989; McNab, 1995). To compensate for their low energy diet, pandas spend a majority of their active time searching for and eating bamboo (Yonzon & Hunter, 1989). In view of the pandas' unusual status as herbivorous carnivores, there has been considerable research investigating the behavioural energetics of lactation in the red pandas and it has been shown that red pandas will increase their feeding behaviours by 200% while lactating (Gittleman, 1988; Gittleman, 1989; Wei et al., 1999b).

General physical characteristics

Body mass ranges from 3.7 kg-6.2 kg in adult (captive) male and female red pandas, and the length of the head and body from 560 to 625 mm, and the length of the tail from 370 to 472 mm; there is no sexual dimorphism in body size or in the colour of the coat (Roberts & Gittleman, 1984). The tail is comparatively long and marked with approximately 12 alternating red and buff rings (Roberts & Gittleman, 1984). Animals of the sub-species *styani* are somewhat darker in colour and have more distinct 'tear track' marking on their face. Red pandas also have a distinct 'thumb' (which is actually an enlarged radial sesamoid bone); this adaptation allows for better manipulation and handling of bamboo (Anton et al., 2006).

Ecology in the wild

Habitat preference

The red panda's current habitat in South-western China overlaps with that of the giant panda, and they compete for resources (e.g. bamboo). Field studies show that although the two species' habitats overlap by 70-85%, they exhibit different patterns of microhabitat use which likely allows them to co-exist (Johnson et al., 1988; Wei et al, 2000a; Zhang et al., 2004; Zhang et al., 2006). Red pandas prefer steeper sites with higher densities of fallen logs and shrubs (Wei et al., 2000). Feeding habits also differ in that red pandas have adjusted to a diet low in digestible energy by careful selection of bamboo leaves whilst giant pandas are relatively non-selective and will eat both leaves and stems (Johnson et al., 1988). Activity patterns also vary in that red pandas are primarily crepuscular and nocturnal and are active only 37% of a 24-h cycle whereas giant pandas are active both during the day and at night for approximately 57% of a 24-h cycle (Johnson et al., 1988). The difference in activity levels between the two species has been attributed to differences in body size and the correlated differences in energetics and feeding behaviours (Johnson et al., 1988). However, red panda activity levels were estimated at 56% of a 24-h cycle in Nepal, suggesting that red pandas in China may have adapted their activity levels in order to better co-exist with giant pandas.

Distribution

Populations are confined to isolated mountain ranges ranging in altitude between 1,500 and 4,800 m (Glatston, 1994; Choudhury, 2001) (see also Figure 1.3); estimated total global population is 8,000-20,000 (Wei et al., 1999c; Choudhury, 2001; Glatston &

Leus, 2005). The sub-species *fulgens* and *styani* are biogeographically separated by both mountains and the Nujiang River (Roberts & Gittleman, 1984; Wei et al., 1999c). Home range sizes in the wild have been recorded to vary between 1.02 and 9.62 km^2, with males averaging larger home ranges than females (Johnson et al., 1988; Yonzon & Hunter, 1989). Home ranges can overlap between sexes and among males, but rarely among females (Yonzon & Hunter, 1989).

Genetic diversity in the wild

In China, the Sichuan population is both larger and more stable than the Yunnan population, implying a southward expansion (Su et al., 2001). Recent phylogenetics has revealed that the current population structure has resulted from habitat fragmentation from glacial refugia. Because of the species' habitat requirements the population has likely undergone bottlenecks and population expansions several times in its history (Li et al., 2005). The genetic diversity of populations in India and Nepal has been less studied, so it is unknown what is the degree of genetic variation of the western population of red pandas; although, given the distinct habitat niches of the red panda, it is unlikely that there is much genetic exchange between the sub-populations.

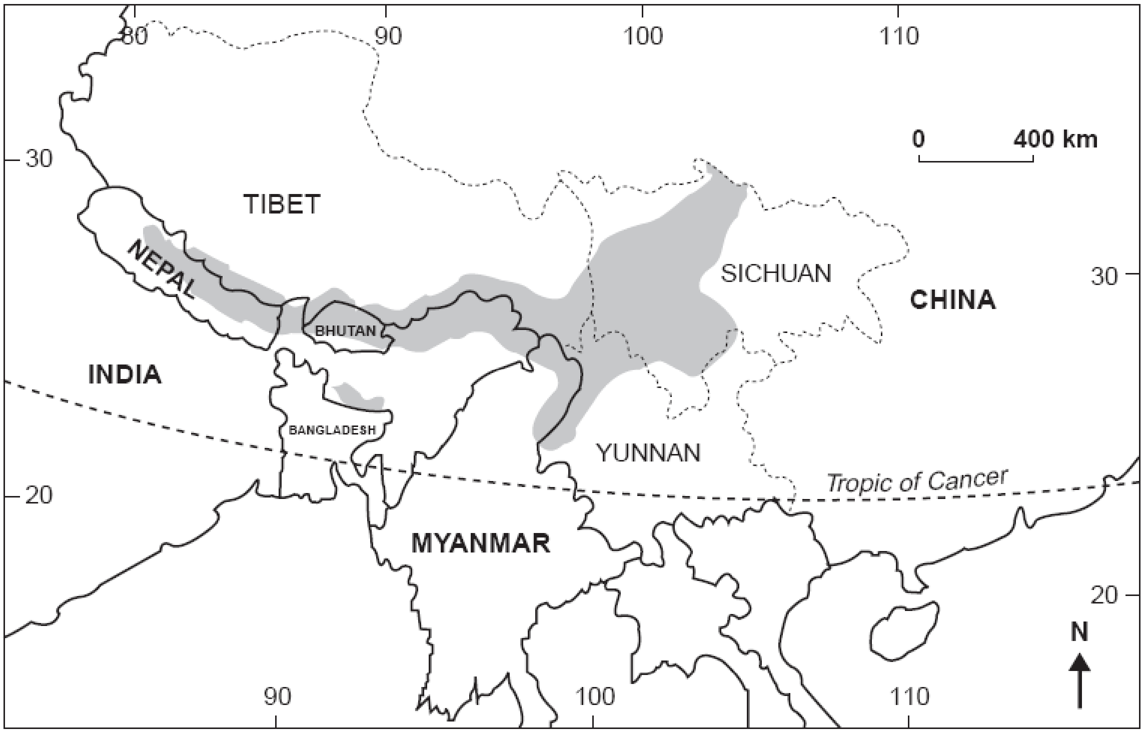

Figure 1.3 Global distribution (shaded area) of the red panda (*Ailurus fulgens*). Figure adapted from Choudhury (2001)

Behaviour

Because of their elusive and arboreal nature, very little is known about the behaviour of wild pandas aside from their feeding and breeding strategies. Mating season has been observed between January and March, outside of this period males and females are asocial (Roberts & Gittleman, 1984; Johnson et al., 1988; Yonzon & Hunter, 1989; Pradhan 1999). Because young develop relatively slowly due to the low-energy milk produced by the mother, the association between mother and offspring is extended, and can last for more than one year (Roberts, 1992). Yonzon and Hunter (1991) report that the mother-young association is the only social behaviour exhibited in red pandas except for mating during the breeding season.

Conservation

Red pandas were listed in Appendix I of CITES in 1995 and EN C2a of IUCN Red List in 1996. The major threats to their existence in the wild are poaching (live or dead) and habitat loss from either the expansion of grazing lands for domestic livestock or bamboo harvesting (Yonzon & Hunter, 1991; Glatston, 1994; Fox, Yonzon & Podger, 1995). It is believed that the sub-species *A. f. fulgens* is under greater threat than *A. f. styani*, although both populations of sub-species are declining.

Red pandas in captivity

The first recorded red panda birth in captivity was a female born on the 23rd June, 1972 at the National Zoo in Washington D.C. There are now seven globally recognised regional breeding programmes – the North American Species Survival Plan (SSP) and the European Endangered Species Breeding Programme (EEP) as well as the Chinese, Japanese, Australian, South African, and Indian programmes, which house a current global population (of both sub-species) of 584 animals (Glatston & Leus, 2005).

The founder population consists of 27 individuals, which prompted Glatston and Roberts (1988) to deem the (then) current situation of the captive population not encouraging. Further to this, Princee (1988) stated that inbreeding depression was likely to occur in the regional populations. However, more recent reports by Glatston and Princee (1993) and Glatston and Leus (2005) suggest that the captive red panda population is currently viable – although genetic diversity has declined in most regions.

The Chinese sub-species (*A. f. styani*) has been less successful in captive breeding efforts, with the first known successful captive breeding outside of China occurring in 1986 (Glatston & Roberts, 1988).

Although little evidence has been found to suggest that red pandas eat meat in the wild, some red pandas in captivity have been recorded to eat mice and chicken eggs.

Limited research has been carried out on red pandas in captivity, but it includes: an investigation into methods to detect pregnancy through the use of progestin concentrations in faecal samples (although this has only been tested at zoos in the United States and has not yet been utilised by any of the other global breeding programmes) (MacDonald, Northrop & Czekala, 2005); seasonal energy utilisation in bamboo (Wei et al., 2000); and single institution reports on the behaviours of red pandas in captivity (Conover & Gittleman, 1989; Holst, 1989).

Thesis rationale and aims

The rationale for this thesis stems from the increasing promotion of captive breeding for conservation by zoological institutions. Considering that conservation has become a widespread marketing strategy utilised by many zoological institutions, it is important to investigate the viability and practical application of using captive-bred animals in conservation efforts. Previous to this thesis, researchers have alluded to differences between the survival of wild-caught and captive-born animals released into the wild, but this has not been statistically nor recently reviewed (Griffith et al. 1989; Beck 1994). Therefore, this thesis begins by asking if this trend is still consistent or whether conservation efforts involving captive-bred animals over the past two decades have improved.

For if captive breeding for conservation purposes is to continue we need to ensure that it is a viable technique. The first aim of this thesis is to determine whether captive-born animals do indeed survive less than wild-caught animals when released into the wild (which is presented in Chapter 2). Once it has been shown that captive-born animals survive less than their wild counterparts, the next aim of this thesis is to study a case study species in captivity in order to determine what aspects of their life in captivity may be contributing to their lower survival rate (Chapters 4 & 5 investigates the red pandas' response to captivity). Based on this investigation, I want to assess if a current captive population of red pandas is viable for release into the wild

Chapter 4 uses the historical studbook to investigate changes in traits associated with fitness across generations in captivity. Chapter 5 investigates the effects of environmental variables on the behaviours of captive red pandas. Once it is better understood how species respond to life in captivity, methods can then be developed to improve individual success rates (e.g. survival and reproduction) in release projects. These methods must utilise what we have learned about the traits needed to survive in the wild as well as the factors contributing to the loss of these traits in captivity; Chapter 6 aims to identify one such method.

A focus on carnivores

Carnivores are a particularly valuable order to study in terms of *ex situ* conservation due to a combination of their extensive decline in the wild and their poor response to captivity (Woodroffe & Ginsberg, 2000; Clubb & Mason, 2003). Due to the taxonomic bias mentioned previously, carnivores are one of the best represented orders in *ex situ* conservation. This is primarily due to their public appeal, which in many ways is in contradiction to their continued and rapid extirpation in the wild. Carnivores in captivity do not tend to fare well – for example, they have relatively poor breeding success and high levels of abnormal behaviours (Clubb & Mason, 2003). Due to their rapid decline in the wild, coupled with the increasing necessity of using captive-born animals in reintroduction, there is an urgent need for research on investigating the effects of captivity in carnivores.

Case study selection

Red pandas were selected as the case study for this thesis due to their phylogenetic placement in the order Carnivora. Red pandas have been listed as endangered since 1995 and one of the goals of their captive management is to provide animals to supplement the wild population (Glatston & Leus, 2005); their well-documented captive breeding history spanning over 40 years provides a substantial amount of longitudinal data to analyse and interpret. In addition, the behaviour of red pandas – both in captivity and the wild – is relatively unstudied and the formation of an ethogram providing further knowledge of their behaviour captivity can only help to improve the conservation of this species. All of these criteria contributed to making this species a suitable case study. However, the methods and practical implications of the findings in thesis should be considered transferable to other species.

Chapter 2

The effects of captive experience on reintroduction survival in carnivores: a review and analysis[*]

Abstract

This review focuses on the success and survivorship of captive-born versus wild-caught carnivores used in reintroductions. Previous reviews have suggested that reintroduction projects using captive born animals are less likely to be successful than projects translocating wild-caught animals. The purpose of this paper is to examine this statistically and investigate how captivity may affect the survival of reintroduced carnivores. We examined results published in previous reviews, and found evidence to support that reintroduction projects using wild-caught animals are significantly more likely to succeed than projects using captive-born animals. We further compiled our own review of 45 case studies in carnivore reintroduction projects (in 17 species across 5 families) to investigate survival rates rather than overall project 'success'. We found that 1) wild-caught carnivores are significantly more likely to survive than captive-born carnivores in reintroductions; 2) humans were the direct cause of death in over 50% of all fatalities; and 3) reintroduced captive-born carnivores are particularly susceptible to starvation, unsuccessful predator/competitor avoidance and disease.

Keywords: reintroduction; carnivores; translocation; captivity; captive-born; wild-caught

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[*] This chapter is a version of the article Jule, K. R., Leaver L.A., & Lea, S.E.G. (2008). Effects of captive experience on reintroduction survival in carnivores: A review and analysis. *Biological Conservation 141(2)*, 355-363.

Brief Introduction and Background

Humans have a long history of translocating animals, whether by intention or not. In the past, intentional translocations of animals have predominantly been for the purpose of supplementing game species. However, more recently, translocation for the purpose of re-establishing endangered animals into their native habitat has become an increasingly popular conservation technique (MacKinnon & MacKinnon, 1991; Stuart, 1991). The IUCN (International Union for the Conservation of Nature and Natural Resources, also known as the World Conservation Union) (1998) defines a translocation as "a deliberate and mediated movement of wild individuals or populations from one part of their range to another" (p. 6) and a "reintroduction (a)s an attempt to establish a species in an area which was once a part of its previous historical range" (p. 6). It is important to note that the IUCN definition of a reintroduction makes no mention of the origin (i.e. wild-caught or captive-born) of the source population. Reintroduction has been seen as a valuable tool for conservation with the potential to save many species from extinction (Kleiman, 1989; MacKinnon & MacKinnon, 1991; Sarrazin & Barbault, 1996; Seal, 1991; Stuart, 1991; Tear, Scott, Hayward, & Griffith, 1993).

However, reviews have found that translocations and reintroductions of endangered species for conservation purposes have widely ranging success rates; the means of the project success values reported in these reviews ranged from11-53% (Beck, 1994; Fischer & Lindenmayer, 2000; Wolf, Griffith, Reed, & Temple, 1996), which suggests that the use of translocations and reintroductions as a conservation tool needs to be further investigated and improved upon in order to ensure that they are viable options.

Previous reintroductions for conservation

There have been a number of well publicised reintroductions carried out for conservation purposes, e.g. golden lion tamarin (Kleiman & Mallinson, 1998), red wolf (Oakleaf et al., 2004), California condor (Toon & Wallace, 1994), black-footed ferret (Russell et al. 1994), and Arabian oryx (Stanley Price, 1989). Most of the animals used in these projects were either captive born or brought into captivity due to their near extinct status. To evaluate the outcome of these projects, many have attempted to define reintroduction success (Fischer & Lindenmayer, 2000; Kleiman, Beck, Dietz, & Dietz, 1991; Kleiman, Reading, Miller, Scott, Robinson, Wallace, Cabin, & Felleman, 2000; Seddon, 1999; Stanley Price, 1991) and a combination of the following four criteria are now generally agreed upon as indicating project success: 1) breeding by the first wild-born population, 2) a three year breeding population with recruitment exceeding adult death rate, 3) an unsupported wild population of at least 500, and 4) the establishment of a self-sustaining wild population.

However, there are difficulties in setting minimum success criteria (Kleiman et al., 1994), as they can lead to assumptions that there is an end-point to which supplemental releases or continued monitoring of projects may no longer be required (Seddon, 1999). Therefore, the success of a reintroduction can only be examined at a specific point in time; which, in the majority of projects, is often shortly after release- since long-term monitoring is infrequent due to time and budget constraints. Also, current reintroduction success criteria do not include success at the level of the individual animal.

Previous reviews have highlighted several factors that appear to contribute to the success or failure of a reintroduction project. A comprehensive evaluation of the factors affecting success in reintroduction projects is beyond the scope of this paper, and many have already been published (e.g. see Seddon, Armstrong, & Maloney 2007; Beck, 1995; Beck, Rapaport, Stanley Price, & Wilson, 1994; Breitenmoser, Breitenmoser-Wursten, Carbyn, & Funk, 2001; Fischer & Lindenmayer, 2000; Griffith et al., 1989; Reading & Clark, 1997; Stanley Price, 1991; Wolf et al., 1996). However, the main biological and ecological factors contributing to project outcome can be summarised as follows: habitat suitability, long-term food availability, the season of release, type of release (soft or hard) and the source (wild-caught or captive-born) of released animals. We are interested in how the source of animals (i.e. whether they were obtained wild-caught from a sustaining wild population or from captive breeding stocks) might affect the success of a reintroduction project. In most cases of translocating game species, the stock comes from a stable wild population. However, reintroduction projects for the purpose of conservation are carried out because wild populations are declining; thus, founder stock are increasingly being sourced from captive populations (Wilson & Stanley Price, 1994).

There are many risks involved when reintroducing captive animals; however, the main concern is that animals in captivity often show a loss of natural behaviours associated with wild fitness. Deficiencies can be seen in foraging/hunting, social interactions, breeding and nesting, and locomotory skills (Rabin, 2003; Snyder, Derrickson, Beissinger, Wiley, Smith, Toone, & Miller 1996; Stoinski, Beck, Bloomsmith, & Maple 2003; van Heezik & Ostrowski, 2001; Vickery & Mason, 2003; Wallace, 2000). Other considerations include captive-born animals' lack of immunities to viruses/diseases prevalent in their wild counterparts (Bush, 1994; Cunningham, 1996; Woodford & Rossiter, 1994). Studies have suggested that projects using captive-born animals are less likely to be successful than projects using wild-caught animals (Mathews, Orros, McLaren, Gelling, & Foster, 2005). A review by Beck et al. (1994) estimated that only 16 out of 145 reintroduction projects using captive-born animals were successful.

Previous reviews and their findings on the effect of source population

Out of the several previously published reviews, three in particular, Griffith et al (1989), Wolf et al (1996) and Fischer & Lindenmayer (2000), have reported differences between the success rates of reintroduction projects and the source of animals used, and in all cases projects using captive born animals averaged a lower success rate than those using wild-caught. Further to their 1989 paper, Griffith et al. (1990) statistically reported that this difference was significant; however, they did not investigate differences in survival rates between sources across species, and therefore do not account for species biases.

Why focus on carnivores?

Carnivores are well represented in reintroduction projects; this can be explained by the taxonomic bias observed in species selected for conservation. Conservation societies often use flagship species, for example the giant panda (*Ailuropoda malanoleuca*), to promote conservation efforts and these are often chosen for their visual appeal, e.g. flagship species are typically large mammals. This preference for animals with 'visual appeal' can also be seen in species selected for reintroduction projects. Bias can be seen towards mammals, and to some extent, birds; and despite their proportionally greater endangered status fish, amphibians, reptiles, invertebrates and plants comprise a much smaller ratio of reintroduction projects. Within mammals, two Orders are particularly over-represented in reintroductions, artiodactylids (e.g. ungulates) and carnivores (Seddon, Soorae, & Launay 2005).

There are many causes of decline in carnivore numbers, such as decreasing prey densities, loss of habitat, and competition with humans. Direct human-carnivore conflicts are generally related to livestock, and as a result carnivores have been heavily persecuted (Clutton-brock, 1996; Johnson, Yao, You, Yang, & Shen, 1996; Woodroffe, 2003). Indirect human-carnivore conflicts, such as the effects of hunting and rising human densities, also heavily affect the decline of carnivore populations (Woodroffe & Ginsberg, 2000; Ginsberg, 2001). Carnivore population densities are particularly sensitive to eco-system changes and are often quite variable (Wildt, Howard, & Brown, 2001). Carnivores are long-lived, have extensive social learning e.g. to gain hunting skills (Gittleman, 1996), and have a relatively long generation time, which means that populations do not quickly recover from extensive decline. These aspects of their natural history have implications for both *ex situ* and *in situ* conservation.

There have been several reviews that specifically examine carnivore conservation (Breitenmoser et al., 2001; Clark, Curlee, & Reading, 1996a, 1996b; Reading & Clark, 1997; Soorae & Stanley Price, 1997; Weber & Rabinowitz, 1996; Hayward et al., 2007), and it is generally proposed that long-term *in situ* efforts, such as habitat protection and ensuring prey densities, are more effective conservation measures than *ex situ* releases. A major difficulty facing carnivore reintroductions is that often the cause behind the initial extirpation (i.e. conflict with humans) is not resolved at the time of proposed reintroduction (Miller, Ralls, Reading, Scott, & Estes, 1999; Wilson, 2004).

Furthermore, there are not many sustainable carnivore populations left in the wild to provide release stock. This necessitates the use of captive populations, either to establish a new population or to supplement existing populations. However, there are particular difficulties unique to captive-born carnivores, which include loss of socially learned skills (e.g. hunting), conditioning to humans, experience feeding on livestock, inappropriate social behaviours (e.g. mating and dominance) and other factors associated with adaptation to captivity (Soorae & Stanley Price, 1997). Wide-ranging carnivores appear to respond poorly (e.g. low breeding success and high levels of stereotypies) to captivity (Clubb & Mason, 2003) and it has been further shown that these stereotypies (or abnormal behaviours) are strong behavioural deficiencies that may have an effect on reintroduction survivorship (Vickery & Mason, 2003; Vickery & Mason, 2005).

Main objectives

There are two main objectives for this paper. One was to statistically verify differences between the success rates of reintroduction projects (obtained from previously published reviews) based on their source of founder stock. The prediction is that projects using wild-caught animals will be more successful than those using captive-born animals (Mathews et al., 2005). The second objective was to provide an updated review and analysis on the survival rates of reintroduced and translocated endangered carnivores. Reading et al. (1997) and Breitenmoser et al. (2001) carried out reviews of carnivore reintroductions; however these reviews are now out of date and did not specifically investigate the effect of source animals. Therefore, we have compiled statistics from the results of reintroductions and translocations of carnivores published since 1990 in order to investigate the survival rates of reintroduced animals in relation to the source of founder stock, wild or captive. We looked at survival percentages of released animals instead of "success" criteria, which can define a project as successful despite the high mortality of released animals. Survival can be used as an assay of animal welfare as well as a tool to assess factors contributing to individual successes or failures; though it is worth mentioning that a successful reintroduction may well be considered to have a worthy outcome in the face of possible extinction, despite mortality costs.

Methods

Literature search

The literature search was carried out on carnivore reintroduction and translocation projects that have been published post 1990. Literature was collected over a 5 month period in early 2005, and included over 25 journals, two of which were particularly applicable- *Biological Conservation* and *Conservation Biology*, and over 30 relevant books and symposium proceedings. Journals were searched via online databases and electronic journals, such as Web of Science, EBSCO, JSTOR, IngentaConnect, and Elsevier ScienceDirect. We also carried out extensive web searches with keywords such as "reintroduction", "translocation", and "carnivore", as well as specific carnivore species. In these web based searches, we were able to find unpublished reports, government run projects, and projects published in lesser known journals, newsletters, and updates. Additionally, articles were collected opportunistically, and in some cases by referral (see Table 2.1 for a list of all projects included in review).

Creation of the data base

Because of our interest in looking at survival rates of the founder stocks, we restricted our search to include only projects that 1) reported actual numbers of animals released, and 2) also carried out some form of post-release monitoring and thus were able to report on the number of mortalities or survivors. Post release monitoring varied across projects, but ranged in time from 6 to 18 months. Given these criteria, we were able to include only 45 projects, some projects using only captive or only wild subjects; some using a combination of both. Combination projects were only included if independent data were available on source of animal and were therefore analysed as separate projects, which for the purpose of analysis brought the N up to 49 (see Table 2.1). The 49 (N_{wild}=29, $N_{captive}$=20) case studies included 17 carnivore species across 5 families (Felidae, Canidae, Ursidae, and Mustelidae and Ailuridae) using a total of 2152 animals (N_{wild}=1169, $N_{captive}$= 983).

Statistical analyses

Fischer and Lindenmayer (2000) reported that wild reintroduction projects were more successful than captive projects but they did not test this difference statistically. We calculated an independent G test to see if this difference was significant. It is important to note that the original authors did not control for species biases and/or over-representation; therefore, it is not clear whether there were any external factors influencing the results, such as different representation of species between the two sources (wild/captive). The purpose of this test was merely to look at previous trends in reintroduction projects using different source populations.

We carried out a nested mixed model ANOVA (using SPSS v. 14) for the independent variables 'species' within 'families', the dependent variable was 'percent survive' and the grouping variable was 'source' (wild or captive). Projects (N=49) were weighted by 'sample size' (number of animals in each project) as a regression weight, which applies an estimated modification to the variance or weights in an effort to control for the differences in representation across species in the projects used for this analysis. Analyses carried out before weighting for sample size suggested an effect of species, thus weighting for the sample size helped to control for effects of projects with either very high or very low numbers of individuals. An ANOVA was used instead of a G-statistic because initial G calculations showed that the samples (species and families) were not statistically independent.

Individual G-tests of independence were carried out on each species where both sources were represented; this was done in order to investigate how consistent the survival trends were within each species (in one case, wild dog, a Fisher's exact test was used because two cells contained numbers less than 5). To investigate whether this was a trend across species, we then carried out a Wilcoxon related samples test on all species where both sources were represented.

Table 2.1 Carnivore reintroduction and translocation programmes (post-1990)

Class: **Mammalia** Order: Carnivora	Species	# of Animals released Captive/Wild		% of founder pop. surviving Captive/Wild		Cause of Death (In order of prevalence)
Felidae	Lynx *Lynx canadensis*[1]	0	96	NA	.59	Starvation, various
	European lynx *Lynx lynx*[2]	19	0	.68	NA	Disease, various
	Lynx lynx[3]	7	0	.42	NA	Human
	Lynx lynx[4]	25	0	.30	NA	Recapture, human, unknown
	Lynx lynx[5]	21	0	.30	NA	Human, recapture, starvation
	Iberian lynx *Lynx pardinus*[6]	0	2	NA	.50	Unknown
	bobcat *Felis rufus*[7]	0	32	NA	.90	Drowning
	Mountain lion *Felis concolor azteca*[8]	0	14	NA	.35	Injuries, disease, human
	Felis concolor[9]	0	7	NA	.57	Unknown, human
	Felis concolor stanleyana[10]	0	7	NA	.57	Human, unknown
	Felis concolor stanleyana[11]	0	8	NA	.63	Human, unknown
	wildcat *Felis silvestris*[12]	6	0	.33	NA	Unknown
	cheetah *Acinonyx jubatus*[13]	0	21	NA	.66	Human
	Acinonyx jubatus[14]	0	3	NA	.33	Human, unknown?
	Amur tiger *Panthera tigris altaica*[15]	0	2	NA	1.0	N/A
Canidae	Swift fox *Vulpus velox*[16]	16	0	.68	NA	Starvation, humans, unknown
	Vulpus velox[17]	108	19	.06	.32	Coyotes, various
	Vulpus velox[18]	365	204	.11	.47	Coyotes
	Wild dog *Lyacon pictus*[17]	8	0	0	NA	Humans
	Lyacon pictus[19]	13	0	0	NA	Lions, rabies, humans
	Lyacon pictus[19]	11	0	0	NA	

						Humans
	Lyacon pictus[19]	9	0	0	NA	Unknown
	Lyacon pictus[19]	0	4	NA	.25	Lions
	Lyacon pictus[19]	0	6	NA	0	Unknown
	Grey wolf *Canis lupus*[20]	0	31	NA	.71	Human, unknown
	Mexican grey wolf *Canis lupus baileyi*[21]	79	51	.18	.35	Human, recapture
	Canis lupus baileyi[19]	0	100	NA	.63	Human, unknown
	Canis lupus baileyi[19]	0	45	NA	.62	Human
	Canis lupus baileyi[19]	0	11	NA	.27	Human
Ursidae	Black bear *Ursus americanus*[22]	0	43	NA	.25	Unknown
	Ursus americanus[22]	0	21	NA	.43	Unknown
	Ursus americanus[23]	0	79	NA	.70	Human, unknown
	Ursus americanus[24]	0	14	NA	.54	Unknown
	Ursus americanus[25]	23	0	.25	NA	Various
	Brown bear *Ursus arctos*[22]	0	3	NA	.66	Unknown
	Ursus arctos[22]	0	3	NA	.66	Human
	Ursus arctos[22]	0	4	NA	.25	Unknown
Mustelidae	European otter *Lutra lutra*[27]	25	11	.42	.79	Various
	River otter *Lontra Canadensis*[28]	0	303	NA	.88	Various
	Lontra Canadensis[29]	0	25	NA	.72	Human/Various
	Black footed ferret *Mustela nigripes*[30]	49	0	.20	NA	Various, Unknown
	Mustela nigripes[31]	94	0	.59	NA	Unknown
	Mustela nigripes[31]	77	0	.32	NA	Unknown
	Mustela nigripes[31]	26	0	.69	NA	Unknown
Ailuridae	Red panda *Ailurus fulgens*[26]	2	0	.5	NA	Predated

[1]Shenk, T. (2001), [2]Anders, O. (pers. contact), [3]Boer et al. (1995), [4]Blomqvist et al. (1999), [5]Vandel et al. (2006), [6]Rodriguez (1995), [7]Warren et al. (1990), [8]Ruth, T. (1994), [9]Ross, I. & Jalkotzy, G. (1995), [10]Belden & Hagedorn (1993), [11]Jansen, D. & Logan, T. (2002), [12]Olmo et al., (1992), [13]Purchase, G. (1998), [14]Phiri, C. (1996), [15]Miquelle et al. (2001), [16]Bremner-Harrison et al. (2004), [17]Woodroffe & Ginsberg (1997), [18]Carbyn, Armbruster, & Mamo (1994), [19]Moehrenschlager & Somers (2004), [20]Phillips, M. & Smith D. (1997), [21]Oakleaf et al. (2004), [22]Clark et al. (2002), [23]Wear et al. (2005), [24]Eastridge & Clark (2001), [25]Stiver et al (1997), [26]Padhan, S. (pers. contact), [27]Sjoasen, T. (1996), [28]Johnson et al. (1999), [29]Johnson & Berkely (1999), [30]Russell et al. (1994), [31]Vargas et al. (1999)

Results

An analysis of success of reintroduction projects using wild-caught versus captive-born

The calculated G statistic on the results from Fischer & Lindenmayer's (2000) review, shows that reintroduction projects were significantly more likely to succeed when a wild source population was used (31% of 45 projects) than when animals from a captive source were used (13% of 52 projects); G= 4.466, df=1, p=0.035).

Survival of wild-caught versus captive-born animals and family differences

The results of the ANOVA show that wild-caught individuals survived significantly more (53%) than captive-born (32%), $F_{(1,4.66)}$=17.697, p=0.01; Fig 1). See Figure 2.1.

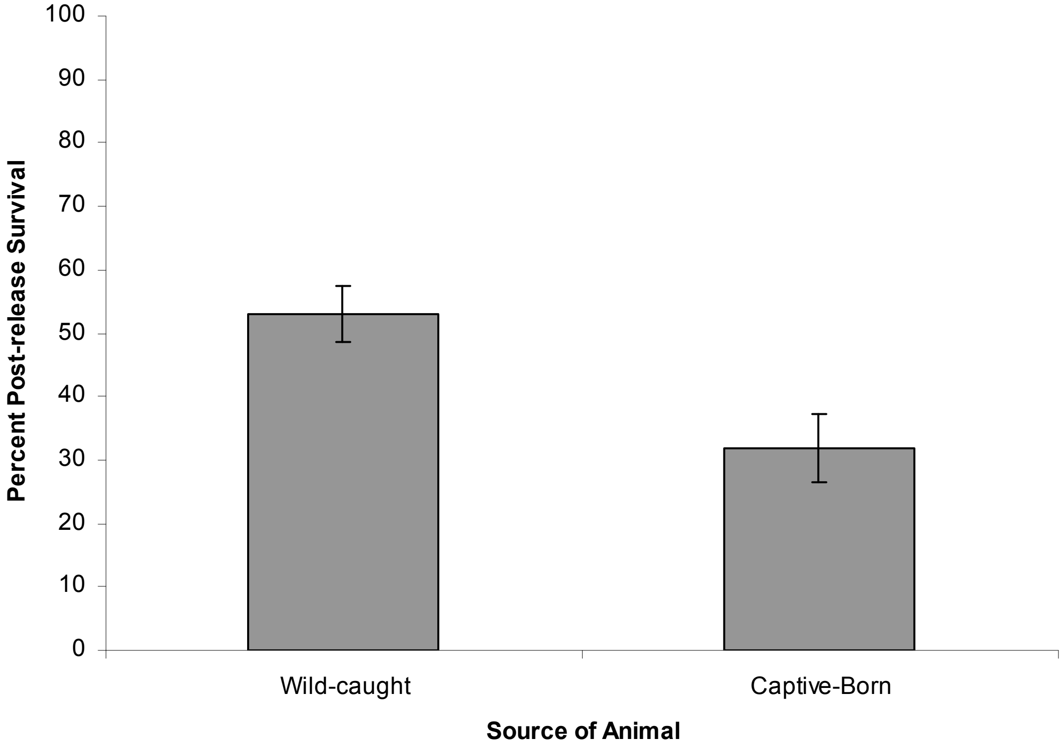

Figure 2.1 Percentage survival rates in reintroductions based on source of animals. Error bars represent the standard errors from the average percentage of survival for each source.

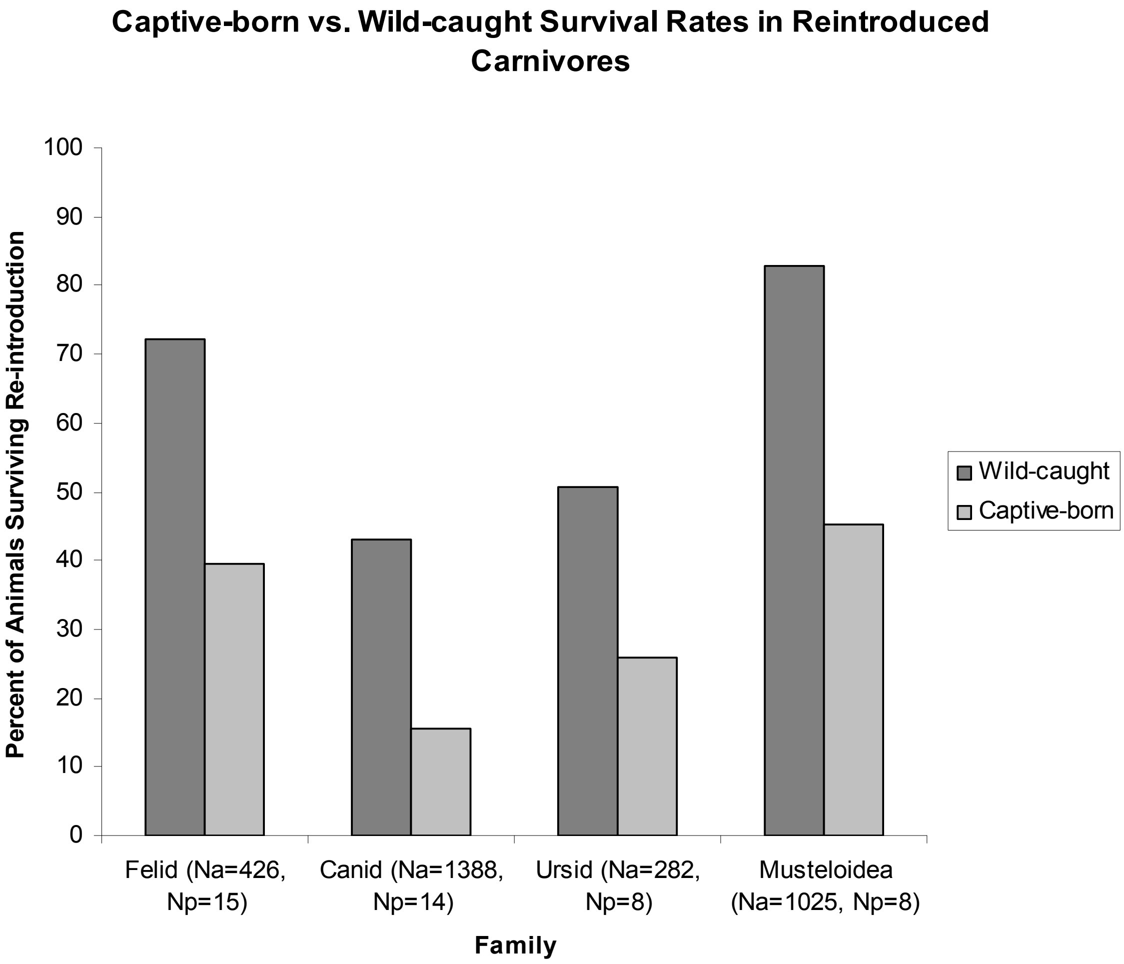

Figure 2.2 Reintroduction survival rates across carnivore families (N_a= number of animals, N_p= number of projects). No error bars present as percentages were calculated from grand totals for each family.

When controlling for sample size, there were no significant differences in post-release survival across families ($F_{(3,0.29)}$=13.140, p>0.05)[*] or species within families ($F_{(12,10.76)}$=0.667, p>0.05). Nor were there any significant interactions between source

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[*] Note that the df in the denominator is less than one. This is unusual, but not impossible. There are situations, in particular when dealing with nested ANOVAs, where this can result in an inappropriate over-conservative test (Hillis, 2007). However, replacing the fractional value by 1.0 and re-calculating the *F* statistic still results in a non-significant effect of family.

survival and families ($F_{(2,14.93)}=0.121$, $p>0.05$) (Figure 2.2) or species within families ($F_{(2,27)}=0.805$, $p>0.05$). We did not include the Ailuridae family in this analysis due to low sample size.

We repeated the analysis, this time removing species that were represented by fewer than 3 animals, the red panda (also removed from previous analysis), the Iberian lynx, and the Amur tiger. This was done in an attempt to eliminate a biased effect from a small sample size. The new project N was 46, and animal N was 2146. The F value for the main effect of source remained unchanged, $F_{(1,4.81)}=17.378$, $p=0.01$ and all other effects remained non-significant.

Analyses were carried out on species where both captive and wild sources were represented, N=5; swift fox (*Vulpus velox*), Mexican grey wolf (*Canis lupus baileyi*), black bear (*Ursus americanus*), European otter (*Lutra lutra*) and wild dog (*Lyacon pictus*). There was a significant effect of source on four of the species tested such that animals from wild sources survived better than animals from captive sources; swift fox (G=96.619, df=1, $p<0.001$), red wolf (G=33.055, df=1, $p<0.001$), black bears (G=5.442, df=1, $p=0.01$), European otters (G=5.714, df=1, $p=0.01$) all results reported are two-tailed. A Fisher's exact test was used (due to low cell values) to calculate effect of survival on wild dogs, and was not significant at $p=0.20$, in this case survival for both wild and captive animals was very low. The Wilcoxon related samples test showed a significant difference between source survival across the 5 species (z= 2.023, N-Ties=0, $p=0.043$) such that survival was better from wild sources (48.5%) than from captive sources (19%).

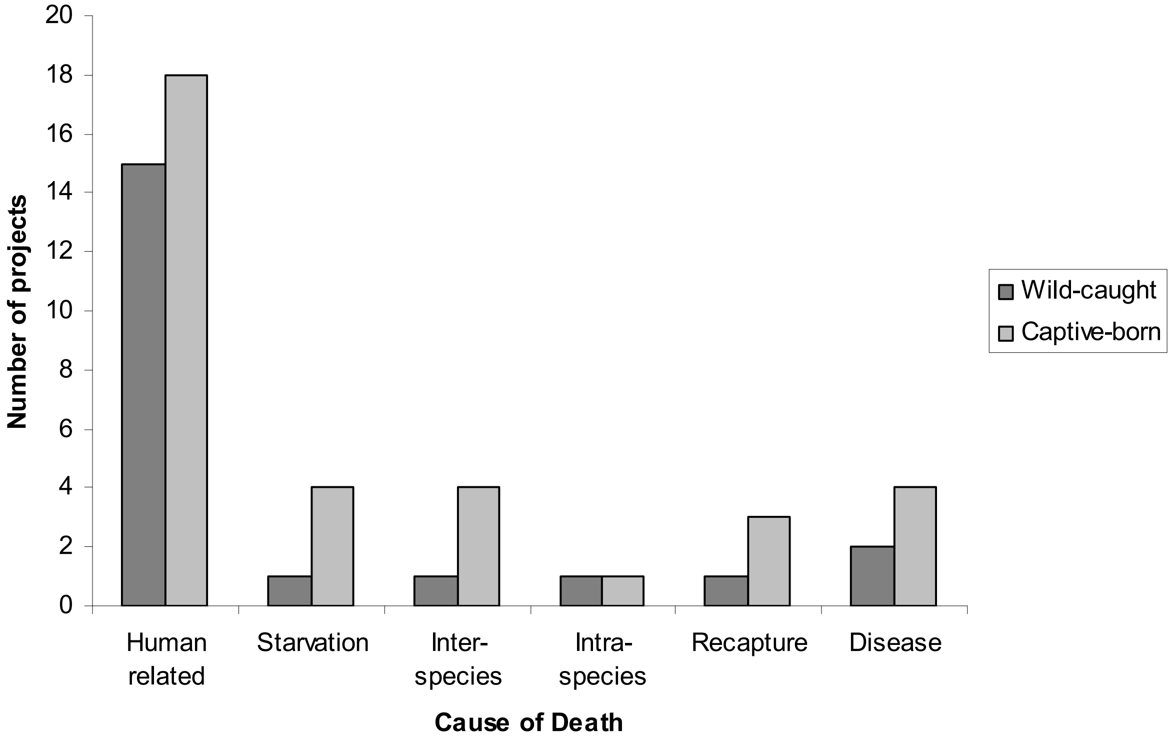

Figure 2.3 Number of cases where main cause of death is known

Investigating cause of death

Regardless of the success or failure of a reintroduction project, the most common cause of death for both wild and captive animals, was by human means (this includes shooting, poisoning, automobile driving accidents , and other related incidences) (refer to Figure 2.3). Starvation, inter-species aggression (e.g. reintroduced wild dogs killed by lions) and disease (such as rabies and distemper) were also prevalent causes of death for captive animals. Recapture was measured as death, since individuals were only removed in cases where they would not otherwise survive.

Discussion

Success of projects based on results of previous publications

Our results confirm that the use of different source populations has an effect on the success of the project and corroborates Fischer & Lindemayer's (2000), Griffith et al's (1989) and Wolf et al.'s (1996) reviews. Considering that captive-born animals are less likely to survive a releases into the wild, presumably there are some aspects of captivity that result in a lack of appropriate 'wild' type behaviours (Rabin, 2003). Other potential factors influencing captive animals' lack of success can range from lack of immunities to diseases present in wild populations and/or to an unnatural confidence towards humans (Woodford & Rossiter, 1994; Woodroffe, 2003). There needs to be further investigation into the factors affecting success rates between wild and captive source populations in order to determine where these differences may lie.

Success at the individual level in carnivore reintroductions

We investigated differences in survival as well as causes of death across families. Ideally, it would be advantageous to statistically evaluate differences between or across species; in our case our non-significant findings across families and species may be due to our relatively small data set containing unequal source and species representation. Despite the data in this review not being robust enough to investigate any species differences, we were still able to look at trends across four of the five families presented. It appears from Figure 2.2 as though captive experience has a particularly negative effect on survival for canids and slightly less so for ursids and mustelids. It would be worthwhile to see if this trend could be supported statistically in a larger data set.

In examining survival rather than success rates, we were able to preliminarily investigate which factors influenced cause of death in captive-born or wild-caught animals. Our results indicate that behaviours associated with tameness towards humans, lack of social influence from con-specifics, and lack of foraging/hunting skills are factors that should be investigated more thoroughly in order to improve upon the survival of captive-born released carnivores. For future studies, we recommend not just evaluating survival, but also breeding success, longevity, and causes of fatality and mortality; however, with the information available from published reintroductions, this is ambitious. Additionally, unsuccessful reintroduction projects are less likely to be published than successful projects (Fischer & Lindenmayer, 2000, Reading et al., 1997) which suggests that our estimates of survival are likely to be conservative.

Problems with the data set and suggestions for future studies

Because of the publication bias, the selection process of species involved in reintroduction projects (i.e. flagship species), and the limited amount of literature and resources available, a more robust and complete data set would be difficult to compile. There are also inherent difficulties in analysing reviews of published literature, such as repeatability likelihood and issues facing the methodological rigour of carrying out literature searches (Fazey et al., 2005; Roberts et al., 2006; Stewart et al., 2005).

An ideal data set should be able to control for effects of species by having a wide range of species represented; relatively equal sample sizes across families and species as well as between source; comprehensive post-monitoring; and information on individual animals. A more exhaustive review of this type of data set including a wider range of species, as well as those outside the order Carnivora, would greatly improve our knowledge of the effects of captivity.

A data set such as this could identify what factors or species characteristics may influence captive survival rates (e.g. home range size or social structure) as well as elucidate factors which could increase the success of reintroduction projects using captive-born animals. This would allow researchers to identify areas where captive animals might benefit from specific training programmes (e.g. see Shier & Owings, 2007). Results could also lead to development of more specific reintroduction guidelines for particular species. Investigating the effects of husbandry and pre-release experience on survival of released animals is equally important. Determining which species were more successful in reintroductions after captive experiences would allow for recommendations on the improvement of both *in situ* and *ex situ* conservation efforts. More reviews should be conducted on reintroduction projects using primarily captive animals (but also projects using a combination), in order to investigate overall trends and to flag important effects or variables influencing the success of individual animals (e.g. effects of hand-rearing versus dam rearing).

Conclusion

Our findings confirm previous reports that reintroduction projects using wild-caught animals are more successful overall than those using captive-born animals. We also found that wild-caught carnivores are more likely to survive than captive-born carnivores in reintroductions and that this trend appears to be consistent across species and families. Further reviews should be conducted on carnivores, as well as other taxonomic groups, in order to improve our understanding of how captivity affects survival in reintroductions.

Rationale for thesis

This chapter provides the rationale and theoretical framework for the remaining empirical studies of this thesis. This chapter provides clear evidence that current *ex situ* conservation techniques using captive-born animals need improvement. These findings necessitate the importance of further understanding of the effects of captivity and their implications for species conservation. The remainder of this thesis aims to investigate how a species (the red panda) responds to life in captivity and how this response may impact upon their suitability for release into the wild; furthermore, based on what is learned regarding the changes that occur in captivity, this thesis aims to devise a technique to improve the likelihood of survival and reproductive success of released captive-born animals.

Chapter 3

The use of a behaviour discovery curve to predict optimal observation time: captive red pandas (*Ailurus fulgens*) as a case study[*]

Abstract

This paper suggests a method of calculating behaviour discovery curves, which provides researchers with a formalised technique to estimate the optimal amount of data to collect when establishing an ethogram. The formation of ethograms furthers our knowledge about species specific traits and requirements and is useful because obtaining reliable catalogues of behaviour on species, both in the wild and in captivity, is critical for improving both *in situ* and *ex situ* conservation efforts. The behaviour discovery curve is fitted to a logarithmic model that predicts the number of behaviours which will be observed in any given length of observation time. To illustrate the methods, 31 captive red pandas (*Ailurus fulgens fulgens*) were observed for 30 h each and a behaviour discovery curve was estimated for each animal based on the rate at which new behaviours were observed. We demonstrate how to use the curve in the evaluation of an ethogram, whilst also providing an indication of how many more behaviours would be observed in a longer observation period. This is an important consideration in the creation of any ethogram, since there are currently no standard methodologies for establishing ethograms, and no guidelines on how much data is 'sufficient' for determining a species' behavioural repertoire. The curve does not allow an estimate of the total size of the

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[*] This chapter is in the format of a manuscript in preparation to *Conservation Biology*, Jule, K.R., Lea, S.E.G., & Leaver, L.A. The use of a behaviour discovery curve to predict optimal observation time: captive red pandas (*Ailurus fulgens*) as a case study.

behavioural repertoire, but does allow a systematic analysis of the likely costs and benefits, to both researchers and animals, of further observation. We also suggest a method for quantifying the degree of idiosyncrasy of a population.

Keywords: *Ailurus fulgens*; behaviour discovery curve; behavioural methodology; ethogram; observation time; red pandas

There are currently no universal methods for determining how much observation is appropriate for the purpose of behavioural research. Very few methodological handbooks set criteria or guidelines for observation length (for example, Martin & Bateson 1993 and Lehner 1996 do not mention length of observation sessions). Altmann (1974) only briefly states that observation sessions should be long enough to adequately obtain durations and frequencies of behaviours, but not over-long in order to avoid observer fatigue, but does not mention overall amount of observation (for example, how many total hours or days). Thus, length of observation time is determined by convenience, or by custom and practice, both of which can vary across types of studies (i.e. field and laboratory research). Additionally, behavioural frequency can vary drastically between species, so the same total observation time can yield very different results for, for example, a very active *vs.* a very inactive species. This could result in the erroneous conclusion that the active species has a wider behavioural repertoire, which may not actually be the case. Particular difficulties in compiling ethograms include rarely performed behaviors and inter-individual variation, both of which will be addressed in this paper. We propose a method that can be used to estimate the time course of observation of further behaviours likely to be shown by a species in any given period of time, based on the rate at which new behaviours have so far been shown by that species over time.

Several methods have been devised to predict the frequency of occurrence of particular behaviours. However, while predicting the frequency of occurrence is important when asking questions regarding the frequency and/or likelihood of observing novel behaviours, it does not give indicate what proportion of the probable observable repertoire of behaviours may already have been observed. Hence, formulas calculating frequency of occurrence do not estimate how much of an animal's behavioural repertoire is 'captured' in any given observation period. Zipf's statistic (Zipf 1965), obtained from a log-log plot of frequency of occurrence of signalling units against their rank order, has been used in animal communication studies to explain the distributional structure of non–linguistic natural communication systems (McCowan et al. 1999; McCowan et al. 2005). However, the use of Zipf's statistic as a linguistic tool has been criticized heavily (Rapoport 1982). Suzuki et al. (2005) has argued that Zipf's technique is not methodologically appropriate for the analyses of animal communication signals. Some of the criticisms of Zipf's statistic are that results are subject to false positives and misinterpretation, and are not internally consistent (Suzuki et al. 2005). Zipf's law has also been used in the prediction of number of species within a genus but was found to only weakly represent the distribution (Hill 1970).

We therefore decided to work from models which predict rates of discovery rather than frequency of occurrence. Discovery rate models have been used to estimate the rate of species detection by plotting trend curves. Species discovery curves have been under discussion at least since Steyskal (1965), and have since been used in numerous reports to estimate the number of undiscovered species, based on current and previous discovery rates (e.g. see results by Soberon & Llorente 1993; Medellin & Soberon 1999; Bebber et al., 2007). This type of trend curve has more recently been used to predict species extinction rates (Pimm et al. 2006). There have been several critiques of the validity of estimating the number of species yet to be discovered based of these curves, primarily due to the large potential for error (e.g. Solow & Smith 2005; Hammond 2005).

However, using the trend curve in behaviour discovery is less problematic than in species discovery as it is not used to estimate the total number of all potential behaviours existent, merely to estimate the rate of behaviour 'discovery' and how it changes over time. This trend curve can be used to estimate an optimal observation time for a species of study based on a specific research question. For example, if researchers are investigating rare, atypical behaviours they may wish to observe a large percentage of a species' estimated behavioural repertoire for any given amount of maximum observation time. In which case, it would be beneficial to know how much further observation is required to observe more rare behaviours. Once the curve has been calculated, the costs and benefits of continuing to look for new behaviours can be highlighted. Since, as the curve levels off, the gain (in terms of observing new behaviours) decreases over time. Increased observation time is never without cost, either in experimenter's time or, where it is a consideration, disturbance to the animals, so the net gain may fall below zero. Alternatively, it could be less than optimal to stop observations at an arbitrary time while the curve remains at a steep incline, when a little further observation would yield a significant number of new behaviours.

As mentioned previously, ‘optimal observation time’ is likely to vary across research questions, e.g. depending on whether researchers are determining behavioural budgets or investigating novel behaviours. The method we propose for estimating observation times is flexible enough to be used across different fields of study as well as a variety of research questions.

The purpose of this study was twofold, namely 1. to calculate a formula that accurately represents the rate at which we observe new behaviours and 2. to utilise this model when creating an ethogram for captive red pandas (*Ailurus fulgens fulgens*) in order to illustrate the applicability of this method.

The behaviour of red pandas has not been extensively observed in the wild (mostly because of their elusive nature) (see Reid et al 1991; Pradhan et al 2001). Despite the existence of an international captive breeding programme spanning nearly thirty years, there has been very little behavioural investigation on captive red pandas (see Glatston 1993). However, they are a unique species both taxonomically and phylogenetically (Slattery & Obrien 1995; Flynn et al. 2000; Su et al. 2000) and are in need of further study.

Methods

Subjects

Thirty one (17 male, 14 female) adult red pandas housed in 13 zoological institutions within the UK were observed for a period of 30 h per individual between the months of April 2006 to October 2006, giving a total of 930 h of observation.

Observations were carried out by one researcher. The pandas were housed in pairs or groups of three and ranged in age from 2 to 11 years. The decision was made in advance not to observe the pandas during their breeding season, which is typically in the months of December and January (Glatston 1989), in order to limit behavioural differences across individuals over the time of data collection. Behaviour was recorded using two methods: state behaviours were recorded via focal point sampling every 60 s, and event behaviours were recorded via all-occurrence sampling.

Model

We expected that as observation time lengthened, the frequency or likelihood of observing new behaviours in an animal would decrease, but would never reach zero. We therefore tested a logarithmic model to see how well it represented the rate at which new behaviours were observed in red pandas. We call the plot of this equation a 'behaviour discovery curve', because it shows how the frequency of observing new behaviours in an individual animal changes over time. We predicted that the slope will have a gradual increase before it begins to level off as behaviours observed reach saturation; however, since, in theory, there can be an infinite number of behaviours, the curve should never reach an asymptote.

The model equation is,

$$y = a + b * ln(t+1) \qquad \text{Equation 3.1}$$

where a and b are constant terms, t is the time in hours of observation (+1 to ensure that predicted numbers of behaviours are always positive). Values for the parameters a and b estimated by nonlinear regressions run on each individual's cumulative observed behaviours; the independent variable was the natural log of hours (+1). Theoretically, it may seem logical to have only one parameter (*b*) in the equation considering that at time zero the number of behaviours so far observed would always be zero. However we found that a constant (*a*) is necessary in ensuring an accurate fit of the model (particularly during early observation periods) as there will always be at least 1 behaviour observed between time 0 and 1 h.

To investigate how much data is sufficient to create an accurate model we plotted the change in variance of *b* across all animals at 5 h, 10 h, 15 h, 20 h, 25 h and 30 h. As observation time increases and the parameter estimates become stable, the variance of *b* across animals should decrease and then level off. Once the variance has levelled off, this suggests that the accuracy of the model would only be marginally improved with increased observation time.

In view of the results of the previous analysis, we wanted to generalise the model to all captive red pandas. We used the means of the *a* and *b* values after 20 and 30 h of observation to calculate a generic representation of the number of behaviours observed in a captive red panda at a given observation time. We then tested the predictive power of this generalised model by comparing the predicted versus the mean of actual behaviours observed over time.

We also wanted to investigate how the focus (i.e. one animal or several animals) of observation influences the number of behaviours observed. For example, which yields the most data- watching 1 animal for 30 h or 30 animals for 1 hour each? We combined the cumulative data at each hour of observation for all animals (N=31), so that observation time started at 31 h and progressed to 62, 93, 124, and so on up to 930 h. We then used a two-parameter model (which for the purpose of reference, is expressed as $y = A + B*\ln(t+1)$, with A and B representing the parameters estimated by a nonlinear regression) in order to predict the numbers of behaviours likely to be observed across all animals. We checked the predictive value of the model by comparing the model generated results to the actual number of observed cumulative behaviours of all animals. The cumulative model was then compared to the previous individual model in order to compare the two sampling techniques and also to investigate the idiosyncratic nature of behavioural differences across the population. If the animals were completely interchangeable, an hour spent watching one animal would have exactly the same effect as an hour spent watching another, so we should expect that $B=b$. If they were completely idiosyncratic, such that each animal showed completely different behaviours, then an hour spent watching each of N animals would yield N times as many behaviours as an hour spent watching one so, to a good approximation, we would expect that $B=Nb$. Accordingly, we can explain the degree of idiosyncrasy by the expression $I° = B/Nb$, which will take the value of 1 given maximum idiosyncrasy and $1/N$ given minimum idiosyncrasy.

Results

Actual number of behaviours at 30 h of observation per animal ranged between 14 and 35, with $\overline{X}$ ± SD = 20.87 ± 4.75. The variance across animals or observed number of behaviours levelled off at 20 h of observation (see Figure 3.1) suggesting that observations on red pandas carried out beyond 20 h do not notably improve the predictive power of the model.

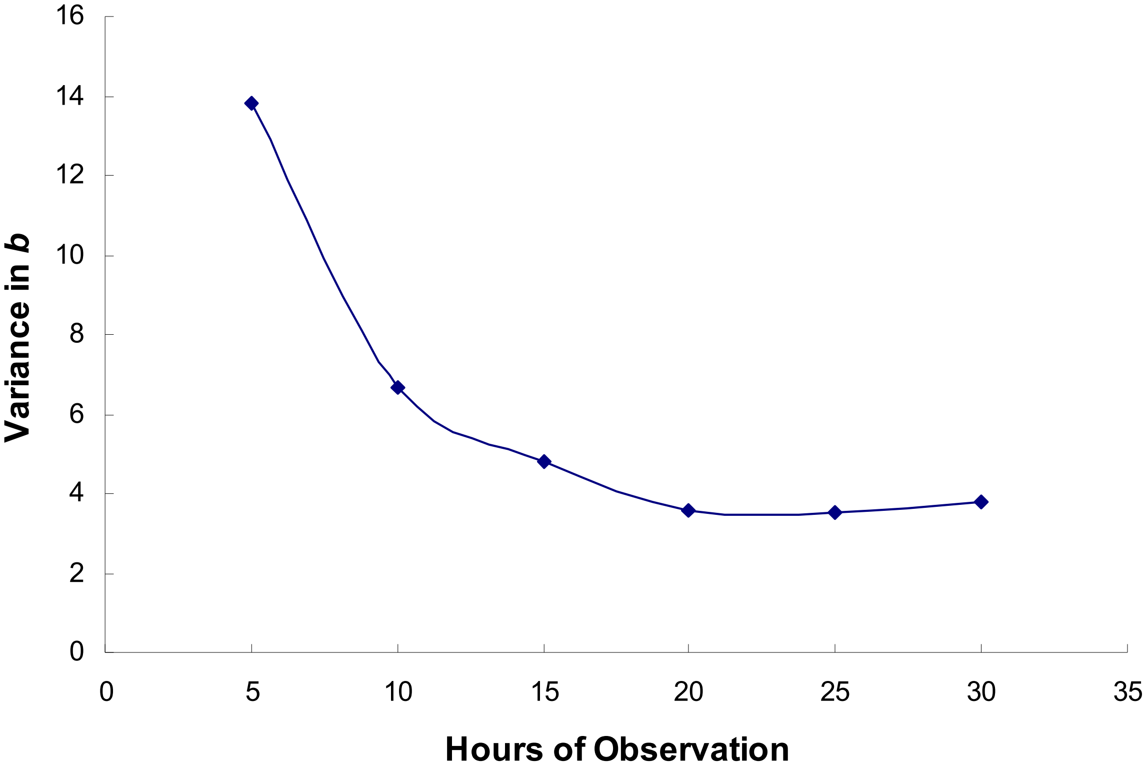

Figure 3.1 Variance of the parameter *b* across individuals

Using a generalised equation based on the 20 h data to represent all animals (where the parameters were found to be 2.9 for *a* and at 5.21 for *b*), we plotted the predicted behaviours from the model against actual observed behaviours (means of all observed animals) on a log plot. Figure 3.2 shows that the observed number of behaviours predicted from the generalised equations derived from 20 h and 30 h observation did not differ systematically from the mean of actual observed behaviours.

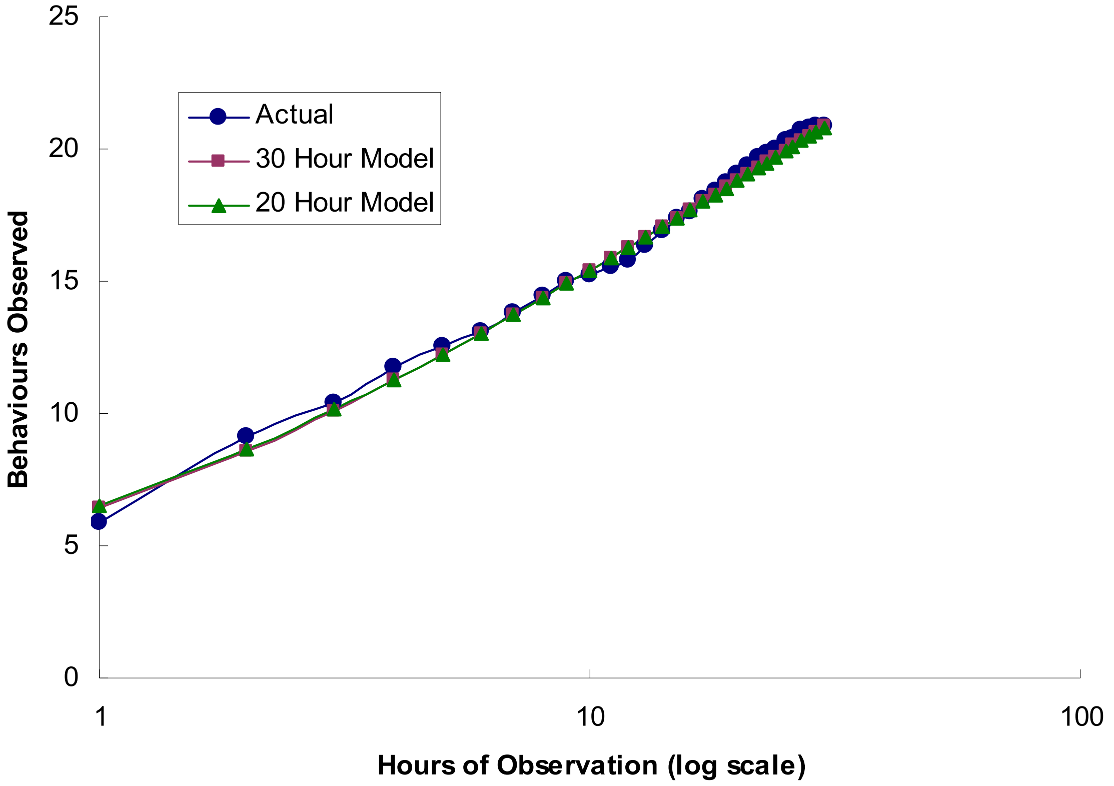

Figure 3.2. 20 hour and 30 hour models plotted against the mean of actual observed behaviours.

The behaviour discovery curve for captive red pandas, generalised from 20 h of observation is shown in Figure 3.3. From this curve, it is possible to estimate, for example, that 30 h of observation on one animal yields approximately 80% of the behaviours that would be observed at 100 h.

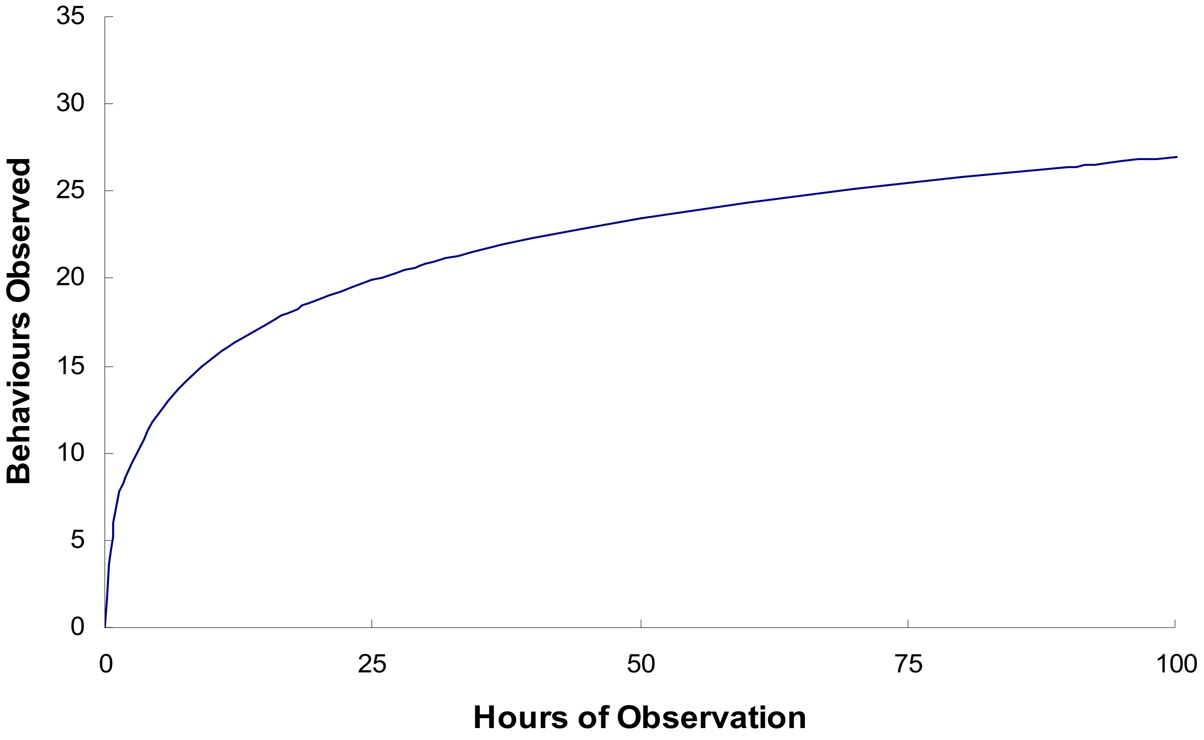

Figure 3.3 Behaviour Discovery Curve- generalised model (from 20 of observation) for the rate of behaviour discovery in observation of a single red panda.

A comparison of the predictions from the model derived from the mean of 20 h of observation per animal and the model derived from cumulative behaviours of all animals per hour is plotted in Figure 3.4 (parameters for the cumulative behaviour model were found to be -28.86 for *A* and 14.11 for *B*). Actual values and mean values are plotted alongside each model prediction. *B*/*b* = 2.71, which shows that the rate at which new behaviours are seen is over two times greater when observing *N* animals than when just observing one. Dividing *B*/*b* by *N* will yield the degree of idiosyncrasy (*I*°) in the population. This population of red pandas has a degree of idiosyncrasy of 0.087, which suggests that red pandas lean more towards inter-changeability than to idiosyncrasy in their display of behaviours.

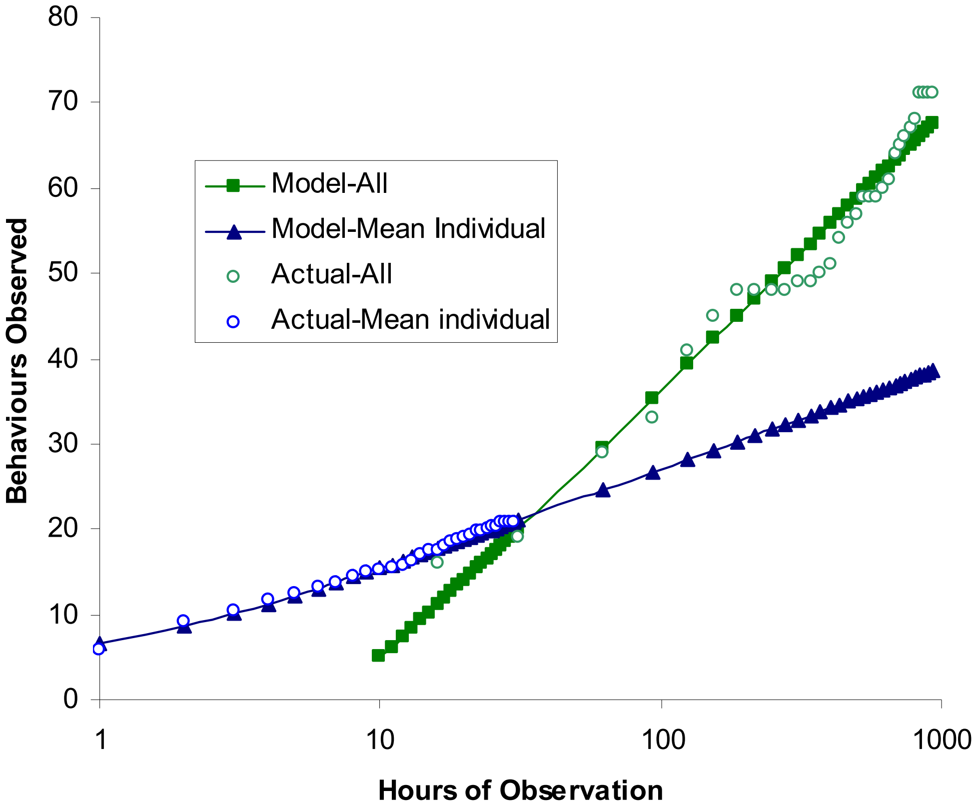

Figure 3.4 Comparison of the Models derived by observation by animal vs. observation by hour.

Ethogram

An ethogram consisting of 74 behaviours was compiled from the 930 h of observations carried out on 31 animals (see Table A1 in Appendix 1). Arbitrary points selected from the model for all animals predict that 96 behaviours would be observed at 2,000 h of observation and 153 behaviours at 5,000 h of observation.

Discussion

By using a simple logarithmic equation, we can effectively summarize the rate at which behaviours can be observed in species, with regard to a particular observation methodology. The resulting plots are species specific and should not be considered transferable but the application of the model *is* potentially transferable to any species. The application of this model is useful in ongoing observational studies on species, either in the wild or in captive settings. For example, in the red panda, 20 h of observation per animal proved to be the minimum amount of observation time required to produce a reliable, accurate estimation of behaviour discovery, since the model prediction did not differ with the addition of another 10 h of observation. Researchers should look for the levelling out of the variance in number of behaviours across animals before running the model as this suggests that individual variation, although still present, has reached a relatively stable point. The model cannot provide a specific endpoint of observation but it does give an indication as to how much gain will be made in increasing observation time. For example, in the red panda, an additional 70 h of observation (from 30 to 100 per individual) yields a rate of approximately one novel behaviour every 14 h. It is up to researchers to determine the value of increased observation time for the purposes of their particular study or the cost of additional disturbance, in the case of field studies, to the animals.

When the two models generated from observations recorded per animal versus per hour were compared, the predictions indicate that when the total number of observation hours exceeds the number of animals observed, the number of behaviours observed per hour increases at a more rapid rate when observing several animals within an observation time than when observing one single animal for the same length of time. In practical terms, this suggests that it is better to observe more animals in any given observation period than one animal for a long period of time. This is true even in animals like the red panda where the degree of idiosyncrasy appears relatively low. However, until degrees of idiosyncrasy are calculated for other species we do not yet know how red pandas compare.

Based on the calculations of this model, we estimated that 30 h of observation yielded an ethogram representative of 80% of the behavioural repertoire predicted for 100 h. For the purpose of the behavioural data collected in Chapter 5, this was deemed an accurate representation of the animal's repertoire, taking into account both individual differences as well as an overall behavioural representation of the species in captivity. Because the animals were observed during their non-breeding time, and since there were no young or juveniles observed, we know there are behaviours that are not included in the ethogram. However, the model could easily be refitted to include these additional behaviours once/if they were recorded. Red pandas are asocial animals, and as such do not have a varied social behaviour repertoire. Species with larger behavioural repertoires might have a steeper initial slope and a larger gain over time than the red pandas.

Conclusion

The value of having a model that predicts optimal observation time is wide-ranging. At a minimum, the model can be used during ongoing observations to estimate how many more behaviours are likely to be observed if observation is continued for an additional amount of time. Whether or not observation should then be stopped will depend on a cost-benefit analysis, as discussed above. Considering the lack of a universal method for determining length of observation time, this generalised straight-forward logarithmic model is useful for the purpose of setting minimum observation criteria ensuring compatibility of data across research.

Chapter 4

Investigating adaptation to captivity and effects of inbreeding in the global captive population of red pandas (*Ailurus fulgens*)

Abstract

Captive breeding has become a common technique used to facilitate the conservation of endangered species. However, behavioural and genetic changes in response to life in captivity over generations have been found to decrease the likelihood of success of animals upon release back into the wild. These changes have been attributed to adaptation to captivity as well as inbreeding depression. Red pandas are red listed as endangered (CITES 1995 and IUCN 1996) and have been part of an established captive breeding programme extending over the past 40 years. In order to investigate the response of red pandas (*Ailurus fulgens*) to life in captivity, information obtained from Glatston and Leus's 2005 red panda master globalplan and the historical studbook was used to assess the state of the current population of red pandas in captivity. I found that the lifetime reproductive success of dams (measured as proportion of successful offspring) increased rapidly (within three generations), hence providing evidence for adaptation to captivity. I also found evidence for the occurrence of inbreeding depression across generations when investigating rates of infant mortality, birth-sex ratios and longevity. Based on these findings, I make recommendations for changes in the captive management of red pandas and discuss the implications of these findings for future *ex situ* conservation efforts.

Captive breeding for conservation

Captive breeding has become a popular *ex situ* conservation technique over the past thirty years, which has been particularly promoted by zoological institutions. Due to the decline in natural habitats, captive breeding is an essential tool used to ensure the survival of many species. The aims of captive breeding programmes include research, education and conservation. The *ex situ* conservation of species is not only important for the preservation of the species in captivity, but for the maintenance of a species held in captivity for eventual release back into the wild. Habitat protection and supporting wild populations, or *in situ* conservation, are the most effective types of conservation techniques (Loftin, 1996). However, wild habitats are continuing to decline, which not only increases the number of species brought into captivity for conservation but also limits where and when captive bred animals can be released. Some estimate that the amount of suitable habitat area will not improve for at least another 100-200 years (coinciding with a predicted human population decline) (Soulé et al., 1986), if ever, which implies that there is high potential need for many species to be maintained in captivity for several generations. The length of time a population remains in captivity has implications for the suitability of the animals utilised in reintroductions.

As discussed in Chapter 2, the survival of captive born animals in reintroductions is significantly lower than the survival of translocated wild animals. The purpose of this chapter is to consider some of the direct and indirect effects of captivity and how these changes affect the animals' suitability for release into the wild. It is critical to investigate how these animals respond and adapt to life in captivity over time, as keeping generations of wild animals in captivity inevitably leads to adaptation to captivity, that is, some degree of domestication. This adaptation to captivity has implications for the survival of animals that may be released back into the wild (Frankham, 2008). Therefore, understanding the processes behind the factors contributing to adaptation to captivity will help in determining methods that might be utilised to minimise the extent of domestication that these wild animals in captivity undergo.

Adaptation to captivity vs. domestication

There are varying degrees of distinction between what is considered adaptation to captivity versus domestication, but the difference between the two can be largely attributed to how domestication is defined. In the short-term, adaptation can refer to the maintenance of immediate fitness. However, in the long-term, at a population level, there must be sufficient genetic variation within the population so that it is able to adapt by selection to changing environmental conditions (Soulé, 1989). Previous definitions of domestication have been as broad as stating that domestication is a condition in which the breeding, care, and feeding of animals are more or less controlled by humans (Hale, 1969). This definition implies that animals should be considered domestic simply by their existence in captivity. However, domestication is more than just the consequence of altering behaviour due to changes in environmental experience, as domestication also involves genotypic changes that occur gradually over generations, and can result in the formation of sub-species. A case in point is the domestic dog (*Canis lupus familiaris*) but the formation of sub-species has also been reported in captive zoo otters (*Lutra lutra*) and captive red pandas (L. Dueck, personal communication, 2nd March 2005). This additional clarification has led to a refined definition of domestication as a ‘process by which a population of animals becomes adapted to man and to the captive environment by some combination of genetic changes occurring over generations and environmentally induced developmental events reoccurring over each generation' (Price, 1984 p3).

Based on the definition outlined above, it is feasible to say that an individual animal taken from the wild might adjust to life in captivity but does not become domesticated, since domestication refers to the result of adaptation occurring over generations. It is tempting to pose the question of how many generations it takes for a population to adapt to captivity to the point of domestication? However, there are many factors influencing the rate of adaptation (for example, the number of animals in the population, the amount of exposure to humans, the enclosure type and so on) and if we consider adaptation to captivity to exist along a continuum, then it is difficult to pinpoint stages.

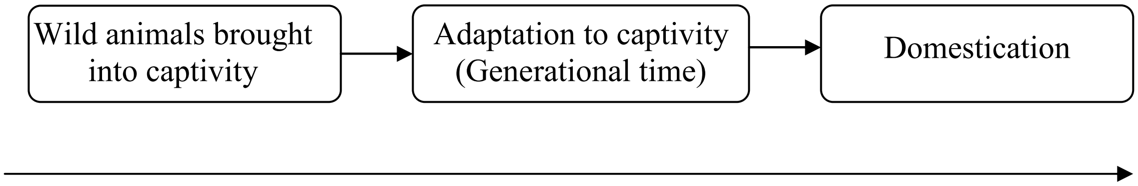

Figure 4.1 Simplified continuum of the domestication process

The continuum begins at the level of individual animals brought into captivity, which then leads into the gradual behavioural and genetic adaptation to life in captivity of the founder animals' descendants (Figure 4.1). Domestication can be considered to be achieved when the overall reproductive fitness of a population is maintained at a stable level and when no further genetic or environmental changes have any significant long-term effects on the population (Price, 1984).

Integrating behavioural and genetic adaptation to captivity

Genetic and behavioural adaptations to captivity are not independent from each other. The well known farm fox experiment elucidates this nicely – the geneticist Dmitry Belyaev selectively bred more tame animals and found that this artificial selection based on behavioural traits resulted in subsequent genotypic and phenotypic changes (Trut, 1999). Therefore, it is essential to investigate both behavioural and genetic changes to captivity.

A loss of wild type behaviours can be seen as animals adapt or adjust to life in captivity (Rabin, 2003; Wallace, 2000). The best studied behavioural aspect of adaptation to captivity is how animals adjust their response to novel stimuli, in particular with regard to their captive environment (Price, 1998). This change involves a decrease in reaction to novel stimuli, both in the intensity of the reaction and in the response time. As animals become accustomed to their captive environment, stress levels decrease and fitness is improved (Price, 1998). The repercussions of this behavioural adaptation to captivity can be seen directly when investigating the common causes of death in reintroduced captive born animals (see Chapter 2).

Loss of genetic diversity in captive populations

Captive populations are essentially small, isolated populations and therefore are subject to rapid genetic deterioration and fitness decline. The decline in fitness of a population can be attributed to loss of genetic diversity, inbreeding depression, increased presence of deleterious mutations and genetic adaptation to captivity (Lacey, 1996; Woodwarth et al., 2002). Loss of genetic diversity can affect a population such that effective population size is reduced, sex-ratios become unequal and inbreeding depression may occur (Briscoe et al., 1992; Frankham et al., 2003). The importance of research on genetics in relation to species conservation is essential in providing a greater understanding of how populations respond to change in environmental conditions (Amos & Balmford, 2001).

Terminology

Inbreeding and inbreeding depression

Before proceeding further into a review of conservation genetics, it is necessary to clarify some key terms and how they relate to captive breeding. Inbreeding occurs when the parents of an individual are related. Inbreeding in small populations is inevitable since all individuals become related by descent over time. Inbreeding is an important issue facing captive breeding programmes as it is known to reduce reproductive fitness by contributing to a decrease in heterozygosity, thereby increasing the likelihood of the expression of deleterious mutations. This reduction in fitness is referred to as inbreeding depression (Olney, 1977). Inbreeding is measured by an inbreeding coefficient (F) which ranges from zero to one, with zero representing completely outbred populations and one representing completely inbred. The relationship between inbreeding and inbreeding depression is linear, so that as inbreeding increases so does inbreeding depression. Inbreeding depression has been shown to adversely affect all aspects relating to reproductive fitness, including number of offspring, life expectancy, mating ability, sex ratios, maternal ability and delayed developmental time (Bryant, Vackus, Clark & Reed, 1999; Frankham et al., 2003). The importance of this in relation to *ex situ* conservation can be seen in the results of a study by Jimenez et al (1994), who showed that outbred animals survived significantly more often and for longer periods than their inbred counterparts in a reintroduction.

Family size

Family size (k) refers to the lifetime production of offspring per individual. The variance in family size is denoted as V_k and the ratio of V_k/k equals one in an idealised population (a population which is maintained through random mating to ensure maximum genetic variation). The value of one represents one offspring for each sire and dam pairing. In a stable population of a randomly breeding monogamous species, the mean family size (k) is 2 (which indicates that each parent is replaced by one male and one female) and the variance (V_k) is 2. From these values the V_k /k ratio can be calculated. When the V_k/k ratio is 1 (the variance (V_k) equals the mean (k)) there is a Poisson distribution which represents a population with an idealised structure (random matings). Most V_k/k ratios in reality exceed 1 and as such denote a variation in family size, which suggests that there is a proportion of individuals not contributing offspring to the next generation. High variance in family size might suggest the presence of either very large or very small families within a population. A high variation in family size reduces the effective population size since only animals which are successfully breeding are genetically contributing to the population (Frankham et al., 2003).

Effective population size

The genetically effective population size (N_e) differs from the actual or census population size (N). N_e is defined as the size of an idealised population that would lose genetic diversity (or become inbred) at the same rate as the actual population (Frankham et al., 2003). In other words, if a real population loses genetic diversity at the same rate as an ideal population of 100, then the real population has an effective size of 100, even if it contains 1000 individuals. Therefore the N_e of a population represents a measure of its genetic behaviour relative to that of an ideal population. The N_e/N ratio is important in determining the factors contributing to loss of genetic diversity and inbreeding. A meta-analysis carried out by Frankham (1995) showed that the most important factors affecting the N_e/N ratio are population size, variation in family size and unequal sex-ratios. If family sizes are equalised so that all individuals contribute equally to the next generation, then N_e is approximates $2N$ and the effective size of the population can essentially be doubled. Consequently, equalisation of family sizes (EFS) is predicted to halve genetic adaptation to captivity as it removes opportunities for natural or artificial selection (Frankham et al., 2000). Management of captive breeding programmes for conservation commonly uses EFS to increase N_e (minimise inbreeding) (Nunney, 2000; Glatston & Leus, 2005; Russello & Amato, 2004).

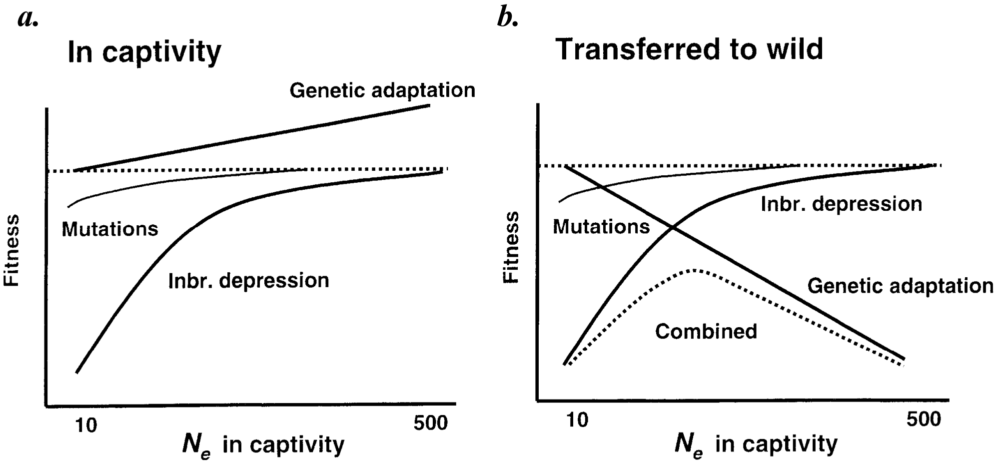

Figure 4.2 Genetic deterioration in captivity and its impact on release back to the wild. The effects are shown for populations maintained for approximately 50 generations under (a) benign captive conditions and (b) when released into the wild. Figure adapted from Woodwarth et al. (2002).

The combined effects of inbreeding depression, the build up of deleterious mutations and genetic adaptation to captivity and their expected relationship between reproductive fitness and population size (N_e) can be seen in Figure 4.2 *a* and *b*. These figures are adapted from a study conducted by Woodworth and colleagues (2002) who also showed supporting evidence that these interactions occur in captive populations. The figures show that changes in relation to the wild or captive fitness of a population are most influenced by genetic adaptation and inbreeding depression, and less so by deleterious mutations. Logically, inbreeding depression has more of an effect on small populations, but the plots also show that genetic adaptation is of great concern in large populations. Plot *b* clearly shows that genetic adaptation to captivity (or an increase in captive fitness) has serious implications for the adaptive abilities (or wild fitness) of animals upon their release into the wild. The curvilinear relationship between wild fitness and population size indicates that genetic adaptation and inbreeding depression are the two contributing factors to the decline in wild fitness of captive animals; the peak of the curve suggests that there is an optimal population size which will yield the least amount of loss in wild fitness. Fitness refers to all aspects associated with reproduction, including the behaviours associated with mating abilities and strategies.

Wild animals in captivity for conservation

It is important to consider the implications of inbreeding for wild animals involved in captive breeding for conservation. Each species responds to captivity to a different extent: factors relating to these differences can include varying mating systems (e.g. monogamy, polygamy, etc…), longevity (i.e. life expectancy and generation time), habitat and social structure in the wild, food source (e.g. carnivore, herbivore, etc…) and so on. These factors not only contribute to how a species adapts to captivity but the rate at which it adapts. Therefore, it is essential to investigate individual species' responses to life in captivity and to investigate the implications of these responses in relation to their conservation. Species that are listed as endangered and involved in captive breeding for conservation programmes should be among the first to be evaluated in such a manner.

Red pandas as a species of study

Red pandas (*Ailurus fulgens*) are listed as endangered and are involved in global conservation breeding programmes; therefore, their adaptation to captivity is important to study for their future conservation. A comprehensive review of red panda ecology and behaviour can be found in Chapter 1, Part 2. For the purpose of this chapter, an outline of their behaviour and life history traits in the wild will be briefly reviewed for the purpose of comparison to their adaptation to captivity. Red pandas in the wild spend a majority of their time searching for and eating bamboo, and due to their low-energy diet and their unspecialised gut, are otherwise moderately inactive (Roberts, 1992). Red pandas are solitary in nature and maintain relatively (for their body size) large home ranges. Home ranges overlap between sexes and among males, but seldom among females (Yonzon & Hunter, 1991). Males and females meet up only during the breeding season. Offspring develop slowly and maternal care continues until six to twelve months of age. Due to their elusive nature in the wild and a lack of longitudinal studies, lifespan in the wild is unknown, but their mean life span in captivity is 8-10 years (Roberts, 1992).

The global captive breeding masterplan for the red panda (Ailurus fulgens fulgens and Ailurus fulgens styani)

The global masterplan is a report on the current condition of the red pandas in zoological institutions. The masterplan also sets the goals for the captive population of red pandas. The current goals for the population are 1) to provide a back up population for wild populations, 2) to educate and improve public awareness, 3) to supply individuals for reintroduction purposes, 4) to maintain a minimum of 90% founder gene diversity in the global captive population after 100 years, and 5) to manage the population at a global level with semi-autonomous regional subpopulations. The global captive breeding masterplan for the two sub-species of red pandas *(Ailurus fulgens fulgens* and

Ailurus fulgens styani) referred to in this study was current up to 2005 (Glatston & Leus, 2005).

There are seven main regional breeding programmes: the North American (SSP) and Chinese breeding programmes work with both sub-species; the European (EEP), Australian, South Africa and Indian programmes focus on the sub-species *Ailurus fulgens fulgens*; and the Japanese programme focuses on *Ailurus fulgens styani*.

The historical list of captive red pandas (both sub-species) housed in accredited zoological institutions dates back to 1964 (Glatston, 2005). The previous global red panda masterplan report came out in 1993 (Glatston & Princee, 1993) and the achievements of the goals and objectives set out in the 1993 masterplan are discussed in the 2005 plan. The main goal of the 1993 masterplan was to retain 90% genetic diversity for a period of at least 100 years without importing individuals from the wild (Glatston & Princee, 1993). Some of the steps defined to achieve this goal were to 1) increase the effective population size by equalising family sizes (limit six offspring per sire and dam), 2) increase numbers of mated pairs housed together, 3) ensure equal sex ratio, 4) avoid inbreeding and 5) reduce infant mortality. The 2005 masterplan highlights that not all of these objectives were met. Despite an increase of 25% in the overall population, the N_e/N ratio has only improved slightly from 0.25 to 0.28 and only small efforts have been made to equalise family sizes (in other words, to improve the number of animals genetically contributing to the population). The 1993 and 2005 global masterplans report that red pandas in inbred groups showed increased infant mortality. Table 4.1 lists some of the statistics published in the Glatston & Leus (2005) report on captive red pandas which will be used for reference and comparison in the discussion.

Table 4.1. Statistics from Glatston & Leus (2005) Global masterplan for captive red pandas (*Ailurus fulgens*)

	Ailurus fulgens fulgens						*Ailurus fulgens styani*		
	Founder representation	SSP	EEP	Australia	S. Africa	India	Founder representation	SSP	Japan
Number of founders	28	23	26	21	20	22	45	31	38
Gene diversity	0.948	0.907	0.927	0.869	0.862	0.850	0.974	0.907	0.970
Potential gene diversity	0.971	0.947	0.96	0.922	0.910	0.899	0.988	0.956	0.987
Predicted 100 year gene diversity (Goal 0.90)	0.90	0.731	0.859	0.527	0.363	0.224	0.719	0.01	0.884
Genetic variation	0.946	0.903	0.925	0.854	0.855	0.843	0.970	0.904	0.966
Mean inbreeding coefficient	0.047	0.043	0.042	0.07	0.082	0.028	0.009	0.04	0.005
Ne/N ratio	0.286	0.312	0.276	0.385	0.247	0.296	0.296	0.196	0.33
Generation length (years)	6	6.9	6.1	5.4	5	5.7	6.5	6.2	6.7
Max. population growth rate	1.05	1.00	1.05	1.09	1.21	1.06	0.982	0.979	1.007
30 day mortality (%)	27.2	40.2	23.6	10.6	25.9	18.4	22.5	21.5	25.5
r (males/females)		0.011/-0.991	0.050/0.055	0.106/0.068	0.247/0.141	0.136/-0.023	0.012/-0.033	0.003/-0.450	0.025/-0.012
Current population	450	105	222	44	35	18	254	40	227
Effective population	126	32.6	66.6	16.7	8.4	5.2	73.7	7.6	74.9

Other parameters for *Ailurus fulgens fulgens*: Mx is a measure of age specific fecundity, which peaks (Mx > 0.3) from 4 to 7 for females and from 3 to 9 for males. First year mortality is around 40%

Aims and Objectives

The overall aim of this study was to assess how red pandas were responding to life in captivity. This study, in particular, investigated generational changes in fitness within the known population of red pandas housed in zoological institutions globally. The data available in the historical studbook (list of all known captive red pandas, both living and deceased) were the base of my analyses. The 2005 global masterplan addresses several issues relating to inbreeding and genetic diversity in the current population. However, it does not provide insight into how these changes have progressed through the generations in captivity or the population's current state of adaptation to captivity and inbreeding depression. Based on previous research investigating the effects of generational time in captivity we decided to focus on parameters associated with captive fitness, such as lifetime reproductive success, infant mortality rates, birth sex ratios and lifespan.

Methods

Data set and subjects

The data set was extracted from the red panda (*Ailurus fulgens*) historical studbook list published in 2005, which was current up to 31st December 2004. The data set contains information on individuals dating back over 40 years (to 1964). Descriptive statistics on the data set are included in Table 4.2. Breeding was measured as success if the offspring either lived to six months or were transferred to another institution, whichever came first, such that death was not associated with parent rearing. Therefore, success is only used as an indicator of the breeding success of the parent (in this case, the dam). Infants who do not contribute to the dam's reproductive success are only counted in infant mortality data throughout the analyses and summary data; all other variables only include surviving infants who are deemed to contribute to a dam's reproductive success.

Table 4.2. Descriptive statistics on the historical studbook list of captive red pandas

	Total	Wild-caught	Captive-born	Surviving captive-born	Currently living
Male	1060	141	919	569	306
Female	1043	158	885	593	290
Unknown	258	4	254	12	7
Totals	2361	303	2058	1174	603

Life expectancy for captive-born animals (who lived past infancy) was found to be 5.95 years, SD± 4.18, with the oldest recorded age of 18.41 years. The mean age of a female at her first birth was 3.29, SD±1.5 (only captive-born females are represented here, as age at first birth is unknown for wild-caught). Generations from wild were calculated via matrilineal lines. There are seven generations from the wild represented in this database. For the purpose of analyses, generations five to seven from the wild were combined because generations six and seven were not adequately represented in the population. I was unable to calculate generation from wild for 161 animals as they could not be traced back to a known founder dam (for example, information was missing on dam 7210, a potential founder dam, and since I could not determine her status (either wild-caught or captive-born), I therefore could not calculate her descendants' category).

Family size and effective population size

Values were calculated for mean family size (k) and the variance in family size (V_k). From V_k, we can also calculate the current effective population size (N_e) from the following equation (Frankham et al 2003):

$$N_e = (4N - 2) / (V_k + 2) \qquad \text{Equation 4.1}$$

Where N represents the number of adults in previous generations.

I also investigated the effect of the variance in family size on the effective population size, which is calculated by the equation:

$$N_e / N \sim 4 / (V_k + 2) \qquad \text{Equation 4.2}$$

Analyses

T-tests were used to look for differences between males and females in lifespan and number of transfers. Regressions were run, using the enter method, selecting for dams only, in order to investigate the effect of generation from wild on reproductive success, infant mortality, and the proportion of female offspring (birth sex ratios). A paired samples t-test was run to see if there were any sex differences in infant mortality. A regression was also used to investigate any changes in life expectancy across generations; only deceased, captive-born animals were included in this analysis.

All analyses were carried out in SPSS v.14.

Results

Average life expectancy was $\overline{X}$ ± SD = 5.79 years ± 4.07 for males and $\overline{X}$ ± SD = 6.19 years ± 4.27 for females, this difference was not significant (t=-1.519, df=989, p=0.129). On average, males were transferred more than females ($\overline{X}_{males}$ ± SD = 2.38 ± 1.09, $\overline{X}_{females}$ ± SD = 2.26 ± 1.07) t=1.973, df=1449, p=0.049.

Family size and effective population size

Mean family size (k) is 2.4037 and variance in family size (V_k) is 14.643, the V_k/k ratio was therefore 6.095 (which exceeds 1, therefore indicating there is variance in family size within the captive population of red pandas). The effective population size (N_e) was calculated to be 206 (see Equation 1). Using Equation 4.2 above, the variance in family size was calculated to reduce the effective size of the red panda population to 24% (the N_e/N ratio is 0.24). When N_e was compared to the actual current number of living red pandas (N=603), then N_e was representative of 34% of the observed number of potential breeders.

Changes across generations

Mean successful offspring

Table 4.3 Predictor variables for mean successful offspring

Predictor Variable	**β**	***p***
Years lived so far	.458	$p<0.0005$
Number of transfers	-.084	$p=0.028$
1 Generation from wild	.164	$p<0.0005$
2 Generations from wild	.152	$p=0.001$
3 Generations from wild	.229	$p<0.0005$
4 Generations from wild	.144	$p=0.002$
5 + Generations from wild	.095	$p=0.043$

Lifetime reproductive success of a dam was significantly predicted by generation from wild, when controlling for age (in years) and number of transfers Adj $R_2 = .195$, $F_{7,636}=23.260$, $p<0.001$. Lifetime reproduction success significantly increased, exceeding founder rates in all generations from wild (see Figure 4.3). Significant variables are shown in Table 4.3. Age had a positive effect on reproductive success and the number of transfers appeared to have a negative effect on lifetime reproductive success.

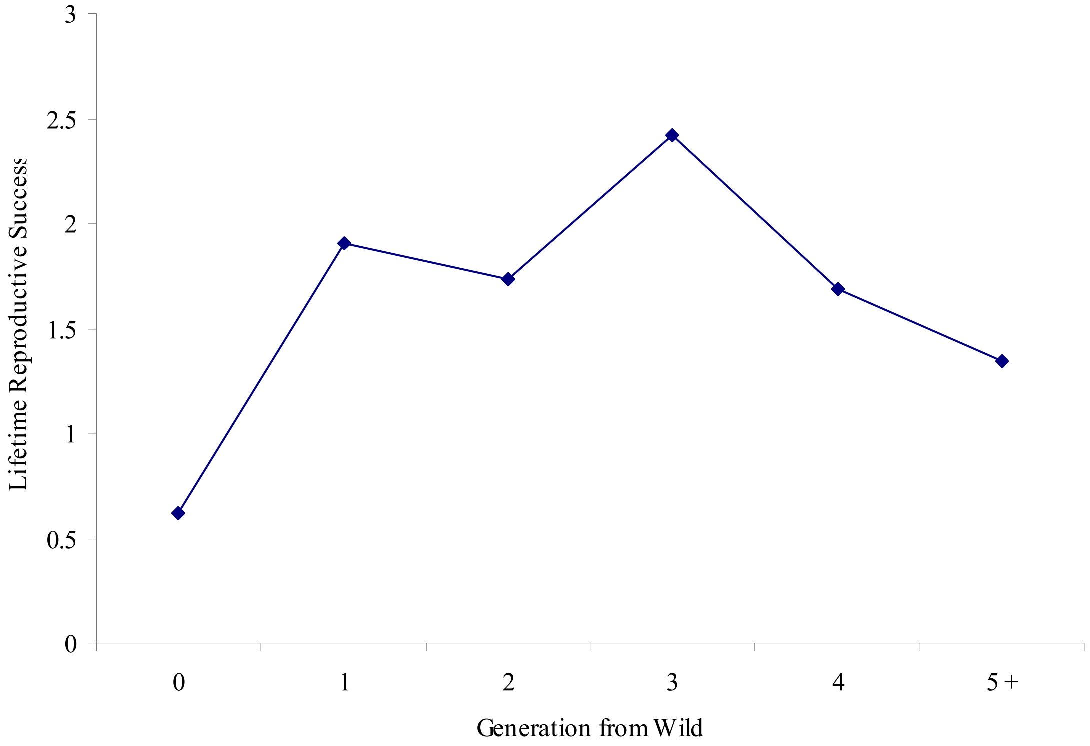

Figure 4.3. The change in mean reproductive success across generations. Means represent the adjusted means calculated for each variable of generation from wild, taking into account years lived and number of transfers.

Infant mortality

Table 4.4. Predictor variables for infant mortality

Predictor Variable	**β**	***p***
Years lived so far	-.016	$p<0.005$
Number of transfers	-.008	$p=0.666$
Generation from wild	.197	$p<0.005$
2 Generations from wild	.224	$p=0.001$
3 Generations from wild	.144	$p=0.036$
4 Generations from wild	.278	$p<0.0005$
5 + Generations from wild	.334	$p<0.0005$

Infant mortality, or the proportion of offspring who died by six months, was significantly predicted by generation from wild (see Figure 4.4). Again, I controlled for age and number of transfers and age appears to have a negative effect on infant mortality, suggesting that older dams have lower rates of infant mortality, Adj R_2 =.117, $F_{7,307}$=6.916, p<0.001. The variables are shown in Table 4.4.

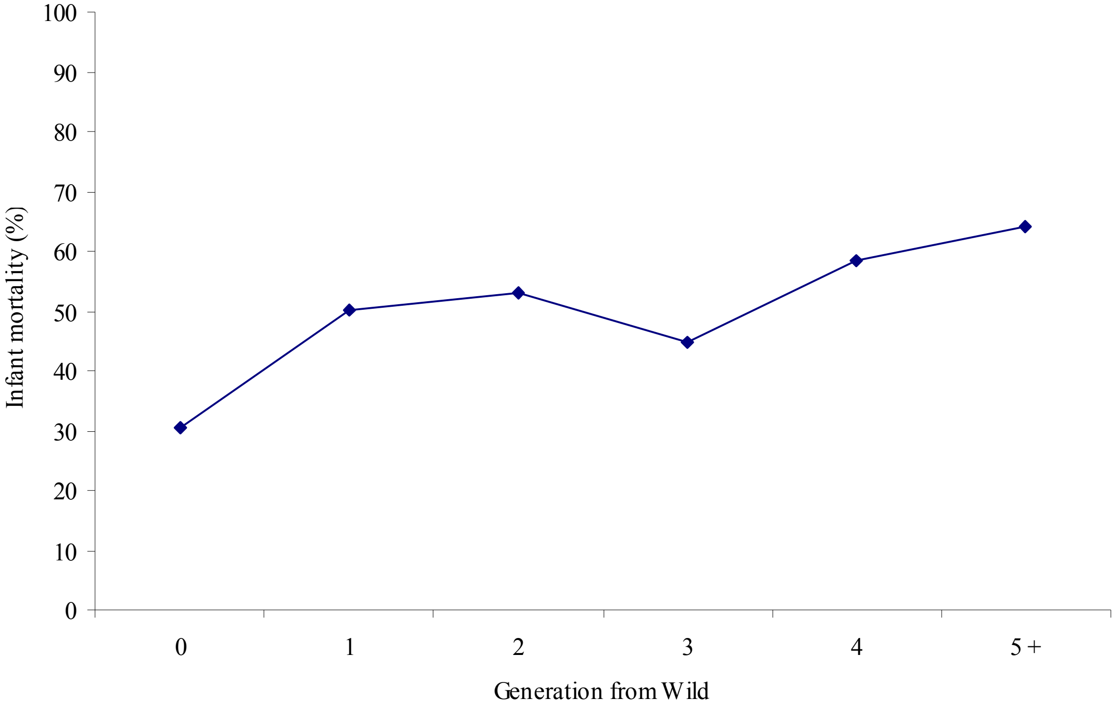

Figure 4.4 The adjusted mean increase in infant mortality across generations.

There were no differences in the rate of infant mortality in male (0.492 ± SD 0.33) and female (0.486 ± SD 0.33) offspring, t=-.128, df=242, p=0.899.

Birth-Sex ratio

Table 4.5 Predictor variables for sex ratio

Predictor Variable	**β**	***p***
1 Generation from wild	-.158	p=0.035
5 + Generations from wild	-.251	p<0.001

The proportion of total female offspring born (regardless of survival) could be predicted by generation. Again, age and number of transfers were controlled for, and the overall regression was significant Adj R_2 = .025, $F_{7,307}$=2.163, p=0.037, with a low adjusted R square. Neither age nor number of transfers were significant predictors, but results show that the drop in the rate of female births between wild and first generation dams is significant. The ratio appears to level out for generations one through four, but goes significantly lower than the founder ratio in generations 5 + (see Table 4.5 and Figure 4.5).

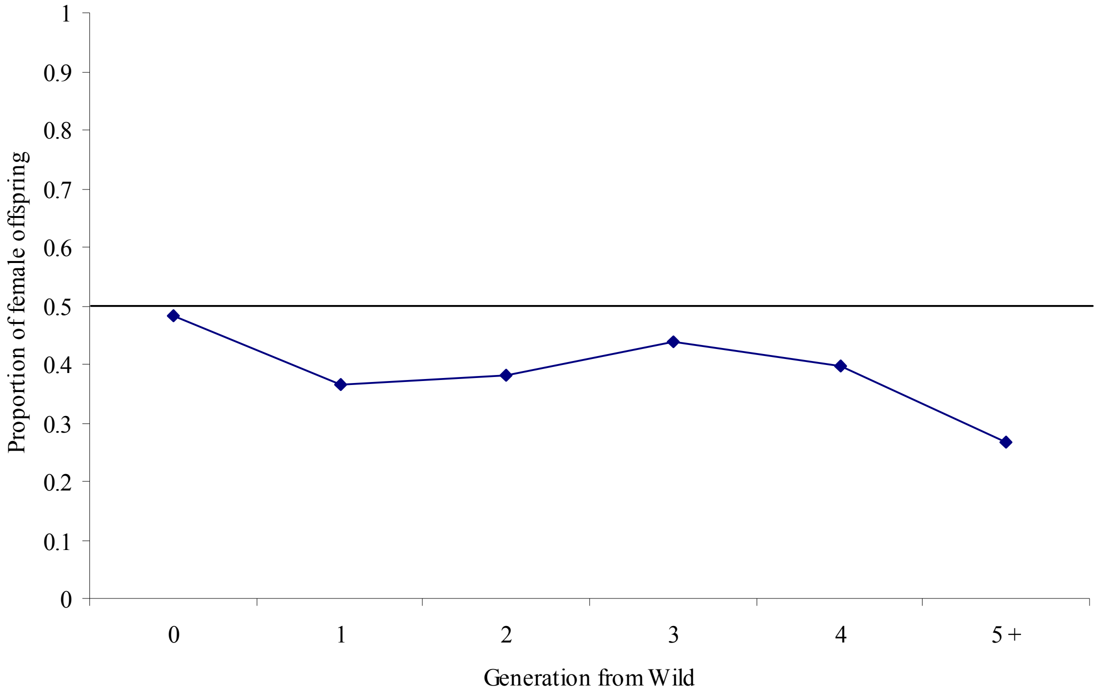

Figure 4.5 The decline in the female sex ratio (adjusted mean) across generations.

Life expectancy

Table 4.6 Predictor variables for life expectancy

Predictor Variable	**β**	***p***
Number of transfers	1.678	$p<0.0005$
2 Generations from wild	-1.363	$p=0.008$
4 Generations from wild	-2.258	$p<0.0005$
5 + Generations from wild	-3.246	$p<0.0005$

Life expectancy was found to be affected by generation from wild and appears to decrease as generations move further from the wild (see Figure 4.6). Adj $R_2 = .257$, $F_{5,483}=34.71$, $p<0.0005$. Wild-caught animals (Generation 0) were excluded from the analysis because their exact age could not be determined. The number of transfers had a positive effect on lifespan, however the direction of the relationship is more likely to be driven by the probability that the longer an animal lives, the more likely it is to be transferred rather than the converse. Significant predictor variables are shown in Table 4.6.

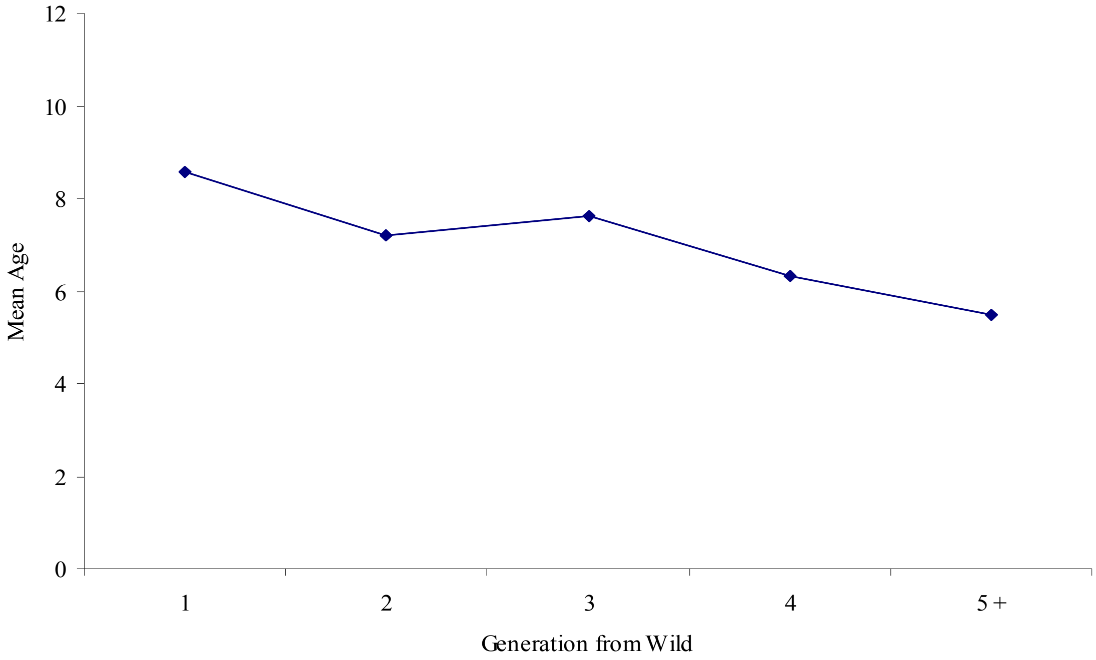

Figure 4.6. Mean adjusted age at death for subsequent generations from the wild.

Discussion

Overall, the number of generations from wild was a strong predictor variable for all factors investigated in this study, namely, dam's reproductive success, infant mortality, birth sex-ratios and longevity. How these findings relate to adaptation to captivity and inbreeding depression will be examined further on in the discussion. The implications of these results on the future captive breeding for conservation will also be addressed.

Family size

Calculations of the family size and variance of family size reveal that the V_k/k ratio is just above six. This suggests that the efforts made by the management programme to equalise family sizes (EFS) by reducing the offspring to six per each pairing of sire and dam have been reasonably successful. However, since EFS occurs at one, the number of offspring could be lowered further in an attempt to decrease the rate of loss in genetic diversity. This variance in family size implies a N_e/N ratio of 0.24, which is similar, although slightly lower then the ratio calculated in the 1993 and 2005 reports (0.25 and 0.28 respectively).

Gene diversity and inbreeding coefficients

Gene diversity in the current population ranged from 0.89 to 0.98 (calculated by Glatston & Leus, 2005, refer to Table 4.1), which isrelatively high. The calculations presented by Glatston & Leus (2005) are slightly confusing as they report that the predicted goal of 0.90 genetic diversity for 100 years is possible for the captive *Ailurus fulgens fulgens* as a whole; however, none of the individual programmes (e.g. SSP) reaches this goal. Mean inbreeding coefficients range from 0.009 to 0.082 in the sub-populations, with an overall population inbreeding coefficient of 0.047.

Changes across generations

Number of generations in captivity appeared to have an effect on all factors associated with fitness. Reproductive success was significantly higher than founder levels (wild caught animals) in all generations in captivity from the wild, the most significant increase occurs at 3 generations from the wild. Reproductive success of third generation dams was four times greater than that of wild-caught individuals; this increase in reproductive success over the generations suggests adaptation to captivity. There are at least two explanations to consider for the cause in decline of reproductive success from the third generation. Firstly, it might be explained as an effect of captive management to equalise family sizes (EFS) and control the number of offspring each sire and dam pairing contribute to the population (i.e. through birth control or male-female separation during the breeding season). As stated above, efforts by zoo management to equalise family sizes have been successful. A second possibility is that the decline in successful offspring after the third generation is explained by inbreeding depression, since a decline in reproductive fitness is a common consequence of inbreeding depression (Laikre, 1999; Cassinello, 2004) and this decline has been observed in just two generations from the wild (Araki, Cooper & Blouin, 2007). It is difficult to discriminate between the two explanations, and perhaps it is a combination of both factors working in conjunction or another factor entirely.

The increase in reproductive success across generations provides evidence for adaptation to captivity; however, results from analyses on infant mortality, sex ratios and lifespan all suggest that there is decreasing reproductive success across generations. Infant mortality increases, sex ratios shift and mean lifespan declines as the generations move further away from the wild. These fitness reducing factors suggest that inbreeding depression may be occurring at some level in the captive population of red pandas. Infant mortality is currently over 60% in generations five to seven from the wild. This figure is much higher than the overall 40% infant mortality rate calculated in the 2005 report, and it is only by investigating the population at the generation level that we see a more accurate picture of the population's change in infant mortality. Steps need to be taken to improve this decline in infant mortality in order to preserve genetic diversity.

The drop in ratio of female offspring is particularly worrying as an equal sex ratio is an important factor in improving the N_e/N ratio. The 2005 management programme has identified this shift in sex ratios in three of the red panda sub-programmes and has highlighted the need to rectify the problem. There are no differences between the sexes in rates of infant mortality, so this decline of females in the population is purely related to sex determining mechanisms such as differential abortion. Varying sex ratios can and do occur in the wild, most often in response to changes in environmental conditions, and are generally associated with the costs or benefits associated with producing male or female offpsring (Clutton-Brock & Iason, 1986). Therefore, it is feasible to suggest that this change in sex ratios may be a result of the captive environmental conditions (e.g. nutritional quality of provisioned food); however, the fact that this occurs in conjunction with decline in infant mortality and longevity is more suggestive of inbreeding depression.

Effects of age and number of transfers

Age was a significant predictor of fecundity and percentage of infant mortality such that annual reproductive success improved and infant mortality was reduced in older females. This finding of increased fitness with age has implications for housing and breeding management. Results from the 2005 report show that female fecundity peaks between four and seven years of age. Red pandas are reported to be sexually mature at 18 months (Roberts, 1984), so it would be worthwhile to investigate what factors influence fecundity in females and if success is associated with experience.

The number of transfers also appeared to have an effect on reproductive success such that fewer transfers predicted an increase in reproductive success. This again has implications for captive management in that transfers should be limited, when possible, and males should be transferred more frequently than females. Analysis shows that males are already transferred more than females, but this difference could be greater, as the current mean for number of transfers per male is 2.38 times in a lifetime and only slightly less at 2.26 times for females.

Red pandas in captivity

It appears that the red pandas in captivity are undergoing genetic adaptations to captivity as well as inbreeding depression. The four-fold increase in lifetime reproductive success in dams in the third generation from wild suggests that there is indeed rapid adaptation to captivity. Previous research reports that captive reproductive fitness can improve by at least 25% in as little as six generations from the wild (Gilligan & Frankham, 2003), so this substantial increase in lifetime reproductive success in as few as 3 generations suggests that adaptation to captivity can occur more rapidly than previously thought.

The decrease in overall fitness with respect to infant mortality, birth ratios and longevity suggest inbreeding depression. The overall current inbreeding coefficient was .047, which is relatively low. However, traits associated with fitness (e.g. infant mortality and longevity) have been found to decrease by as much as 6 – 31% at an inbreeding coefficient (*F*) of 0.1 (Laikre, 1999), so inbreeding depression in this population is feasible. Moreover, Figure 4.2 implies that an effective population size (N_e) of 200 can still produce a substantial decrease in genetic diversity over generations just a few generations. Both the effects of genetic adaptation and the potential for inbreeding depression in the captive red panda population have implications for the fitness of these animals upon a release into the wild.

Based on the results of the studbook analyses, efforts should be made to increase the effective population size (*Ne*) by reducing the rates of infant mortality (particularly of individuals who are currently not genetically contributing to the population) and reversing the shift in sex ratios (by investigating factors that may be influencing this shift, e.g. diet). These efforts should help increase the potential number of individuals reproductively contributing to the population. Possible recommendations for practice include decreasing the number of transfers for each female.

Implications of findings

Considering the rate of adaptation to captivity and the possibility of inbreeding depression in the red panda's population, it is worthwhile to consider the implications for *ex situ* conservation, such as when it is feasible for individuals or populations in the wild to be moved into captivity for conservation. The red pandas' generation time has not yet exceeded seven from the wild in 40 years of captivity. As found in the red pandas, adaptation to captivity (or captive fitness) can increase substantially in just three generations and inbreeding depression can appear in an effective population size of over 200 in just five generations. Therefore, bringing populations into captivity from the wild for conservation prematurely (or before they need to be) decreases their wild fitness, and hence limits that species' likelihood of success when released back into the wild. These findings stress the importance of promoting *in situ* conservation, such as protection of natural habitats, for as long as possible since adaptation to captivity and inbreeding depression are strong forces acting against the fitness of captive populations being conserved in captivity.

Chapter 5

An investigation on the behaviours of captive red pandas (*Ailurus fulgens fulgens*) housed in the UK

"follow the trail of the fox
to tell the tale of the fox…"

From the poem 'Untitled Couplets' by Paul Aird

Abstract

Behavioural investigation of animals in captivity is important not only in regard to learning more about the species in question, but also in improving the captive conditions under which animals are kept. The purpose of this study was two-fold: one aspect was to explore the effect of various aspects of the captive environment and how these may have an effect on the behaviours of captive red pandas; the other aspect was to further investigate behavioural indicators of adaptation to captivity. The model presented in Chapter 3 provided the rationale for the amount of behavioural data collected in this study. 31 captive red pandas (*Ailurus fulgens*) in 13 zoo institutions were observed for a total of 930 hours. Regression analyses were run using individual level data on behaviours (such as stereotypies and frequency of scent marking) when investigating the effects of generational time in captivity and relatedness. In the exploratory regression on environmental effects, analyses were run using enclosure level data. Generation time in captivity was not a significant predictor of behaviours. Some environmental variables such as amount of keeper contact, size of usable area, enclosure complexity did affect behaviours, such as activity levels and stereotypies. Recommendations are made for improving husbandry techniques and the implications of these findings on our understanding of response to life in captivity are discussed.

Why Research Animals in Captivity?

There are intrinsic difficulties in researching animals at zoological parks. Researchers must contend with small sample sizes, unavoidable extraneous variables, interruptions and lack of baseline data (Maple et al., 1995). However, the benefits of zoo research are far-reaching and have great potential for furthering our knowledge about many species (Gittleman & McMillan, 1995). Benefits of carrying out zoo research include the opportunity to obtain life history information on individual animals, information on the global population (from the studbook) and the opportunity to study animals that are elusive in the wild - which allows for research to be conducted on a much smaller budget.

Researching animals in captivity can also tell us a great deal about how animals respond to life in captivity and has the potential to give us insight into long-term effects of captivity on behaviour and genetics (see Chapter 4). Captive animal research done in conjunction with field work on wild counterparts is important in helping to assess which 'natural' behaviours are essential for an animal's well-being as well as in gaining a greater understanding of the process of adaptation to captivity that species are undergoing.

For the purpose of conservation (species survival and reintroduction), research must be carried out on captive animals to determine effects of captivity and methods for improving welfare, particularly on endangered species which are bred in captivity for potential release into the wild (Gippoliti & Carpaneto, 1997). Captive breeding has a selective effect on genetic and behavioural traits of captive animals; these non-random selective mechanisms have been defined by Price (1984) as artificial selection (traits desired by humans), natural selection in captivity (animals best able to adapt to captive conditions), and relaxation of selection (the maintenance of traits that would have been selected against in the wild). Chapter 4 includes further discussion and explanation of these processes. Many of these short-term (e.g. abnormal behaviours) and long-term (e.g. genetic changes) effects of captivity can be detrimental to the conservation of a species, thus zoo research on investigating effects of captivity is critical to improving the chances of species survival and *ex situ* conservation efforts (Sutherland, 1998).

Behaviours in Captivity

Activity budgets

Behavioural budgets of animals in captivity can differ drastically from those of their wild counter-parts. The size and complexity of the enclosure, the provisioning of food, the amount and type of social housing, and the lack of prey or predators all have an effect on the activity budgets of animals in captivity (Mellen et al., 1999). Therefore, it is important to assess the behavioural budgets of animals in captivity and to investigate how these behaviours may or may not indicate well-being, as well as gaining an understanding of the process by which animals are adapting to captivity.

Activity budgets represent how much time a captive animal spends active versus inactive and also provide information on an animal's behavioural repertoire. Some researchers report that behavioural variance increases in captivity, primarily due to the lack of selection pressures (e.g. predator impact) so animals are more able to engage in novel types of exploratory behaviour (McPhee, 2004). However, it may be more accurate to say that behavioural repertoires shift in captivity, because although novel, less fearful and more bold-type behaviours may increase (see Chapter 6), this can coincide with a loss of more wild type behaviours, such as avoidance and appropriate social behaviours. Activity budgets can be important indicators of animal welfare (Blasetti et al., 1988). Field data on the species' wild counterparts is also useful for comparison and is an essential aspect of using activity levels as a welfare indicator (Lindberg, 1988). For example, reduced exploratory behaviour in captive leopard cats (*Felis bengalensis*) was reported as a response to chronic exposure to aversive environmental conditions (Carlstead et al., 1993). Decreased activity can also suggest apathy, which can indicate the poor mental state of an animal (Shepherdson, 1999). However, not all differences in activity budgets between wild and captive animals indicate poor well-being, since animals can adjust their activity budgets based on what opportunities they have available; and these opportunities differ substantially between wild and captive settings.

Abnormal behaviours

Abnormal behaviours may indicate detrimental effects of captivity. Many types of abnormal behaviours have been observed in captive animals, such as stereotypic pacing, self-mutilation and lethargy (Shepherdson, 1999). There is some debate as to how abnormal behaviours can be used as indicators of poor welfare and to what extent they should be considered reliable indicators (Mason & Latham, 2004). Deprivation studies on laboratory animals have shown associations between an increase of abnormal behaviours and poor social stimulation, stimulus deprivation and minimal environmental complexity (Shepherdson, 1999).

Stereotypical behaviour is estimated to be displayed by over 85 million captive animals worldwide (Mason & Latham, 2004). Considering that it has not been observed in wild animals, the occurrence of stereotypical behaviour is clearly associated with some aspect (or aspects) of captivity. Stereotypical behaviour refers to behaviour that is repetitive, invariant in form, and has no obvious goal or function (Odberg, 1978; Mason, 1991). There are many variant forms of stereotypies and they have been associated with social and environmental deprivation (Carlstead 1998, Mason & Latham, 2004). Cage stereotypies are the most common form observed in zoo animals and environmentally induced stereotypy suggests that the animal is not mentally impaired (i.e. as has been observed in autistic humans) but that the environment in which it lives is less than optimal (Carlstead, 1998). Cage stereotypies include locomotory stereotypies, such as pacing and weaving, which can be accompanied by various head movements (e.g. head dipping) and oral stereotypies, which can involve repeated self-licking, tongue flicking and curling (Vickery & Mason, 2003). Research suggests that the direction and type of stereotypy can be indicative of causal factors. For example, Carlstead (1998) found that black bears (*Ursus americanus*) paced outward towards the public area more often during the summer breeding season and only paced inward towards the inside of the enclosure during the fall; it was speculated that pacing during the summer may be motivated by mate-seeking and pacing whereas during the fall may be motivated by the need to forage.

The use of stereotypies as an assessment of animal welfare is a controversial issue, for it remains difficult to discern whether, or to what degree, stereotypies indicate poor welfare (Appleby, 1999; Mason & Latham, 2004). Stereotypies are associated with levels of stress (Wielebnowski et al., 2002). However, lack of stereotypical behaviours does not necessarily indicate good animal welfare (Mellen et al., 1998), since increased levels of stress hormone (e.g. cortisol) levels have been found in individuals performing few or no stereotypical behaviours (Carlstead et al., 1993). One explanation for why animals might perform these stereotypical behaviours is that animals are using them as a coping mechanism in order to manage stress (Vickery & Mason, 2003).

An alternative investigation into the persistence of stereotypies, which has been extensively studied in the field of psychopharmacology, shows that stereotypies can be induced by the use of psychoactive drugs (e.g. amphetamine) (Creese & Iversen, 1974). Since stereotypies can be induced by increased concentrations of dopamine (a neurotransmitter associated with the expression of motivation and stress behaviours) (Iversen & Iversen, 2007). Stereotypies, in this context, are more suggestive of a reactive mechanism to increased levels of stress rather than a coping mechanism. However, the precise mechanism or function of stereotypies is still unclear, and therefore its use as an indicator of well-being is somewhat contentious.

Regardless of this contention, reducing stereotypies remains one of the most common goals of environmental enrichment programmes in zoos (Carlstead, 1996; Shepherdson, 1999; Shepherdson, 1998; Young, 2003). However, some stereotypies have been reported to become centrally controlled (habit-like) and may continue to persist even after aspects of the animal’s welfare have been improved (Mason & Latham, 2004). Stereotypies have only been observed in captive animals, but not in their wild counterparts (Carlstead, 1998, Vickery & Mason, 2003). Similarly, cortisol levels have been found to decrease as generations move further from the wild (Kunzl et al., 2003) and the association between cortisol and stereotypy are clearly indicative of some response to life in captivity.

The presence of stereotypies (Carlstead, 1993, Vickery & Mason, 2003, Mason & Latham, 2004), the amount and repertoire of active behaviours (Mellen et al., 1998; Broom 1999; Margulis et al., 2003) and breeding success (Carlstead et al., 1999; Clubb & Mason, 2003) are the most frequently used behavioural variables. It is important to note that the levels of such behaviours can vary between species (i.e. lions are active for fewer hours a day than zebras) and individuals.

Behaviours measured in this study

General measures of behaviour included in this study were behaviour diversity and activity level. Behavioural state categories within activity level were also individually investigated such as: locomotion, vigilance, exploration, social and feeding related behaviours (feeding budgets are particularly relevant to pandas considering how diet affects their activity budget in the wild). Stereotypy was considered abnormal behaviour and as such was kept in a separate category from normal activity level; stereotypical behaviour was exhibited in two manners, state-like stereotypy (such as pacing) was measured as proportion of time spent whereas event-like stereotypy (such as tongue flicking) was measured as frequency.

Other supplemental behavioural measures were also used in this study to add breadth to the behavioural investigation. These included measures such as: behaviour diversity, enclosure utilisation, spatial distribution, height preference and branch use. Height preference and width of branch use are behaviour measures relevant to skills necessary in the wild for arboreal animals. Loss of climbing skills has been reported as an important factor affecting success in released captive-born arboreal animals, particularly in relation to foraging (Stoinski et al., 2003). Refer to Table 5.5 for a full list of behavioural variables analysed in this study.

Factors influencing behaviours in captivity

In Chapter 4, I discuss the effect of generation time on factors related to genetic adaptation to captivity. Behavioural adaptation is concurrent with genetic adaptation and the two are inexorably intertwined (Ballou & Briscoe, 2002). One aim of this study is to investigate if any behavioural changes in a captive population of red pandas can be observed across generations. Based on the evidence found in Chapter 4 that red pandas are undergoing adaptation to captivity, it is hypothesised that generation time in captivity will act as a predictor for certain aspects of behaviours expressed in captivity. As generations move further away from the wild, it is predicted that stereotypies will decrease, behaviours associated with exploration will increase, height preference and width of branch use will decrease and behavioural repertoire will either increase or decrease.

Captive Environments

There are components of captive environments that can affect the behaviour of captive animals (Shepherdson, 1998; Shepherdson, 1999). An animal's environment consists of who it lives with (social housing), where it lives (e.g. enclosure- size, shape, furnishings, and weather conditions), and how it lives (e.g. enrichment, husbandry and feeding regimes). Each of these environmental aspects can be enriched to help improve the well-being of captive animals (Poole, 1998), and all aspects of enrichment techniques can be used in conjunction with each other. For the purpose of this study, environmental measures were broken down into three main categories – physical environment, husbandry, and social environment.

Physical Environment

It is not viable to provide captive species that are naturally wide-ranging with enclosure areas equal to their home ranges in the wild. However, space limitation does affect an animal's well-being. Clubb and Mason (1993) have found that the size of a species' home range in the wild is a predictor of how that species responds to captivity in both breeding success and presence of stereotypies. For example, a polar bear's (*Ursus maritimus*) home range can be as large as 550,000 km² (Ferguson, 1999) and polar bears respond particularly poorly to captivity in that they have high amounts of stereotypies and relatively poor breeding success. Therefore, the size of an enclosure should have an effect on the behaviours displayed by captive animals.

However, the complexity and the design of the enclosure are also important features. David Shepherdson (1999 p146), one of the pioneers of environment enrichment, states that 'it is quality rather than quantity of space that is important' although he clarifies this by adding that 'a larger area is one aspect of improving the quality of the space'. A study on captive grey wolves (*Canis lupus*) found that their behaviour was not affected by an increase in enclosure size (Kreeger et al., 1996), so it is important to consider not only the size of an enclosure, but also the features within the enclosure. Knowledge about ecological aspects of a species' preferred habitat in the wild can help in exhibit design (Seidensticker & Doherty, 1996).

For example, field research has revealed that wild cheetahs (*Acinonyx jubatus*) use elevated areas to observe prey (Caro, 1994). Lyons et al. (1997) subsequently found that captive felids significantly preferred elevated areas when these were provided, so, integration of elevated areas in the design of some felid enclosures is important. Additionally, Renner & Lussier (2002) found that behavioural diversity and overall enclosure use increased in captive spectacled bears (*Tremarctos ornatus*) after the addition of a climbing structure – this indicates that it is important to have a physical environment that provides opportunities for the exhibition of natural behaviours. The number of dens available in the enclosure, particularly during breeding, has been found to influence breeding success (von Schmalz-Peixoto, 2003). The percent of enclosure perimeter which has visitor access and percent of perimeter that is made of solid barrier (where visual, olfactory, and/or auditory cues are reduced) also has been found to have an effect on the behaviour of captive animals (Carlstead et al., 1999; Hancocks, 1995).

Enclosure complexity is important for providing good animal welfare (Maple et al., 1995; Shepherdson, 1998; Shepherdson 1999) and for encouraging the display of more natural behaviours (Vargas & Anderson, 1999). Many types of enrichment, both permanent and semi-permanent, are employed by zoos to increase complexity; they can include the introduction of manipulable objects, varied bedding materials, olfactory and auditory stimulators, and various other additions to the enclosure area (Maple & Perkins, 1996).

Another aim of this study is to investigate how aspects of the captive environment may influence the behaviours of this population of captive red pandas. It is hypothesised that physical environmental variables will have an effect on the behaviour of animals, such that larger, more complex enclosures should be associated with more ‘normal-type’ behaviours and reduced abnormal behaviours (stereotypies). The investigation into the effects of individual aspects of complexity (e.g. perimeter type) is exploratory.

Husbandry

An important aspect of husbandry enrichment includes keeper and animal interaction. Research has shown that greater interaction between caretakers and animals reduces the presence of abnormal behaviours, reduces aggression, and increases activity levels and exploratory behaviours (Mellen et al., 1998; Carlstead et al., 1999), such that overall well-being and welfare (or captive fitness) while in captivity are improved. In addition to influences of the physical captive environment, this study will investigate if amount of keeper contact has an effect on behaviour. It is predicted that an increased amount of keeper contact will be associated with better captive well-being (e.g. increased normal activity and increased enclosure utilisation).

Another aspect of husbandry enrichment is feeding regime. Abnormal behaviours have been shown to decrease when feeding regimes are varied and diet diversity is increased (Shepherdson et al., 1993; Carlstead, 1998; Mellen et al., 1998). The presence of stereotypic behaviours has been associated with feeding, either occurring at pre- or post-feeding times (Shepherdson et al., 1993; Carlstead, 1998; Mellen et al., 1998) and has been reported to increase on non-feed days (Lyons et al., 1997). It is suggested that providing food in a way that minimises predictability while maximizing foraging behaviour is an important enrichment technique that helps to reduce abnormal behaviours in captive animals (Shepherdson, 1993). This study will also investigate if time of day and frequency of feeding will have an effect on behaviours of red pandas in captivity. It is hypothesised that the amount of bamboo provisioning will affect activity budgets.

Social environment

Social enrichment with conspecifics has been observed to reduce abnormal behaviours and facilitate more normal ‘natural’ behaviours in captive animals (Mellen et al., 1998; Kreger et al., 1998); although, conversely, overcrowding or inappropriate social housing has been shown to cause stress (De Rouck, Kitchener, Law & Nelissen, 2005). Therefore, application of knowledge about the natural history of a species in the wild can help to determine which type of social environment best suits a species in captivity. Appropriate social enrichment would ideally match group size, group hierarchy, sex ratio, and age ratio of the species’ wild counterparts (Kreger et al., 1998).

The presence of other species in mixed-species exhibits also affects behaviours. In some cases there is improved well-being of species housed together while in other cases there can be an increase in stress related behaviours (Thomas & Maruska, 1996). This study will investigate if social housing has an effect on behaviours. Since there has been no previous research on social housing (both *inter* and *intra* species) in red pandas, it is hypothesised that aspects of social housing will have an effect on behaviours but no specific predictions are made.

Methods

Subjects and Study Locations

Thirty one adult red pandas (*Ailurus fulgens fulgens*) (17 males, 14 females), ranging in age from 2-11, years were observed in the study. The subject number represents 60% of the captive red panda population housed at accredited institutions within the UK (N=53, Glatston, 2007). Individuals were housed in 15 enclosures across 13 zoological institutions in the United Kingdom. None of the animals observed in the study bred or reared young during the course of the observations. See Tables 5.1 to 5.3 for numbers of individuals, institutions and enclosure sizes.

Observations of thirty hours per animal were carried out in this study, totalling 930 hours of observation. Chapter 3 reveals that this amount of observation represents approximately 80% of the behavioural repertoire which would be observed in 100 hours of observation. The model generated in Chapter 3 predicted that further observation would yield roughly a rate of one new behaviour every 14 hours. Based on these criteria, I deemed this amount of observation gave a reasonable representation of the animals' behavioural repertoires. Additionally, this length of observation did not exceed a period where continued disturbance to the animals yielded limited gain.

Data Collection

Data were collected from 7th July 2005 to the 19th November 2006 (observations were not carried out between December and March in order to avoid recording behaviour during the mating season). Instantaneous point sampling every 60 s (Altmann 1974) was used to record state behaviours, location in the enclosure, distance from nearest neighbour and branch width. Individuals were observed simultaneously, when possible, for 30 min sessions. Observations were carried out between the hours of 07:00 and 18:00. Individual animals were observed for a total of 30 h each. Event behaviours, such as scent-marking and sniffing, were recorded via all-occurrence sampling between each point sample (for a duration 60 s).

Individual (Independent) variables

Generation from wild was determined via matrilineal lines and ranged from third generation from the wild to seventh. Relatedness was determined by assigning family groups, consisting of a dam and her offspring. There were seven identified family groups represented in the observed population with seven of the animals observed not placed in a family group (see Table 5.1).

Table 5.1. Family groups based on dam location

Family	N (of individuals)
Bristol	6
Cotswold	4
Paignton	4
Dublin	4
Galloway	2
Hayle	2
Whipsnade	2
Total	24

Table 5.2. Zoo Enclosure information

Enclosure number	Institution	# of animals observed (♂/♀)	Relationship	Nearest neighbour	Time housed together	Mixed species exhibit	Keeper contact	Feed time	Amount of bamboo
1	Birmingham Nature Centre	2/0	Brothers	Dispersed			Medium	AM and PM	Low
2	Bristol Zoo Gardens	1/1	Mated pair	Dispersed			High	AM	Medium
3	Cotswold Wildlife Park and Gardens	1/1	Mated pair	Random			Medium	AM and PM	Low
4	Dublin Zoo	0/3	Mother with two daughters	Random			High	AM	High
5	Dudley Zoo	2/0	Unrelated males	Dispersed			High	PM	Low
6	Fota Wildlife Park	2/0	Father with son	Random			Low	AM and PM	Medium
7	Galloway Wildlife Conservation Park	1/1	Mated pair	Dispersed	New	Yes	Low	AM and PM	Medium
8	Paradise Park- Hayle	1/1	Mated pair	Random	New		Medium	AM and PM	Medium
9	Marwell Zooloigical Park- enclosure 1	1/1	Mated pair	Dispersed			Low	PM	Low
10	Marwell Zooloigical Park - enclosure 2	1/1	Mated pair	Dispersed			Low	PM	Low
11	Newquay Zoo	1/1	Mated pair	Random	New		Low	AM	Low
12	Paignton Zoo Environmental Park	1/1	Mated pair	Dispersed		Yes	Medium	AM and PM	High
13	Port Lympne- enclosure 2	1/1	Mated pair	Dispersed			High	AM and PM	Medium
14	Port Lympne- enclosure 2	1/1	Mated pair	Dispersed			High	AM and PM	Medium
15	Welsh Mountain Zoo, Colwyn Bay	1/1	Mated pair	Dispersed	New	Yes	Low	AM	Low
Overall Total		17/14							

Environmental (Independent) Variables

Twelve environmental conditions were used as variables in captivity (see Table 5.4.). Variables included enclosure size (both two dimensional and usable area), enclosure complexity, percent of perimeter with visitor access, percent of perimeter made of solid wall, number of dens accessible, amount of keeper contact, exhibit type (whether it was mixed species), feed time (AM, PM or both), amount of bamboo given, relationship of housed animals and previous length of time spent. Enclosure complexity has been shown to influence captive behaviours, but it has not previously been quantified.

Other variables, such as husbandry and some aspects of feeding regime, which have also been found to be of environmental importance to carnivores (Mellen et al. 1998), were not included in this study due to high variations across zoos. See Table 5.3 for the descriptive statistics of environmental variables at the institutions.

Table 5.3. Descriptive statistics on enclosures

Variable	N (Enclosures)	Mean	SD	Range
Area (m^2)	15	163	95.58	50-390
Usable area (m^2)	15	273	155.56	70-650
Enclosure complexity score	15	36.9	18.03	15-79
% perimeter visitor access	15	48.67	30.20	0-100
% perimeter solid wall	15	71.0	35.36	10-100
# of dens accessible	15	2.73	0.96	1-4

Table 5.4. Environmental variables

Name of Variable	Description of variable
Area (m^2)	Estimated two dimensional area of enclosure.
Usable Area (m^2)	Estimated usable area, including trees and or climbing areas.
Enclosure complexity score	Total number of permanent enclosure enrichment furnishings, e.g. trees, bushes, substrates, logs, climbing structures, etc... Enclosure complexity has been shown to influence captive behaviours; however it has not previously been quantified.
Percent perimeter visitor access	Estimated percent of enclosure perimeter where public visitors had direct access to view animals.
Percent perimeter solid wall	Estimated percent of enclosure perimeter that consisted of a solid wall- such as brick, cement, or wood.
Number of dens	Number of dens accessible to the animals at all times
Keeper contact	Subjective measurement designated as either low, medium or high. Based on researcher rating of amount and type of interaction.
Exhibit type	Mixed species enclosure (yes or no). Other species included Asian short-clawed otters, pudus, and a free-ranging lemur.
Feed time	Feeding time of day (AM, PM, or AM and PM).
Bamboo	Amount of bamboo (low, medium or high).
Social relation	Mated pair, related, or unrelated same sex pair.
Social time	Previous length of time housed together (old or newly housed together). In three cases, time housed together was less than 1 month.
Dominance rank	Dominance rank (dominant, neutral or submissive). Dominance was measured by number of encounters (e.g. displacement) won.

Table 5.5. Dependent variables

Name of Variable	Description of variable and scoring method
Spread of Participation Index (SPI)	Spread of Participation Index (Plowman 2003)- Measurement of enclosure utilisation, scaled between 0 and 1, where 0 is maximum use (equal use of all zones) and 1 is minimum use (use of only 1 zone).
Behaviour Diversity Index (BDI)	Calculated by dividing the number of behaviours observed in each animal by the total number of behaviours observed in all animals.
Branch use	Mean representation of the branch width used by an individual when climbing
Height	Time spent at greater than 6 metres (height preference is controlled for by availability)
Nearest neighbour distance	Dispersal arrangement of conspecifics in the enclosure
% time spent active	Overall time spent active performing normal behaviours, including- locomotion, social interaction, grooming, vigilance, foraging, etc…
Locomoting	Time spent locomoting- including climbing, walking, etc…
Explore- general	Exploring the enclosure- often accompanied by sniffing, but *not* scent marking
Explore- territory related	Exploring enclosure- often accompanied by sniffing and scent marking.
Vigilance- general	Awareness and observation (often directed) of surroundings
Vigilance of conspecific	Awareness and observation of conspecific
Feeding	Includes eating, drinking, browsing, and foraging for food
Social behaviours	Observed frequency of time spent engaging in social behaviours with con-specifics, i.e. playing, grooming, fighting, etc…

Scent marking (all occurrence)	Frequency of scent marking (represented in table 5.6 as frequency per hour)
Sniff (all occurrence)	Frequency of smell investigation (represented in table 5.6 as frequency per hour)
Stereotyping	Percentage of time spent on abnormal behaviours or performance of a repetitive activity with no obvious goal or function.
Stereotyping (all occurrence)	Frequency of all occurrence stereotyping, e.g. small circles and tongue flicking (represented in table 5.6 as frequency per hour)

Table 5.5 continued

Behavioural (Dependent) variables

Behaviours were categorized as either active or inactive. Inactive behaviours were classified as ‘lying sleeping’, ‘lying alert’ or ‘out of sight’ (out of sight was deemed inactive if the animal was not visible for more than 15 minutes *and* the behaviour observed before moving out of sight was not highly active). A list of behavioural variables included in analyses can be seen in Table 5.5, but includes ‘locomoting’ and ‘exploration’. Table 5.6 and Figure 5.2 give the descriptive statistics on dependent or behaviour variables. All variables except for nearest neighbour distribution were used in the analyses (e.g. generation and relatedness) carried out on individuals because distribution was calculated per enclosure. Only 10 of the 17 behavioural variables were used in the analyses carried out per enclosure due to the appropriateness of using a combined representation of a given behavioural variable for the enclosure. For example, combining the behaviour diversity index of two animals housed in an enclosure would not accurately represent this behavioural measure.

Table 5.6. Descriptive statistics on the dependent variables not shown in figure 5.2

Variable	N (Individuals)	Mean X	SD ±	Range
SPI	31	0.71	.14	.68
BDI	31	0.29	.06	.34
Branch use	31	3.94	.96	3.48
Total active	31	37.60	14.59	63.56
Stereotyping (event)	31	0.96		17.06
Scent marking (event)	31	2.35		10.40
Sniffing (event)	31	11.03		37.53

Spread of Participation Index (SPI)

Spread of Participation Index (SPI) is a measurement of enclosure utilisation which is scaled between 0 and 1, where 0 is maximum spread (equal use of all zones) and 1 is minimum spread (use of only 1 zone). To determine enclosure utilisation, a modified equation of the spread of participation index which allows for unequal zones was used. The modified formula, taken from Plowman (2003) is as follows:

$$\mathrm{SPI} = \frac{\sum|f_o - f_e|}{2\,(N - f_{e\,min})}$$

where f_o is the observed frequency of observations in a zone, f_e is the expected frequency of observations (based on percentage estimate of zone size assuming even use of the whole enclosure), $|f_o - f_e|$ the absolute value of the difference between f_o and f_e for each zone, N is the total number of observations in all zones, and $f_{e\,min}$ is the expected frequency of observations in the smallest zone.

This modified SPI formula allows for the zones in an enclosure to be defined by other parameters (e.g. resource) rather than equal areas. The size of each zone is estimated as a percentage of the entire enclosure to allow for calculations of SPI.

Nearest neighbour index

Clark & Evan's (1954) dispersion index, R, was calculated to determine how pandas dispersed themselves within an enclosure. This was calculated from the following equation:

$$R = \frac{R_O}{R_E}$$

The observed mean nearest neighbour distance (in metres), R_O, is calculated using the expression:

$$R_O = \frac{\sum r_i}{n}$$

where r_i = the nearest neighbour distances and n= the number of observations.

The expected mean nearest neighbour distance, R_E, for a random dispersion pattern is calculated using the relationship:

$$R_E = \frac{1}{2\sqrt{d}}$$

where d is the density of individuals/m^2.

If $R= 1$, then the dispersion is random, if $R < 1$ then the dispersion is clumped and if $R > 1$ then the pattern is uniform (with a maximum value of 2.149).

A z statistic was used to determine the significance of the deviation of the observed from the expected. The standard error of the expected mean, s, is first calculated using the expression:

$$s = \frac{0.261}{\sqrt{nd}}$$

where n is the number of observations contributing to R_O and d is the density.

The z statistic is then calculated from the equation:

$$z = \frac{|R_O - R_E|}{s}$$

The difference between the observed and the expected distance between individuals is statistically significant if $z > 1.96$. Nearest neighbour dispersal was defined per enclosure rather than per animal and was ranked 1 for clumped, 2 for random and 3 for dispersed.

Behaviour Index

A simplified behaviour diversity index (BDI) was calculated for each animal using the following equation:

$$\text{BDI} = \frac{B_I}{B_N}$$

Where B_I is the number of behaviours observed in that individual and B_N is the total number of behaviours observed in the population (N=31).

Data analyses

Inter-observer reliability

In order to test the reliability of recording methods used, the author and a research student carried out simultaneous recordings of the same two individuals, for 120 min. Three methods of observational recording were assessed - recording behavioural state, location within the enclosure, and the distance to the nearest conspecific. A Kappa statistic was used for comparing inter-observer reliability for behaviour state and location in enclosure as these variables were nominal and categorical. A Spearman's rho correlation was used to analyse distance from nearest neighbour since this measure (in metres) was continuous and the data were not normally distributed.

Generation from wild and relatedness

Multiple regressions using the forced entry method were run on behaviours (see Table 5.4) of individual animals (N=31). Both generation time in captivity and family group were treated as dummy variables for the purpose of analysis, age was also included as a covariate.

Effects of environmental variables

Multiple regressions using the forward stepwise method were run on a representation of the combined behaviours of individuals housed within the same enclosures (N=15). Analyses were run on enclosures rather than individuals because individuals housed in the same enclosure were not independent of each other, as they share the same environmental conditions. Because a representation of the combined behaviours was used for the dependent variables, not all behavioural variables listed in Table 5.6 were appropriate to use when investigating effects of environmental variables. Stepwise regressions were used since the purpose of analysis was exploratory.

Non-parametric correlations (Spearman's rho) were run on all independent and dependent variables used in the regressions.

All statistical analyses were carried out using SPSS v. 14.

Results

Inter-observer reliability

Results from the analyses on the inter-rater observations suggest that the recording methods used in the observations are reliable. The Kappa statistic was 0.955, N=120, $p<0.0005$ for the recording of behavioural states and 0.975, N=120, $p<0.0005$ for recording location within the enclosure. Recording distance to nearest neighbour was also reliable, Spearman's rho = 0.966, N=118, $p<0.0005$.

Dispersal

Nearest neighbour distance indicates that the distribution of animals was significantly dispersed in 10 out of the 15 enclosures, and random in the remaining 5 enclosures. There were no enclosures where individuals were clumped.

Red panda activity budgets

There were no consistent diurnal activity patterns. Red pandas were active for approximately 37% of their time, 36% engaging in normal types of behaviours and 1% engaging in abnormal, stereotypic behaviours (see Figure 5.1). Refer to Appendix 2, Table A5.1 for a comprehensive ethogram of the captive behaviours of red pandas.

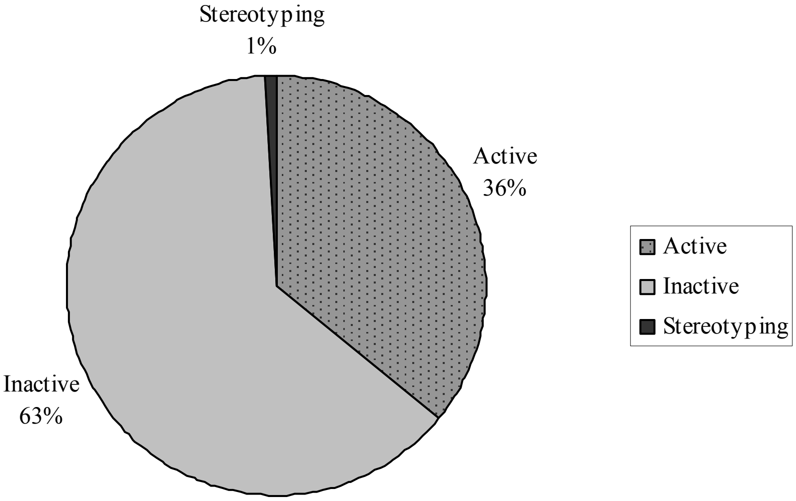

Figure 5.1. Overall activity budget

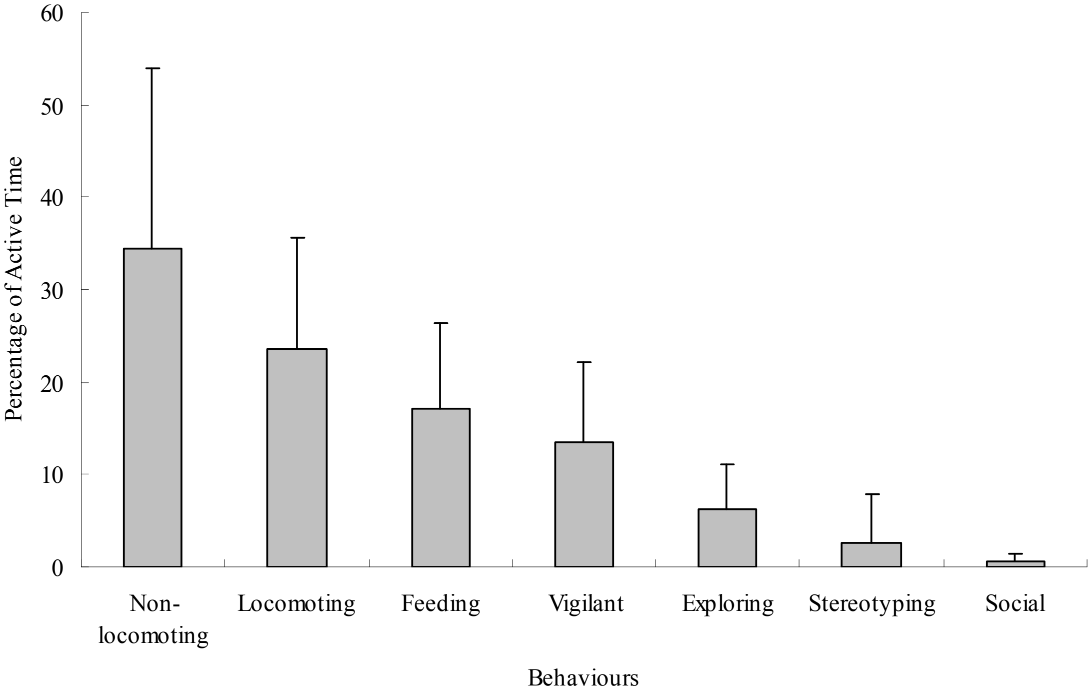

Figure 5.2 Simplified ethogram of behaviours with standard deviation bars

Captive red pandas spent a majority of their active time in non-locomotory behaviours, such as grooming (refer to Figure 5.2 for a simplified ethogram of behaviours). After locomotory behaviours, red pandas spent a large portion of their active time associated with feeding and foraging behaviours, which included eating provisioned food (e.g. fruits and vegetables) and bamboo, as well as browsing on trees and grass.

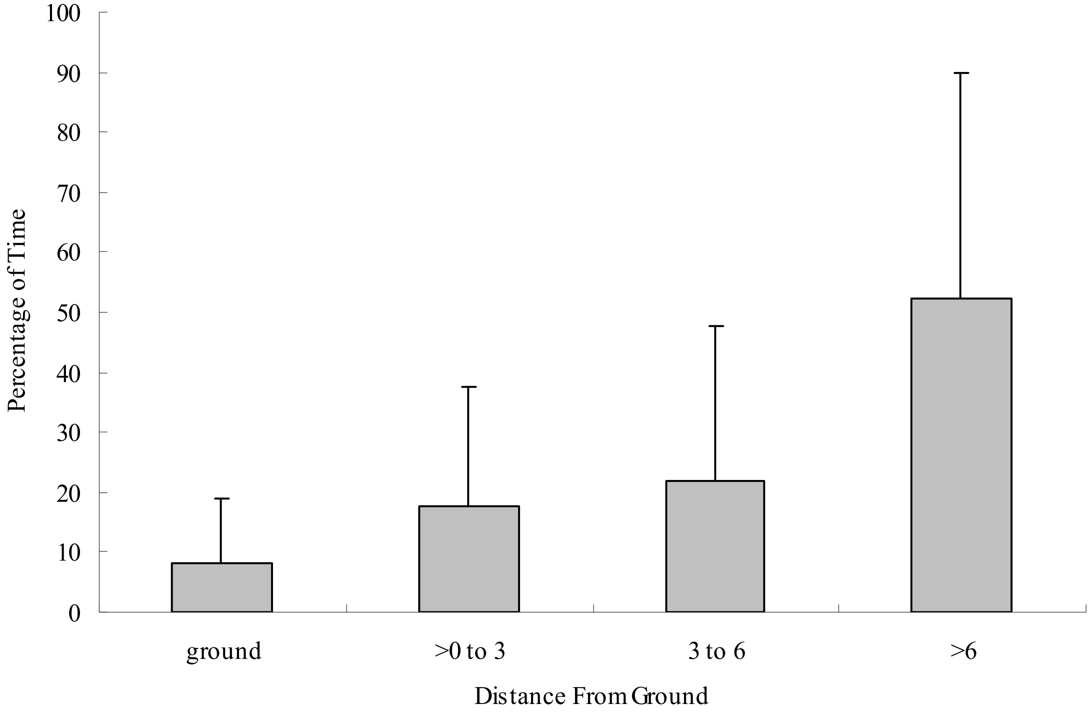

Figure 5.3 Height Preference

Pandas spent over 90% of their time above the ground, and when possible, spent up to 90% of their time above 6 metres (see Figure 5.3).

Sex and dominance rank

Behaviours did not differ significantly between the sexes (see Table 5.7 and Appendix 2, Table A5.1). Dominance rank (see table 5.4 for a description of how this was determined) did not correlate with any of the recorded behaviours (see all non-significant results in Appendix 2, Table A5.1).

Table 5.7 Mean differences in behaviours between the sexes

	Male (N=17) $\bar{X}$ ± SD	Female (N=14) $\bar{X}$ ± SD
SPI	.67 ±.15	.75 ± .12
BDI	.28 ± .05	.30 ± .08
Branch use	3.75 ± 1.00	4.23 ± .86
Active	37.90 ± 12.32	37.35 ± 17.44
Locomotion	11.35 ± 9.53	6.42 ± 3.54
Explore	2.16 ± 1.88	1.95 ± 1.80
Explore (territory)	.56 ± .64	.46 ± .49
Vigilant	3.91 ± 3.11	5.19 ± 3.23
Vigilant (conspecific)	.60 ± .49	.81 ± .77
Feed	5.26 ± 2.49	8.17 ± 6.66
Social	.21 ± .25	.32 ± .42
Stereotypy	.81 ± 1.23	.88 ± 1.71
Stereotypy (event)	.65 ± 2.52	1.35 ± 4.53
Scent marking (event)	2.68 ± 3.08	1.95 ± 2.59
Sniffing (event)	11.57 ± 8.85	10.38 ± 8.73

Factors influencing behaviour in captivity

Generation from wild

Generation from the wild did not have an effect on the frequency or occurrence of normal or abnormal behaviours (see all non-significant regressions in Appendix 2, Table A5.2). There was a non-significant trend in locomotory behaviours (Adj R_2= .221, $F_{7,22}$= 2.363, p=0.061) such that the amount of locomotion increased as generations got further from the wild.

Effect of environmental variables on behaviours

Correlations were carried out on all environmental and behavioural variables to investigate relationships within the variables. Complexity and usable area were positively correlated, both enclosure complexity and usable area were negatively correlated with amount of visitor access and perimeter type. Not surprisingly, activity levels were positively correlated with several other behaviours (however, they did not correlate with stereotypies). Refer to Tables 5.8 and 5.9 for a complete matrix of correlations for the independent and dependent variables.

Table 5.8 Correlation table of all independent variables (Spearman's Rho)

	Usable area	Complexity	Mixed species	Feed time	Bamboo	Visitor access	Perimeter solid wall	Time with conspecific	Relation
Usable area									
Complexity	.793**								
Mixed species	.388	.310							
Feedtime	.456	.556*	.064						
Bamboo	-.019	.188	.211	.245					
Visitor access	-.467	-.595*	-.234	-.407	-.213				
Perimeter	-.666**	-.597*	-.122	-.016	.232	.100			
Time with conspecific	-.088	-.035	.452	-.154	-.115	-.300	.203		
Relation	.503	.167	.149	-.114	-.298	-.482	-.292	.180	
Keeper contact	.343	.364	-.349	.054	.416	-.031	-.479	-.482	.113

Table 5.9 Correlation table of all dependent variables (Spearman's Rho)

	Neighbour distance	Activity	Enclosure use	Exploration	Locomotion	Social	Behaviour diversity	Feeding	Stereotypies
Neighbour distance									
Activity	-.379								
Enclosure use	-.264	-.264							
Exploration	-.456	.629*	-.445						
Locomotion	-.243	.614*	-.304	.459					
Social	-.105	.472	-.326	.537*	.510				
Behaviour diversity	-.411	.624*	-.404	.609*	.533*	.629*			
Feeding	-.468	.696**	-.007	.452	.396	.384	.698**		
Stereotypies	-.357	.370	-.371	.633*	.375	.233	.510	.230	
Stereotypies (event)	-.067	-.055	.122	-.009	-.278	-.099	.174	.090	.521*

** Correlation significant at the .01 level (two-tailed)
*Correlation significant at the .05 level (two-tailed)
All correlations have an N of 15

Table 5.10 The effects of environmental variables (with β values) on captive behaviours of red pandas. The direction of the influence is described in the selection below.

	Usable area	Complexity	Mixed species	Feed time	Bamboo amount	Visitor access	Perimeter solid wall	Time with conspecific	Relation	Keeper contact
Neighbour distance Adj R_2 =.229, $F_{1,13}$ = 5.155, p=0.041							-.533*			
Activity Adj R_2 =.444, $F_{1,13}$ = 12.192, p=0.004										.696**
Enclosure use Adj R_2 =.718, $F_{3,11}$ = 12.900, p=0.001		.342*						.652**	-.785**	
Exploration Adj R_2 =.555, $F_{3,11}$ =6.828, p=0.007	.741*	-.949**								.632**
Locomotion Adj R_2 =.283, $F_{1,13}$ = 6.529, p=0.024	.578*									
Social Adj R_2 =.739, $F_{2,12}$ = 20.856, p<0.0005				-.678**						.541**
Behaviour diversity Adj R_2 =.490, $F_{2,12}$ = 7.739, p=0.007			-.490*		.672**					
Feeding Adj R_2 =.631, $F_{2,12}$ = 12.947, p=0.001								-.397*		.750**
Stereotypies Adj R_2 =.842, $F_{12,2}$ =7.240, p=0.128			-1.893		4.092					-3.672

** β value significant at the .01 level (two-tailed)
* β value significant at the .05 level (two-tailed)

Results for the regression analyses are described below for each dependent variable:

Nearest neighbour distance

Perimeter wall was the only predictor for spatial distribution. The overall model was significant, Adj R_2 =.229, $F_{1,13}$ = 5.155, p=0.041. Distance between nearest neighbours decreased as the proportion of solid perimeter wall increased (β =-0.533, *p*=0.041).

Activity levels

Keeper contact was the only predictor of activity level, Adj R_2 =.444, $F_{1,13}$ = 12.192, *p*=0.004, such that normal activity levels increased with increased keeper contact (β = 0.696, *p*= 0.004).

Enclosure utilisation

The regression run on enclosure utilisation (SPI) highlighted three predictor variables - relationship of animals housed together, amount of time housed with conspecific(s) and enclosure complexity. The overall model was significant, Adj R_2 =.718, $F_{3,11}$ = 12.900, *p*=0.001. Enclosure utilisation decreased when individuals housed together were related (β = -0.785, *p*<0.0005), increased when individuals had been housed together longer (β =0.652, p=0.001) and increased as enclosure complexity increased (β = 0.342, *p*=0.043).

Exploratory behaviour

Three predictor variables were found for exploratory behaviours – usable area, enclosure complexity and keeper contact. The overall model was significant, Adj R_2 =.555, $F_{3,11}$ =6.828, p=0.007. Exploratory behaviour increased in larger usable areas (β =0.751, p=0.016), exploration decreased as complexity increased (β = -0.949, p= 0.005) and exploration increased as keeper contact increased (β = 0.632, p=0.007).

Locomotion

Usable area was the only predictor for locomotion, Adj R_2 =.283, $F_{1,13}$ = 6.529, p=0.024. Locomotion increased in larger usable areas (β = 0.578, p=0.024).

Social behaviours

Amount of keeper contact and feed time were predictors of social behaviours. The overall model was significant, Adj R_2 =.739, $F_{2,12}$ = 20.856, p<0.0005. Social behaviours increased as keeper contact increased (β = 0.541, p=0.002) and social behaviours also appeared to be influenced by the time of feeding, such that animals engaged in more social behaviours when they were fed only once in the morning rather than fed only in the afternoon or fed in the morning and afternoon (β = -0.678, p<0.0005).

Behaviour diversity

The regression run on behavioural repertoire found two predictor variables - amount of bamboo and mixed species. The overall model was significant, Adj R_2 =.490, $F_{2,12}$ = 7.739, p=0.007. Behavioural repertoire increased with increased provisioning of bamboo ($\beta = 0.672$, $p=0.005$) and decreased in the presence of other species ($\beta = -0.490$, $p=0.027$).

Feeding related behaviours

There were two predictor variables on feeding related behaviours (e.g. eating, foraging and browsing) – keeper contact and relation of housed conspecifics, Adj R_2 $=.631$, $F_{2,12} = 12.947$, $p=0.001$. The amount of feed time increased with increased keeper contact ($\beta = 0.750$, $p<0.001$) and feed time increased when individuals housed together were related ($\beta = -0.397$, $p=0.031$).

Stereotyping

There were no significant environmental predictors on state stereotyping behaviour, therefore the stepwise method returned no results. A forced entry regression revealed an overall non-significant result, Adj $R_2 = .842$, $F_{12,2} = 7.240$, $p=0.128$. The only predictor variables approaching significance were mixed species enclosures, the amount of bamboo, and keeper contact. Stereotypies increased when pandas were housed in mixed species exhibits ($\beta = -1.893$, $p=0.059$), stereotypies decreased as the amount of bamboo given increased ($\beta = 4.092$, $p=0.070$) and sterotypies increased as keeper contact increased ($\beta = -3.672$, $p=0.067$).

Effect of environmental variables on all occurrences of stereotypies was also not significant, a forced entry regression revealed no predictor variables (Adj $R_2 = -1.421$, $F_{12,2} = 0.253$, $p=0.961$).

Discussion

There was little evidence of behavioural adaptation to captivity in this population of red pandas. However these findings could result from limitations in the data set which will be discussed below. There were several aspects of the physical environment that affected behaviours, including: size of usable area, enclosure complexity, type of perimeter wall and mixed-species housing. Aspects of husbandry such as amount of keeper contact and feeding regime also had an effect on behaviours. Results are discussed in more detail below.

Red panda activity budgets in captivity

Captive red pandas spend, on average, 37% of their time active, with a majority of their active time spent grooming and locomoting. This corroborates previous research on red pandas in captivity that found that pandas were active for approximately 40% and spent a majority of their active time locomoting and grooming (Holst, 1989). There was no consistent diurnal activity pattern, which suggests that their activity levels throughout the day in captivity are affected more by husbandry practices than by ecological preference.

Behavioural repertoire for each animal averaged only 30% of the overall behaviour observed for the population (N=31). In other words, individuals displayed approximately one third of the behaviours expressed by the population as a whole. Stereotyping ranged up to 22% of overall time spent active, but was only observed in 16 out of 31 animals.

Captive red pandas used, on average, only 30% of their enclosure space and preferred to use three-dimensional space (i.e. off the ground), particularly (when provided) heights above 6 m. Thus, provisioning of climbing structures is important for red pandas. The preference for height and time spent off the ground has been found in previous research on captive red pandas (Holst, 1989; Stevenson et al., 1989).

Red pandas are reported as asocial in the wild (Roberts & Gittleman, 1984; Johnson et al., 1988; Yonzon & Hunter, 1989; Pradhan 1999) and evidence from this study suggests that they are also relatively unsociable in captivity. Individuals maintained an either dispersed or random distance from conspecifics housed in the same enclosure, suggesting that individuals position themselves around the enclosure to ensure minimal contact. They also spent less than 1% of their active time engaging in social interactions. Despite an animal's dominant or submissive status within the enclosure, there were no differences in behaviours in animals of varying dominance rank, suggesting that there is little maintenance of a social hierarchy. There were also no behavioural differences between males and females.

Effect of generation

There were only some trends to suggest that generation time from the wild had an effect on behaviours expressed by captive red pandas. Predictions were that stereotypies would decrease, exploratory behaviours would increase, width of branch use would decrease and behavioural repertoire would increase as generations in captivity increased. None of these predictions was statistically supported. Findings from the behaviours measured in this study did not suggest the kind of adaptation to captivity that was shown in Chapter 4. However, it is important to note that only generations three to seven from wild were investigated in this chapter, whereas all generations were included in the historical studbook analyses (Chapter 4). In addition, the largest changes usually occurred between generation 0 (wild-caught) and generation 1. Since behavioural adaptation to captivity is likely to be expressed prior to genetic adaptation (May 1991), it is possible that the analysis in this chapter missed a critical stage (generations 0 to 2) in the change of behaviour across generations. It is also possible that not all behaviours associated with adaptation to captivity were included in the analyses; for example, changes across generations in behaviours associated with mating displays have been observed (Kunzl et al., 2003). Based on these missing data, there should be further investigation into behavioural adaptation to captivity in other species, ideally beginning at the point at which a species is initially brought into captivity. In addition, several types of behavioural measures should be included in order to investigate how and when (in generation time) behavioural changes in any given captive population may be occurring.

Exhibit design and complexity

The effects of exhibit design and its complexity on behaviours is an area in need of further research in many species. Individual species requirements call for extensive evaluation of various aspects in the physical environment (Seidensticker & Doherty, 1997). Red pandas appeared to be most affected by the amount of usable area, enclosure complexity and the amount and type of perimeter. The amount of usable area provided in an enclosure was related to an increase in both locomotory and exploratory behaviours. Previous research has highlighted the importance of size of enclosure on the behaviours of captive giant pandas (*Ailuropoda melanoleuca*) (Liu et al., 2003) and found that stereotypies decreased in larger, more complex enclosures.

A more complex enclosure appears to promote greater enclosure utilisation, but the amount of exploratory behaviours decreased in more complex enclosures. This is somewhat contradictory, but enclosure use and exploration were not correlated and perhaps exploration involves specific types of resources (e.g. climbing structures) and therefore not all types of complexity are equal. This suggests that the measure of complexity should be further investigated and aspects of complexity (i.e. resources or enclosure furnishings) should not be considered as equal in their desirability to the animals.

The amount of solid perimeter wall also appeared to have an influence on the social spacing of red pandas in that distance between individuals decreased as proportion of solid perimeter wall increased. This can perhaps be explained by the potential visual limitation resulting from a greater amount of solid barrier, such that a conspecific may be less visible than when there is a more open area thus resulting in less awareness of the location of the conspecific. The specific nature of the effect of perimeter on the behaviours of red pandas should be considered in greater detail; however, in any case the design and use of perimeters and barriers and their effect on the behaviours of both animals and visitors is an issue of general importance to zoos (Hancocks, 1997).

The number of dens in the enclosure was not a reliable predictor of behaviour, but previous research suggests that this is important in breeding success (Von Scmalz-Peixoto, 2003). Since the rearing of young was not observed in this study, it is recommended that the effect of the number of dens of rearing success be investigated.

Social grouping

The amount and type of social housing (both inter and intra species) was found to have an effect on behaviours of red pandas. Related animals and animals housed together for longer periods of time had increased enclosure utilisation, this might suggest that an increased association, either in relatedness or length of time housed together, promotes confidence with the enclosure area.

The presence of other species appeared to negatively influence the behaviours of the pandas in that pandas housed with other species had decreased behavioural repertoires and there was also a trend towards increased amount of stereoptypies. Mixed-species enclosures have been reported to have both positive (e.g. increase in overall activity) and negative (increase in agonistic or aggressive encounters) effects on animals, so it is important to evaluate each enclosure individually, as well to consistently monitor interactions between the species (Thomas & Maruska, 1997). Red pandas may not do well in mixed-species enclosures and this should be integrated into their husbandry protocol.

Keeper interaction and husbandry

The amount of keeper interaction was the most consistent variable affecting the behaviours of captive red pandas. Increased keeper interaction was associated with increased activity levels, the amount of exploration and the amount of social (conspecific directed) behaviours. Based on these findings, the amount and type of keeper contact clearly has a noticeable impact on the well-being and welfare of red pandas in captivity, possibly due to a lower amount of stress associated with the presence of humans. These results support previous findings (Mellen et al., 1998; Wielebnowski et al., 2002) that increased keeper contact decreases amounts of abnormal behaviour as well as environmental stressors in small felids. An interesting trend found that increased keeper contact was also associated with increased stereotypy in the red panda, which may suggest that increased human contact may not always be beneficial for the animals' well-being. Additionally, and perhaps more importantly, in the context of a species in captivity for the purpose of conservation and release into the wild, increased human contact is not desirable as it has been shown to reduce the likelihood of survival when released into the wild (Van Manen, Crawford & Clark, 2000) (elaborated further in Chapter 6). The inherent conflict between what is best for an animal's current welfare versus an animal's future welfare needs to be addressed when the species is initially brought into captivity. For species brought into captivity for display and/or education, positive keeper interaction should be strongly encouraged; alternatively, for species brought into captivity for conservation and eventual release back into the wild then keeper interaction should be minimised.

The amount of feed (in this case, bamboo) and feed time had a positive effect on the behaviours of red pandas. The amount of bamboo provisioned appeared to increase behavioural diversity and decrease stereotypies, suggesting that providing red pandas with more amounts of their preferred natural diet is important in encouraging more natural behaviours.

Limitations of data set

As is common in zoo research, this data set is limited in size with only 31 individuals and 15 enclosures, so the potential for detailed exploratory analyses, particularly on the environmental variables, is limited. Considering that the investigation into the effects of the captive environment was so exploratory, it recommended that further research investigating particular aspects of the captive environment (e.g. keeper contact, mixed species exhibits, etc…) should be carried out. Data are also missing on the mating and breeding behaviours of captive red pandas. Therefore, discussion points should be tempered with the proviso that the results should be considered as trends and that any recommendations made based on these results should be carefully considered further. Subject to that proviso, some recommendations can be made on the basis of the present data, see below.

Recommendations for changes to husbandry

- Climbing structures and three-dimensional usable areas (with varying branch and/or bar widths) should be provided in all red panda enclosures.
- Red pandas distribute themselves around an enclosure in order to reduce contact with conspecifics. Therefore the number and relatedness of animals housed together should be taken into consideration when housing red pandas.
- Provisioning of bamboo and foods that promote foraging and browsing should be encouraged.
- Enrichment that encourages more exploration, (e.g. olfactory stimulation) should be put into practice to improve enclosure utilisation.
- For the welfare of animals housed in captivity for education or research, positive keeper interaction should be encouraged. However, for animals housed in captivity for potential release into the wild, husbandry regimes and the amount

and type of keeper contact should be re-evaluated and most likely kept to a minimum.

Conclusion

Findings from Chapter 2 suggest there are aspects of captivity which negatively influence the likelihood of survival. Findings from Chapter 4 imply that although there is adaptation to captivity, there is also evidence of inbreeding depression. Unlike Chapter 4, this chapter did not yield any revealing results regarding the red pandas' behavioural adaptation to generations in captivity. However, this chapter does elucidate how aspects of the captive environment, both physical (e.g. size and complexity) and interactive (e.g. keeper and conspecific) affect the behaviour of captive red pandas. Application of these findings suggests that there should be amendments to current husbandry regimes, particularly with regard to the underlying purpose for which the species is being held in captivity (e.g. display or conservation). A worthwhile next step would be to investigate further how the captive environment influences not only behaviours, but also traits associated with fitness, like those investigated in Chapter 4.

Chapter 6

Fortune favours the bold, or does it? Examining the use of behavioural assessment in captive-born animals to identify traits associated with reintroduction success[*]

Abstract

Using captive-born animals in reintroductions is becoming an increasing trend in conservation. However, given that reintroduction projects using captive-born animals are significantly less likely to be successful than those using their wild counterparts (see Chapter 2) methods need to be developed to help improve the likelihood of survival for captive-born animals. Using assessment of personality traits has recently been promoted as a method for selecting animals most suitable for reintroductions. This study examines a captive population of red pandas, *Ailurus fulgens,* and determines which animals would be least suitable for use in a hypothetical reintroduction programme. Animals were recommended for exclusion from a reintroduction if their scores fell outside of the range of behaviours associated with wild fitness. Methods of Behavioural Assessment (MBA) questionnaires were completed by the animals' keepers and covered three contexts of captive life: interaction with humans, interaction with conspecifics, and interaction with their environment. Results show that boldness traits were relatively consistent across contexts. Based on criteria found in previous literature to be associated with adaptation to captivity or poor survival in reintroduction, 13 out of 38 animals were recommended for

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exclusion from a hypothetical reintroduction. We further found that exclusion was positively predicted by generational time in captivity. We recommend testing this technique in practice to determine its viability in improving the success rates of reintroduction projects using captive born animals.

Keywords: *Ailurus fulgens*, captivity, temperament, personality, red pandas, reintroduction

Reintroduction for the purpose of restoring endangered species back into their native habitat is an increasing trend in conservation. However, reintroduction success rates are relatively low, estimated at between 26% and 66% (Wolf et al. 1996; Fischer & Lindenmayer 2000) and success rates for projects releasing captive-born animals into the wild are even lower (estimated range between 16% to 38%: Beck et al. 1994; Griffith et al. 1999; see also Chapter 2). This lower success rate in reintroductions using captive-born animals has been linked to a loss of natural behaviours associated with an adaptation to captivity. Traits such as a natural fear of humans, predator avoidance and appropriate mating behaviour have all been observed to decrease while in captivity, particularly over increased generational time in captivity (Gilligan & Frankham, 2003; Araki et al. 2007). After several generations of captive breeding (through intentional and unintentional selection), successful animals (assessed by breeding success and overall well being) will have adapted to be less aggressive towards humans and less fearful of novel stimuli (Carlstead 1996; McPhee 2004).

On average, only one in three captive born animals survives a reintroduction. Causes of death commonly reported include contact with humans, starvation, and inter-species aggression (Jule et al. 2008). Therefore, identification of temperament traits that are associated with adaptation to captivity or a loss of more wild-type behaviours might be useful in selecting animals that are not suited for reintroductions. The appropriate selection of captive-born animals for release into the wild, in conjunction with pre-release experience and/or training, could decrease individual mortality and morbidity and hence, improve the welfare of the animals released and ultimately increase reintroduction success (Bauer, 2005; Mathews, Orros, McLaren, Gelling & Foster 2005; McDougall, Reale, Sol & Reader 2006).

Adaptive significance of temperament

Differences in behaviour between individuals have been documented in mating strategies, predator avoidance, foraging behaviour, and risk sensitivity; usually these differences in behavioural traits are considered to be linked to fitness and are therefore maintained through natural selection (Dingenmase & Reale 2005; Reale, Reader, Sol, McDougall & Dingenmase 2007). It has been proposed that individual personalities can be selected for if there is a fitness payoff, either based on the frequency of competing strategies and/or an individual's behavioural history (Dall, Houston & McNamara 2004).

Temperament and personality are synonymous with the term 'behavioural syndrome', often used in evolutionary biology literature. Behavioural syndrome refers to suites of correlated behaviours which are consistent across different contexts and situations (Sih, Bell & Johnson 2004a; Sih, Bell, Johnson & Ziemba 2004b).

The shy-bold continuum, which refers to an animal's propensity to take risks, is considered to be one of the most important and stable measurements of behavioural variation in non-human animals (Kagan 1994). In captivity, behavioural phenotypes, in particular those associated with terms 'shy' and 'bold', are subject to intentional and unintentional artificial selection (Price 1984). In this context, consideration of individual behavioural characteristics can be used to improve *ex situ* conservation techniques through the identification of animals that are either *most* or *least* suitable for reintroduction (Curio 1996; McDougall et al. 2006).

In the process of domestication, certain temperaments are selected for in order to make working with animals easier: for example, there is selection against aggression and towards a reduced responsiveness to changes in the animal's environment (Price 1984). Individuals with more 'tame' behaviours are not only artificially selected to breed but also most likely to fare well in captivity and, hence, breed of their own initiative. Not only does domestication make animal husbandry and working with the animals easier, it can improve the animals' welfare by decreasing stress levels and increasing their coping abilities (Faure & Mills 1998; Koolhaas, Kort, de Boer, Van Der Vegt & Van Reenan 1999; Trut 1999). However, the behavioural and genetic traits that are adaptive in the domestic situation are likely to be maladaptive if or when the animal is restored to its natural environment.

Behavioural profiling: consistency and reliability

Gosling (1998) suggests that an animal's personality can be seen as an accurate representation of its propensity for certain behaviours. However, there is some conflicting evidence regarding the consistencies of temperament across contexts, i.e. if an animal is bold towards predators, can it be assumed that the animal will also be bold in its mating strategy? Most literature supports the idea that personality remains consistent across contexts (e.g. Kooij et al., 2002; Sih et al. 2004a; Ward, Thomas, Hart & Krause 2004; Svartberg, Tapper, Temrin, Radersater & Thorman 2005); however, some evidence suggests that individual differences do not always correlate across contexts (e.g. Coleman & Wilson 1998) and this either brings into question the methods used to assess personality (Miller, Garner & Mench 2005) or the types of contexts under consideration (Reale, Gallant, Leblanc & Festa-Bianchet 2000). However, on the whole, the organisational principles underlying behavioural profiling can be considered to be fundamentally sound across species, as has been demonstrated by Groothius & Carere (2005) in their cross-species comparisons of behavioural differences. Contexts themselves, however, can be multi-faceted, and it is essential to incorporate as many factors as possible that may contribute to individual variation. A factor analytical approach often reveals that that there are several factors contributing to personality or temperament within a specific context (Mather & Anderson 1993; Koolhaas et al 1999) and therefore should be explored for each assessment.

Temperament traits associated with success in the wild

Animals become bolder, in particular towards humans and their reaction to novel stimuli, as they adapt to captivity (McDougall et al. 2006). This unnaturally high confidence in exploratory behaviour and reaction to novel stimuli has been found to be a predictor of fatality in reintroduced captive born animals (Bremner-Harrison et al. 2004).

The increased propensity to display bolder behaviours in captivity can perhaps be explained by the reduced cost of performing these risky behaviours (i.e. the lack of natural predators which would select against wild animals that were *too* bold). This shift in boldness of traits associated with decreased reaction to humans and/or novel stimuli is representative of behavioural adaptation to captivity which increases captive fitness, and hence decreases wild fitness.

It is important to highlight the distinction between boldness and aggression in terms of behavioural adaptation to captivity, as boldness does not have to be associated with aggression or dominance. Rather it can simply refer to the latency of an animal to investigate or react to novel stimuli. For our purposes, boldness refers to an animal's coping strategy in captivity, which Koolhaas et al. (1999) has termed to be either '*proactive*' or '*reactive*'. Since there is less reprisal for bold type behaviours displayed in captivity, and in some cases, even rewards for increased boldness or confidence towards humans and exploratory behaviour, then bolder and more confident animals should be selected for in captivity (Svartberg, 2002; Sundstrom, Petersson, Hojesjo, Johnsson & Jarvi 2004; Coleman et al. 2005). In cases such as fish reared in hatcheries, where there is captive breeding but minimal human contact (hence a lack of need for selection towards tameness and/or handling), this increased boldness has been seen to turn into increased aggression among conspecifics (Berejikian, Tezak, Schroder, Flagg, & Knudsen 1999; Kelley, Magurran & Macias-Garcia 2006). Due to the influence of captive environment on behaviour, it would be worthwhile to investigate which particular aspects of the environment, such as enclosure size and/or complexity, affect an animal's boldness towards its environment.

Red pandas as a case study

Because of the endangered status of red pandas (*Ailurus fulgens*) (Appendix 1, CITES in 1995 and EN C2A, IUCN Red List in 1996), they have been involved in global captive breeding programmes extending over the past 30 years (Glatston & Leus 2005). One of the objectives of this captive breeding programme is to maintain this species in captivity for the purpose of conservation and potentially to supply individuals for reintroduction purposes. Although, there are no current plans for reintroducing red pandas into the wild, there has been one (unpublished) reintroduction project using two captive female red pandas. One female was killed by a leopard within a few months and the other went on to breed, although there was no further monitoring after this was observed (Pradhan, personal communication).

Methods

The purpose of this paper is to devise and refine a method using temperament traits to select captive-born animals that are least likely to survive a release into the wild. In order to achieve this, a questionnaire was conducted with zoo keepers in multiple institutions to quantify individual variation on the shy-bold continuum, explore the consistency of personality in individual red pandas across situations, and to investigate whether generation time (number of generations in captivity) was a predictor of whether or not an animal would be suitable for reintroduction.

Questionnaires and subjects

The questionnaire used in this study was an adapted version of the one used by Carlstead (1999), who designed the Methods of Behavioural Assessment questionnaire (MBA) for the purpose of improving captive breeding success by attempting to identify suitable mating partners for breeding and also to improve husbandry techniques by investigating trends in animals' behaviour towards their keepers and their environment. The questionnaire focuses on three aspects of captive experience: interaction with humans, interaction with conspecifics, and interaction with environment.

The questionnaire was adapted for this study in order to obtain behavioural profiles that reliably represent how red pandas behave or react in any of the three given contexts (human, conspecific, and environment). The main purpose was to identify which traits or behaviours expressed in captivity could be associated with either an increase in captive fitness and/or a decrease in wild fitness and then to determine each animal's respective suitability for a 'hypothetical' reintroduction. Once these traits were identified and quantified (see *analyses of questionnaires and the creation of scores* below), the aim was to apply criteria based on previous research to select animals who rated either too high or too low on traits associated with either poor survival in reintroductions or increased adaptation to captivity (refer to Table 6.1 for a comprehensive list of the factors highlighted in the PCA and the previous research findings associated with these traits).

Questionnaire responses were obtained for 38 red pandas (*Ailurus fulgens fulgens*) housed in zoological institutions within the UK and Ireland, which accounts for approximately 75% of the population listed at accredited zoological parks in the UK and Ireland (Glatston 2007). Questionnaires were filled out by the animals' keepers (N=20).

One keeper assessed all animals housed in the same enclosure (in most cases, N=2 animals). It would have been preferable to have one rater per subject, but this was not feasible given the husbandry regimes at the institutions.

Prior to distribution, we obtained approval and support for the questionnaires from the British and Irish Association of Zoos and Aquariums (BIAZA).

Inter-rater reliability

The inter-rater reliability of the questionnaire was tested by comparing four keepers' ratings across four animals, i.e. all four keepers rated the same four animals. The inter-observer reliability was analysed using Kendall's coefficient of concordance *(W)* as this is the most appropriate method for calculating inter-rater reliability using ordinal level data when multiple raters assess multiple objects (Lehner 1996). Agreement of the raters was analysed for each question and average Kendall's *W* values were estimated for each category (human, conspecific and environment).

Analyses of questionnaires and the creation of scores

Principal Component Analyses (PCA) were run on each category (human, conspecific and environment) covered in the questionnaire in order to identify the constructs within the questionnaire. There is an argument for using the Principal Axis Factoring (PAF) technique, but previous related research in MBA used PCA and therefore, for the purpose of comparison, PCA was used in this study. However, in order to validate the results of the PCA, we also ran PAF and the results were consistent. A direct oblimin rotation was selected because the measures were not expected to be independent from each other (Field 2005). Reliability analyses (Cronbach's α) were used after the PCA to assess the internal consistency of factors.

Using the factors isolated by PCA, we generated a score for each animal within each context. The score represented the mean of the combined ratings for each of the characteristics that formed a factor. These scores represented a quantification of a particular aspect of their temperament within one of the three specified contexts (human, conspecific, and environment). Pearson's correlations on the factor scores were used to assess the consistency of traits across contexts.

Effect of independent variables on scores

The relationships between the factor scores and independent variables were investigated. The independent variables considered were keeper age, keeper gender, keeper experience, amount of contact, number of keepers, animal age, animal sex and when known (N=31), enclosure size and enclosure complexity. See Table 6.2 for a description of independent factors and which statistical tests were used.

Table 6.1. Criteria for exclusion (N of animals =38)

	Criteria for exclusion (*with reference(s)*)
Keeper Interaction	
Factor 1. Boldness- (friendly)	Exclude animals with a score above 3 as too confident towards humans (Grandin 1998; Price 1999; Coleman et al 2005)
Factor 2. Boldness- (aggressive)	Exclude animals with a score 1 and below as too tame (Groothuis & Carere 2005; Price 1984,1999)
Conspecific Interaction	
Factor 1. Sociability and proximity	Exclude animals with a score below 3 because these are likely to be too unsociable and exclude animals 6.5 and above as this suggests inappropriate social traits * (Faure & Mills 1998)
Factor 2. Bold/ Dominant †	Exclude animals with a score below 2 as not bold enough (de Boer et al 2003; Malmkvist et al 1997)
Factor 3. Submissive†	Exclude animals with a score above 5 as too submissive (Godin & Dugatkin 1996)
Environment Interaction	
Factor 1. Boldness	Exclude animals with a score below 2 as not bold enough and above 5 as too bold (Greenberg & Mettke-Hoffman 2001; Bremner-Harrison et al 2003; Kelley et al 2005)
Factor 2. Trepidation	Exclude animals with a score 2 and below as too bold and above 5 too timid (Travis & Dytham 1999; Hellstedt & Kallio 2005)

*This applies to individuals housed together that are unrelated, as highly sociable traits can be seen as more acceptable in related individuals (e.g. D1 and D2)

†These two factors were negatively correlated, therefore an animal was not recommended for exclusion if two of three exclusions were from conspecific interaction factors 2 and 3.

In all cases (except for factor 2 in Keeper Interaction, boldness- aggressive), the criteria for selection was outside at least one standard deviation ± of the mean.

Effect of generation on scores

ANOVA was used to test the effect of generation time in captivity on individual animal scores. Two animals' generational time in captivity were unknown, so *N*=36 for this analysis. Generational time in captivity was measured via matrilineal family lines.

Application of scores for the purpose of selection criteria

Histograms were plotted for each of the factors highlighted by the PCA (see Appendix 2, Figures A6.1-A6.7). There were seven factors across the three contexts: two in keeper interaction (labelled human boldness *friendly* and *aggressive*), three in conspecific interaction (labelled *sociability*, *dominance*, and *submission*) and two in environment interaction (labelled *boldness* and *trepidation*). Based on previous literature and the distribution of scores for each factor, criteria were set to *exclude* animals whose scores fell outside one standard deviation from the mean (this was true in all but one case, aggression towards keeper). Table 6.1 gives a more detailed explanation of the criteria used to select animals. To ensure a conservative measure for exclusion from a reintroduction, animals were identified as unsuitable for reintroduction if they were excluded by 3 or more factors provided the factors were selected from *at least* two categories (human, conspecific, and/or environment); it should be noted that this selection criterion is arbitrary.

Table 6.2. Independent factors

Independent factors	**Description and *statistical test used***
Keeper gender	Gender of keeper filling in questionnaire (*T-test*)
Keeper age	Age (in years) of keeper filling in questionnaire (*Regression*)
Keeper experience	Experience working with the animal (in years) of keeper filling in questionnaire (*Regression*)
Number of keepers	Number of keepers in contact with animal (*Regression*)
Keeper contact	Amount of contact per day in hours (range 1-3) (*ANOVA*)
Social Housing	Relationship of animals housed together (related, unrelated same sex, or mated pair) (*ANOVA*) note* all animals were housed in pairs or in one case, a trio
Animal sex	Sex of animal (*T-test*)
Animal age	Age of animal (in years) (*Pearson correlation*)
Enclosure size (when known)	3-Dimensional space (e.g. climbing space) rated as small, medium, or large (*ANOVA*)
Enclosure complexity (when known)	Enclosure complexity- rated as low, medium, or high (*ANOVA*)

Also of interest was the effect of generational time in captivity on the exclusion of animals from a reintroduction to the wild. A binary logistic regression was used to test this relationship, with age and sex also included in the analysis. Two animals' generational time in captivity were unknown, so *N*=36).

All statistical analyses were carried out using SPSS v.14.

Results

Inter-rater reliability

Table 6.3. Agreement across raters for each question

	Kendall's *W*	***p***	
Keeper Interaction			
1a. Friendly	0.836	0.018	*
1b. Playful	0.546	0.087	NS
1c. Touch	0.899	0.013	*
1d. Vocalise	0.333	0.261	NS
1e. Spontaneously approach	0.525	0.098	NS
1f. Approach when called	0.663	0.047	*
1g. Aggressive	1.000	0.000	**
1h. Ignore	0.250	0.392	NS
1i. Fear	0.891	0.014	*
Conspecific Interaction			
2a. Aggressive	0.333	0.261	NS
2b. Assertive	0.926	0.011	*
2c. Depressed	0.250	0.392	NS
2d. Social	0.625	0.058	AS
2e. Timid	0.425	0.165	NS
2f. Sociable	0.543	0.089	NS
2g. Dominant	0.953	0.010	**
2h. Submissive	0.757	0.028	*
2i. Self-defensive	0.679	0.043	*
2j. Cautious	0.639	0.053	AS
Environment interaction			
3a. Curious	0.508	0.107	NS
3b. Vigilant	0.358	0.231	NS
3c. Anxious	0.899	0.013	*
3d. Hesitant	0.844	0.018	*
3e. Active	0.280	0.339	NS
3f. Excitable	0.958	0.009	**

Results for the inter-rater reliability using Kendall's *W* test ranged from 0.250 to 1.0 in keeper interaction, from 0.250 to 0.953 in conspecific interaction and from 0.280 and 0.958 in environment interaction (see Table 6.3). Overall keeper interaction was the most reliable measure across the four keepers (mean Kendall's *W*= 0.660), closely followed by environmental interaction (mean Kendall's *W*= 0.641) and then conspecific interaction (mean Kendall's *W*= 0.613). The Kendall's *W* means calculated for each measure suggests there is partial agreement across raters. Figure 6.1 shows the mean rating and range across each section of the questionnaire.

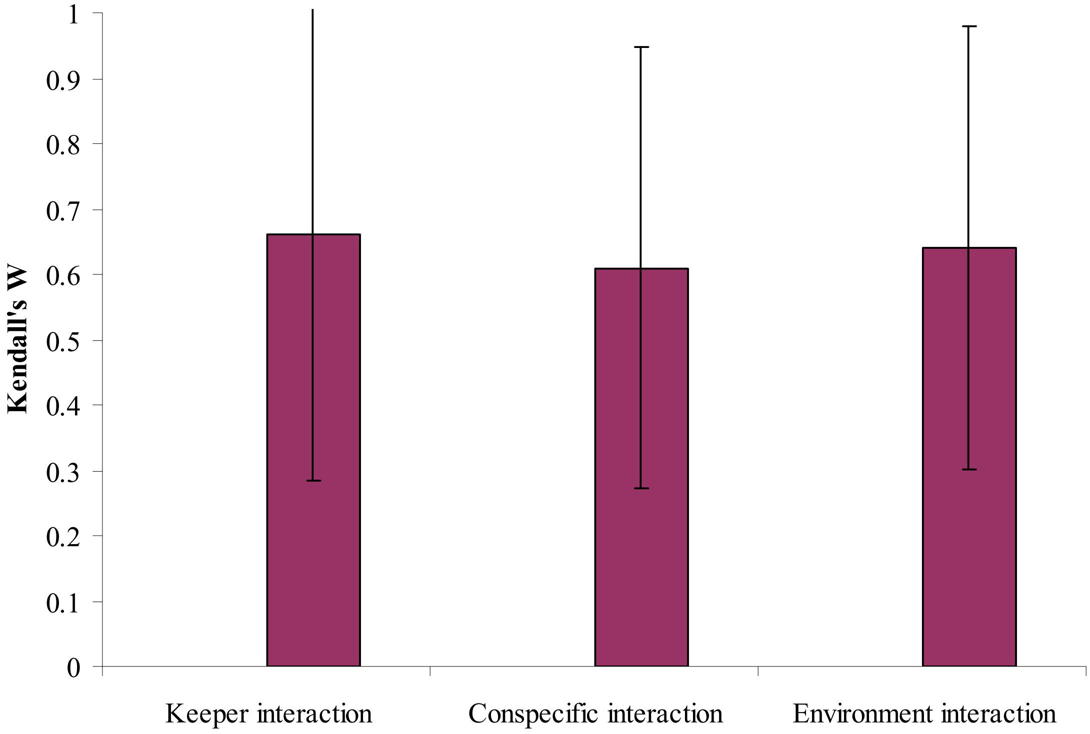

Figure 6.1. Average value for Kendall's Agreement across raters (with range) in each section.

Analyses of questionnaires and creation of scores

Tables 6.4-6.6 show the Principal Component Analyses (PCA) run on each of the three contexts.

Table 6.4. Factor analysis of keeper interaction

Item	Factor	
	Boldness (*friendly*)	Boldness (*aggressive*)
Friendly towards keeper	.98	
Approaches when called	.88	
Allows keeper to touch	.82	
Spontaneously approaches	.81	
Playful with keeper	.58	
Vocalise to keeper		.90
Aggressive towards keeper		.89
Eigenvalues (before rotation)	4.41	1.55
Explained variance after rotation (%)	44.8	13.6
Cronbach-alpha for scale	.89	.79
KMO measure of sampling adequacy	.771	
Bartlett's test of sphericity	171.57, df=36, p<0.001	
Overall variance explained (%)	66.2	

Note. Factors were analysed by using a principal-components analysis with Direct Oblimin rotation (because variables are not independent). The PCA generates a correlation between each variable and each factor; this value represents how strongly a variable loads onto a factor. Only loaded values for the pattern matrix (rotated) >.50 are displayed.

*The variables fear and ignore did not load highly in the PCA and therefore are not included

Table 6.5. Factor analysis of conspecific interaction

Item	Factor Sociability and social proximity	 Submissive	 Boldness/ Dominance
Asocial - prefers spending time alone (Recoded)	.81		
Sociable - maintains proximity	.77		
Timid - reluctant to approach (Recoded)	.69		
Depressed - inactive and unresponsive (Recoded)	.69		
Cautious - approaches with care and wariness (Recoded)	.63	(.59)	
Submissive - willing to give way		.89	
Self-defensive (Recoded)		.74	
Assertive - behaves boldly, with confidence			.93
Dominant - able to displace or control others			.80
Aggressive - initiates fights			.53
Eigenvalues (before rotation)	3.15	1.28	2.3
Explained variance after rotation (%)	31.6	12.7	23.1
Cronbach-alpha for scale	.78	.80	.72
KMO measure of sampling adequacy		.633	
Bartlett's test of sphericity		127.71, df=45, p<0.001	
Overall variance explained (%)		67.2	

Note. Factors were analysed by using a principal-components analysis with Direct Oblimin rotation (because variables are not independent). The PCA generates a correlation between each variable and each factor; this value represents how strongly a variable loads onto a factor. Only loaded values for the pattern matrix (rotated) >.50 are displayed.

Table 6.6. Factors of environment interaction

Item	Factor	
	Boldness	Trepidation
Active - readily explores and investigates surroundings	.86	
Vigilant - attentive to its surroundings; watchful and observant	.86	
Curious - not hesitant to seek out novel objects	.84	
Excitable - easily affected by unexpected actions, people, or events	.67	
Anxious - vacillates between approaching and withdrawing from novel objects or situations; interested, but fearful and uneasy		.86
Hesitant - Does not approach novel objects or situations		.80
Eigenvalues (before rotation)	2.83	1.61
Explained variance after rotation (%)	47.2	26.8
Cronbach-alpha for scale	.82	.60
KMO measure of sampling adequacy	.706	
Bartlett's test of sphericity	78.82, df= 15, p<0.001	
Overall variance explained (%)	74	

Note. Factors were analysed by using a principal-components analysis with Direct Oblimin rotation (because variables are no independent). The PCA generates a correlation between each variable and each factor; this value represents how strongly a variable loads onto a factor. Only loaded values for the pattern matrix (rotated) >.50 are displayed.

Both types of human boldness (*friendly* and *aggressive*) were correlated with conspecific *sociability* and *boldness* as well as environment *boldness*. Conspecific *sociability* and *boldness* were also correlated with environment *boldness*. Also, conspecific *boldness* was inherently negatively correlated with conspecific *submissiveness*. Table 6.7 reports the list of correlations of all factor measures.

Table 6.7. Correlations of factors (*N*=38)

	Keeper Boldness *friendly*	Keeper Boldness *aggressive*	Conspecific *sociability*	Conspecific *boldness*	Conspecific *submissiveness*	Environment *boldness*
Keeper Boldness-*aggressive*	.266					
Conspecific *sociability*	.545**	.314*				
Conspecific *boldness*	.339*	.339*	.163			
Conspecific *submissiveness*	.036	-.195	-.169	-.403*		
Env't *boldness*	.464**	.372*	.343*	.343*	.105	
Env't *trepidation*	-.153	.139	-.278	-.077	.367	.075

** correlation significant at the 0.01 level
* correlation significant at the 0.05 level
All correlations are Pearson's two-tailed

Interaction between scores and independent variables

There was no significant effect of keeper sex or age on either of the human boldness factors (*friendly* or *aggressive*). A multiple regression showed no significant effects of keeper age, number of keepers, or keeper experience on either of the two human boldness scores. There was, however, a significant relationship between the amount of keeper contact and the animals' human boldness *friendly* score, in that a high amount of keeper contact coincided with a high human boldness *friendly* score, $F_{(2,28)}=4.510$, $p=0.018$. There was no significant relationship of keeper contact on human boldness *aggressive* scores.

There was no significant effect of social housing (mated pair, same sex pair, or related pairs) on any of the three conspecific factors (*sociability*, *boldness*, or *submissiveness*).

There was an effect of enclosure size on the environmental factor *confidence*, and a contrast test revealed that animals housed in medium sized enclosures had the highest confidence scores, $t=2.777$, $df=26$, $p=0.01$. There was no effect of enclosure size on the environmental factor *fear*. There was no significant effect of enclosure complexity on environmental confidence, although there was a slight trend to suggest that the most complex enclosures yielded animals with the lowest scores $t=-1.685$, $df=26$, $p=0.104$. There was no effect of enclosure complexity on the environmental factor *trepidation*.

All non-significant results are reported in Appendix 2, Table A6.1.

Effect of generation on scores

There were no significant effects of generation on individual factor scores (see Appendix 2, Table A6.2 for a list of all non-significant results).

Table 6.8. Proposed exclusion of animals by selection criteria

Animal number	Sex	Keeper friendly	Keeper aggressive	Con-specific sociability	Con-specific boldness	Con-specific submissive	Env't Boldness	Env't Trepidation	Generation from wild	Not recommended for reintroduction
G1	F		✓					✓	Unk	
66	F		✓						5	
70	F		✓			✓			4	
73	M								7	
74	M		✓		✓	✓		✓	7	✓
150	F				✓				4	
151	M	✓						✓	5	
152	M		✓						5	
154	M	✓	✓			✓	✓	✓	6	✓
157	F		✓		✓	✓			6	✓
246	M		✓	✓	✓		✓		6	✓
247	F	✓	✓				✓	✓	6	
248	F							✓	5	
250	M	✓							6	
253	M		✓				✓	✓	4	
320	M		✓						6	
321	M		✓		✓		✓	✓	6	✓
337	M		✓				✓		6	
496	F	✓					✓		6	
497	F	✓					✓		6	
9547	M		✓				✓		4	
9556	F						✓		5	
9558	M		✓			✓			5	
9559	F	✓	✓			✓	✓		5	✓
9636	F		✓	✓					5	
9644	F				✓				3	
9658	M		✓		✓			✓	7	✓
9659	M	✓	✓	✓	✓	✓			6	✓
9666	M						✓		3	
9760	F	✓	✓						4	
9829	M		✓		✓	✓			4	✓
9847	M	✓	✓		✓			✓	6	✓
9849	F		✓						6	
9860	M		✓		✓				6	
9936	M		✓						3	
9947	M		✓	✓				✓	6	✓
9948	M		✓				✓		6	
9959	F	✓	✓					✓	6	✓
9960	F	✓	✓	✓				✓	6	✓
9966	M								6	

Application of scores for the purpose of selection criteria

In total, 13 animals out of 38 (approximately 34%; 9 male, 4 female) were recommended for exclusion from a hypothetical reintroduction programme. Table 6.8 shows the animals recommended for exclusion from a hypothetical reintroduction based on their scores.

A logistic regression analysis was performed with exclude or not exclude as the dependent variable and age, sex and generation from wild as predictor variables. A total of 37 cases were used and the full model was significant (χ^2=10.505, df=3, *p*=0.015) (Cox & Snell R_2 =0.24 and Nagelkerke R_2= 0.34). 53.8% of the not-excluded animals were successfully predicted, and 87.5% of the excluded animals were accurately predicted, with an overall prediction accuracy of 75.7%. Only generation from wild was found to be a reliable predictor for exclusion from a reintroduction (B=1.46, Wald= 5.18, *p*= 0.023) and the value of the coefficient reveals that an increase of one generation is associated with an increase in the odds of exclusion by a factor of 4.305. Neither age (B=0.11, Wald=0.56, *p*=0.45) nor sex (B=-0.53, Wald=0.42, *p*=0.58) were predictors for selection.

Discussion

Reliability of assessment and trait consistency

The inter-observer reliability agreement shows that the questionnaire was a reasonably reliable keeper assessment of personalities across animals. However, it is important to note that the four keepers' ratings of the four different animals were not statistically independent, as each of the four keepers were familiar with each other as well as the animals. However, having statistical independence would be difficult to ensure, as the questionnaire needed to be completed by keepers who were familiar enough with the animals to rate their behaviour in different contexts, and therefore the respective keepers will have a higher likelihood of acquaintance with one another.

The factor analyses revealed multiple factors within each of the three contexts, namely, human, conspecific and environment interaction. This suggests that there are multi-faceted aspects to personalities even within contexts. Results from the correlation analyses between the individual factor scores showed that some traits, but not all, were consistent across the three domains. Boldness traits were particularly well correlated across all domains: in particular, environmental boldness was an especially strong predictor of boldness across other domains, which supports results found in previous research and validates the use of boldness as a reliable measure. However, timidity and fear did not correlate well with other factors, and some other research also suggests that these may be less reliably measured, as these behaviours can be more inconsistent (Miller, Garner & Mench 2005).

Effects of captive environment on temperament

Some of the independent variables analysed appeared to have an effect on the factor scores. The amount of keeper contact predicted the human friendliness score, in that more contact was related to a higher score; however it is difficult to show from this analysis what is driving this interaction (i.e. is the keeper initiating contact with the animal or vice versa?). Temperament did not appear to be related to animal sex or age, which corroborates findings by Reale et al. (2000). Three-dimensional enclosure size was found to influence environmental boldness and reaction to novel stimuli scores in that medium-sized enclosures elicited the highest amount of exploratory behaviour and confidence towards the environment. As stated previously, an unnaturally high confidence in exploratory behaviour and reaction to novel stimuli has been found to predict fatality in reintroduced captive born animals (Bremner-Harrison et al. 2004). Therefore size and complexity (as implied through the measure of three-dimensional space) of enclosure space should be considered when designing enclosures for housing animals involved in captive breeding. It would be worthwhile to further investigate the causal nature of this interaction and how enclosure size and/or complexity may affect an animal's boldness and confidence towards its environment.

These findings emphasize the inherent conflict between what is best for an animal's well-being while in captivity versus what is best for maintaining wild traits in captivity for an animal involved in captive breeding for conservation. Research has shown that increased keeper interaction and increased enclosure complexity are correlated with a decrease in stress associated behaviours (such as pacing) and an increase in breeding success (Mellen, Hayes & Shepherdson 1998; Wielebnowski 1999).

It is important to recognise that these contradictory priorities exist. It follows that the purpose of the captivity, whether for zoo recreation and/or education or for captive breeding for eventual reintroduction, needs to be clarified as early on in the captive process as possible, and separate guidelines should be created to suit each of the requirements for both circumstances of captivity.

Aside from setting guidelines for the care and husbandry of animals in captivity for the purpose of conservation, there are other techniques that can be used to improve animals' chances of survival upon release into the wild. If possible, rearing conditions should be set up to prepare animals for life in the wild, such as appropriate social grouping, surrogate parenting, and environmental enrichment, as these have been shown to increase success rates (Vargas & Anderson 1999; Kelley et al 2005; Nicholson, Mayer, Staedler & Johnson 2007). Pre-release experience and/or training have also been found to improve chances of survival upon release. Successful manipulations have included dis-habituation to humans (Bauer, 2005), predator recognition/avoidance training (McLean, Lundie-Jenkins & Jarman 1996; McLean, Holzer, Studholme, 1999; Griffin, Blumstein & Evans 2000), predator/hunting training (Shier & Owings 2006), and environment experience (exposure to large pens or more naturalistic enclosures) (Biggins, Vargas, Godbey & Anderson 1999).

Application of results for the purpose of conservation

The criteria for selection of animals that were not suitable for reintroduction was based upon previous evidence and research that either focuses on aspects of adaptation to captivity or important behaviours associated with success in the wild. There were 13 animals in total that were recommended for exclusion from a ‘hypothetical’ reintroduction programme based on these criteria. It would also be possible to rank animals based on their suitability in order to maintain the number of animals needed for a reintroduction.

Generational time in captivity was a significant predictor of exclusion from a reintroduction, with the odds of exclusion increasing considerably with every generation in captivity. This highlights the fact that captive breeding for conservation needs to be under constant surveillance, not only to reduce inbreeding (Gilligan & Frankham, 2003; Hedrick & Fredrickson 2008), but to monitor the rate of adaptation to captivity, which can thereby reduce the viability of using captive-born animals in reintroductions. This result also confirms that our selection criteria were appropriate in that we were selecting *against* traits associated with adaptation to captivity, and therefore promoting the use of animals in reintroductions that show temperament traits associated with lower captive fitness, and consequently, a higher wild fitness (see Chapter 1).

Conclusions

Adaptation to captivity is correlated with an increase in boldness towards humans, the environment, and in reaction to novel stimuli. Increased boldness is associated with behavioural adaptation to captivity which indicates a decreased wild fitness and unsuitability for release into the wild. These traits can be influenced by the amount of contact with humans and the size and complexity of their enclosures. Personality traits across three contexts (human interaction, conspecific interaction, and environment interaction) appear to remain relatively consistent. Based on the results from this study, suitability for reintroduction is strongly predicted by generation time in captivity, with the odds of becoming unsuitable for release increasing by a factor of approximately four for every one generation away from the wild.

Future application of results

Determining valid criteria for selection based on temperament traits is an important step forward in improving the use of captive-born animals in reintroductions (Bremner-Harrison et al. 2004; Mathews et al. 2005; McDougall et al. 2006). The methods discussed in this paper need be tested in practice, to see if using temperament as a selection criterion is valid for identifying the suitability of an animal in a release into the wild. In particular, the significant predictor of generation time in captivity is worthwhile to consider as a criterion for selection. If this technique is found to be a reliable method for predicting the likelihood of survival, then used in conjunction with pre-release training and/or experience, individual survival and welfare of the animals released could potentially be improved, as could the overall success rates of reintroductions.

Chapter 7

Effects of captivity and implications for *ex situ* conservation

"*wild animals in the stars*
wild animals in the clouds

what will we see in them
when the wild ones are gone?"

From the poem Signs, Paul Aird

This concluding chapter begins by restating the overall themes laid out in this thesis and how these chapters have contributed to improving *ex situ* conservation. An updated schematic representation summarising the findings of the empirical chapters and how these findings relate to captive breeding concerns in conservation will be presented. Techniques are suggested for improving the success of reintroduction projects using captive born animals and suggestions are made for the direction of future work investigating the effects of captivity and its implications for *ex situ* conservation.

Summary of results

The findings in Chapter 2 highlight that current *ex situ* conservation techniques have not improved enough over the past few decades to ensure high levels of reintroduction success. The results show that there is reduced survivorship of captive-born animals in comparison with their wild counterparts when released into the wild, suggesting that captivity reduces the 'wild fitness' of captive-born animals. Most common causes of death are human-related incidents, followed by starvation, inter-species aggression and disease. It is important to reiterate one of the discussion points from this chapter, that the review in Chapter 2 is not all inclusive and further, more comprehensive reviews of the success of reintroductions and releases of other mammalian and non-mammalian species are strongly encouraged. Nonetheless, this chapter provides a clear rationale for the research carried out in the following empirical chapters because if conservation efforts to release captive-born animals into the wild are to continue, then methods need to be improved upon in order to ensure that this is a viable and worthwhile technique.

Before methods can be improved, it is vital to gain further knowledge of the behaviour of animals in the wild as well as in captivity. Obtaining this data with the least amount of disturbance to the animals and costs to the researchers is the most effective way to accomplish this. In Chapter 3, I suggested a method that formalises a technique to determine the optimal amount of behavioural observation by estimating the rate of behaviour discovery. In addition, I also suggested a method for calculating the degree of idiosyncrasy within a population of animals. If this technique is utilised in the study of other endangered and non-endangered species it may well advance our overall knowledge of what ecological factors contribute to behaviour diversification in species.

Response to life in captivity

The behavioural and genetic changes in response to captivity and the environmental factors influencing this change were investigated in Chapters 4 and 5. In conjunction, these chapters reveal how red pandas respond to captivity, which is discussed in more detail below.

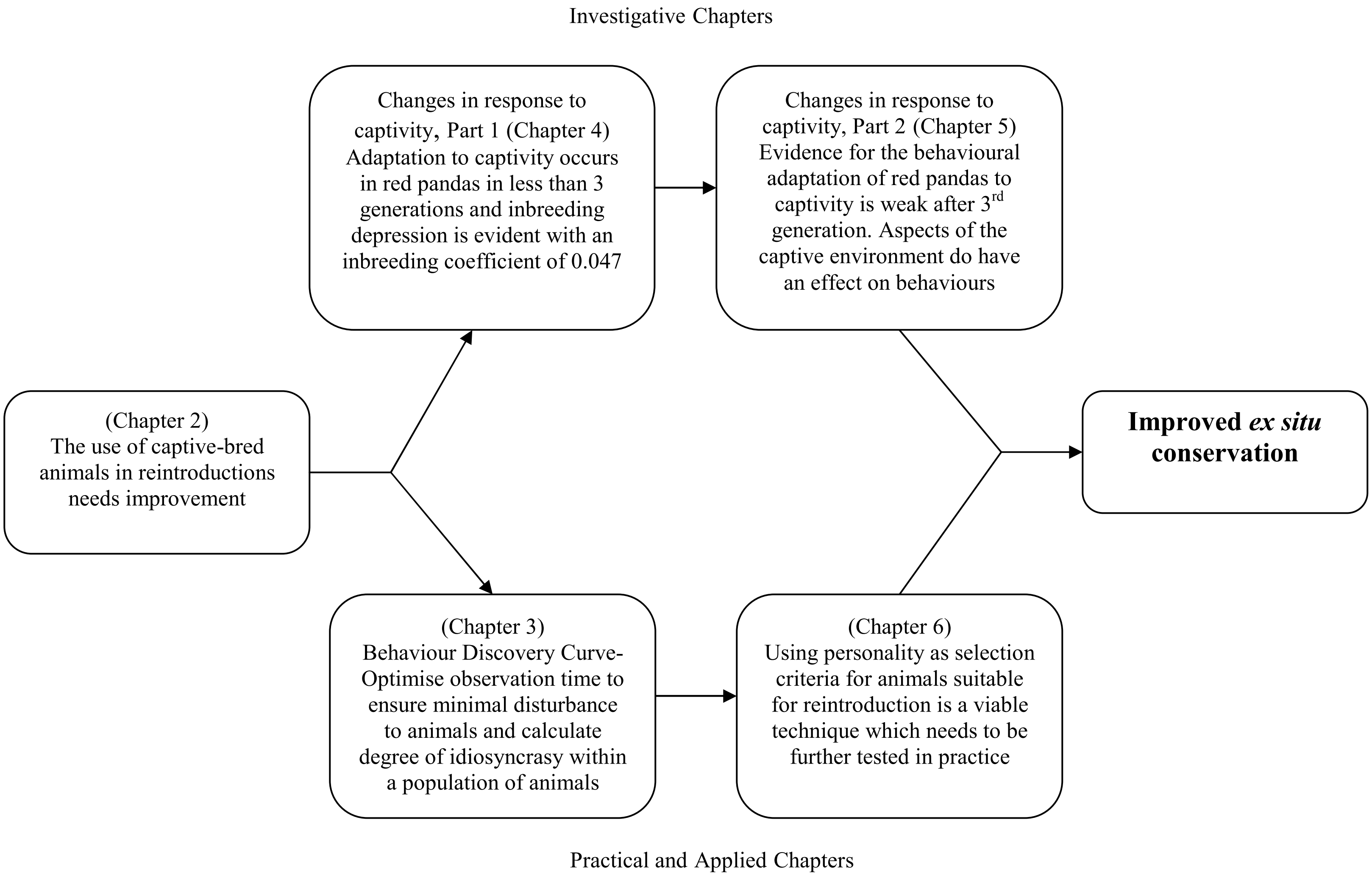

Figure 7.1. Schematic representation of the outcomes of aims laid out for this thesis

Adaptation to captivity and inbreeding depression

Chapter 4 found that lifetime reproductive success significantly increased in the first three generations from the wild, and although reproductive success declined over consequent generations, it still remained above founder levels. This provides evidence that red pandas gain captive fitness. Research into adaptation to captivity in the common fruit fly *Drosophila melanogaster* has found that an approximate 25% gain in captive fitness can occur in 6 generations or less, with an overall 50% gain in captive fitness in just 15 generations from the wild (Gilligan & Frankham, 2003). Other factors investigated in Chapter 4 showed trends that are more associated with inbreeding depression and these will be discussed below.

Findings from Chapter 4 suggest that inbreeding depression is acting on the captive population of red pandas (causing, for example, decreased longetivity, increasing infant mortality and shifting sex ratios across generations from the wild). Given that the inbreeding coefficients (F) ranged between 0.009 and 0.082 across the global captive red panda population, this evidence of inbreeding depression is somewhat surprising given the relatively low F coefficient values. However, traits associated with fitness (e.g. infant mortality and longevity) in carnivores have been found to decrease by as much as 6 – 31% at an inbreeding coefficient (F) of 0.1 (Laikre, 1999), so inbreeding depression occurring in this population is feasible.

The findings from Chapter 4 indicate that both inbreeding depression and adaptation to captivity are of equal concern for populations held in captivity for conservation purposes. There have been several methods developed to improve the reproductive management of species held in captivity for conservation, including techniques such as insemination, genetic resource banks and *in vitro* fertilisation (Ptak et al., 2002; Bainbridge & Jabbour, 1998). If trends continue as they are in the captive population of red pandas, it may be worthwhile to consider employing some of these techniques.

Considering the rapid change in the red panda population over just a few generations, I recommend that the population's suitability for release into the wild should be reviewed. Efforts should be made to 'step up' the conservation efforts since inbreeding depression is only likely to increase as generations move further from the wild. Despite the fact that one of the main goals for this captive population of red pandas is to provide individuals for reintroduction, the only foreseeable plans for future releases are for a small number of red pandas bred in Darjeeling Zoo, India (Pradhan, personal communication).

Behaviours in captivity

Chapter 5 was not as strong in providing evidence that behavioural adaptation was occurring. However, the inherent drawback in the data from Chapter 5 is that they only represent generations three to seven from the wild and if the adaptive process is initially driven by behaviour, then Chapter 4 suggests that behavioural adaptation is likely to be observed earlier than genetic adaptation (May, 1991; Trut, 1991). It is also possible that the behavioural traits most associated with adaptation to captivity were not measured in this study, since evidence has been found to show that boldness and mating behaviours are associated with adaptation to captivity (Trut, 1999; Kunzl et al., 2003). This data set was also not large enough for me to omit any outliers; therefore, it is suggested that future research into the behavioural adaptation to captivity uses a larger data set with relatively equal representation of all generations (including founder animals) and includes more reliable measures of behaviour associated with adaptation to captivity.

Changes in behaviours of red pandas across generations in captivity were not observed in Chapter 5; however, findings from Chapter 6 suggest that behaviours associated with boldness do change over generations in captivity. In Chapter 6, generation acted as a predictor of unsuitability for release into the wild, since the criteria used to determine suitability were based temperament and behavioural traits. This reveals that there are aspects of the behaviour of red pandas which are currently changing across generations in captivity.

The importance of stereotypical (or abnormal) behaviours has also yet to be appropriately determined. Stereotypy may either represent a reaction mechanism to stress or an individual coping mechanism for managing stress, and it would be interesting to further investigate whether personality correlates with certain behaviours (such as stereotypies, activity levels and scent marking) and if there is a relationship between personality and coping style or the expression of stress (e.g. whether individuals react proactively or reactively). Personality in association with cortisol levels has been studied to some extent (Bryne & Suomi, 2002; Capitanio, Mendoza, & Bentson, 2004). Evidence suggests that high cortisol reactivity was associated with lower levels of activity and that exploration and traits associated with boldness (e.g. aggressive, confident and curious) were negatively associated with cortisol levels whilst fearful traits were correlated with high cortisol levels.

Further research into the area of stereotypies and stress indicators might reveal that particular animals may be more prone to responding to captivity in a predictable way and hence, it may be possible to determine how (or if) the presence of stereotypy can provide an indication regarding an animal's suitability for release. Investigation into these trends would also further help our understanding of the origins of stereotypy. Preliminary analyses from data compiled for this thesis investigating the relationship between personality and measurable behaviours (e.g. stereotypies and activity levels) were inconclusive, but more directed research might yield more fruitful results.

Effects of environmental and husbandry variables on behaviour

Aside from factors associated with the physical aspects of the captive environment (e.g. enclosure size and complexity), Chapter 5 also found that that the amount of human contact contributes to higher overall activity levels and more exploration. These findings suggest that the amount and type of human contact can improve the well-being and welfare of animals in captivity. This is unsurprising as human interaction has previously been found to improve captive well-being (Wielebnowski et al., 2002; Carlstead et al., 1999; Mellen et al., 1998). However, it is important to reiterate that improved well-being and welfare in captivity may be associated with increased captive fitness, which is presumed to be negatively correlated with traits associated with wild fitness. Therefore, there is an inherent conflict between breeding animals for life in captivity versus breeding animals for conservation and eventual release into the wild. These results highlight the necessity to clearly establish the role of the captive population (e.g. education or conservation) as soon as it is brought into captivity.

Improving survival rates of released animals and developing techniques to improve overall reintroduction success

Chapter 6 examines the use of a selection technique for captive-bred animals using temperament traits as criteria to determine their suitability for release. Criteria were based on findings from published research in order to identify skills necessary for survival and success in the wild. Based on the designated selection criteria, 13 out of 38 red pandas were recommended for exclusion from a hypothetical reintroduction. Chapter 6 additionally showed that as generation time in captivity progresses, the likelihood of being recommended for exclusion also increases. The primary limitation of the findings of this chapter is that it has yet to be tested in practice. Only practical application of this technique will determine if this is a viable method for determining an animal's likelihood of success.

In addition to setting guidelines for the care and husbandry of animals in captivity for the purpose of conservation, there are other techniques, which have not been directly addressed in this thesis, which can be used to improve animals' chances of survival upon release into the wild. Other factors associated with reintroduction survival and success include providing naturalistic rearing environments (both physical and social) and training predator avoidance and/or prey recognition – including providing hunting opportunities (Box, 1991; McLean et al., 1996; Biggins et al. , 1999; McLean et al., 1999; Griffin et al., 2000; Beck et al., 2002). Rearing conditions can and should be designed to prepare animals for life in the wild through appropriate social grouping, surrogate parenting, and environmental enrichment, as these have been shown to increase success rates (Vargas & Anderson, 1999; Kelley et al., 2005; Nicholson, Mayer, Staedler &

Johnson, 2007). Techniques are also being developed to help animals dishabituate from humans (Soorae & Stanley Price, 1997); this is important considering that over 70% percent of carnivore reintroduction projects list human related deaths (e.g. trapping, shooting and poisoning) as the main cause of death in released animals (Chapter 2).

The value of post-release monitoring is strongly emphasised by many researchers (Fischer & Lindenmayer, 2000; Clark, 1996). Better and longer monitoring will provide greater amounts of data on what contributes to individual success or failures, as well as providing more detailed information on how the population is sustaining over time.

The importance of captive animals in reintroductions and problems facing supplementation of wild populations

Luo et al. (2008) argue that captive-bred populations, in their case tigers (*Panthera tigris*), have great conservation value. The authors state that there is greater genetic representation of sub-species in captivity than in the wild and that this should be utilised in supplementation efforts to increase genetic diversity in the wild populations (Luo et al., 2008). The authors raise an important consideration and I maintain that captive populations bred for release back into the wild should be released as soon as feasible in order to reduce the amount of wild fitness lost in captivity (i.e. due to adaptation to captivity and inbreeding depression). However, the authors do not address any concerns regarding the deleterious effects of inbreeding depression in the current global captive population of tigers, nor do they consider adaptation to captivity (either behavioural or genetic) or the suitability of the animals for release. Nevertheless, Luo et al.'s argument emphasises the critical need to improve the genetic representation of inbred populations in the wild. How can we begin to address this need?

For even the translocation of wild animals is not without risk when supplementing an existing population. To elaborate, a population of inbred wild lions was supplemented with translocated animals in order to increase the population's genetic diversity (Trinkel et al., 2008); the translocated females did not bond into the native female prides and only a small portion of the translocated males were successful in securing their own prides. Consider this scenario if captive animals were released instead – captive-bred males would likely not withstand the intra-species aggression and females would not be able to integrate into the existing female prides. If the individuals released survived, the captive born animals would be more likely to breed amongst themselves, and therefore not directly contribute genetically to the wild population.

Indeed, this has been found to be the case in practice. Arrendal et al. (2004) found that the genetic effects of an otter (*Lutra lutra*) release appeared to be restricted to areas in the immediate vicinity of the release sites, which implies that the supplemented animals did not contribute to the genetic diversity of the existing population. However, these conclusions are limited by the extent of post monitoring, as the effect of releasing new genetic stock may be indirect (i.e. from 2nd or 3rd generation wild-born animals) – provided that the captive-born animals successfully breed and the habitat remains sustainable.

Application of findings and suggestions for future work

Based on the current rate of adaptation to captivity and the evidence of inbreeding depression in the captive red panda population, I recommend that greater efforts should be made to begin integrating the current captive population into the wild as individuals' suitability for release into the wild is only likely to decrease in further generations in captivity.

Findings from Chapter 6 suggest that a third of the current population of red pandas housed in the UK may unsuitable for release and unless husbandry regimes, captive breeding techniques, and pre-release experience and training are greatly improved, this proportion can only be expected to increase as generations in captivity move further from the wild. Given that the current probability of a captive carnivore surviving in the wild is only one in three (Chapter 2), this further reduces the 'release viable' captive population size (although it is anticipated that the use of selection criteria will improve survival rates). I use the term 'release viable' population size to refer to the proportion of the captive population likely to survive and breed upon release into the wild, although this is not a term used in the literature.

To elaborate, a population of 100 captive-born red pandas might have 66 animals selected for suitability in a release; subtract from this number the likelihood of survival. Likelihood of survival of captive-born animals in current reintroductions is approximately 30% (Chapter 2), but with the addition of selection criteria there is more of a chance that survival rate will increase. If survival rate increases to 50%, then roughly 33 captive born animals are likely to survive a release into the wild. A 'release viable' population size is effectively a third of the captive population. Other methods to improve selection criteria and pre-release experience need to be developed in order to have the utilisation of animals from a captive source as a sustainable method for *ex situ* conservation.

For future benefit, it would be practical to devise a method of calculating a captive population's effective genetic contribution upon release into the wild – taking into account likelihood of suitability for release, probability of surviving and odds of successfully reproducing. However, to be able to calculate this statistic, further knowledge would be needed regarding all three of these parameters. Therefore, additional research is critical for creating and testing suitable selection criteria and greater post-monitoring should be carried out to provide more detailed information on survivorship as well as breeding success after release, as this data is sorely lacking in current literature.

Direction of future research efforts

Investigation into adaptation to captivity in other species, particularly species that are currently being reintroduced or those bred in captivity for potential release into the wild, is crucial in evaluating the direction of suitable conservation techniques.

Researchers and conservationists should investigate trends from order level to species level in order to gain a greater understanding of how animals respond to captivity. For species that respond poorly to captivity or adapt at a faster rate, conservation efforts should be focussed more on *in situ* techniques. Something similar to the checklist presented in Table 1.2, but for captive breeding for release into the wild would benefit many conservation plans (Balmford, 2000; Kleiman et al., 2000; Ostermann, Deforge & Edge, 2001; Earnhardt, Thompson & Shad, 2004; Tenhumberg, Tyre, Shea &

Possingham, 2004). The checklist should include information about the species in the wild such as population counts, rate of decline and factors contributing to the rate of decline. The checklist should also include critical information about the species in captivity such as length of time in captivity, rate of loss of genetic diversity, presence and intensity of inbreeding depression and the rate of adaptation to captivity (i.e. the effect that loss of behaviours has on the likelihood of survival upon release, which can vary across species). This information can be used to create appropriate species action plans. Techniques such as this will help to ensure that clear guidelines are set when a captive breeding programme is initiated, which is critical when considering that even when a captive population is appropriately managed, there will still be an inevitable decline in fitness over time. A captive population should be utilised as early on in the captive breeding process as possible and *ex situ* conservation should always occur in conjunction with *in situ* conservation (Frantzen, Ferguson, & de Villiers, 2001).

Concluding statements

One of the main aims of this thesis was to investigate the current captive red panda population's potential for release into the wild. I recommend that efforts to release animals from the captive population to the wild should be instigated, for those animals suitable for release into the wild. In addition, changes in husbandry techniques should be implemented as soon as possible in order to better prepare them for life in the wild; this particularly includes dis-habituation to humans along with an extended preparatory period.

The overarching aim of this thesis was to identify practices that can be utilised to improve and/or examine current *ex situ* conservation techniques across all species of endangered carnivores, as well as other animals. Current techniques need to be improved in order to ensure that *ex situ* conservation is a successful option. Considering the rate of global species' declines, there will be a growing need for better *ex situ* conservation techniques in the near future. Therefore, I stress the importance of considering how amenable a species is to captive breeding and reintroduction before full scale *ex sit*u conservation efforts are put into place. I also recommend that species action plans be carefully constructed at the time when a species is initially brought into captivity for conservation. This thesis contributes to our understanding of the effects of captivity and proposes methods which have the potential to improve future *ex situ* conservation efforts for many species.

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Appendix 1

Table A1. Captive red panda (*Ailurus fulgens*) ethogram

Behaviour	Description
Inactive	
lying- alert	Head up, eyes open, reaction to surroundings in some manner (head or ear movement)
lying- sleeping	Lying sleeping (either curled in ball or lying flat out)- unresponsive to noise/activity
cooling?	Lying flat out, limbs spread- only done in moderate up to very warm temperatures
Out of sight	Continuous stretch of time out of sight (believed to be inactive)
Active	
Locomotive	
walking	Using all four limbs walking on ground
jogging	Using all four limbs jogging on ground
running	Using all four limbs running or bounding on ground
climbing	Moving along vertical or horizontal plane provided it is off the ground and not wider than one metre
fast climbing	Running or bounding on non-horizontal plane or off ground, but no wider than one metre
self play	Purposeless activity with self (i.e. rolling, tale chasing), but not Repetitive
out of sight	Briefly out of sight while moving
hunt/stalk	Hunting/stalking of bird or other animal
carry object	Carry object (e.g. bamboo, peacock feather) in mouth or hand while traveling (e.g. walking or climbing)
out of sight	Believed to be active, but out of sight
Non-locomotive	
standing	Standing on all fours
sitting	Sitting with front paws on the ground

sitting- paws up	Sitting with front paws off the ground
standing	Standing upright on two legs
scratching self	
grooming self	
hanging	Hanging from tree or enclosure furnishing
Vocalisation	Usually to con-specific or keeper
quack-snort	Harsh, broad-band, polysyllabic
grunt	Short, deep
Territorial	
vigilance- in	Observation within enclosure (of a non con-specific)
vigilance- out	Observation outside enclosure
exploratory	Exploratory/territorial investigation of enclosure, can involve sniffing, digging, interaction with furnishings within enclosure
scenting	Rubbing of genital regions either sideways or front to back
scratching	Using claws to rake across ground or object
rubbing- muzzle	Rubbing of muzzle on ground or object
rubbing	Rubbing of dorsal/lateral sides on ground or object
sniffing	Olfactory investigation of an object or a non-animal
licking	Olfactory investigation of an object or a non-animal
tactile	Tactile investigation using paws to manipulate item
digging	Extensive digging in ground, can include ‘rooting’ with muzzle in loose soil
Social	
eye contact	Two individuals making eye contact (stare)
vigilance-con	One individual watching another (con-specific vigilance)
physical avoid	Physical avoidance from a “reasonable” distance away
displace- init.	Initiate physical displacement behaviour

displaced- recip.	Recipient of displacement behaviour
displacement-w	Displacement of another with no contact –Win
displacement-l	Displaced by another with no contact – Lose
initiate fight	Initiate physical aggression
recipient fight	Recipient of physical aggression
phys. fight-w	Winner of physical fight
physical fight-l	Loser of physical fight
chase	Chasing a con-specific
chased	Being chased by a con-specific
grooming other	Initiate grooming session
mutual groom	Mutual grooming session
being groomed	Recipient of groom
mutual touching	Close proximity or touching (while awake or sleeping)
touching	Touching another con-specific
being touched	Being touched by another con-specific
smelling other	Sniffing another con-specific, note* olfactory examination is amongst the most common type of social behaviour
being smelled	Being sniffed by another con-specific
paws up	Standing up on hind paws- initiate
Keeper Interaction	
vigilant	Vigilance/observation of keeper
approach-f	Approach keeper- friendly
approach-a	Approach keeper- aggressive
take item (food)	Take an item from keeper (most likely food)
touched	Allow being touched by keeper
touch-f	Touching keeper friendly/voluntarily

touch-a	Touching (biting/scratching) keeper aggressively
climb	Climbing on keeper (friendly)- (not personally observed, but described)
Consumption	
drinking	
eating browse	Eating provisioned bamboo or browse in enclosure
eating provision	Provisioned food- fruits, vegetables, pellets
food forage	Foraging in enclosure (e.g. permanent trees, grass), can include digging
digging	Digging with front paws
Stereotypies	
stereotypy-1	Purposeless locomotion (including walking and climbing), mostly repetitive, throughout the enclosure often in a figure-8 style, although route can vary to some extent
stereotypy-2a	Repetitive in a localized area- facing out towards public
stereotypy-2b	Repetitive in a localized area- facing in towards enclosure
stereotypy-3 circle	Repetitive walking/running in a tight circle, can be done on its own or within a pacing/stereotypic routine (Event behaviour)
stereotypy4	Excessive mouth movements, i.e. tongue flicking
stereotypy5	Excessive grooming/licking
stereotypy6	Repetitive route in enclosure- predictable pattern, limited response/awareness to outside stimuli. In this case, accompanied by scent marking at repetitive locations but with no investigation (e.g. sniffing)

Table A5.1. Non-significant results of correlations with dominance and sex differences

Dependent variable	Correlation with dominance rank (Spearman's, two tailed)	Sex differences (t-test, two-tailed)
SPI	rho= -.317, *p*=.094	t=-1.692, df= 29, *p*=.101
BDI	rho=.082, *p*=.671	t=-.628 , df=29, *p*=.535
Time spent active	rho= -.247, *p*=.196	t=-1.114, df=18, *p*=.280
Branch use	rho=.339, *p*=.156	t=.102, df=29, *p*=.920
Time spent locomoting	rho=.050, *p*=.798	t=1.830, df=29, *p*=.078
Time spent exploring	rho= -.111, *p*=.567	t=.307,df=29, *p*=.761
Time spent exploring (territory)	rho= -.243, *p*=.222	t=.467,df=27, *p*=.644
Time spent vigilant	rho= -.077, *p*=.691	t=-1.117, df=29, *p*=.273
Time spent vigilant (conspecific)	rho= -.117, *p*=.547	t=-.907, df=29, *p*=.372
Time spent feeding	rho= -.300, *p*=.114	t=-1.674, df=29, *p*=.105
Time spent socialising	rho=.284, *p*=.136	t=-.951, df=29, *p*=.350
Time spent stereotyping	rho= 280, *p*=.141	t=-.147, df=29, *p*=.884
Frequency of stereotyping (all occurrence)	rho=.332, *p*=.079	t=-.548, df=29, *p*=.588
Frequency of scent marking	rho=.064, *p*=.743	t=.710, df=29, *p*=.484
Frequency of sniffing/exploring	rho= -.022, *p*=.909	t=.374, df=29, *p*=.711

Table A5.2. Non-significant results of multiple regressions on individuals (N=31) using the forced entry method, with age included as a variable

Dependent Variable	Generation from wild	Family Group
Spread of Participation Index (SPI)	Adj R_2= .171, $F_{7,22}$= 1.857, p=.126	Adj R_2= .063., $F_{9,20}$=1.217, p=.338
Behaviour Diversity Index (BDI)	Adj R_2= -.186, $F_{7,22}$= .351, p=.921	Adj R_2=.279, $F_{8,21}$=2.405,p=.051[1]
Branch use	Adj R_2= .191, $F_{6,12}$= 1.707, p=.203	Adj R_2= 063., $F_{9,9}$=1.094, p=.448
% time spent active	Adj R_2= -.077, $F_{7,22}$= .703, p=.669	Adj R_2= -.046, $F_{9,20}$=.857, p=.576
Locomoting	Adj R_2= .221, $F_{7,22}$= 2.177, p=.063[2]	Adj R_2= -.145, $F_{9,20}$=.591, p=.790
Explore- general	Adj R_2= -.156, $F_{7,22}$= .440, p=.866	Adj R_2= -.121, $F_{9,20}$=.607, p=.762
Explore- territory related	Adj R_2= -.112, $F_{7,22}$= .612, p=.740	Adj R_2= -.214, $F_{9,20}$=.471, p=.876
Vigilance- general	Adj R_2= -.117, $F_{7,22}$= .567, p=.774	Adj R_2= .174, $F_{9,20}$=1.678, p=.160
Vigilance of conspecific	Adj R_2= -.096, $F_{7,22}$= .636, p=.721	Adj R_2= .078, $F_{9,20}$=1.273, p=.310
Feeding (eating, foraging and browsing)	Adj R_2= .042, $F_{7,22}$= 1.181, p=.354	Adj R_2=.241, $F_{8,21}$=2.152, p=.076[3]
Scent marking (all occurrence)	Adj R_2= .008, $F_{7,22}$= 1.034, p=.436	Adj R_2= -.036, $F_{9,20}$=.872, p=.554
Sniffing (all occurrence)	Adj R_2= -.111, $F_{7,22}$= .586, p=.760	Adj R_2= -.030, $F_{9,20}$=.896, p=.537
Stereotyping	Adj R_2= .143, $F_{7,22}$= 1.689, p=.164	Adj R_2= -.028, $F_{9,20}$=.911, p=.535
Stereotyping (all occurrence)	Adj R_2= -.146, $F_{7,22}$= .471, p=.845	Adj R_2=.095, $F_{9,20}$=1.382, p=.261
Social behaviours	Adj R_2= -.040, $F_{7,22}$= .841, p=.566	Adj R_2= .036, $F_{9,20}$=1.119, p=.394
Height	Adj R_2= .167, $F_{6,16}$=1.735, p=.177	Adj R_2=-.130, $F_{8,14}$=.684, p=.699

[1] Dublin family group had a higher BDI than other groups

[2] Locomotion increased as generations from wild increased

[3] Dublin family group spent more time in feeding behaviours than other groups

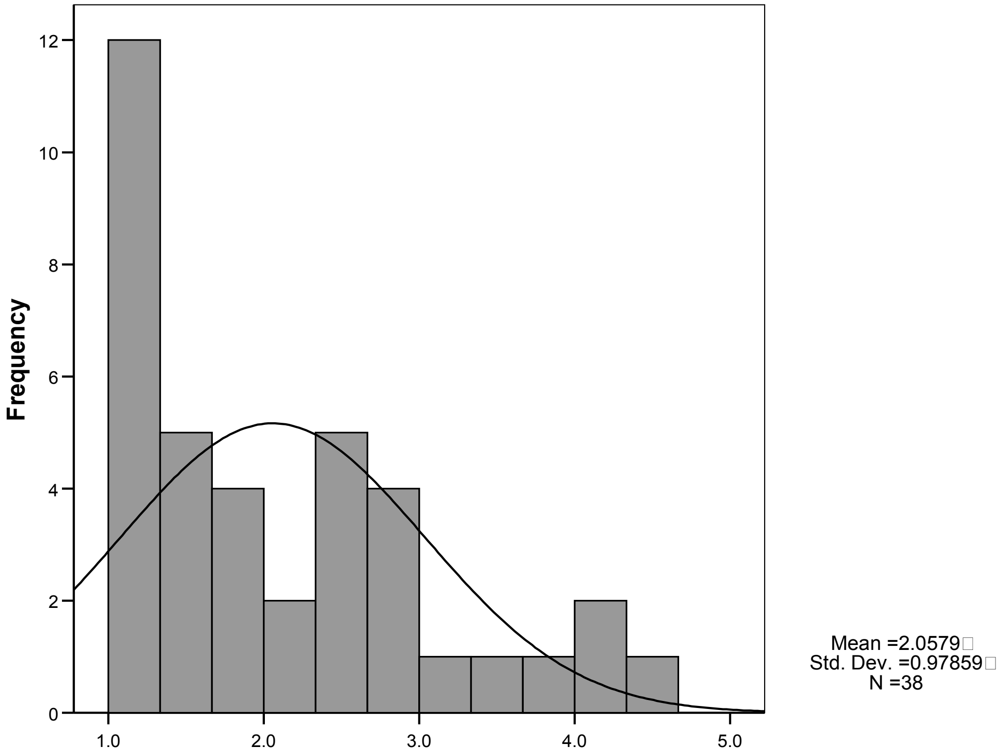

Figure A6.1. Histogram (with distribution curve) of Human Factor 1- friendly

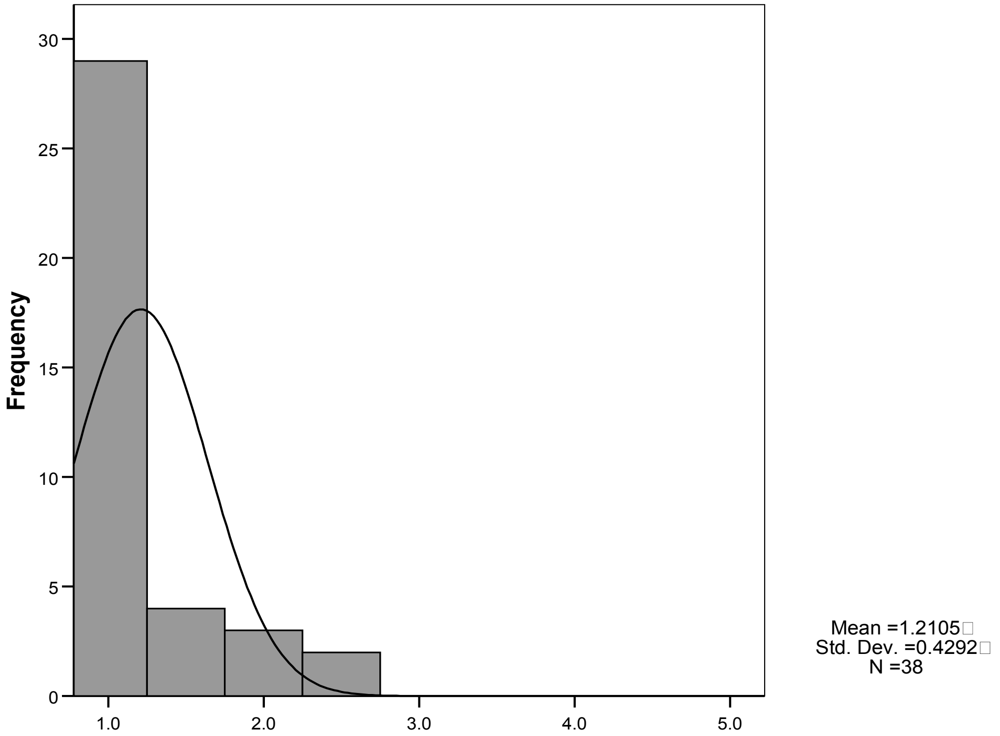

Figure A6.2. Histogram (with distribution curve) of Human Factor 1- aggressive

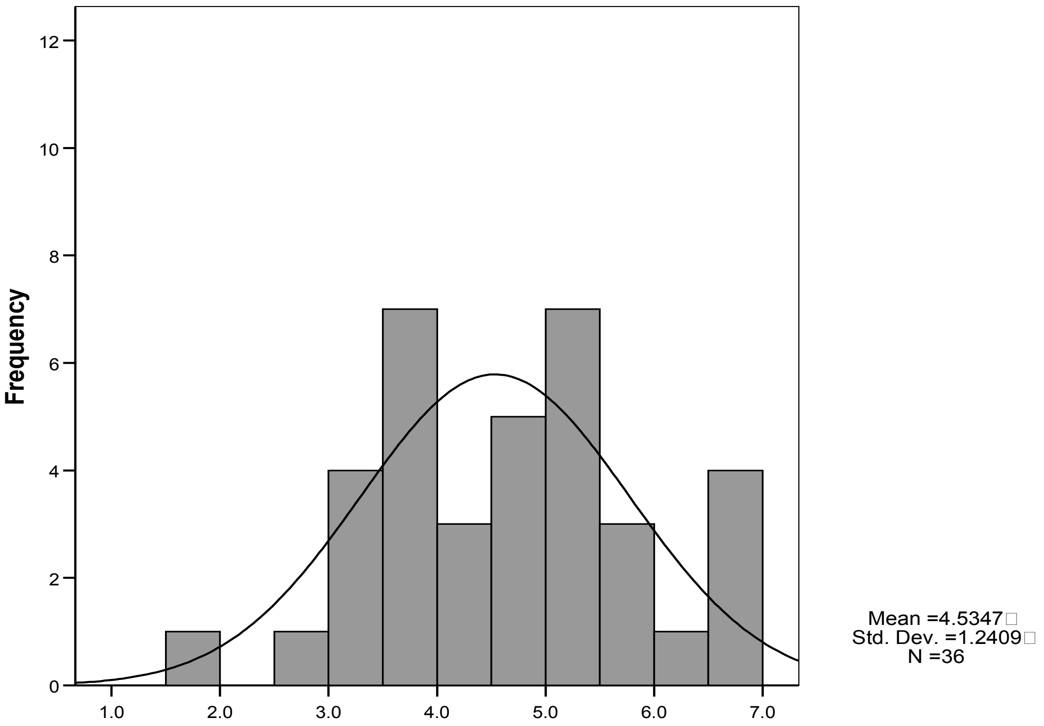

Figure A6.3. Histogram (with distribution curve) of Conspecific Factor 1- Sociability

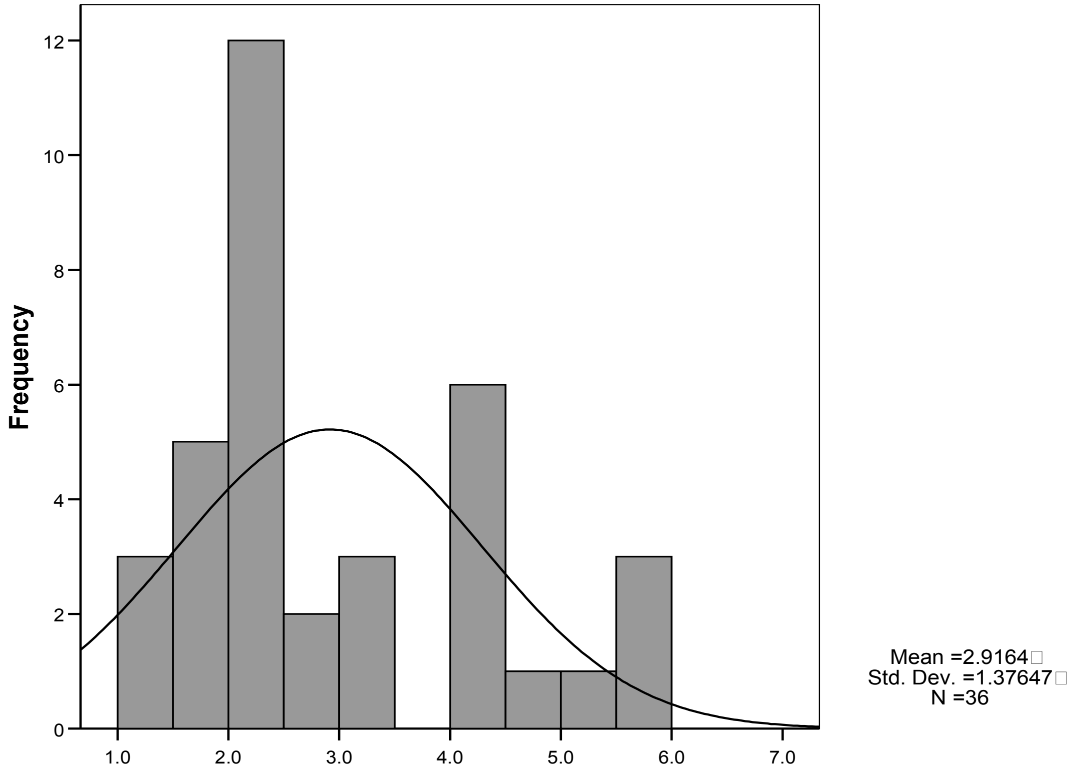

Figure A6.4. Histogram (with distribution curve) of Conspecific Factor 2- Boldness

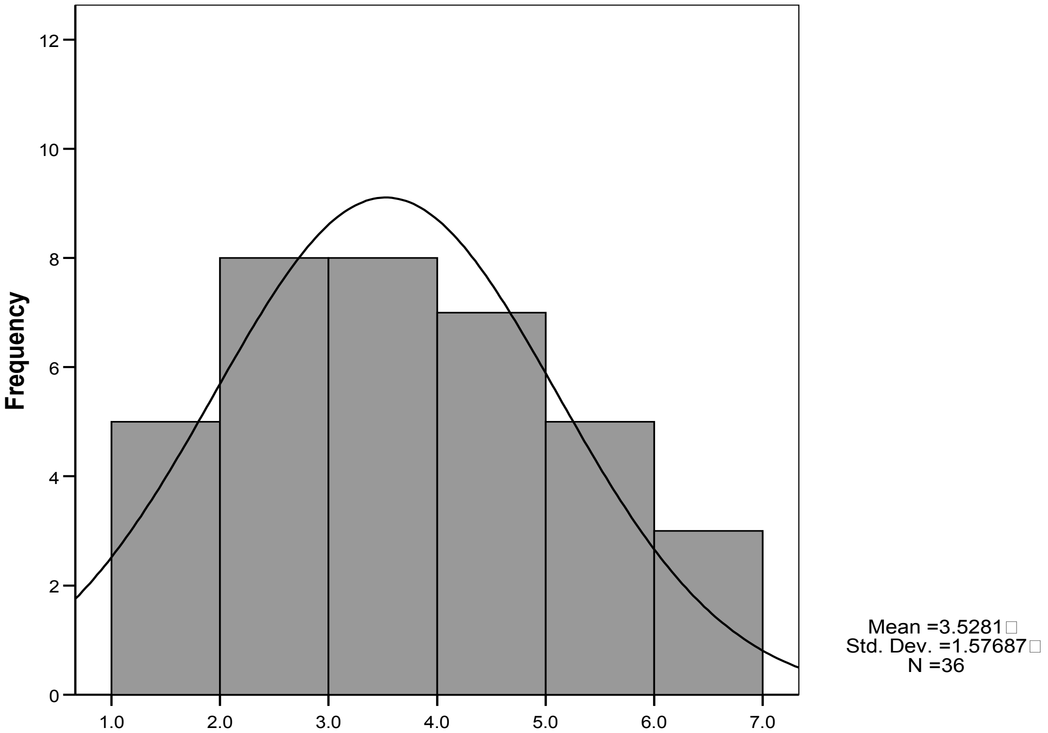

Figure A6.5. Histogram (with distribution curve) of Conspecific Factor 3-Submissiveness

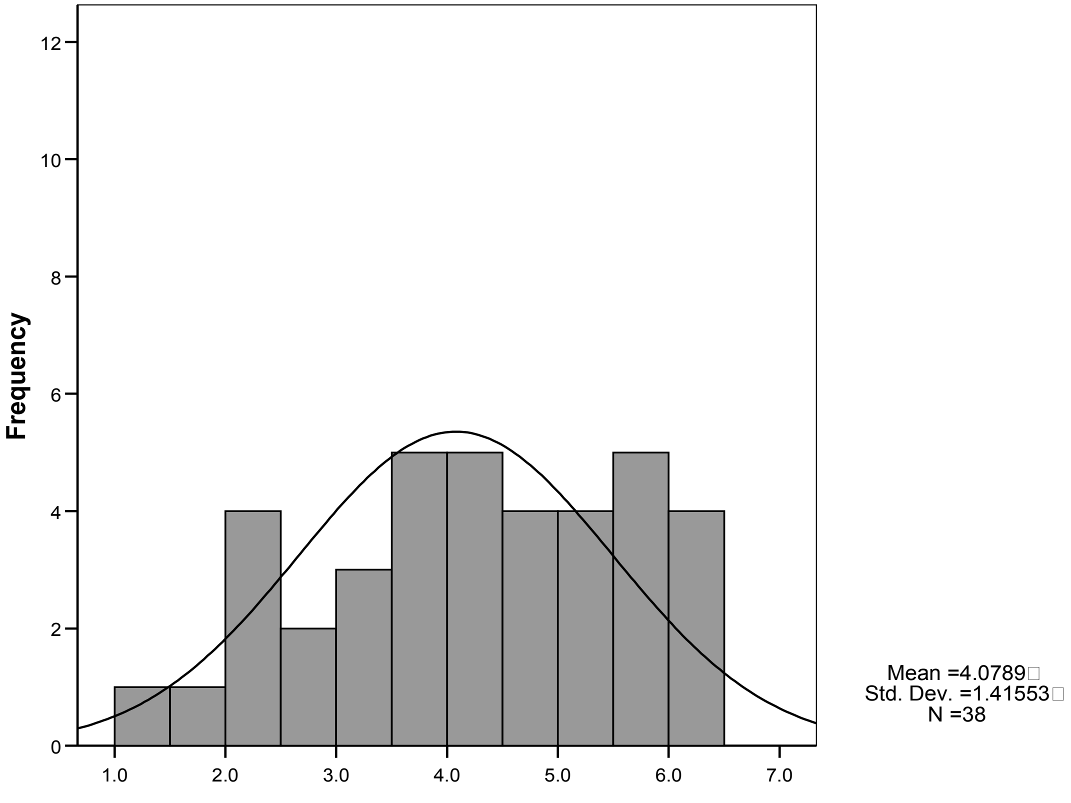

Figure A6.6. Histogram (with distribution curve) of Environment Factor 1- Boldness

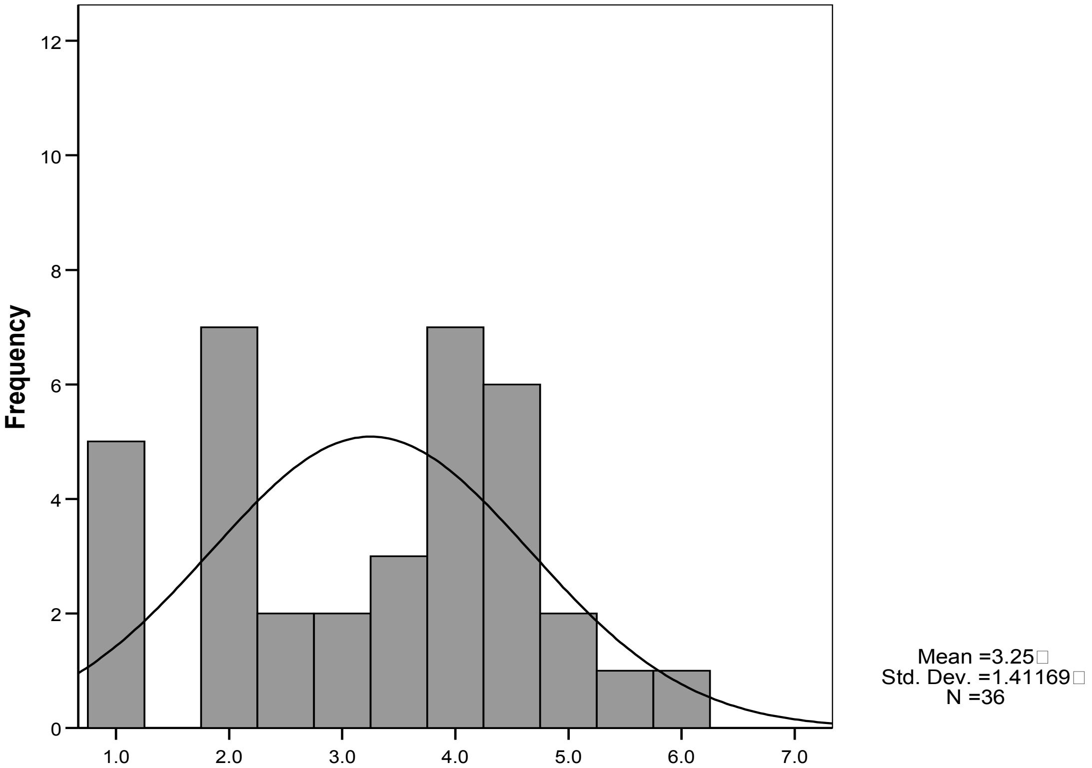

Figure A6.7. Histogram (with distribution curve) of Environment Factor 2- Anxiety

Table A6.1. All significant and non-significant results on effects of independent variables on scores

	Temperament factors						
Independent variable	Human-friendly	Human-aggressive	Conspecific-sociability	Conspecific-boldness	Conspecific-submissive	Environment-boldness	Environment-trepidation
Keeper gender	t=.615	t=.301	–	–	–	–	–
Keeper age	*Beta* =.182	*Beta* =.334					
# of keepers	=.216	=.305	–	–	–	–	–
Keeper experience	=-.053	=-.071					
Keeper contact†	F=4.510* t=2.503*	F=.710 t=-1.161	–	–	–	–	–
Social housing	–	–	F=.804	F=.378	F=.291	–	–
Animal sex	t=1.425	t=-1.083	t=-.710	t=-.100	t=.766	t=.078	t=-.764
Animal age	r=.054	r=-.149	r=-.224	r=-.029	r=.062	r=.167	r=.003
Enclosure size†	–	–	–	–	–	F=4.571* t=2.777*	F=.889 t=-1.142
Enclosure complexity†	–	–	–	–	–	F=1.476 t=-1.685	F=.449 t=-.852

†ANOVAs were run on the independent variable keeper contact as well as both enclosure variables, both the F values and the t values generated from contrast tests are reported.

Table A6.2. All significant and non-significant results on effect generational time in captivity on scores

	Temperament factors						
Independent variable	Human-friendly	Human-aggressive	Conspecific-sociability	Conspecific-boldness	Conspecific-submissive	Environment-boldness	Environment-trepidation
Generation from wild (degrees of freedom =4)	F=.959	F=1.075	F= 5.635, *p*=0.054	F=1.262	F=1.646	F=.030	F=1.522
Contrasts (*if applicable*)		t=-2.007, *p*=0.054 (3rd Gen considerably more aggressive)	t=1.964, *p*=.097 (6th and 7th Gen more sociable)		t=3.740, *p*=.068 (3rd Gen less submissive)		

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