Feline Stress

Methodological Considerations for Non-Invasive Assessment of Cats Housed in Groups and Singly

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Abstract

Group-housing of domestic cats (*Felis silvestris lybica*) may induce a stress response with consequences such as cats developing infectious disease or problem behaviours. Still, there is no validated behavioural protocol to assess stress in cats. The aim of this thesis was to investigate the effect of group-housing of cats, and how this can be assessed non-invasively, by advancing a behavioural assessment tool.

In Study I, frequency of group-housing and related issues such as management was investigated using a survey sent to Swedish shelters. The majority of shelters practised group-housing and had routines and/or protocols for management and care. Despite a high rate of group-housing, many shelters reported low occurrence of disease.

In Study II suitability of saliva sampling as a non-invasive method to assay cortisol in naïve awake shelter cats was investigated by association with plasma cortisol levels and prevalence of respiratory disease. Few samples yielded enough saliva for analysis and there was no correlation with plasma cortisol levels. Few cats tested positive for respiratory agents.

In Study III cats housed in groups or singly were observed to investigate which stress related behavioural elements (BEs) can predict time from available for adoption until adoption (*Time at Shelter*). Fourteen BEs could predict short and nine long time until adoption. Significantly fewer BEs were recorded in single-housed cats, so housing in itself seems to have an effect on the possibility to use the BEs to assess cats.

In Study IV research cats kept under stable conditions, in stable groups, were observed using repeated measures to investigate stability of the BEs found to predict *Time at Shelter*. Close to 80% were stable in 75% of the cats.

Group-housing is common in Swedish shelters, but does not necessary result in negative consequences. Salivary cortisol was not suitable for studies on cats not trained for sample collection. The majority of the BEs associated with *Time at Shelter* were stable within an individual and were used to develop a first version of the further advanced assessment tool to determine coping in group-housed cats.

Keywords: Assessment, Behaviour, Domestic Cat, Group-housing, Shelter, Stress

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Dedication

In loving memory of my grandfather, Tore Ardeskog, whose dream I have been living.

Stressors, like beauty, lie in the eye of the beholder.

(Everly & Lanting, 2013).

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Hirsch EN, Andersson M, and Loberg J (2014). Swedish cat shelters: a descriptive survey of husbandry practices, routines and management. *Animal Welfare* 23, 411-421.

II Hirsch EN, Loberg J, Hydbring-Sandberg E, Lidfors L, Berg C, and Andersson M. Cortisol Measurements and Investigation of Upper Respiratory Disease in Shelter Cats: methodological considerations (manuscript).

III Hirsch EN, Andersson M, and Loberg J. A Further Development of a Scoring System to Assess Behavioural Stress in the Cat (manuscript).

IV Hirsch EN, Loberg J and Andersson M. Stability of Behavioural Elements in Cats Housed in Stable Groups (manuscript).

The contribution of Elin N Hirsch to the papers included in this thesis was as follows:

I Planned the study together with the co-authors. Performed data collection and preparation of data. Analysed data with help from the co-authors. Main responsible for writing the manuscript with input from the co-authors.

II Planned the study together with the co-authors. Performed data collection together with one co-author and five additional veterinarians. Analysed data with help from the co-authors. Main responsible for writing the manuscript with input from the co-authors.

III Initiated the idea for the study and planned it with input from the co-authors. Discussed set-up and received input from Dr Candace Croney and Dr Judi Stella, Purdue University. Performed data collection and preparation of data. Analysed data with help from a statistician, the main-supervisor and the co-authors. Main responsible for writing the manuscript with input from the co-authors.

IV Initiated the idea for the study and planned it with input from the co-authors. Discussed set-up and received input from Dr Bonne Beerda, Wageningen University. Performed data collection and preparation of data. Analysed data with help from the co-authors. Main responsible for writing the manuscript with input from the co-authors.

Abbreviations

ACTH	Adrenocorticotropic hormone
ANS	Autonomic nervous system
BE	Behavioural element
C:Cr	Cortisol-to-creatinine ratio
CatBeWell	Cat Behaviour and Well-being tool
CRH	Corticotrophin-releasing hormone
CSS	Cat-Stress-Score
eSA	extended Stress Assessment
FHV-1	Feline herpesvirus-1
FIC	Feline interstitial cystitis
GAS	Global Assessment Score
GCs	Glucocorticoids
HPA axis	Hypothalamus-pituitary-adrenal axis
IQR	Interquartile range (a measure of statistical dispersion)
LUTD	Lower urinary tract disease
PSNS	Parasympathetic nervous system
SNS	Sympathetic nervous system
sSA	shortened Stress Assessment
URD	Upper respiratory disease

1 Background

As the most numerous and popular companion animal in Sweden (SCB, 2012) and one of the most popular in the United States, Canada and Northern Europe (Lyons & Kurushima, 2012) the domestic cat would be assumed to have a relative high standing in society. Unfortunately, the reality is a different story. Growing numbers of abandoned domestic cats are roaming cities (Lyons & Kurushima, 2012) or are being relinquished to shelters or euthanised (Dantas-Divers *et al.*, 2011; Scarlett *et al.*, 2002). There also seem to be issues relating to providing of proper housing and care for cats. Issues relating to lack of knowledge about basic cat behaviour (Kass, 2007), for example agreeing to statements such as *cats misbehave out of spite* or that *cats do not mind sharing house with other cats* (Salman *et al.*, 1998) as well as basic behavioural needs, for example relating to 'environmental enrichment' (Alho *et al.*, 2016).

In a study of housing and enrichment provided by cat guardians, the majority supplied only a 'moderatley enriched environment', and none reached the set requirements for level of 'excellent environment', for example, by providing one litterbox per cat, plus one extra, or separating water and food bowls (Alho *et al.*, 2016). These can be seen as indications that guardians are in need of further knowledge concerning cats' behavioural and environmental needs. The American Association of Feline Practitioners (Rodan *et al.*, 2016) recently issued a position statement concerning the impact of lifestyle choice (indoor or outdoor) on cats. One issue raised was that most cat guardians are not aware of the cat's environmental, nor emotional and social, needs. There are different issues related to indoor and outdoor housing of cats, and indoor only cats are reported to be more sensitive to certain diseases, as well as the confinement itself, which can have negative effects on cats (reviewed by Buffington [2002]). In a survey of Swedish cat guardians, we found a significant difference in reported temperament and behavioural problems between guardians that provided cats with outdoor access and those who did

not. Guardians that provided cats with outdoor access reported experiencing significantly fewer problems than expected (Hirsch *et al.*, 2015). Besides related to fewer actual problems, this could be related to guardians not being aware of the problems, or not observing them, as cats remain partially outdoors. However, if these problems are *problem behaviours*, in other words behaviours that are natural for cats but not accepted by guardians or the society (e.g., marking behaviours) and not *behavioural problems*, that is behaviours risking the animals' welfare, outdoor access would still be beneficial from the cat's perspective as it will likely decrease the risk of abandonment and relinquishment.

Most cat guardians turn to their veterinarian or veterinary nurse for guidance concerning provisions for their cat's needs. A previous study comparing veterinarians and veterinary nurses (professionals) knowledge concerning behavioural needs with that of cat guardians found that it did not differ statistically between areas (Da Graça Pereira *et al.*, 2014). That many veterinary professionals lack knowledge about cats needs to maintain welfare was also suggested by Rodan *et al.* (2016) as one important issue within cat welfare. Providing an unsuitable physical or social environment can result in cats experiencing fear and stress and subsequently developing undesired (unwanted) behaviours, such as elimination problems and aggression (Levine, 2008). Elimination problems are the most common behavioural conditions and make up 40-75% of guardian reported conditions (Seksel, 2012). Still, simply playing with your cat for bouts of 5 minutes has been found to be related to fewer guardian reported problems such as aggression and inappropriate elimination (Strickler & Shull, 2014).

Previous studies have determined undesired behaviours as leading causes for relinquishment to animal shelters (Salman *et al.*, 2000). Relinquishment results in cats being at risk of experiencing negative emotions associated with changes in both the physical (Gooding *et al.*, 2012; Dybdall *et al.*, 2007; Griffin & Hume, 2006) and social (Ottway & Hawkins, 2003) environment. These environments often involve space restrictions (Gouveia *et al.*, 2011; Kessler & Turner, 1999a), having to share resources with unknown individuals (Gourkow & Fraser, 2006; Hurley, 2005), the presence of dogs (McCobb *et al.*, 2005) or unpredictable handling and routines (Stella *et al.*, 2011; Carlstead *et al.*, 1993b).

2 Introduction

The domestic cat (*Felis silvestris catus*) originates from the African Wildcat (*Felis silvestris lybica*) (Driscoll *et al.*, 2007) an opportunistic territorial predator (Casey & Bradshaw, 2007). Not much is known about the general behaviour or sociability of the African Wildcat. There has been no historical evidence of social groupings, besides queens and kittens, and due to modern time hybridization with the domestic cat, observations of social living during modern time could as well be linked to a domestic ancestor (Bradshaw, 2016).

2.1 Behaviour of the domestic cat

The *domestic cat* (hereafter, *cat)* shares many morphological and behavioural characteristics with its wild ancestor (Montague *et al.*, 2014) such as remaining a solitary opportunistic predator (Driscoll *et al.*, 2009; Casey & Bradshaw, 2007; Corbett, 1979). Cats still hunt small prey (Bradshaw, 2016) such as small mammals, birds and herpetofauna (Calver *et al.*, 2007) but are known to take down rabbits, especially younger rabbits, (Corbett, 1979). Cats can be preyed upon by larger predators, such as coyotes (*Canis latrans*) (Grubbs & Krausman, 2009) and domestic dogs (*Canis lupus familiaris*) (Stella *et al.*, 2014). The marking behaviour of cats is similar to that of solitary wildcats in that cats will leave scratch marks and scent mark using urine, faeces and pheromones (Macdonald *et al.*, 2010). This flexibility in hunting behaviour and the opportunistic hunting style has allowed the cat to survive in most regions around the world besides the North Pole and South Pole. However, due to their hunting abilities, and flexibility, cats are considered 'pests' in several parts of the world where there are no indigenous predators such as Australia, New Zealand (Farnworth *et al.*, 2010) and some isolated islands, and where eradication takes place using for example traps, poison and introduction of diseases, primarily viruses (Nogales *et al.*, 2004). Less severe actions

suggested to spare the impact on fauna are restrictions and curfews for when cats have outdoor access (e.g., Barratt, 1997), as well as different types of collar-mounted prey protectors, for example, pounce protectors (neoprene bib or fabric [e.g., Hall *et al.*, 2015; Calver *et al.*, 2007]).

2.1.1 Sociability of the domestic cat

The domestication of the cat started around 10 000 years ago (Vigne *et al.*, 2012) and it is during this time that social behaviours seen have emerged, a very short time from an evolutionary perspective (Bradshaw, 2016). There is no evidence of social living in the African Wildcat, besides that of the queen and kittens (Bradshaw, 2016) but the same social signals, as seen in cats, have been found in four undomesticated small felines (Geoffroy's cat, *Oncifelis geoffroyi*; Caracal, *Caracal caracal*; Asiatic wildcat, *Felis silvestris ornata* and Jungle cat, *Felis chaus*) (Cameron-Beaumont, 1997). As proposed by Cameron-Beaumont (1997), it is therefore likely that the social behaviours derives from the African Wildcat, and not the domestication process, and that interactions either stem from sexual or mother-young interactions or are present but not utilised in the African Wildcat due to solitary lifestyle. The one exception found was *tail-up* as used during affiliative interactions, which might have evolved during the domestication process. For example, in mother-young interaction, kittens greet mothers using tail-up followed by *head rubbing* during food solicitation (Cafazzo & Natoli, 2009). In the cat, tail-up has been found to be a signal of affiliative intent, and research has shown that cats approach cat silhouettes displaying tail-up faster and with less hesitation than silhouettes with the tail down (Cameron-Beaumont, 1997).

Despite a short time, from an evolutionary perspective, the domestication process has now enabled cats to function in groups under certain contexts (Casey & Bradshaw, 2007) that is, cats have been observed to form social groups with affiliative relationships, recognize colony compared to non-colony members, and cooperate with raising of kittens by for example, allo-suckling (Crowell-Davis *et al.*, 2004). Under free-ranging conditions, for example feral or farm cats, groups are formed around matrilineal relations (Macdonald *et al.*, 2000). More closely related females have been observed to interact more, and adult males largely live solitary lives without close permanent social ties to groups (Macdonald *et al.*, 2000). Solitary or group-living have been related to distribution and availability of resources (Corbett, 1979). However, for this group-existence, cats need to learn how to interact with other cats which is something they learn by interacting (e.g., playing) with other kittens around the age of 12-14 weeks (Bradshaw, 2013). Cats can also learn to live together with other species such as humans and dogs. Still, each cat needs to be socialised to

accept living in proximity to humans. This means that cats need to be handled during the sensitive period, between 2 and 7 weeks of age (Karsh & Turner, 1998) for a subsequent successful relationship with humans (McCune, 1995) without which cats may never come to feel completely comfortable in the presence of humans. However, later studies have found that the relationship with humans can continue to develop during the first 4 months of life (Lowe & Bradshaw, 2002) after which it seems to stabilise at least during the first few years (Lowe & Bradshaw, 2001). Interestingly, there is also evidence of an effect of the sociality of the sire (McCune, 1992). Friendly fathers were seen to have litters of more sociable kittens.

Looking at the requirements for cats to live social lives it is clear that sociability is individual and depends not only on previous experience but also on early life events, for example, separation from the littermates and group compositions, as well as genetic factors.

2.2 Group-housing and housing requirements

A common issue concerning feline friendly husbandry relates to the social abilities of cats. As seen, in free-living cats, group-living is dependent on resource availability (Corbett, 1979) and groups are formed around matrilineal relations (Crowell-Davis *et al.*, 2004; Macdonald *et al.*, 2000). These conditions however are seldom fulfilled when groups are composed by humans. When groups are made up of unrelated or unknown individuals group-housing can become problematic (Ottway & Hawkins, 2003). Despite this, cats are often group-housed for example in catteries, cat shelters, research colonies and in private homes (e.g., Hirsch *et al*., 2014; Ramos *et al.*, 2013; Kessler & Turner, 1999b). Shelters primarily group-house due to space restrictions (Gouveia *et al.*, 2011; Kessler & Turner, 1999a), lack of availability of resources and current thinking about behavioural needs of cats. Previous studies have found that adoption rates are higher in cats housed in groups compared to standard single cages (Gourkow & Fraser, 2006) so the notion that group-housing increases adoption rates likely also affect the choice of housing in shelters. However, other factors, such as providing toys, have also been shown to increase viewings and adoption rates (Fantuzzi *et al.*, 2010).

To what degree cats are effected by group-housing varies, and depend not only on the group density but also on the quality of the housing (Hurley, 2005). Gourkow and Fraser (2006) found that group-housing does not always result in more negative consequences, but a husbandry that allows cats to avoid each other (discrete) is better than a communal housing that promotes interactions between cats. Discrete housing resulted in fewer recorded negative encounters.

In group-housing it is important to provide enough resources so as to minimise the risk for competition or resource guarding. Recommendations are to provide the same amount of resources, for example, litterboxes and food and water bowls, as cats, plus one additional (Möstl *et al.*, 2013). Providing appropriate materials for scratching (marking and claw maintenance) is important as cats will likely find something less suitable to scratch on otherwise. Using the three-dimensional environment with shelves and elevated platforms provides not only additional space but also escape routes in case of group tension or conflict. Stand-alone shelves, with screened off compartments, have been found to be a popular and well-utilised resource for laboratory cats and seem to decrease agonistic interactions after feeding (Desforges *et al.*, 2016).

That the cat is a predator is often considered by providing cats with toys promoting hunting related play, as well as windows to observe their surroundings, for example, the outdoors including potential prey. In contrast, that the cat is also a prey species is sometimes overlooked. Hiding has been shown to be an important behaviour for cats when feeling threatened (Vinke *et al.*, 2014; Kry & Casey, 2007; Carlstead *et al.*, 1993b), and hides should be provided for all cats.

Other important factors in the physical environment concern keeping good air quality, humidity as well as temperature (Hurley, 2005). Recommendations for housing of cats in shelters or laboratories include 10-12 air exchanges per hour (Möstl *et al.*, 2013). The thermal neutral zone for a cat has been suggested at 30-38° C (National Research Council, 2006) which is several degrees higher than normal indoor temperatures. Providing cats with the opportunity to self-regulate the micro-climate by providing insulated hides and warm surfaces such as blankets can help cats cope with temperatures more suited for humans. Provision of soft resting places have also been seen to decrease the likelihood of cats resting in inappropriate places (e.g., litterboxes) and to increase REM sleep (Crouse *et al.*, 1995).

These issues, concerning the physical and social environment, are all related to the cats' welfare, and when not considered, potentially subject cats to negative emotions. This increases the risk of the relationship between the cat and the guardian becoming disrupted, increasing the risk for relinquished or abandoned of the cat. For cat shelters, whose aim is to rescue society's unwanted cats, there is a risk that they instead become arenas where cats are being subjected to aversive environments. Animal shelters not only provide potentially aversive social environments but also a high turnover of animals, which in combination with crowding provides more opportunities for transmission of pathogens involved in feline respiratory disease (Cohn 2011). Practices that can increase

the risk for a cat being euthanised for welfare reasons. The euthanasia rate at shelters differ between countries and have been estimated to be 10% in Sweden (Eriksson *et al.*, 2009), 33% in Australia (RSPCA Australia, 2015) and 40-50% in the United States and Canada (Turner *et al.*, 2012).

2.3 Stress, stressors and the stress response system

Stress, may be defined in several different ways. In this thesis, stress is defined as any challenge, interpreted as a threat by an individual, which results in changes in behaviour and/or physiology (McEwen, 2000). Stress is a normal and adaptive response that promotes adaptation by activation of defences (i.e., the *stress response system* [Möstl & Palme, 2002]), meaning that stress is not inherently bad (Dawkins, 1998). The stress response system includes numerous coordinated responses (Lupien *et al.*, 2009; Sanchez, 2006) which can be divided into three general biological responses; behavioural, physiological and immunological. These responses all aim at keeping the body's systems that are essential for life in homeostasis by *allostasis*, that is, adjustments of the organism related to re-establishment of stability (McEwen, 2005).

The cerebrum and hypothalamus are critical in the body's behavioural and physiological response to stress, and indirectly influences the immune system. As the cerebrum includes structures related to personality, there are individual differences in responses to the same event. This means that different *stressors*, that is, stimuli (situations and environmental factors) activating a stress response (Everly & Lanting, 2013) can affect individuals differently, inducing stress in some while not in others. Whether a stressor is perceived as harmful or not is critical, and influenced by a myriad of factors called *modifiers* (Moberg, 2000) such as early experience, genetics and social relationships. The hypothalamus and limbic system act like links between emotions and physical reactions. The hypothalamus also controls the endocrine system, the autonomic nervous system and behaviour.

The stress response system involves adaptations that work primarily via two physiological pathways, the *hypothalamus-pituitary-adrenal axis* (HPA axis) resulting in release of *glucocorticoids* (GCs), for example, *cortisol*, and activation of the *autonomic nervous system* (ANS), mainly the *sympathetic nervous system* (SNS) resulting in, for example, cardiovascular adaptations such as increased blood pressure and heart rate. Stress can arise in situations where the individual has the cognitive perception of not being able to control and/or predict the environment. This occurs when the animal cannot *cope*, that is, manage the perceived stressful event by modifying its behaviour and/or physiology (Koolhaas *et al.*, 1999). This relates to the *motivation* of an animal.

In scientific terms, motivation has an operational definition stating that if an animal is motivated to perform an action, it likely will, independent of if it relates to approaching (appetitive motivation) or avoidance (aversive motivation) (Kirkden & Pajor, 2006). If an animal is highly motivated to perform a behaviour, for example, escape something aversive, but prevented from doing so, the animal remains in a high motivational state and *suffering* (i.e., prolonged or acute unpleasant subjective states) may occur (Dawkins, 1990). The inability to perform a motivated behaviour in cats, for example, escape from confinement, has been linked to frustration (Gourkow & Phillips, 2016). Within captive environments, where animals are kept, to some extent, under unnatural conditions, strategies shaped by evolution to handle threatening situations might become ineffective (Morgan & Tromborg, 2007). Failure to cope may reduce an animal's welfare (Broom, 2006) by inducing suffering (Dawkins, 1990) and result in a state of stress (Ottway & Hawkins, 2003). In the following thesis, *welfare* will be discussed not only as relating to physical health, but also in relation to feelings of an animal (Duncan, 2005). As feelings are subjective, they can be accessed by for instance observations of signs of stress, as well as disease (Duncan, 2005). One consistent finding is that if the environmental stressor is too demanding, and the individual cannot cope with the change, the health of the animal is at risk (Koolhaas *et al.*, 1999). So, it is not only the physical nature of the stressor, but the perception, and predictability and/or controllability that determines the actual effect of a stimulus (Weiss, 1968). This means that if the animal feels threatened, it may suffer independent of actual danger (Dawkins, 1990).

Differentiation between responses are usually made based on the duration of the stress response. *Acute* (short-term) stress, provides energy for the body to cope with challenges (Sapolsky, 2002) and can be countered by an animal taking behavioural and/or physiological actions. *Chronic* (long-term) stress, is when defence mechanisms fail, or are activated during a prolonged time period (Toats, 1995). When stress is truly threatening for an animal, it experiences *distress*, however when stress moves into becoming *distress* can be difficult to determine as both acute and chronic stress can cause distress (Moberg, 2000). This presents a major challenge during animal welfare assessments.

The stress response system can also become desensitised, or habituated to a stressor, after repeated exposure resulting in loss of stimulation of the stress response system. In response to repeated exposure to the same stressor, the system can also become sensitised meaning that exposure to a new different stressor can induce a stronger stress response (Aguilera, 1998).

Del Giudice *et al.* (2013) have proposed that stress, as a complex biological mechanism, should be approached from multiple perspectives, preferably with a basis in Tinbergen's 'four questions'. To understand a biological system, Tinbergen (1963) proposed four complimentary 'approaches' (i.e., ways of problematising an observed phenomenon) nowadays known as the 'four questions'. In the modern study of animal behaviour these are divided into two major groups, *proximate* and *ultimate* questions. Proximate questions relate to how internal and external factors control a behaviour (*causation*) and how it develops during an individual's lifetime (*ontogeny*). Ultimate questions relate to the evolutionary perspective of a behaviour, what the survival value of the behaviour is (*function*) and how it evolved from a historical perspective (*phylogeny*). This thesis focuses primarily on the proximate causes behind stress and stress related behaviours, as in captive environments, suffering has been proposed to be primarily related to proximate (here and now) mechanisms underlying a behaviour (Dawkins, 1990). The need for an understanding of the evolutionary perspective is not ignored but approached more in the discussion.

2.4 Biological responses to stress

During recent years it has become evident that stress physiology is integrative and that there is also an effect of the social environment on both physical and mental health. This is due to the two-way communication between the brain and body working through the ANS, endocrine and immune systems (McEwen, 2005). Stress can be measured using different physiological mediators of allostasis (McEwen, 2005) such as primary mediators (e.g., cortisol), secondary outcomes, for example, increased ventilation, or tertiary outcomes such as a reduction in the immune system's efficiency (McEwen & Seeman, 1999). That the biological responses (behaviour, physiology and immune system) are connected is clear. In cats, stress have been connected to anorexia (behavioural response) which in turn can result in the disease hepatic lipidosis, a fatty liver syndrome (Amat *et al.*, 2015). There are also indications that cats with feline idiopathic cystitis (FIC), that is, recurrent clinical symptoms of lower urinary tract disease (LUTD), may have more severe symptoms in response to stressors (Westropp *et al.*, 2006). Cats with LUTD often also have comorbid disorders such as behavioural problems for example, fearfulness and aggression (Buffington *et al.*, 2006). Such behaviours have previously been found as risk factors for a breakdown in the relationship with the guardian, increasing the risk of euthanasia and relinquishment to shelters (Salman *et al.*, 2000).

It is important to remember that many of these signals of reduced welfare are adaptations, shaped by evolution, to protect the organism from threats to fitness, meaning that they might reduce well-being temporarily, but will enhance fitness in the long-run (Dawkins, 1998).

2.4.1 Behavioural

The behavioural response, in some literature referred to as the *primary response* to stress, is generally considered biologically cost-effective especially when the animal can escape the stressor (Moberg, 2000). The behavioural response to stress is in part controlled by the hypothalamus and limbic system (reviewed more in detail under the section 'The HPA axis'). Due to differences in stressors and environments, especially artificial environments provided by humans, behavioural responses might not always be effective. Running away, or hiding, from another cat in one's social group might not always be possible during confinement, for example in shelters.

During aversive situations, cats may resort to different aggressive behaviours (Levine, 2008). Behaviours relating to aggression in cats can be both *overt* (active) and *covert* (passive) (Levine, 2008). Guardians might recognise overt aggression, for example, physical fighting, biting and scratching correctly (Levine *et al.*, 2005) but might miss more subtle signs of covert aggression (Levine, 2008) such as blocking access and staring. Cats can express fear through aggressive behaviours (Moffat, 2008), especially when escape is not an option (Ramos & Mills, 2009; Levine, 2008). Fear and pain have been suggested as the two most common causes for aggression seen in cats at veterinary clinics (Rodan, 2010).

Inability to cope with a stressor can with time result in chronic stress. Chronic stress has been seen to result in decreased exploration in captive leopard cats (*Felis bengalensis*), however abnormal behaviours were not always associated with changes in cortisol levels (Carlstead *et al.*, 1993a). In laboratory cats, the main responses were increased hiding and vigilance behaviour as well as a reduction in general activity and exploratory behaviour (Carlstead *et al.*, 1993b).

2.4.2 Physiological

The Autonomic Nervous System

The ANS supply nerves to (*innervate*) smooth muscle, heart muscle and glands and consists of not only the SNS but also the *parasympathetic nervous system* (PSNS). Most organs are controlled by both the SNS and PSNS through dual innervation. During threats and stress situations, it is primarily the SNS that is activated. One function of the SNS is to activate the cardiovascular system

through generalised arousal via the *Fright-Fight-Flight* response increasing, for example, heart rate and ventilation (Sjaastad *et al.*, 2010). It is worth mentioning that it has been suggested that physical fighting or fleeing is not adaptive for females of all species, often caring for immature offspring, and that females instead utilise a *Tend-and-Befriend* strategy, calming offspring or getting them out of harm's way as well as forming alliances with other females (reviewed by Taylor *et al.*, [2000]). The PSNS is primarily activated during times of rest, and promotes for example, digestion. PSNS activation has been found in connection to *reactive* (i.e., passive) coping styles in response to stressors, where freezing is often seen as a response to predators or inescapable stressors (Koolhaas *et al.*, 1999). So it seems that for reactive coping animals, if there is no opportunity to fight or flee for instance due to confinement, or the response is ineffective, the PSNS can be activated and the animal may become seemingly passive (freezing).

The SNS stimulates the adrenal medulla to release adrenaline and noradrenaline. Noradrenaline is also released as a neurotransmitter in the SNS. Both adrenaline and noradrenaline enhances the effect of the SNS and has approximately the same effect as a sympathetic nerve stimulation (Sjaastad *et al.*, 2010). Activation of the SNS has been related to alterations in the immune function and seem to take place before stimulation of a cortisol release in humans (Herbert & Cohen, 1993).

The HPA axis

Stress usually also activates the hypothalamus, pituitary and adrenal cortex, the HPA axis. The hypothalamus releases *corticotropin-releasing hormone* (CRH) which in turn regulates the release of *adrenocorticotropic hormone* (ACTH) from the anterior pituitary. ACTH is released into the general circulation where it in turn regulates the release of GCs, in cats cortisol, from the adrenal cortex. The HPA axis is regulated through a negative feedback system, where the end product inhibits the initiating substance. Cortisol (the end product) affects both the hypothalamus and the anterior pituitary (the Long-loop feedback), inhibiting the production of CRH (initiating substance) and ACTH and where also ACTH inhibits its own secretion by acting on the hypothalamus (Short-loop feedback) (Sjaastad *et al.*, 2010). CRH has been reported to play an integrative role in regulation of the stress response by acting as a gatekeeper, initiating and inhibiting responses to stress (Miller & O'Callaghan, 2002).

Cortisol is a multitasking hormone, meaning that it not only increases during stress but also in non-threatening situations such as in response to general activity and metabolism. Increased cortisol concentrations results in increased levels of blood glucose, energy to be utilised by the brain and

skeletal muscles during responses to danger and threat. In cats, increased cortisol concentrations have been measured in individuals showing behaviours relating to both 'friendliness' and 'aggression' (Gourkow *et al.*, 2014b).

Due to the large congregation of CRH receptors found in the amygdala, CRH has been suggested to be involved in mediating stress induced emotion-related behaviours, such as activity and exploration in open field tests in rats (Liang & Lee, 1988).

2.4.3 Immunological

Stress has a clear connection to the immunological response and can, when acute, enhance the immune system (Pruett, 2003) but when chronic, lead to suppression (Pruett, 2003; Toats, 1995; Griffin, 1989). Chronic stress, with elevated cortisol levels, may result in a reduction of the immune system of an individual, rending it more susceptible to disease (Pruett, 2003; Toats, 1995; Griffin, 1989), by stress induced immunosuppression (Gourkow *et al.*, 2013). As the immune system is reduced and the animal become more susceptible to disease, smaller amounts of infectious agents are needed for an animal to become infected (Sapolsky, 2004). Immunosuppression can increase the risk of latent viruses clinically manifesting in an individual, for example, with reactivation and shedding of viruses (Kennedy & Little, 2012; Day *et al.*, 2010; Lappin *et al.*, 2009; Pontier *et al.*, 2009; Edwards *et al.*, 2008) and result in the individual becoming sensitive to secondary infections (Sykes, 2010).

2.5 Factors reported to relate to stress in cats

Housing and handling are factors known to affect stress in cats. Building on the scoring system established by Sandra McCune, first described as the *Global Assessment Score* (GAS) (McCune, 1992) and later summarised in McCune (1994) under the *Cat Assessment Score*, Kessler and Turner (1997) developed the *Cat-Stress-Score* (CSS). The CSS describes 7 possible stress levels from *Fully relaxed* to *Terrorized* based on behavioural and postural elements. Previous research by Kessler and Turner (1999a) has shown that there is a correlation between the number of cats in a group and the stress level of an individual, as shown by differences in behavioural stress, assessed using the CSS protocol. When studying the association between urinary cortisol levels and housing (single- or multi-cat housing) in privately owned cats, Lichtsteiner and Turner (2008) found that available space (m^2), the human density and number of humans per household affected the cortisol levels. The basal cortisol levels were also compared in samples from shelter cats, where no effect was found for housing style (group or single). Also, in a more recent study of

privately owned cats by Ramos *et al.* (2013), the faecal GCs concentration did not differ between single- or group-housed cats. Kessler and Turner (1999a) found that CSSs increased when densities were more than 0.6 cats per m^2 during group-housing. Gouveia *et al.* (2011) studied differences in behaviour of sheltered cats held in groups with different sex ratios, cat densities and time spent in the shelter. What they found was that cats having spent longer time in the shelter were less active and participated more in negative encounters. Also, in rooms with high densities, over 0.5 cats per m^2, cats were more inactive.

Stress levels in group-housed cats vary, and depend not only on the group density but also on the quality of the housing (Hurley 2005) seen in a study by Loberg and Lundmark (2016) where there was no effect on the CSSs when available space was 1 m^2, 2 m^2 or 4 m^2 per cat, but the resources remained the same.

Within non-functioning groups competition for resources, resulting in aggression, can occur (Crowell-Davis *et al.*, 2004), and group-housing in general can negatively affect some cats (Kessler & Turner, 1997). When studying single- and group-housing of cats, Kessler and Turner (1999a) found that group-housing induced stress in cats not well socialised towards conspecifics, as measured by a higher score on the CSS. Cats not well socialised to humans were the most stressed, independent of housing condition. Further on, the study showed that a stressed individual could influence and increase the stress levels of the other cats in the group by becoming more active and thereby disturbing the other cats.

Unpredictability, in human handling as well as environmental changes, has previously been shown to increase stress in cats (Stella *et al.*, 2013; Carlstead *et al.*, 1993b). For example, experiencing a novel environment (Stella *et al.*, 2013; Gooding *et al.*, 2012; Griffin & Hume, 2006) such as moving from a known environment when surrendered to a shelter has been shown to induce stress in cats (Dybdall *et al.*, 2007). In their study of cats entering a shelter, Dybdall *et al.* (2007) found that guardian surrendered cats had higher behavioural stress levels (CSSs) compared to cats entering as strays. As these cats were kept in single cages, the stress was not caused by group-housing but other factors in the environment. The cats with higher stress scores were also those cats that in the end were not adopted.

In a study, comparing the home environment with the environment at the veterinary hospital during a veterinary examination, it was found that 30 apparently healthy cats showed a significant increase in respiratory rate, heart rate and blood pressure when measured at the hospital (Quimby *et al.*, 2011). However, in the home environment, the cats reacted with more struggling and vocalisation which has been found to be indicators of stress in cats (Iki *et al.*,

2011). In support of these findings, Nibblett *et al.* (2015) found that cats had higher plasma glucose levels when examined at a clinic, and where they attempted to hide more, compared to the home environment. Also, during a second examination, plasma cortisol levels were lower in cats' whether examined in the home or at the clinic. These are additional indications that novel environments, or even novel handling in a previously known environment, can activate a stress response in cats.

Other factors related to stress in cats are housing together with unknown cats (Ottway & Hawkins, 2003), overcrowding (Möstl *et al.*, 2013), small cages an inadequate environment (Rees & Lubinski, 2008) as well as housing in proximity to dogs (McCobb *et al.*, 2005).

As seen, keeping cats healthy, both physically and psychologically, requires that we look at aspects both in the social and the physical environment. Problems relating to group-housing of cats can be reduced as long as there are enough resources at hand (Crowell-Davis *et al.*, 2004) and the groups are kept stable (Bernstein & Strack, 1996). Regroupings and changes in existing groups (even removal of a cat) can disrupt previously functioning groups (Overall *et al.*, 2005). How well the cat is socialised seems to be a factor for how well the cat handles the group setting (Kessler & Turner, 1999b), and socialisation with other cats is necessary for the cat to learn appropriate responses and intraspecific communication to function in a group (Crowell-Davis *et al.*, 2004). Minimising stress through husbandry routines is possible; for example providing hiding boxes, as previous studies have shown that cats spend much of their time in hiding (e.g., Rochlitz *et al.*, 1998) and try to make hides if they are not provided by for example turning the litterbox upside down (Gourkow & Fraser, 2006). Further on, providing consistent handling is important, unpredictable handling by humans has been found to affect cats negatively (e.g., Stella *et al.*, 2011; Kessler & Turner, 1999a; Carlstead *et al.*, 1993b). Modifying husbandry according to the individual's previous experiences (e.g., socialised or not towards other cats) during group-housing can also decrease the stress response (Kessler & Turner, 1999a). In general, if group-housing is considered it is important to make sure that enough resources are provided to avoid resource guarding or competition between cats, and that the environment allows individuals to avoid each other and claim space of their own as shown by for instance Gourkow and Fraser (2006).

Stress also affects the cats' behaviour (Kessler & Turner, 1999b) for example by cats developing (by guardians) undesired behaviours, resulting in risk of the cat being relinquished (or abandoned) and ending up in a shelter (Bernstein, 2007) or being euthanised. If the shelter practices group-housing,

this would likely result in additional exposure to stress (Ottway & Hawkins, 2003) as well as infectious load (e.g., Möstl *et al.*, 2015). Minimising stress in shelters is important not only to decrease and control disease transmission and recrudescence of latent viruses, but also to improve the welfare of the animals and shorten time to adoption (Patel *et al.*, 2010) as only healthy cats are put up for adoption. Adopters have been suggested to avoid selecting cats they believe are prone to behavioural problems (de-clawing) (Fritscher & Ha, 2016), so keeping cats mentally healthy should also be an important aspect to consider. One of the major issues with assessment based on a stress response is that different stressors do not provide unique behavioural or physiological responses, so responses only show that there is something wrong, not what is wrong (Morgan & Tromborg, 2007).

Unfortunately, there is still no easy-to-use assessment tool available for cat caretakers to determine how cats are faring, nor their likely outcome (e.g. time spent at shelter before adoption) in for example a shelter setting.

2.6 Measurements of stress in the cat

2.6.1 Behavioural measurements of stress

The CSS is a standardised, and well used, method for behavioural assessment of stress, and according to literature searches, it is also the most commonly used protocol (e.g., Loberg & Lundmark, 2016; Rehnberg *et al.*, 2015; Vinke *et al.*, 2014; Broadley *et al.*, 2013; Moore & Bain, 2013; Gooding *et al.*, 2012; Tanaka *et al.*, 2012; Patel *et al.*, 2010; Dybdall *et al.*, 2007; Kry & Casey, 2007; Gourkow & Fraser, 2006; McCobb *et al.*, 2005; Kessler & Turner, 1999a; Kessler & Turner, 1999b). Despite this, the CSS has so far not clearly been validated against other signs of stress, such as physiological measurements, for example, cortisol concentrations (Rehnberg *et al.*, 2015; McCobb *et al.*, 2005). Several users (e.g., Gooding *et al.*, 2012; Dybdall *et al.*, 2007), including the developers themselves (Kessler & Turner, 1997), have proposed the need for further validation. The scoring is subjective, and static, building on behaviours displayed under short time intervals (Broadley *et al.*, 2013), that is, according to the original methods, 1 minute observations (Kessler & Turner, 1997). It seems that using the CSS provides different results for potential confounding factors (e.g., age, sex, neutering status) depending on studies (Table 1), which might be connected to differences in methods, such as time observing a cat before providing a score, or other unknown factors, for example individual experiences of the cats, housing or resources provided.

Table 1. *Basic demographic data for subjects in reviewed studies using the CSS.*

Parameter		Reference
Age		
	No effect	Dybdall *et al.*, 2007; Kry & Casey, 2007; Kessler & Turner, 1997
	Older cats had lower CSSs	Broadley *et al.*, 2013
	Older cats had higher CSSs	Rehnberg *et al.*, 2015
Sex		
	No effect	Dybdall *et al.*, 2007; Kry & Casey, 2007
	Females had lower CSSs	Rehnberg *et al.*, 2015
Neutering status	No effect	Broadley *et al.*, 2013; Dybdall *et al.*, 2007
	Neutered males had higher CSSs	Rehnberg *et al.*, 2015

If the CSS is as sensitive to these confounding factors as it seems, caution should be used when interpreting studies relying solely on the CSS as a measurement of [behavioural] stress.

McCobb *et al.* (2005) could not find any correlations between scores on the CSS and corresponding urinary cortisol-to-creatinine ratio (C:Cr). Neither was there a correlation between CSS and faecal cortisol metabolites (Rehnberg *et al.*, 2015). Further, CSSs were not found to relate to outcome (adoption, euthanasia) in McCobb *et al.* (2005) or Moore and Bain (2013) but higher averaged CSS was related to euthanasia in Gourkow and Fraser (2006) and cats that were deemed suitable for adoption had lower CSS in Dybdall *et al.* (2007). In contrast, Tanaka *et al.* (2012) found an association between higher levels of CSS and development of upper respiratory disease and decreased food intake in shelter cats.

Hiding is one behaviour that in several studies has been found to increase in cats in response to stressors such as unpredictable environments (e.g., Carlstead *et al.*, 1993b). Hiding seem to be an important coping strategy for cats (Vinke *et al.*, 2014; Kry & Casey, 2007), especially when entering a new environment (Rochlitz *et al.*, 1998). Time spent hiding decreased with time spent at the facility in a study of quarantine cattery cats (Rochlitz *et al.*, 1998). Using the CSS Rehnberg *et al.* (2015) found that it was the cats with the highest scores that spent most time in hiding, and Kry and Casey (2007) and Vinke *et al.* (2014) found that having the opportunity to hide reduced the cats' scores on the CSS. In cases where opportunity to hide is not provided by caretakers, cats have been observed trying to create hides by for example turning litterboxes upside down (Gourkow & Fraser, 2006) or hiding under towels provided to rest upon (personal observation, Figure 1).

Figure 1. Cat attempting to construct a hide using the towel provided to rest upon. (Photo: EN Hirsch)

Cats attempting to hide behind the litterbox had lower urinary cortisol levels (Carlstead *et al.*, 1993b), and cats that did not have a hiding box spent 45% of the total observed time behind the litterbox (Vinke *et al.*, 2014).

However, as there has been discussions if the CSS really relates to stress or underlying fear (McMillan, 2012). It would be assumed that cats with different latency would also show differences in CSSs. Gooding *et al.* (2012) did not find a clear correlation between the CSS and latency to approach a novel object, a commonly used test of fear.

2.6.2 Physiological and immunological measurements of stress

SNS activation results in several physiological reactions. As mentioned in cats, Quimby *et al.* (2011) found that respiratory rate, heart rate, blood pressure and rectal temperature were lower in cats when measured in the cats' home environment compared to when measured in a more stressful situation at a veterinary hospital. A similar study by Nibblett *et al.* (2015) found higher blood glucose levels and attempts to hide during examination at the clinic.

In a series of publications, Gourkow and colleagues (Gourkow & Phillips, 2016; Gourkow & Phillips, 2015; Gourkow *et al.*, 2014a; Gourkow *et al.*, 2014b) measured mucosal immunity and signs of URD in relation to welfare in shelter cats. Looking at the connection between behaviour, faecal cortisol metabolites and immunoglobulin A (S-IgA) measured from faeces, Gourkow *et al.* (2014b) found that cats displaying calm behaviours and acting in a friendly way towards humans had higher levels of S-IgA than cats that did not.

However, they did not find a connection between faecal cortisol metabolites and S-IgA levels. Increased levels of S-IgA were also present in cats that experienced positive human interactions compared to control cats and resulted in fewer instances of URD when compared to cats rated as both anxious (Gourkow *et al.*, 2014a) and content (Gourkow & Phillips, 2015) upon admission to the shelter. Cats rated as frustrated upon admission, but receiving cognitive enrichment (training) also showed an increase in S-IgA levels compared to control cats (Gourkow & Phillips, 2016).

Most physiological studies of stress in cats however, measures activation of the HPA axis and changes in circulating concentrations of cortisol (e.g., Mazzotti & Boere, 2009; Accorsi *et al.*, 2008; Lichtsteiner & Turner, 2008; Genaro *et al.*, 2007; McCobb *et al.*, 2005). Plasma GCs (e.g., cortisol) measurements are commonly used for welfare assessment in most species (e.g., Iki *et al.*, 2011; Genaro *et al.*, 2007; Mormède *et al.*, 2007). In cats, different media has been used to quantify cortisol. Acute stress has been quantified using cortisol assayed from plasma (Iki *et al.*, 2011; Genaro *et al.*, 2007) and serum (Mazzotti & Boere, 2009; Carlstead *et al.*, 1992; Sparkes *et al*., 1990). Chronic stress has been quantified from urine, using C:Cr, (Uetake *et al.*, 2013; Lichtsteiner & Turner, 2008; McCobb *et al.*, 2005), faeces (cortisol metabolites) (Ramos *et al.*, 2013; Ramos *et al.*, 2012; Accorsi *et al.*, 2008) and hair (Finkler & Terkel, 2010; Accorsi *et al.*, 2008). It is important to consider that changes in cortisol concentrations are dependent on several factors besides the stressor, such as the sampling procedure and restraint put on the individual (Mormède *et al.*, 2007), especially when measuring acute stress where there can be a large effect of temporary fluctuations for example due to handling. For the more invasive blood sample it is important to consider the effects of the procedure itself on cortisol levels. Peak cortisol concentrations in plasma have been measured at 5-15 minutes after a stressor (Iki *et al.*, 2011; Genaro *et al.*, 2007) but after 30-180 minutes in serum (Carlstead *et al.*, 1992; Sparkes *et al*., 1990) in cats. However, it takes longer to reach other media such as the saliva. In cows this time lag between peak concentrations in plasma and saliva has been measured at 10 minutes (Hernandez *et al.*, 2014). To reduce the effects of handling, it has been suggested that a vascular access port can be implanted which would allow multiple samplings without having to repeatedly puncture the cat's skin (Iki *et al.*, 2011). Another option working around the effect of handling is using a non-invasive medium such as hair, urine or faeces, but this is only possible for the measurement of long-term stress.

Depending on the research question and aim of study, the set-up can require measurement of either short or long term stress, for which there are different options as some biological samples provide more direct measurements of

circulating cortisol levels while other provide more of an average level for a certain time period. There are strengths and weaknesses with each medium for physiological measurement of stress. For measurement of acute stress, for example, from plasma or serum, the potentially large effect of handling and sampling itself on stress levels is problematic. Still, the media allow for the measurement of the short term effects of an *event*. When measuring chronic stress, for example by analysing cortisol levels in faeces, urine and hair, the cortisol concentrations are not affected by handling or sample collection. However, effects of shorter stressors will, in such media, be diluted by a specific time, depending on media used, and not show up as peaks. These types of media are therefore not suitable when studying effects of shorter and more acute events.

When comparing different studies it is also important to consider if the total cortisol concentration (bound and free hormone) or only the free cortisol fraction (the biologically active part) has been measured.

Plasma cortisol

Measurements of cortisol levels in plasma have the general benefit that samples reflect momentary central circulating concentrations, meaning that it is considered an accurate measurement of actual active levels in the body. A few minutes after the initiation of the stressor, the levels of cortisol in the blood increases (Mormède *et al.*, 2007). In cats, previous studies have found that peak concentrations of cortisol in plasma occur between 5 minutes (Genaro *et al.*, 2007) and 15 minutes (Iki *et al.*, 2011) after an initial stressor, using ACTH stimulation tests. This rapid change allows for measurements of short term stressors and events such as handling. The disadvantage of collection of plasma (as well as serum) is primarily that the procedure is invasive, requires preparation of the animal such as shaving an area for puncture, using a stasis and keeping the animal still during the entire blood collection. In cats, administration of anaesthesia before blood collection (Genaro *et al.*, 2007) or a venous access port system (permanent catheter) (Iki *et al.*, 2011) have been used in attempts to reduce stress during blood collection which, however, also may influence the results. The advantage that cortisol levels collected from plasma and serum reflect shorter events also connects to the disadvantage that changes in stress response due to the sampling procedures itself might affect the results (Schatz & Palme, 2001).

Urinary cortisol-to-creatinine ratio

Urinary C:Cr have the advantage of being collected non-invasively in cats, that is, there is no effect of the sample procedures on the cortisol levels measured.

Samples can be collected straight from the litterbox using double-layered litterboxes and plastic non-absorbent litter. The C:Cr provides an average of the cortisol levels circulating during the time the urine was produced. C:Cr in cats have been found to decrease with time after a cat has entered a new environment (quarantine cattery) (Rochlitz *et al.*, 1998), and has been found to be negatively correlated to hiding (Carlstead *et al.*, 1993b). There are some issues relating to sample procedures. For instance, collection of individual urine samples would be very difficult in group-housed cats and requires cats to be individually housed during collection. In cats, stress has been found to be related to refraining from urination during the initial 24-48 hours, which can then render the method unsuitable for measurements of initial stress (Stella *et al.*, 2011; McCobb *et al.*, 2005). Previous studies of shelter cats found that 25% of urine samples contained traces of blood (*hematuria*) (McCobb *et al.*, 2005), which introduces uncertainty into the results. The urinary cortisol assay can also be affected by certain syndromes such as FIC, where previous studies have found that cats effected with FIC to a higher degree had hematuria (30%) than healthy control cats (7%) (Westropp *et al.*, 2006). Other issues relate to the metabolism of cortisol, as cortisol is excreted via urine or faeces in different proportions depending on species. Previous studies found that in cats, only a small portion, approximately 15% (Graham & Brown, 1996) to 18% (Schatz & Palme, 2001) of cortisol was excreted via urine. Therefore it has been proposed that it is better to measure cortisol metabolites from faeces (Schatz & Palme, 2001).

Faecal cortisol metabolites

Approximately 82% of cortisol is excreted via faeces, enabling assay of faecal cortisol metabolites as a useful tool for cortisol determination in cats (Schatz & Palme, 2001). As with collection of urine, faecal sampling is non-invasive, and the procedures related to collection do not affect the results. Cortisol metabolite levels reflect a mean for the time of production of faeces and, in cats, peak concentrations have been found after 22 $\pm$ 6 hours after administration of [^{14}C]cortisol (Schatz & Palme, 2001) as well as ^{3}H-Cortisol (Graham & Brown, 1996). Faecal cortisol metabolites have been used for investigation of the social environment on cats, finding no effect on cats living singly, in pairs or small groups (3-4 cats) (Ramos *et al.*, 2013). One potential issue with faecal cortisol metabolites is that it is difficult to know the process through the gastrointestinal system and it is therefore more difficult to determine what the measured levels actually reflect. Samples should also preferably be collected and prepared fresh to make sure that they still contain

the biological representative levels of cortisol metabolites (Millspaugh & Washburn, 2004).

Hair cortisol

Hair assay for determination of cortisol levels is a non-invasive technique. Cortisol levels reflect the time during which the hair has grown (retrospectively), and as hair is only collected from areas previously shaved, the time period which the cortisol reflects is known. The advantage is then not only that the collection is non-invasive, but also that it can be collected individually from free-roaming animals even during group-housing. Assaying cortisol from hair is a relatively new technique so despite findings of positive correlations between faecal cortisol metabolites and cortisol levels from hair (Accorsi *et al.*, 2008) and that intact feral female cats, displaying more aggression, have higher levels than both less aggressive intact and neutered females (Finkler & Terkel, 2010), there is need of further validation of the method before it can be considered fully recognised. For instance, there has been some discussion as to whether cortisol levels either reflect actual central (circulating) levels of the body or only local levels as the hair follicle, in humans, has been found to have a structure similar in function to the HPA axis (Ito *et al.*, 2005). Still, reviewing the literature, Stalder and Kirschbaum (2012) found indications that hair cortisol reflects the systemic cortisol levels well and seems only slightly affected by the local follicle cortisol production. Nevertheless, there has been some evidence that the colour of the hairs might have a confounding effect on the cortisol level, at least in dogs (Bennett & Hayssen, 2010). This would need to be further investigated also in cats, and likely taken into consideration during sampling and comparison of results.

Salivary cortisol

Salivary cortisol is a more non-invasive metric than blood (plasma or serum), but will still reflect circulating cortisol levels, in a much lower concentration, as it is an ultra-filtrate of blood plasma. Salivary cortisol includes only the free unbound fraction which constitutes about 2-15% of cortisol in blood (Kirschbaum & Hellhammer, 2000). McCune (1992) attempted to validate salivary cortisol with behavioural elements of stress in cats. Likely due to lack of suitable assay techniques at the time, the attempt was unsuccessful. Siegford *et al.* (2003) successfully collected and assayed salivary cortisol in cats, but did not find a significant correlation with scores on a feline behavioural temperament profile. Still it has potential as it reflects short term stress, in the same way as plasma cortisol levels, and is less invasive than collection of blood. Potential issues relating to collection of saliva for assay is that during

times of stress, the SNS has a negative effect on saliva production which could make it difficult to collect enough volume. Training and habituating the animal to the procedures would likely diminish the issue, but might not always be practically applicable.

The optimal medium for assessment of physiological stress would need to both reflect the central concentrations of cortisol, preferably reflect short-term events, and still not be affected by sampling (i.e., non-invasive collection). A good candidate fulfilling these criteria has in cats been suggested to be saliva. Despite an unsuccessful attempt to utilise salivary cortisol McCune (1992) noted that salivary cortisol could be a future option. Still, there are very few studies utilising salivary cortisol in cats.

2.6.3 Viral infections as a measurement of stress

Due to the clear connection between stress and the immune system, herpes simplex virus (HSV) has been suggested as useful in testing the immune systems function as there is a clear positive association between stress and herpesvirus antibody titres in humans (Herbert & Cohen, 1993). As reactivation of herpesvirus is a signal that immunity is compromised, for instance, due to a stressor, it can be used as a measurement of immune system activation (Kiecolt-Glaser & Glaser, 1987). Therefore, quantification of presence of HSV can be used as a measurement of a negative effect on the immune system in humans. In contrast, other findings on humans carrying HSV indicate that recurrence is likely due to local immunological changes and not a general depression of the immune function (Dalkvist *et al.*, 1995).

In cats, stressful events such as ending up in a new environment, can induce reactivation of feline herpesvirus-1 (FHV-1) in cats infected with the virus (Hellard *et al.*, 2011) resulting in shedding of the virus (Kennedy & Little, 2012; Day *et al.*, 2010; Lappin *et al.*, 2009; Pontier *et al.*, 2009; Edwards *et al.*, 2008).

FHV-1, feline calicivirus and feline coronavirus have all been mentioned as affected by and connected to stress and they are listed as important viruses infecting cats (Hellard *et al.*, 2011; Pontier *et al.*, 2009). As FHV-1 is transmitted through direct contact (Lim & Maggs, 2012), that is, social interactions between animals (Hellard *et al.*, 2011), it can be especially problematic during crowded conditions and group-housing.

URD is common in multi-cat environments and has been suggested to be affected by physiological stressors (Maggs *et al.*, 2007). Illustratively, 58% of cats taken into a shelter developed URD within 21 days (Tanaka *et al.*, 2012). According to Griffin (2012), URD is the most common endemic disease and as

shelters often have a high turnover of animals with unknown infectious disease histories, it can be a very challenging disease to control in these types of settings (Maggs *et al.*, 2007). URD can have several underlying causes among others FHV-1 (Maggs *et al.*, 2007), *Chlamydophila felis* and *Mycoplasma felis* infections (Sykes, 2010). As clinical signs are known to overlap (Schulz *et al.*, 2015), differentiation is made through laboratory tests (Sykes *et al.*, 1997). Respiratory diseases are of major concern in group-housing of cats, especially in shelter environments, as they often are contagious and can be triggered by stress (Cohn, 2011).

2.6.4 Sickness behaviours as a measurement of stress

Stress in cats has been determined looking at *sickness behaviours* (Stella *et al.*, 2011). Sickness behaviours are physiological and behavioural responses to pathogens recognised by the immune system and have in mammals been shown to include organised strategies aimed at facilitating recovery (Dantzer, 2001). These responses include reduced food and water intake as well as a decrease in activity (Dantzer, 2001) and are maintained by glucocorticoids (Dantzer, 2004). Sickness behaviours, such as decreased food intake, can be used as signs of stress and have been found also in cats (Stella *et al.*, 2011). For example, overall decrease in activity, commonly seen in cats in response to stressors (Gooding *et al.*, 2012) allows the individual to save energy which instead can be used to enhance immune activity (Schneiderman *et al.*, 2005). In cats, the most common sickness behaviours in response to unusual external events (stressors) were vomiting of hair, food, or bile, urination outside of the litterbox and decreased food and water intake (Stella *et al.*, 2011). These behaviours could all be used as indications of stress in cats.

3 Aims of the Thesis

The overall aims of this thesis were to (1) investigate the effect of group-housing on the domestic cat, measuring effects on behaviour, physiology and prevalence of disease and (2) further the development of a behavioural assessment tool, a non-invasive protocol, to be used to determine the effect of housing by predicting cats' outcome. Specific questions relating to group-housing and the assessment tool addressed in the four papers included in the thesis were;

- How common is group-housing in Swedish cat shelters? (Paper I)
- What issues relating to the welfare of cats do Swedish cat shelters experience? (Paper I)
- What is the prevalence of common infectious agents related to URD? (Paper II)
- Is salivary cortisol a suitable non-invasive metric for cortisol determination in shelter cats? (Paper II)
- Are there differences in the recorded behaviours in group-housed and single-housed cats? (Paper III)
- What behaviours best predict cats' time spent at shelter, from available for adoption until adopted? (Paper III)
- Are the behaviours found relating to time spent at shelter stable and suitable for inclusion in a future, further developed assessment tool? (Paper IV)

4 Material and Methods

This chapter provides an overview of material and methods used in the papers included in the thesis. Full descriptions and further details can be found in Papers I-IV. Study I consisted of a survey distributed via regular mail during October 2012 to Swedish cat shelters (Paper I). Study II took place during January – April 2013 and samples were collected from 89 cats from 11 of the shelters participating in Study I (Paper II). Study III contains behavioural observations and demographic data collected at a cat shelter in the United States during August and September 2014 (Paper III). Study IV took place during October and November 2015 at a university research colony in the Netherlands (WUR) and includes behavioural observations of cats housed in stable groups (Paper IV).

4.1 Ethical statement

For Study I, shelters participating in the survey were informed about the purposes of the study and no personal data were collected. Respondents were further informed that no information about specific shelters would be traceable from the publication. Study II included a collection of samples from cats which followed a protocol approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden (RN 256-2012). As shelter cats are considered privately owned cats according to Swedish legislation, an exemption from the use of privately owned cats was approved by the Swedish Board of Agriculture (RN 256-2012). Study III included only behavioural observations and information about the participating cats gathered from cat records already collected by the shelter. No personal data were processed. The shelter manager signed an informed consent form agreeing to the aim and set-up of the study. Study IV included behavioural observations and activity data and was incorporated in a pre-existing approved ethical application held by Dr Bonne

Beerda at the Department of Animal Sciences, Wageningen University (WUR), approved by the Animal Experiments Committee (2014.101.b Umbrella project proposal for assessments of personality, food appraisal and welfare of cats).

4.2 Specific aims and objectives

4.2.1 Study I

As there has previously only been one study investigating Swedish cat shelters (Eriksson *et al.*, 2009), the aims of this study, based on previous knowledge about potential issues in shelters were to: (1) investigate and describe policy, husbandry practices and routines at Swedish cat shelters as reported by shelter staff, (2) investigate how common group-housing was and (3) what group sizes were used.

4.2.2 Study II

The main aim of this study was to investigate the suitability of saliva sampling as a non-invasive way of determining cortisol levels in naïve non-sedated shelter cats as this has previously been suggested as a viable option in need of further investigation (McCune, 1992). Additional aims were to: (1) explore the relationship between salivary and plasma cortisol levels, (2) investigate the effect of group size on cortisol level, and (3) look for associations between cortisol levels and infectious disease (FHV-1, *C. felis* and *M. felis*). The aim was further to investigate if salivary cortisol could be used to validate behavioural elements during the advancement of the behavioural stress tool during Study III and Study IV.

4.2.3 Study III

Together with Study IV, Study III aimed to further the development of a behavioural scoring system to assess cats. In this study the aim was to determine which behavioural elements should be included in a new protocol to best predict *Time at Shelter* (time spent at shelter from available for adoption until adopted). The secondary aim was to investigate potential differences between observed behaviour in cats housed in groups or singly. The protocol used, the *extended Stress Assessment* (eSA) (Appendix 1) was based on the CSS (Kessler and Turner, 1997) with 20 additional behavioural elements (BEs) of stress or indications of more positive states (e.g., groom) taken from the original GAS (McCune, 1992) and the wider cat literature. The additional BEs, such as, *Activity - grooming*, *Activity - hiding*, *Legs – paws turned in*, were included as it has previously been suggested to be useful to not only look for

signs of stress, but also to include absence (here presence) of normal behaviours (McCune, 1994).

4.2.4 Study IV

This study built on the results from Study III and here the aim was to look at the stability of the stress related BEs found related to *Time at Shelter*. Only behaviours recorded during Study III from the eSA, and that were deemed to be recorded evenly between observations, were included in the protocol used, the *shortened Stress Assessment* (sSA). By making repeated measures on cats housed in groups under stable (temporal and social) conditions, comparisons were made to determine robustness of the BEs found to be indicative of short as well as long time until adoption in Study III.

4.3 Animals and husbandry

Study I aimed to include all rescue shelters accepting and adopting cats in Sweden. Information about 64 potential shelters was obtained searching the Internet. Of these, 39 (61%) responded to the survey.

Study II included 89 cats, 49 males and 40 females aged between 0.7 and 13 (mean $\pm$ SD, 3.8 $\pm$ 2.6) years, housed at 11 of the 39 shelters that participated in the survey of Study I. The shelters were visited between 22 January and 25 April 2013. The goal was to collect samples from 10 cats at each shelter but due to unforeseen circumstances (euthanasia, adoption) all shelters did not have 10 cats at the day of visit (median = 10, min. = 4, max. = 10). Shelters were spread over the south of Sweden but were situated max. 7.5 hours from the University laboratory where samples were prepared for analysis. All cats were above the age of 6 months.

Study III took place between 18 August and 15 September 2014, at a medium sized animal shelter located in Indiana, United States, with an annual adoption rate of approximately 1600 cats per year. All cats included were housed at the adoption floor, meaning that they were neutered and considered healthy enough by veterinary care staff to be available for adoption. Some cats were, however, adopted before data could be collected. In total, 83 cats housed in either one of five group rooms (n = 70) or singly in one of eight cages (n = 13) participated in the study. Group-housed cats consisted of 50 females and 20 males between 9 months and 120 months (mean $\pm$ SD, 45.8 months $\pm$ 3.4) and single-housed cats were 6 females and 7 males between 6.5 months and 119 months (mean $\pm$ SD, 54.2 $\pm$ 10.5 months).

Study IV was carried out between 30 October and 12 November 2015, at the WUR animal facility *Carus*. At the time of the study, Carus kept 32 cats

housed in four age and sex separated groups: Group 1, 8 female cats age 1 year 2 months; Group 2, 8 male cats age 1 year 2 months; Group 3, 8 female cats age between 3 years 5 months to 3 years 10 months (mean ± SD, 43.1 ± 0.85 months) and Group 4, 8 male cats aged between 3 years 5 months to 3 years 10 months (mean ± SD, 42.9 ± 0.66 months), at the time of the study. All cats were neutered and had lived in the stable groups for at least 9 months prior to the study.

4.4 Study design and data collection

4.4.1 Study I

A survey was sent to all shelters found in Sweden. Surveys were distributed via regular mail and included a pre-stamped self-addressed envelope to return the survey in. The survey was distributed during October 2012 and non-respondents were reminded twice. Data consisted of a convenience sample and since 39% (25/64) did not respond, general conclusions about Swedish cat shelters were drawn with caution. The survey consisted of nine major questions, with sub-questions, concerning: husbandry practices, routines, received animals, euthanasia, the cats' health and occurrence of disease.

4.4.2 Study II

All shelters from Study I were approached about participation but all were not willing to, therefore data represents a convenience sample and results are interpreted with caution. All shelters willing to participate and where a time for a visit could be booked were visited (n = 11). Selection of cats, when more than 10 cats were available, were made randomly among cats that the shelter staff believed could be handled for the full sample procedure. The sample collection (full procedure) for each cat took approximately 10 minutes, and all samples were collected on awake non-sedated cats. Order of sample collection were; saliva sample (cortisol assay), buccal swab (metagenomics analysis), conjunctival swab (presence of FHV-1, *M. felis* and *C. felis*) and a blood sample (plasma cortisol assay). The saliva sample was collected using the Salimetrics Infant's Swab (Salimetrics ® USA) where cats were allowed to chew on the swabs for up to 3 minutes. The buccal swab was collected using a sterile cotton tip that was rolled against the mucosal membrane of the cheek of a cat. The cotton tip was then placed in a cryotube containing 1 ml of Franks transport medium (HBSS without phenol red), and stirred for 5 seconds. Conjunctival swabs were collected by gently sweeping a sterile swab (ESwab™, Copan Diagnostics, Inc., Italy) along the ventral fornix of one eye, according to instructions from the National Veterinary Institute (SVA). The blood sample

was collected from the cephalic vein of one front leg of each cat and collected in two 600 µl EDTA tubes (Multivette® 600, SARSTEDT AG & Co). All samples were immediately stored on ice after collection and kept in a cooler for transport to the laboratory for preparation and temporary storage in a -20° C freezer until long-term storage in -70° C awaiting analysis.

4.4.3 Study III

Cats were selected in a pseudorandomised order before each observation, using a random number generator (Random Number gpv1.0.11 by Saranomy) to include as many unique individuals as possible from each of the three groups or from the single-housed cats that would be observed on a specific day. Direct observations were performed as cats were used to people moving around in the rooms during daytime. Additional information about the participating cats, such as demographics, disease history and treatments, were collected from the cat records kept by the shelter after the study had finished. The observer was therefore blind to length of stay of cats during data collection. Data on sickness behaviours were also collected in the morning before cleaning. Three single-housed cats, or groups of cats, were observed twice a day during a morning (am) and afternoon (pm) session. Each observation took 40 minutes as to keep the original CSS methodology of scoring cats twice on the am and pm session with 15 minutes in-between (Kessler & Turner, 1997). For group-housed cats, a selection of 5+2 cats from the group was made for each day of observation. Sessions consisted of observations of social interactions and activity as well as registration of the behavioural elements included in the *extended Stress Assessment* (eSA). Each session started with 10 minutes habituation. This was followed by five 1 minute eSA observations (5 first cats), 10 minutes social interactions and activity (all 7 cats), repeated twice. For single-housed cats, session consisted of observations of activity and registration using the eSA. Each session started with 10 minutes habituation followed by one 1 minute eSA, 14 minutes activity, repeated twice for eSA and activity.

4.4.4 Study IV

All cats housed in the *Carus* research colony (WUR) at the time of the study were included. The observational order was pseudorandomised before the study started, to make sure that groups were observed according to the social interaction protocol and activity protocol in all available time slots, during both the first and second time slot of the day. All cats in a group were observed during the same session during a morning (2.5 hours) and afternoon (1.5 hours) session. Each group was observed on three days. Sessions consisted of observations of social interactions and use of space and recorded according to

the shortened version of the eSA, the *shortened Stress Assessment* (sSA). Each session started with 14 minutes habituation, followed by eight 1 minute sSA, 60 (am) or 30 (pm) minutes activity or social interactions, repeated twice for the sSA, activity and social interactions. Recording of activity and social interactions was alternated between the first and second time slot, so that both were observed on each day of observation.

4.5 Data editing and statistical analyses

4.5.1 Study I

All returned surveys were used, but due to missing replies on some questions, analysis and results are presented as number of respondents for each question. The closed questions were transferred to Excel ® directly while the open-ended questions were classified into comprehensive categories before analysis. Data were prepared using Microsoft ® Excel ® 2010 which was used for calculations of percentages and counts. Minitab ® (Statistical software version 16.1.0 © 2010 Minitab Inc.) was used for a Pearson correlation between number of reported diseases, shelter size and maximum group size.

4.5.2 Study II

Salivary samples, buccal swabs and conjunctival swabs were collected from all 89 cats, blood samples were collected from 85 cats. One cat's blood sample was excluded from analysis as the collection was delayed by 10 minutes. Data were prepared using Microsoft ® Excel 2010 and basic statistical analysis was performed using Minitab ® Statistical software version 16.1.0. (© 2010 Minitab Inc.). As data were not normally distributed, nonparametric statistics were used. Comparing cortisol levels in relation to sex (n = 83, 36 females and 47 males) was performed using the Mann-Whitney test, and comparing cortisol levels in relation to group size, (groups containing 1, 2-5, 7-13 or 17-25 cats, with n = 16, 36, 12 and 19, respectively) was performed using the Kruskal-Wallis test. Statistica 12 (StatSoft Inc.) was used for Spearman Rank Correlation to test for a relationship between plasma and saliva cortisol concentrations (n = 10).

4.5.3 Study III

Basic calculations were performed using Excel (Microsoft ® Excel ® 2013) and Minitab (Minitab ® statistical software version 17 © 2016 Minitab Inc.), including summaries for registered behavioural elements from the eSA, activity and social interactions, mean temperature and median length of stay at the shelter.

The behavioural elements that best predict *Time at Shelter* was calculated based on the data from each cat's first day of observation, four eSA, using the Survival Analysis based on the Cox proportional hazards regression model using a stepwise regression analysis (**proc phreg** package, SAS ® 9.4). Each of the four scorings of the eSA was calculated separately. The Survival Model estimates parameters which describe the relationship between the *Time at Shelter* and our predictors (the behavioural elements). The model stepwise finds the most important behavioural elements by using the parameter estimates of the Hazard Ratios used to predict *Time at Shelter*. To calculate which behavioural elements that describe long time until adoption, the cat with most days until adoption that is, longest *Time at Shelter* (T_x), for group- and single-housed cats respectively, had its time set as a starting point (T_0). T_0 was then used to calculate a new alternative Time (T_y) for each cat according to $T_y = T_0 - T_x$.

Social interactions were recorded according to a social matrix adapted from a previously used ethogram for group-housed shelter cats (Loberg & Lundmark, 2016). Social behaviours clearly directed towards humans (visitors, volunteers, staff or observer) were removed from analysis as the aim was to describe the interactions between cats.

Data for sickness behaviours were removed from all analysis as during the study it was noted that volunteers cleaned litterboxes and removed vomit from cages during the day.

4.5.4 Study IV

One cat was excluded from Group 3 and from the sSA and activity analysis as she had broken her leg before the study and was confined to a large dog crate within group room 3 during the entire study. Descriptive data for social interactions, *In-Contact* and demographic data were compiled using Excel (Microsoft ® Excel ® 2013) and Minitab (Minitab ® statistical software version 17 © 2016 Minitab Inc.).

All statistical tests were performed using Minitab. To investigate stability in the groups, all social interactions clearly not directed at another cat in the group but directed towards staff or the observer, were removed before analysis. Confirmation of stability of the different groups was based on behaviours seen in stable groups as well as the ratio between affiliative (Positive Social, Positive Vocalisation) and agonistic interactions (Negative Social, Negative Vocalisation). Comparison between clearly positive and negative interactions were compared as percentages for each group of cats. *In-Contact* was compared as a percentage of scans as data were missing for one observation for cats housed in Group 4. Normal distribution of *In-Contact* was confirmed

using the Probability Plots (p = 0.04) and differences between groups were compared using the General Linear Model in Minitab. Comparison between activity levels were performed using Wilcoxon Signed Rank Test.

Stability of the behavioural elements from the SSA were compared descriptively, and summarised for all 12 recordings from each group's three days of observation for each cat and behavioural element. Stability was looked at on three levels: *Stable Absent*, 0-3 recordings of 12; *Unstable*, 4-8 recordings of 12; *Stable Present*, 9-12 recordings of 12. Results are presented as summaries for all cats for each behavioural element.

5 Summary of Results

This section includes a summary of the results from Study I-IV. For full descriptions and further details of the results please see the individual papers (Paper I-IV). Group-housing were practised by 82% of the responding shelters, but few instances of disease were reported (Study I). The low occurrence of disease was later confirmed in Study II where only 5.6% of the conjunctival swabs came back positive for FHV-1, *M. felis* or *C. felis*. Issues with collection of saliva samples related to difficulties with collection of enough volume for analysis as well as blood contamination of the samples due to oral health issues in the cats. Study III found 16 behavioural elements (BEs) correlating with short *Time at Shelter* and 14 BEs correlated with long *Time at Shelter* in group- and single-housed cats. Of the BEs found to correlate to long and short *Time at Shelter* in group-housed cats, 11 BEs were *Stable Present* or *Stable Absent* in all cats during Study IV.

5.1 Study I. Swedish cat shelters: a descriptive survey of husbandry practices, routines and management

The majority, 32 shelters (82%) housed cats in groups while one shelter provided only solitary housing. Thirty-one shelters (80%) provided a combination of single-, pair- and group-housing. The most common group size was 3–5 cats (59%). Ninety-two percent of responding shelters had routines and/or protocol(s) for the management of the cats, 90% of shelters had healthcare routines and 77% of shelters had routines for the admission of cats. All shelters with the exception of one had quarantine, and 22 shelters (58%) vaccinated cats prior to admittance. There was a significant positive correlation between shelter size and number of reported diseases ($p < 0.01$; $r_p = 0.47$) but not for reported disease and maximum group size (ns; $r_p = 0.15$). Several shelters reported having no occurrences of disease in the month preceding the

survey, September 2012 (n = 17), the year (n = 13) or three years preceding the survey (n = 12). The most commonly reported diseases were URD (referred to as *cat 'flu*) and eye infection/inflammation both reported by 7 shelters. In Sweden, shelters provide cats with plenty of resources, relating to, for example, the sub-categories: *physical* (e.g., hides, toys and climbing structures) and *olfactory* (e.g., catnip) often providing outdoor access and a more 'home-like' environment including soft resting places (Figure 2). Providing a 'home-like' environment was reported by 3 shelters as the aim of the *enrichment*.

5.2 Study II. Cortisol Measurements and Investigation of Upper Respiratory Disease in Shelter Cats: Methodological Considerations

Plasma cortisol was analysed from 83 cats, 47 males and 36 females (median 123 nmol/l). No difference was found in cortisol concentration between male and female cats ($p = 0.29$) which were therefore combined for further analysis. Enough volume of saliva for individual analysis was collected from 11 of the 89 cats, 6 males and 5 females, (median 5 nmol/l).

Figure 2. Example of more 'home-like' environment provided by Swedish shelters participating in Study I and II.

There was no correlation between plasma and saliva cortisol concentration for the cats that had individual samples analysed from both media (n = 10) (r_s = 0.18; p = 0.63). Group size did not affect plasma cortisol concentrations (p = 0.17).

Five out of 89 conjunctival swabs were positive, 2 for FHV-1 and 3 for *C. felis*, no co-infections were detected. Due to the low number of positive swabs no further statistical analysis was possible.

5.3 Study III. A Further Development of a Scoring System to Assess Behavioural Stress in the Cat

Of the 85 behavioural elements (BEs) included in the eSA, 26% were never recorded for group-housed and 42% were never recorded for single-housed cats. This difference between the number of recorded BEs between group- and single-housed cats was significant ($p < 0.05$). BEs not recorded belonged to all seven stress levels from the CSS as well as the additional BEs (Table 2). Sixteen BEs (Table 3) were found to best predict short time until adoption using the survival model. Of these, 14 BEs were found to best predict short time until adoption in group-housed cats and 2 in single-housed cats. Of the BEs, two were negatively correlated to short *Time at Shelter*, meaning here that presence of BEs is related to longer time until adoption. Looking at BEs indicative of decreased chance of quick adoption (long *Time at Shelter*), there were 14 additional unique BEs (Table 4), 12 in group-housed cats and 3 in single-housed cats (*Body: standing* was included in both).

Table 2. *Behavioural elements (BEs) from the extended Stress Assessment (eSA) not recorded at all during the behavioural observations of group (GH, n = 70) or single (SH, n = 13) housed cats, as well as BEs not recorded in either GH or SH cats (Identical).*

Scoring level	Number of BE on level	GH	SH	Identical
CSS level				
1	16	2	5	2
2	30	2	9	2
3	24	1	5	1
4	26	3	6	2
5	21	5	7	4
6	16	5	8	5
7	15	5	8	4
GAS	15	10	12	9
Literature	5	2	2	2

The same BE could belong to multiple stress levels on the original CSS protocol

Table 3. *Predictions using Analysis of Maximum Likelihood Estimates, behavioural elements (BEs) only included when p < 0.05, presence of BEs correlated with increased chance of spending shorter time at the shelter and having a quick adoption in Study III.*

Housing	Session No	Behavioural Element	DF	n	Parameter Estimate	Hazard Ratio	Pr>ChiSq
Group	1	Body: sitting	1	59	0.88	2.42	0.01
		Head: moving	1	59	1.44	4.22	0.00
		Eyes: half open	1	59	1.00	2.71	0.01
		Eyes: closed	1	59	0.80	2.23	0.03
		Ears: erect to front	1	59	0.81	2.24	0.01
		Vocalisation: none quiet	1	59	1.60	5.0	<0.00
	2	Head: moving	1	58	1.56	4.75	<0.00
		Eyes: pressed together	1	58	1.98	7.25	0.00
		Eyes: closed	1	58	0.74	2.1	0.03
	3	**Legs: standing extended**	**1**	**59**	**-1.77**	**0.2**	**0.03**
		Tail: loosely wrapped around body	1	59	3.34	28.4	0.01
		Head: on plane of body	1	59	1.48	4.41	0.02
		Pupils: partially dilated	1	59	6.40	603	<0.00
		Vocalisation: none quiet	1	59	1.29	3.63	0.00
	4	Legs: fully extended, stretched out	1	56	3.25	25.7	<0.00
		Legs: front legs laid out	1	56	2.35	10.5	0.00
		Legs: standing extended	1	56	5.40	219	<0.00
		Tail: loosely downward	1	56	2.75	15.6	0.00
		Head: moving	1	56	2.24	9.35	0.00
		Eyes: half open	1	56	1.15	3.15	0.01
		Eyes: closed	1	56	1.12	3.06	0.00
Single	1	-	-	-	-		-
	2	**Eyes: normal**	**1**	**12**	**-2.25**	**0.11**	**0.01**
		Ears: prickled	1	12	2.76	15.8	0.02
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-

Bold denotes negative correlation, presence of BE is indicative of longer *Time at Shelter*

Table 4. *Predictions using Analysis of Maximum Likelihood Estimates, behavioural elements (BEs) only included when p < 0.05 presence of BEs correlated with decreased chance of spending short time at the shelter and having a quick adoption in Study III.*

Housing	Session No	Behavioural Element	DF	n	Parameter Estimate	Hazard Ratio	Pr>ChiSq
Group	1	**Body: Sitting**	**1**	**59**	**-0.99**	**0.37**	**0.02**
		Legs: paws turned in	**1**	**59**	**-1.16**	**0.32**	**0.00**
		Head: moving	**1**	**59**	**-1.00**	**0.37**	**0.01**
		Ears: erect to front	**1**	**59**	**-1.13**	**0.32**	**0.00**
		Ears: erect to back	1	59	1.24	3.44	0.03
		Whiskers: normal	1	59	2.94	18.9	0.00
		Vocalisation: none quiet	**1**	**59**	**-1.131**	**0.32**	**0.00**
	2	Body: standing	1	58	2.28	9.74	0.00
		Legs: hind legs laid out	1	58	2.29	9.85	0.03
		Head: over body	1	58	5.53	253	0.00
		Eyes: slow blink	1	58	4.34	76.6	0.00
		Ears: erect to back	1	58	1.78	5.91	0.00
		Ears: partially flattened	1	58	1.42	4.13	0.03
	3	-					
	4	Ears: erect to back	1	59	1.46	4.31	0.02
Single	1	-	-	-	-		-
	2	-	-	-	-		-
	3	**Activity: Sleeping/resting**	**1**	**12**	**-1.86**	**0.16**	**0.03**
	4	Body: standing	1	12	3.30	27.1	0.04
		Belly: not exposed	**1**	**12**	**-2.80**	**0.06**	**0.01**

Bold denotes negative correlation, presence of BE is indicative of shorter *Time at Shelter*

Seven BEs were negatively correlated with *Time at Shelter* meaning that presence of BEs relates to short time until adoption. The BEs predictive of *Time at Shelter* in group-housed cats that could be scored evenly between cats were saved for further investigation of stability and robustness in Study IV.

The BEs related to short or long *Time at Shelter*, by being positively or negatively correlated, belonged to all seven levels of stress in the original CSS (Table 5).

Table 5. *Corresponding stress level from the Cat-Stress-Score (CSS*) for the behavioural elements (BEs) related to short and long Time at Shelter.*

CSS level	short *Time at Shelter*		long *Time at Shelter*	
	BEs + correlated	BEs - correlated	BEs + correlated	BEs - correlated
1 *Fully relaxed*	9	-	2	2
2 *Weakly relaxed*	14	1	5	6
3 *Weakly tense*	6	2	7	5
4 *Very tense*	4	-	7	4
5 *Fearful, stiff*	2	-	4	2
6 *Very fearful*	-	-	-	1
7 *Terrorized*	-	-	-	1

*CSS, Kessler & Turner (1997)

The majority, 83%, of the BEs positively correlated to short *Time at Shelter* belonged to levels of stress of 3 or lower on the original CSS protocol, which was also true for the BEs negatively correlated to long *Time at Shelter* (62%). BEs positively correlated to long *Time at Shelter* also belonged mostly to levels of stress of 3 or lower, however, the majority of BEs from levels of stress of 4 or higher, 54%, were positively correlated to long *Time at Shelter*.

Recordings of social interactions revealed that most interactions were vocalisations (Table 6). There were few instances of social play which were only observed in one group (Group 1). Group 5, housed on the second floor and not open for access to the public, had most negative social interactions recorded (0.47 per cat and day) as well as most positive (0.41 per cat and day).

Activity, calculated by number of recorded movements (*Moves*) during the 10 minutes × 2 observations, during the am and pm session of each cats first day, was low with median *Moves* of zero for all groups. Results per group are presented as median (IQR, max.). Group 1 containing most active cats with 0 (0-2, 20), followed by Group 3: 0 (0-2, 15), Group 5: 0 (0-1, 9), Group 4: 0 (0-1, 5) and Group 2 containing the least active cats 0 (0-0, 4).

Table 6. *Number of recorded social interactions for the five group rooms calculated as number of interaction per day of observation divided by number of cats in the group during that day.*

Group	Positive Social	Negative Social	Positive Vocalisation	Negative Vocalisation	Social Play	Play
1	0.12	0.12	0.83	0.55	0.13	0.42
2	0.12	0	0.13	0.15	0	0.09
3	0.06	0.17	0.24	1.04	0	0.26
4	0.40	0.03	1.07	0.98	0	0.53
5	0.41	0.47	1.20	1.07	0	0.11

Single-housed cats were more active, calculated by the number of *Moves* during the 14 minutes × 2 observations for the am and pm session of each cats first day. The activity differed between individuals with median (IQR, max.) of 3 (0-10), and the most active cat 16 (3.3-20, 28) and least active cat 0 (0-1, 8).

5.4 Study IV. Stability of Behavioural Elements in Cats Housed in Stable Groups

Stability of the groups was determined based on the ratio of affiliative and agonistic interactions within groups. Affiliative interactions consisted of 86% of recordings of social interactions (Table 7). Cats were seen resting *In-Contact* a median (IQR) of 69.5% (52.5-94.3) of scans. The scans spent *In-Contact* differed slightly within groups (Table 8) as well as between individuals with the cat spending the least recorded scans *In-Contact* 9.9% (F, Group 1) and the cat spending most scans *In-Contact* 94.3% (M, Group 4). *In-Contact* was confirmed as normally distributed using the Probability Plot (p = 0.04), and there was a significant difference between groups for *In-Contact* (F = 7.02, $p < 0.00$).

The median (IQR) activity for all cats, calculated as number of *Moves* between scans over 30 minutes were 2 (0-4). The median (IQR) activity differed between the groups; Group 1 (2.5, 0.63-5), Group 2 (1.75, 0-4), Group 3 (2.25, 0-4.5) and Group 4 (1.25, 0-3). The activity level differed significantly between the am and pm session ($p < 0.01$), compared for 30 minutes scans.
Only the BEs found to predict *Time at Shelter* in group-housed cats in Study III, that were deemed to be recorded evenly between cats, were tested for stability during the study.

Of the BEs related to short *Time at Shelter* (Table 9), 5 were *Stable Absent* in all cats (n = 31). Of these, 4 were positive and 1 negative correlated. One BE, positively correlated to short *Time at Shelter*, was found to be *Stable Present* in all cats. Additionally, three BEs were *Stable Absent* and one *Unstable* in over 75% (n = 24) of the cats, all positively correlated to short *Time at Shelter*.

Table 7. *Interactions registered as Affiliative (Social and Vocalisation) and Agonistic (Social and Vocalisation) according to ethogram used in Study IV.*

Group	Sex*	Age (months)	Affiliative Interactions	Agonistic Interactions
1	F	14	43	26
2	M	14	93	12
3	F	43 (mean)	95	11
4	M	43 (mean)	68	9

* F, Females; M, Males

Table 8. *Median and Interquartile range (IQR) of scans for Activity observations where cats were found In-Contact with another cat or not, calculations based on each groups all three days of observation.*

Group	Median scans in contact	IQR (Q1 – Q3)
1 (Females)	50.0	28.5 - 55.7
2 (Males)	60.3	56.7 - 80.9
3 (Females)	79.4	68.8 - 85.8
4 (Males)	85.8	78.7 - 87.9

Six BEs correlated to long *Time at Shelter* (Table 10) were *Stable Absent* in all cats, 5 positive and 1 negative correlated. One BE positively correlated was found *Stable Present* in all cats. Two BEs negatively correlated to long *Time at Shelter* were *Unstable* in over 75% of the cats.

Table 9. *Stability of behavioral elements (BEs) related to short Time at Shelter as a summary of all recordings for all cats (n =31). Stable Absent: 0-3 of 12, Unstable: 4-8 of 12 and Stable Present: 9-12 or 12 for all 12 observations.*

Behavioural Element	Stable Absent	Unstable	Stable Present
Vocalisation: no, quiet	0	0	31
Tail: loosely wrapped around body	31	0	0
Tail: loosely down	31	0	0
Pupils: dilated	27	4	0
Legs: standing extended	**31**	**0**	**0**
Legs: extended, stretched out	31	0	0
Legs: front legs laid out	31	0	0
Head: on plane of body	29	2	0
Eyes: pressed together, closed	2	26	3
Eyes: half opened	27	4	0
Body: sitting	18	13	0

Bold denotes negative correlation with short *Time at Shelter*.

Table 10. *Stability of behavioural elements (BEs) related to long Time at Shelter as a summary of all recordings for all cats (n =31). Stable Absent: 0-3 of 12, Unstable: 4-8 of 12 and Stable Present: 9-12 or 12 for all 12 observations.*

Behavioural Element	Stable Absent	Unstable	Stable Present
Vocalisation: no, quiet	**0**	**0**	**31**
Ears: back	31	0	0
Head: moving	**5**	**25**	**1**
Legs: paws turned in	**7**	**24**	**0**
Body: sitting	**18**	**13**	**0**
Head: over body	31	0	0
Eyes: slow blink	31	0	0
Body: standing	31	0	0
Legs: hind legs laid out	30	1	0
Ears: partially flattened	31	0	0

Bold denotes negative correlation with long *Time at Shelter*

6 General Discussion

In relation to the aims of this thesis, in the following chapter group-housing will briefly be discussed according to findings in Papers I-III. Assessment of stress, and subsequent welfare, will be discussed in relation to findings in Paper II-IV with focus on methodological issues relating to measurements of stress, ending with a presentation of a further developed tool to assess cats. At the end of the chapter, methodological considerations of the studies will be discussed briefly.

6.1 Group-housing

Of the responding Swedish shelters in Study I, 82% provided some form of group-housing. This is a high proportion when compared to a non-representative survey of North American shelters where 13% were reported to provide group-housing (Spindel *et al.*, 2013). Despite this, few respondents reported presence of, for example, infectious disease, known to be problematic in shelter environments, especially during group-housing (e.g., Möstl *et al.*, 2013; Thiry *et al.*, 2009; Pedersen *et al.*, 2004). Of the responding shelters, 17 shelters reported no occurrence of disease in the month preceding the survey (September, 2012), 13 reported no occurrence of disease the year before the survey and 12 shelters reported no occurrence of disease the three years preceding the survey. Seven shelters reported experiencing URD. The low reporting of common symptoms related to URD in cats (caused by e.g., FHV-1, *M. felis* and *C. felis*) were later investigated in Study II were shelters were visited and cats sampled using a conjunctival swab. According to the samples collected in Study II, only 5.6% of cats tested positive to any of these three infectious agents. This would be in support of the low reporting of disease in Study I and far from numbers reported in studies from other countries. Reports from two shelters in the United States found that upon admission, 4% of the

cats were shedding FHV-1, however, within 1 week, this number was 52% (Pedersen *et al.*, 2004). Looking at shelter cats from one Belgian shelter, 20% of the cats tested positive for FHV-1 (Zicola *et al.*, 2009). In another study from the United States approximately 58% of cats developed URD within 21 days of entering the shelter (Tanaka *et al.*, 2012). As cats spend on avrage 3 months in Swedish shelters (Eriksson *et al.*, 2009) some cats would have been assumed to develop URD during this time since results from Pedersen *et al.* (2004) indicate that some cats are already carriers when entering the shelter. Why this is not the case could, besides lack of actual infection, and successful use of quarantine and vaccination routines, also have other causes, for instance that small signs of URD might have been missed, or not noted into records kept by shelters. This could likely be the case, at least, for reporting from the last year and 3 years.

Occurrence of disease in relation to group size could, from the data in Study I, only be analysed for the shelters maximum group size used (ns; $r_p = 0.15$) as shelters often kept more than one group size and all questions were answered on shelter level. However, there was a significant positive interaction between reported number of diseases and number of cats at a shelter ($p < 0.01$; $r_p = 0.47$). This is likely caused by a higher intake of cats, introducing more infectious agents into the shelter as well as having a larger turnover of animals.

Low reports of disease could also be connected to Swedish shelters providing a more 'enriched home-like' environment (Figure 2). Providing cats with the opportunity to better cope with the shelter environment, for example, by supplying opportunity to express more behaviours could be a way to decrease stress and frustration. In turn, this could decrease the occurrence, reactivation and transmission of infectious diseases. Providing animals with opportunity to express highly motivated behaviours (e.g., hiding in cats) is also important from a welfare perspective, as even if captive animals do not have the need to perform a behaviour from an ultimate perspective (e.g., for survival), the proximate mechanism (here and now need) might still be present (Dawkins, 1983). The animal might perceive itself to be in danger, and being prevented from taking action to remove itself (e.g., by hiding [e.g., Rochlitz *et al.*, 1998]), the animal is at risk of experiencing suffering (Dawkins, 1990). Previous studies found that 'enrichment' (hides, positive handling etc.) can reduce stress, as measured by the CSS, as well as increase adoption rates (e.g., Gourkow & Fraser, 2006) and that cognitive enrichment (training) resulted in better mucosal immunity in cats (Gourkow & Phillips, 2016).

The number of shelters having routines and/or protocols for the management (92%), healthcare (92%) and admission (77%) of cats was high compared to the survey of shelters in North America, where 56% provided

routines for management of URD (Spindel *et al.*, 2013). However, the fact that the question asked in Spindel *et al.* (2013) were much more specific might explain part of this difference. The use of specific routines for cats at shelters are important and can help keep the environment and maintenance more consistent when care is provided by several different members of staff (including volunteers). Keeping the unpredictability of the environment and husbandry at a minimum can help in reducing environmental stressors (Stella *et al.*, 2011; Gourkow & Fraser, 2006; Carlstead *et al.*, 1993b).

Previous studies (e.g., Gourkow & Phillips, 2015; Gourkow *et al.*, 2014a) have found a positive effect of positive human interactions (petting, grooming, playing etc.) on the mucosal immunity and development of URD (fewer developed) in shelter cats. Swedish shelters, based on differences in shelter sizes, might provide more consistent interactions from the cats perspective. This could be a possibility as Swedish shelters are often quite small, according to the results from Study I, with room for a median of 28 cats per shelter (min. = 4, max. = 90) (Hirsch *et al.*, 2014). Average intake at a Swedish shelter has been estimated at 120 cats/year (Eriksson *et al.*, 2009) compared to North American shelters with median intake of 1444 cats (Spindel *et al.*, 2013). Smaller shelters would require less staff, and subsequently likely also provide interaction with fewer staff, and therefore likely less unpredictability, for the cats. This might render time for more interactions with the same familiar person for each cat. As unpredictability has a large negative effect on cats (e.g., Stella *et al.*, 2011; Carlstead *et al*., 1993b), a more predictable environment, could result in less stress and subsequently less occurrences of disease.

Still, low reporting of disease and a high proportion of shelters having routines and or protocols could be a consequence of the methodology of the study. Maybe only the well-managed shelters had time or desire to reply to the survey. If the 25 non-responding shelters had replied the results might have turned out differently.

There were no differences in plasma cortisol levels in relation to group size in Study II ($p = 0.27$). However, lack of difference in relation to group size could be due to methodological issues in the study as only a single sample was collected from each cat, meaning that peak levels might have been collected in some but not all cats, skewing the results and hiding potential differences due to group size. Differences at the 11 shelters might also have hidden potential differences between group sizes. But due to the fact that several shelters had few cats sampled (< 6), this could not be taken into consideration during the statistical analysis. As blood collection is rather invasive, and can be assumed to affect cats negatively, this negative effect could differ between cats due to

individual differences, for example, in socialisation and use of being handled. As handling in itself can affect levels of GCs, handling provides a source of error (Dawkins, 1998) especially in a study like this, as shelter cats have a wide range in background and use of handling. Also, in several cats it was noted that the shaving machine, used to prepare for puncture, affected the cats more than the actual blood collection. So, how used cats are to unusual noise could also be a confounding factor, besides effects in relation to housing and husbandry.

Individual basal cortisol levels differ significantly between cats (Siegford *et al.*, 2003) which together with different socialisation status of the cats, and use of being handled, likely were major sources of error in the study.

Looking at the activity and social interactions of the group-housed cats in Study III these were generally low. The median activity, reported as number of *Moves* during the sessions (10 minutes × 2) was zero for all groups. Most social interactions did not include actual physical interaction, but instead vocalisations (Table 3), and there were no observations of for example, allo-grooming. Potential reasons for the general low activity and few social interactions, in these socially and temporal unstable groups, could be that cats seem to minimise tension in groups by strategies to avoid interactions (Gourkow & Fraser, 2006; Bernstein & Strack, 1996). There were generally few recordings of signs seen in stable groups such as staying in proximity of each other and resting in physical contact (e.g., Crowell-Davis *et al.*, 2004). As the shelter was not open admission, the selection of the cats could also affect the interaction between cats and the general activity. Cats selected might be cats that are generally calmer, and not necessary socialised towards conspecifics. In Swedish shelters, it is generally believed that cats less socialised towards humans are often more socialised towards conspecifics and therefore benefit from living together with another cat (personal observation). This is often used as an argument for group housing. Kessler and Turner (1999b) did find that cats not socialised towards conspecifics had higher CSS levels during group-housing. However, CSSs did not differ significantly in cats from multi- or single-houses when placed in a shelter (Broadley *et al.*, 2013).

Both the activity (median [IQR] 2 [0-4] for 30 minutes observations), and frequency of social interactions were higher in the stable groups of Study IV, although due to methodological differences between the studies, this could not be tested statistically.

6.1.1 Group-housing in relation to the behaviour of the domestic cat

Group-housing, although not occurring under the same presumption in captivity as under free-ranging conditions, where groups are formed by

matrilineal relations (Crowell-Davis *et al.*, 2004) and cats are free to move from a group, can still be made less disruptive for cats. Even in shelters. Still, there are several issues relating to group-housing in shelters, such as, temporal and spatial stability that needs attention.

Looking at the results from Study I-III, providing an environment with plenty of resources (e.g., opportunities to hide and get away from other group members) and express more of a cats behavioural repertoire seem to allow cats to cope better with the environment, seen for instance by low reporting of infectious disease (Study I and II). Previous studies have found that stress-related behaviours can be reduced when the environment is shaped to allow cats to avoid each other, by minimising need for interactions, compared to environments set-up to promote interactions (Gourkow & Fraser, 2006). There is no evidence of stable, or general, social dominance hierarchies in cats, instead cats avoid social conflict by spatial distribution and time-sharing, that is, avoidance behaviours (van den Bos, 1998; Bernstein & Strack, 1996; van den Bos & De Cock Buning, 1994). However, *tail-up* has been suggested as a signal of amicable interaction in cats (Cafazzo & Natoli, 2009; Cameron-Beaumont, 1997). Cats are therefore likely highly motivated to escape and avoid potentially threatening social situations. Preventing, or restricting, cats to perform this highly motivated avoidance behaviour, that in a natural environment could decrease the risk to their fitness, might result in suffering (Dawkins, 1990). Dawkins (1998, 1988) argue thus that suffering does not only related to signs of disease and injury, but also to prevention of motivated actions, either related to aversion (i.e., lack of opportunity to get away when motivated) or deprivation (i.e., lack of suitable conditions to perform behaviours when motivated). Previous studies have also found that cats with opportunity to hide have lower urinary concentrations of C:Cr (Carlstead *et al.*, 1993b) and lower behavioural stress scores on the CSS (Vinke *et al.*, 2014; Kry & Casey, 2007). Time spent hiding and CSSs of 4 or greater were significantly higher in cats housed communally (with unknown cats) compared to discrete-unit housing where cats were housed singly or with previously known cats (Ottway & Hawkins, 2003). In this respect, hiding can be seen as an indication that a group is not stable. Unfortunately cats in Study IV did not have any hides to compare previous findings with.

Stability of the groups (Study IV) is based on results from previous studies and knowledge of interactions within free-living cat groups. Affiliative interactions (86%) outnumbered the agonistic. The majority of cats were also seen *In-Contact* most of the scans (median 69.5%). Groups 3 and 4, with older cats having lived longer together spent more time *In-Contact* than Group 1 and 2 of

younger cats (Study IV). There were more interactions and more affiliative interactions in the male groups (Group 2 and 4), compared to female groups of the same age category (Group 1 and 3). So sex seem to also effect stability within the group when housed in same sex groups. This is in support of previous findings that cats in male/male dyads spends more time in close proximity to each other than other pairs (female/female or female/male) and that time living together is negatively correlated with aggressive interactions (Barry & Crowell-Davis, 1999). This differs from observations of free-living sexual intact cats, such as farm cats, where females, from the same family lines, were seen to interact more than males (Macdonald *et al.*, 2000). However, this difference could be due to the sexual status of the cats, and that intact females cooperate with raising of young and therefore need to keep group cohesiveness of which being in physical contact is part (Crowell-Davis *et al.*, 1997). This could then relate to the *Tend-and-Befriend* coping strategy (in response to stress) suggested in females of some species (Taylor *et al*., 2000), building alliances with other females as a protection for offspring.

Following the suggestion made by van den Bos and De Cock Buning (1994) for interpretation of cat interactions where proximity between individuals is related to affiliation, Macdonald *et al.* (2000) found that closely related females stayed closer together and could therefore be interpreted as having closer ties. Similar results were found in a study of a colony of cats, where interactions and staying in physical contact was seen more between related cats familiar with each other than non-related (Curtis *et al.*, 2003). Applying the same theory on the results from Study IV, it can be assumed that the males, especially older males having lived together longer (Group 4) had closer social ties.

Previous studies have found that regroupings can be disruptive (Griffin & Hume, 2006) and result in agonistic interactions in a group (Overall *et al.*, 2005). So lack of regroupings is likely also a contributing factor to the difference in the stability of the cat groups from Study III and IV. The calmer environment, with less agonistic interactions, in the stable groups in Study IV could also be effected by the practise of feeding cats away from the group in individual cages in a separate room. That feeding can result in competition has previously been discussed in relation to differences in agonistic interactions in group-housed cats where Loberg and Lundmark (2016), providing food in one bowl for each cat, had lower levels of aggression compared to van den Bos and De Cock Buning (1994) providing food communally with 3-4 bowls for 10 cats. As the domestic cat still is a solitary hunter, even when living in colonies (Casey & Bradshaw, 2007), competition around feeding is likely to occur, and modifying feeding routines could help reduce tension within groups.

Applying Dawkins (1998, 1990) arguments about motivation, suffering and animal welfare on housing of cats in relation to highly motivated behaviours (according to the literature), we can see that there are potential issues with both group- and single-housing. In group-housing, preventing cats from escaping from conspecifics, by not providing enough space and resources (e.g., hides) or forcing interactions, can lead to suffering. In the same sense that lack of opportunity to perform highly motivated behaviours, due to space restrictions, will in single-housing using 'traditional cages'.

6.2 Further development of an assessment tool

There was no correlation between salivary cortisol and plasma cortisol levels in Study II. There are several potential confounding factors such as few individuals (n = 10), blood contamination of saliva samples, and issues with collection of enough volume of saliva. Due to these findings, the conclusion was drawn that saliva would not be suitable for use in a study with a similar set-up where there would be no opportunity to train or habituate the cats to the procedures relating to saliva collection. Habituation and training for saliva collection have previously been shown to increase the success rate of sample collection in cats (Siegford *et al.*, 2003). However, this was not possible in Study II or III conducted at up-and-running shelters where cats were available for adoption, and therefore might not remain at the shelter from one day to the next. Blood contamination of the saliva samples likely related to oral health issues. In a random sample of 96 Swedish cats visiting the veterinarian, 32% had resorptive lesions (Pettersson & Mannerfelt, 2003). 'Dental and oral health diseases' was also found to be the most prevalent disease category in a survey of over 8000 Finnish cats, especially in non-pedigree cats (33%) (Vapalahti *et al.*, 2016). Oral bleeding could for instance have been caused by gingivitis (Frost & Williams, 1986). As previously mentioned, differences in basal cortisol levels could have been one factor behind lack of correlation between plasma cortisol level and group size. However, this would not have affected the lack of correlation between plasma and salivary cortisol as these were compared on individual level.

Other issues known to relate to differences in cortisol concentration is time of day when samples are collected as many mammals have circadian variation in cortisol concentrations (Albrecht & Eichele, 2003). However, cats differ somewhat compared to many domestic species in that they are opportunistic predators, ready at a moment's notice to hunt down an available prey. In cats, no circadian variation has been found for cortisol, instead most cats seem to

show episodic variation in cortisol concentration (Kemppainen & Peterson, 1996). In addition, as cortisol is related to activity, it is not surprising that no clear circadian pattern has been found in cats, especially since activity levels in cats are highly sensitive to for example human activity. Feral cats avoid human activity by being active during times when human activity is at its lowest (Haspel & Calhoon, 1993), whereas cats living in human homes synchronise their activity to their guardian, more so if they have restricted outdoor access (Piccione *et al.*, 2013). Cats living in shelters under clear environmental restrictions are likely to be effected more by shelter activity than time of day. Therefore, it is unlikely that the slightly different sampling times for the cats in Study II had a major effect of the results, especially since visits were booked to occur after morning feeding and cleaning at all shelters (i.e., the same 'time' based on shelter activity). However, it is possible that the human activity effected the more or less well-socialised individuals differently and influenced the results.

Previous attempts to validate behavioural measurements of stress, for example, the CSS, against physiological stress such as C:Cr (McCobb *et al.*, 2005) or faecal cortisol metabolites (Rehnberg *et al.*, 2015) have been unsuccessful. This might be related to the methodological set-up of the study, for example use of media for cortisol assay or the CSS as behavioural tool. However, lack of any clear correlation between behaviours and cortisol has been found in other studies as well (Gourkow *et al.*, 2014b). Could these difficulties instead lie in the fact that we are studying a solitary hunter and prey species evolved not to reflect health (physical or mental) in behaviour? Should we even, as a potential threatening predator, be able to determine behavioural stress in a cat we do not know, that likely is fearful and/or stressed? Or are we looking for the wrong behaviours? Still, there are indications that there is a relationship between the CSS, low quality environments and lower adoption rate (Gourkow & Fraser, 2006). Dybdall *et al.* (2007) found that cats deemed suitable for adoption were the cats with lower CSSs. Therefore, in Study III, we chose to instead of looking at behaviours related to other measurements of physiological stress, investigate which behaviours seem to be indicative of cats spending shorter or longer time at a shelter. This would then access either stress related behaviours or behaviours attractive to adopters (possibly both). Either way, this would provide information about behaviours related to if cats will be at risk of spending longer time at the shelter, and possibly be in need of additional resources to cope with the situation. The end product would nevertheless represent a behavioural assessment tool that can differentiate between cats that seem to be coping, or will at least stay for a short time at the shelter, and cats

that might be at risk of ending up spending longer time at the shelter and experiencing poor welfare.

As chronic stress has a negative effect on the immune system, occurrence of infectious disease can be used as an indirect measurement of stress. However, in our study (Study II) only 5 out of 89 samples came back positive for FHV-1, *C. felis* or *M. felis*. The low outcome of positive conjunctival swabs made it impossible to draw any conclusions about prevalence of infectious disease as measurement of this tertiary outcome of stress. However, it did raise questions about the methodology used. To investigate if the collection, or analysis, of samples were involved in the low positive outcome, a post-hoc study of data from samples analysed at the laboratory during 2013 from cats with clinical signs of URD, was initiated.

Approximately 30% of the swabs sent in during 2013 and investigated were positive (Ivarsson, 2015). A previously published study using the same facility and methodology by Ström Holst *et al.* (2010) detected *C. felis* in three and *M. felis* in two out of 20 private owned cats, from 20 households with signs of URD. For symptom free same-household cats the number was 3/20 for both *C. felis* and *M. felis*. FHV-1 was only detected in control households with cats not displaying signs of URD (2/40). Detection rate in the present study from symptom free cats were even lower (5/89 cats). These two studies indicate that even in cats with clinical signs of URD, detection rate is low using the present methodology, and that at least for FHV-1, symptom free cats may test positive. Together with the results from Study II, these results suggest that further investigation of the methodology is necessary. It may for example be hitherto unknown infectious agents causing these problems, or that we are looking for the wrong symptoms.

The fact that only 13 cats were single-housed during Study III, and that one was excluded due to outcome (*returned to guardian*), renders any conclusions about suitability to use the same behavioural assessment tool for group- and single-housed cats uncertain. However, significantly fewer BEs were recorded for single-housed cats in Study III which can be seen as an indication that cats housed in traditional cage systems (Figure 3) might not be able to express the stress related behaviours in the same way as group-housed (Figure 4) cats with opportunity to move around more freely and thereby, more easily express all of the active behaviours included in the *extended Stress Assessment* from the *Cat-Stress-Score*. It could also relate to differences in ease of recording and observing stress depending on housing. It could be that it is more difficult when cats are housed under clearly restricted housing conditions. This should

especially be considered as the single-housed cats observed during Study III where allowed twice the space as traditional caging systems by connecting two adjacent 70×70 ×70 cm cages via a circular hole.

Of the total behavioural elements (BEs) included in the eSA, 24% were never recorded, 26% in group-housed and 42% in single-housed cats (Study III). This difference in non-recorded behaviours was statistically significant ($p < 0.05$). Low levels of activity, such as sleeping or resting, have when using the CSS been found to often result in lower scorings regardless of true emotional state (McCobb *et al.*, 2005). The general low activity, independent of underlying motivation, could have affected the recording of BEs that in the CSS relate to calm and relaxed cats, missing signs of stress, fear and frustration. Few behaviours from Study III belonged to the higher levels of stress (> level 3) in the CSS (Table 3). Lack of recording of BEs related to higher levels of stress could also, as previously discussed, be related to the shelter not being open access and therefor screening cats before intake. The sample population studied might have already been excluding the most fearful and stressed cats.

Figure 3. Illustration of the single-housing used in Study III. Observe that the shelter provided cats with double cages connected via a circular hole allowing the litterbox to be placed in one section and the resting area and food and water bowls placed in the other. (Photo: EN Hirsch)

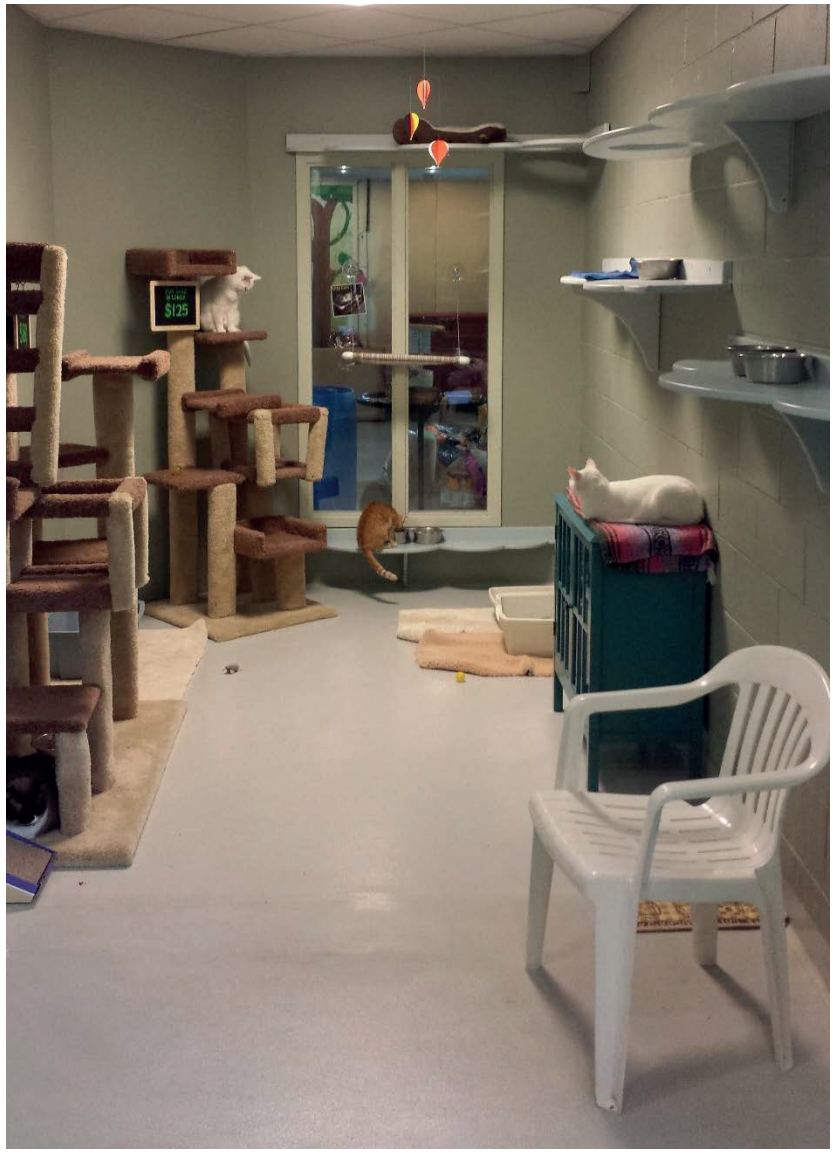

Figure 4. Picture showing part of one of the group rooms from Study III. (Photo: EN Hirsch)

Of the BEs, 16 came out related to short *Time at Shelter* and 14 to long *Time at Shelter* for both group- and single-housed cats. The fact that only four BEs came out significant from single-house data, and that only one BE was the same for both housing styles, together with the significant difference in non-recorded behaviours, leads us to conclude that housing has an effect on the recording on behaviours using the eSA. This was not expected as the CSS is developed to

function in all housing forms as long as the temperature is above 15° C (Kessler & Turner, 1999a). However, it is worth to mention that due to low number of single-housed cats (n = 12) there is need of further investigation to validate these results.

Some of the behaviours seen in single-housed cats might have been related to *frustration*. *Frustration* have previously been described in cats showing behaviours such as persistent vocalisation, pacing and bar biting (e.g., Kessler & Turner, 1997; McCune, 1992) and has been related to lower s-IgA levels (Gourkow *et al.*, 2014b). Frustration have been seen in cats as a consequence of caging in traditional caging systems (Gourkow & Phillips, 2016). These behaviours previously related to frustration were all observed in some of the single-housed cats. Frustration has also been seen to relate to risk of apathy, with increased time spent sleeping and reduction of normal behaviours such as feeding and grooming (Gourkow & Phillips, 2016). This in turn would, based on previous discussion about activity, result in lower scores on the CSS. In Sweden, this type of housing in 'standard cages' approximately 70×70×70 cm is not used in shelters and not allowed for permanent keeping. Instead the minimum space required by Swedish animal legislation is 6 m^2 (ceiling height: 1.9 m), with a minimum of 2 m^2 per individual, (SJVFS 2008:5, chapter 3, section 11).

For test of stability in Study IV the underlying stress that cats were subjected to should be assumable to be stable. This would occur in groups that can be rated as stable. In previous studies 'stable control groups', based on temporal but not social stability, have been defined as groups without change over the last 5 days (Kessler & Turner, 1997) and groups where members have lived at least 2 weeks and where no cats have left during the last 3 days (Kessler & Turner, 1999a). Compared to these requirements, the groups in Study IV, having lived at least 9 months together in the same facility, we can with a fair amount of certainty deem them both temporal and socially stable. Still, in a study investigating the response of cats to group-housing in a shelter Monk (2008) found that some cats had still not adapted to the environment after 8 months.

The stability of the BEs related to short and long *Time at Shelter*, tested according to the three criteria of *Stable Absent*, *Unstable* and *Stable Present*, was fairly similar within the groups with 10 BEs *Stable Absent* and 2 BEs *Stable Present* out of 19 unique BEs in all 31 cats. Looking at stability in at least 75% of the cats, an additionally 4 BEs were found *Stable Absent*. The fact that 15 out of 19 unique BEs were either *Stable Absent* or *Present* in over 75% of the cats is an indication that these BEs can be seen as relatively stable over time in cats housed in stable groups. The difference between individuals for stability in

some behavioural elements could be related to individual differences in coping styles (Koolhaas, 2008). These individual differences, when consistent over time and/or context, can be defined as 'personality' (Dall *et al.*, 2004), also termed for example, 'coping styles', 'behavioural syndromes' and 'temperament'. There has been suggestions that stress is effected by individual differences, that is, the temperament of the cat (Amat *et al.*, 2015). However, Siegford *et al.* (2003) found no correlations between temperament and salivary cortisol levels and neither did Iki *et al.* (2011) for temperament scores and plasma cortisol levels. Coping styles were not found consistent between context in cats based on guardian completed surveys (Kiddie & Casey, 2010), but cats reacted differently to a mild stressor (spray bath) allowing Iki *et al.* (2011) to divide cats into *proactive* and *reactive* copers. Proactive coping cats were found to react with increased locomotion and reactive coping cats with increased vocalisation and cortisol levels. These results were compared to previous findings of the reactive coping style being associated with increased cortisol levels (Koolhaas *et al.*, 1999). Still, due to conflicting results, individual differences in response to stress need further investigation in cats as this can also have an effect on an assessment tool as cats will react differently to different housing styles. Further investigation is required to see if there is a need to account for not only housing, but also coping styles, in a new assessment tool.

Of the BEs positively correlating to short *Time at Shelter*, 83% belonged to stress levels of 3 or lower, and 44% of BEs positively correlating to long *Time at Shelter*, belonged to stress levels of 4 or higher on the original seven-level CSS. Stress levels of 3 or lower on the CSS has previously been deemed acceptable (Kessler & Turner, 1999a) and representing unaffected welfare (Ottway & Hawkins, 2003), so these cats can be assumed to be handling the situation acceptably. What this indicates is that there is a connection between *Time at Shelter* and stress as described by the CSS, and these BEs could therefore be interesting in a future assessment tool for cats. However, 56% of the BEs positively correlated with long *Time at Shelter* also belonged to stress levels of 3 or lower. This makes the interpretation of the results less clear, still, most BEs found on stress levels of 4 or higher in the original CSS were positively correlated with long *Time at Shelter*. One explanation for these seemingly conflicting results can be that despite previous studies indicating that adopters primarily select cats based on behavioural characteristics such as 'friendly', 'relaxed' and 'playful', physical characteristics such as 'coat length' is also weight in during the selection process (Gourkow & Fraser, 2006). This could be effecting the results even though there were no obvious differences in

physical characteristics between the cats selected quickly and those not in Study III. However, this was not tested statistically. In Sweden, shelter personnel often comment on adopters selecting cats based on physical characteristics and that black short haired females often are selected last (personal observation). In the U.S. shelter (Study III) it seemed more like orange tabby females were least popular (personal observation).

6.2.1 *Cat Behaviour and Well-being tool* (CatBeWell)

The behavioural elements (BEs) originating from Study III and tested for stability in Study IV can be used in the advancement of an assessment tool to determine, from an anthropocentric view, how cats are coping with the shelter environment. Coping in this sense would mean that cats are displaying behaviours that make them desirable for adoption and therefore seem to cope better with the man-made and controlled environment. Including both BEs associated with short and long *Time at Shelter* allows categorisation of cats into two general groups: (1) cats that are likely to become adopted fast, and (2) cats that will likely take longer time to become adopted.
Consequences would be that cats in category two might require additional resources, or different environments, to better cope and hopefully increased their chance of a faster adoption. This tool should not be seen as a method to select cats for intake at shelters or euthanasia since all cats were eventually adopted and no BEs recorded related to risk of disease or euthanasia.

This proposal, the *Cat Behaviour and Well-being tool* (CatBeWell) (Table 11) should not be seen as a complete tool, but more of a first step in the advancement of a behavioural assessment tool, as of now, for assessment of cats housed in shelters. Since the time between observations in the stability study (Study IV) were on median 4.5 days, stability of the BEs over longer time would need to be investigated further for conclusions about long-term stability. At what time, if only performed once, observations should take place also need to be studied. Previous studies have found that adaptation to a new environment takes over 2 weeks in the majority of cats (Kessler & Turner, 1997), and that stress levels, as measured by the CSS is elevated at least the first 4 days in shelters (Broadley *et al.*, 2013; Kessler & Turner, 1997). Observations should therefore not be performed during the first few days of entering a new environment. One suggestion, applicable to the Swedish shelter practises, where cats are housed in quarantine areas before entering the adoption ward, is to perform the observations at the very end of this time, usually at least 10 days after intake (Hirsch *et al.*, 2014).

As previous extensive studies of cats have revealed that behaviours related to stress can be divided into three categories, of which one is inhibition of

'normal' behaviours (McCune, 1992), it is also important to include 'normal' behaviours indicative of cats doing well in a future protocol (McCune, 1994). It is necessary to remember that opportunity to express 'natural behaviours' are no guarantee of good welfare. However, lack of opportunity to perform certain behaviours can indicate possible poor welfare (Dawkins, 1998). Reviewing the literature, the most important, and easily scored, behaviours relating to good welfare in cats seem to be: *eating*, *using the litterbox* (Stella et al., 2011), *tail-up* (Cameron-Beaumont, 1997; Cafazzo & Natoli, 2009) and *grooming*. Grooming is both a 'normal' behaviour and a behaviour seen to indicate a more socialised cat (Slater *et al.*, 2013). These behaviours should then be included as potential signs that cats are coping with the environment. It can be discussed if tail-up should only be recorded when presented towards conspecific, however, presence in general can be interpreted as positive. Behaviours more indicative of poor welfare in the literature, besides absence of behaviours of doing well, seem to be: *hiding* or attempting to hide (e.g., Rehnberg *et al.*, 2015; Vinke *et al.*, 2014; Carlstead *et al.*, 1993b), *presence of vomit* (Stella *et al.*, 2011) and behaviours related to *frustration* (e.g., excessive vocalisation, trying to escape) (Kessler & Turner, 1997; McCune, 1992). These behaviours should be included as potential signals that a cat is not coping with the environment. Behaviours relating to frustration have not been included in the CatBeWell at this point, as it has not been determined how to define these behaviours so that they can be scored in a standardised way.

Behaviours included in the CatBeWell were compared to the standardised ethogram for felids described in Stanton *et al.* (2015). However, few BEs included in the CatBeWell were described in the standardised ethogram as behaviours from Kessler and Turner (1997) were not incorporated due to not being titled in the publication. Therefore, few behaviours could be classified according to the behavioural categories described. The few behaviours present in the standardised ethogram, and relating to long *Time at Shelter* in the CatBeWell, belonged to categories 'agonistic', 'aggression' and 'fear'. The behaviours relating to short *Time at Shelter* belonged to categories 'feeding', 'active', 'maintenance', 'calm', 'inactive' but also 'fear'. As only behaviours relating to long *Time at Shelter* were found in the categories 'agonistic' and 'aggression', and that previous studies have found that negative affect in cats often is expressed through aggression/agonistic behaviours (e.g., Rodan, 2010; Levine, 2008; Moffat, 2008) this could be a further indication that the BEs relate to coping.

Table 11. *Suggestion for a further developed behavioural assessment tool based on results from Study III and IV as well as literature. Depending on relationship to Time at Shelter or in literature other signs of stress, behaviours get points for either being present or absent.*

Behavioural Element	Present (point)	Absent (**no** pont)
Vocalisation: no, quiet	1	0
Legs:		
Stretched out	1	0
Front legs laid out	1	0
Tail:		
Loosely wrapped around body	1	0
Loosely down	1	0
Head: on plane of body	1	0
Eyes: half opened	1	0
Pupils: dilated	1	0
Eating	1	0
Used litterbox	1	0
Tail-up	1	0
Grooming	1	0
	Present (**no** point)	Absent (point)
Body: standing	0	1
Legs:		
Standing extended	0	1
Hind legs laid out	0	1
Head: over body	0	1
Ears:		
Back	0	1
Flattened	0	1
Eyes: slow blink	0	1
Hiding	0	1
Presence of vomit	0	1
	Sum:	Sum:
		Total sum:

BEs in italic were not found in the thesis, instead taken from the wider cat literature, but deemed important to include due to previous findings of relationship with stress

The higher the score (sum of points) on the CatBeWell the higher the chance of a fast adoption of a cat. Where the 'dividing line' should be made needs to be investigated by validation of the tool, as well as weighing of the BEs as some might be more important than other. This is a necessary step in making sure that the tool is applicable to the shelter environment as well as suitable for use in practise. Weighing of the BEs included could be related to the Hazard Ratio of each behaviour, relating to how well a behaviour explains difference in time until adoption.

As previously discussed, there is likely also an effect of physical attributes of the cats (e.g., coat length and colour) on the time until adoption (Gourkow & Fraser, 2006), although these were not investigated statistically in the present studies, they might affect the validity of the tool, and should therefore be investigated further during validation tests.

6.2.2 Additional options attempted or considered for validation of behavioural elements

Other options that were tested to be used for validation of BEs for Study III were observations of sickness behaviours. Sickness behaviours have previously been shown to decrease during enriched environments, and increase during more aversive environments, and can therefore be usable for determination of negative effects on cats (Stella et al., 2011). Unfortunately, during the observations at the shelter in Study III, it was noted that volunteers cleaned the cages during the day, meaning that absence of sickness behaviour during the morning registration before cleaning could not be trusted. However, in a more controlled environment this would have been an option for validation of stress related behaviours.

During the planning of the project we considered several different media used for cortisol assay in cats instead of saliva in relation to findings from previous studies. Faecal cortisol metabolites have previously been validated (Schatz & Palme, 2001; Graham & Brown, 1996) and used (e.g., Ramos *et al.*, 2013; Ramos *et al.*, 2012; Accorsi *et al.*, 2008) for cortisol determination in studies on cat welfare. The main advantage of the media is that it can be collected non-invasively. Still, there are some potential issues relating to the use of cortisol metabolites such as age of the collected sample when prepared, where the optimum is to collect fresh samples that still contain biological representative levels of cortisol metabolites (Millspaugh & Washburn, 2004). This was considered a major issue as there was no opportunity to access the cats at all times at the shelters which together with the fact that it would be difficult to individually differentiate between samples from the litterboxes

during group-housing made us reject the use of faecal cortisol metabolites despite having good potential for studies using different set-ups.

Collection of urine for assay of C:Cr was also considered, as this can be collected non-invasively using double underside litterboxes with non-absorbent litter. However, with this media there are also issues with individual differentiation between samples during group-housing. The main issue for rejection of the use of C:Cr was results from McCobb *et al.* (2005) finding that 25% of urinary samples collected from shelter cats in their study contained traces of blood (haematuria) which can be a confounding factor for cortisol concentrations.

Hair cortisol was the last media considered for validation of BEs, but despite finding two studies using cortisol levels assayed from hair (Finkler & Terkel, 2010; Accorsi *et al.*, 2008) the method was considered too new and in need of further validation since previous research has discussed if the cortisol levels measured reflect global or local levels of cortisol, as in humans, the hair follicle has a structure similar to the HPA axis (Ito *et al.*, 2005). Also, since one of the aims of the study was to find a media that reflect 'here and now' levels of stress, measurements of more acute stress seemed to be better options.

6.3 Methodological considerations

The response rate in Study I was 61% and to be able to draw more general conclusions about Swedish cat shelters, a higher response rate would have been preferred. However, the response rate was enough to provide an insight into practices and issues at cat shelters in Sweden.

The main aim for Study II was to investigate the suitability of salivary sampling for determination of cortisol levels in cats by testing the possibility to collect saliva from cats. There are several limitations with the study. Salivation is regulated by the ANS and decreases in response to activation of the SNS. The SNS is activated during stress, so, with this in mind and the fact that blood collection can result in a stress response in cats, it was decided that blood would be collected last to make sure that all samples would be collected from all cats, and to increase the chance of collecting enough saliva for analysis. This might however have introduced limitations in the study, relating to the release of cortisol to different media as cortisol increases faster in plasma than saliva. In an attempt to avoid plasma samples having reached peak levels, and not being comparable to saliva samples, sample collection took approximately 10 minutes. This was believed to be appropriate since peak levels in plasma has been measured at between 5 and 15 minutes (30 minutes in serum) after a

stressor in cats. Still, there is a chance that cortisol concentrations in plasma, but not in saliva, was affected by the handling procedure and that this is reflected in the lack of correlation between plasma and saliva samples. So to find correlations between salivary and plasma levels it would have been preferential to collect multiple samples from several time points as to not access peak levels in only plasma or saliva.

Behavioural observations would have been a good addition to further be able to interpret differences in cortisol levels. However, due to time constrains and the fact that the main aim was to investigate if collection of saliva was suitable, the choice was to instead to collect samples from more individuals. If repeated, due to difficulties with sample collection of saliva in cats not trained for the procedure, the study would have been focused instead on finding ways to collect saliva from naïve cats.

In general when measuring stress, physiological measurements are 'required' to determine if behaviours should be interpreted as potentially harmful or not in validation studies. On the other hand, physiological measurements of stress, for example cortisol, require that we have information about the corresponding behaviours to be able to interpret the physiological response (aversion or not). This is because of the generality of the response of GCs (i.e., stress hormones) to situations that require action, that is, that cause 'excitement' (Dawkins, 1998). As argued by Dawkins (1998) the use of 'stress hormones' alone as indicators of welfare is not reliable due to the clear overlap between responses connected to action, independent of relation to pleasure or aversion. This somewhat circular argument can be difficult to work around and might be the reason why none of the studies reviewed here has found clear correlations between stress related behaviours and for example cortisol.

In Study III, the majority of cats (77%) ended up being female, this could have affected the results, but since we observed cats at an actual shelter we could not control for sex in the cats. This skewed sex ratio can have affected the recording of stress related behaviours as there are sex differences in the response to stress. It has previously been stated that this is important to keep in mind as these sex differences may result in different physiological and behavioural regulations of stress (McEwan, 2005). This comes back to issues raised previously in relation to differences in the adaptive value of coping with stressors with different behavioural responses in males and females, following the *Fight-Flight* (males) or *Tend-and-Befriend* (females) (Taylor *et al.*, 2000). Additional observations with more males could rectify this methodological weakness and strengthen the results to be valid for males as well as females.

There were room for fewer single-housed cats at the shelter, and the fact that cats had a tendency to take longer before adoption from single-housing, there were fewer individuals observed in single-housing. Due to the low number of cats housed singly (n=13) no generalisations were possible to conduct. It would have been preferential to have data from more single-housed cats.

The shelter at which the study took place was not open admission, meaning that the shelter screened the cats, this might have resulted in cats that were all somewhat used to humans and might have excluded the most un-socialised fearful and stressed cats. Therefore, the results for which behavioural elements to include in a final assessment tool cannot be considered complete.

From the start the aim was to use time until *Outcome* (euthanasia, adoption or other) as the hazard rate in the Survival analysis. However, since all cats besides 3 became adopted in the end (2 where euthanised and 1 returned to guardian) this was not possible. In an open admission shelter, this might have been possible, which would then have allowed a possible differentiation between cats at risk for euthanasia allowing shelters to intervene and make changes before this point.

Only group-housed cats were available in Study IV which unfortunately resulted in inability to draw conclusions about behavioural elements related to *Time at Shelter* observed in single-housed cats. However, as only few BEs were found for single-housed cats, likely due to low number of included cats, this was deemed acceptable. However, this means that there is need of further validation of the use of the behavioural elements on single-housed cats as well as test of the stability of the behavioural elements for single-housed cats.

Due to highly controlled setting during Study IV, there is need to validate these BEs and the CatBeWell also in a shelter setting for results on practical use and value.

7 Conclusions

- Group-housing (82%) was the most common housing type in Swedish cat shelters. Despite most shelters providing group-housing, the reporting of occurrence of disease was low, with several shelters reporting to be disease free during the last 3 years (n=12).
- Only 5 of the collected 89 conjunctival swabs came back positive for either FHV -1, *M. felis* or *C. felis*. The low outcome of positive results could be due to low occurrence of disease or that the assay method used was unsuitable. To determine which, further investigation is required.
- Few saliva samples yielded enough volume for the cortisol assay (11/89), which in addition to presence of traces of blood, related to oral health issues, allowed us to conclude that collection of saliva was not a suitable option for shelter cat studies using a similar set-up.
- Of the 85 behavioural elements (BEs) included in the *extended Stress Assessment* tool, 24% were never recorded, meaning that a further developed assessment tool can be made more condensed and simplified.
- The low number of single-housed cats (n = 13, 12 for analysis) did not allow us to draw any conclusions about the suitability of using the same assessment score for both group- and single-housed cats.
- 14 BEs were found to relate to short time until adoption (*Time at Shelter*) and 12 to long time until adoption in group-housed shelter cats.
- Five BEs related to short *Time at Shelter* were found *Stable Absent* for all cats housed in stable groups. One BE was found to be *Stable Present* for all cats.
- Five BEs related to long *Time at Shelter* were found *Stable Absent* for all cats housed in stable groups. One BE was found to be *Stable Present* for all cats.
- BEs found in Study III and IV were used to design a first version of a new assessment tool for cats housed in groups, the CatBeWell tool.

8 Future Perspectives

As the cat shelter used in Study III, as previously discussed, was not an open admission shelter this could have resulted in a biased sample of cats, excluding the most stressed cats that might have displayed a wider range of stress related behavioural elements. Therefore, performing a similar study as Study III, but in an open admission shelter, with more even sex distribution, to make sure that cats of a wider range of stress, fear and socialisation levels are included. There would also be need of collecting data from a larger sample of single-housed cats. This would complement and increase the practical implications of the results. In addition to this, there would then be a need for performing a new stability study similar to Study IV to be able to test the stability of the behavioural elements as well as for data from single-housed cats.

Instead of using stress hormones such as cortisol it would have been interesting to validate behavioural elements for the assessment tool using outcomes of stress such as effects on the immune system using non-invasively collected samples for analysis of S-IgA or finding ways to observe sickness behaviours in both single- and group-housed cats at shelters. This would have provided an additional level of validity for the test of suitability of BEs to assess coping in cats.

The results from the studies presented here, although not complete, were used in the development of a first version of the assessment tool to assess coping in shelter cats, the CatBeWell. This tool, as it is a first proposal, is in need of further validation, such as, intra- and inter-reliability. As one potential area of use, besides within research, is for self-control at shelters, after the initial validation and likely modification, the tool would need to be tested at actual shelters. The idea would be to distribute the tool to shelter staff for further tests of usability and ability to differentiate between cats. One of the aims for the further validation is to keep the tool simple, and self-explanatory, to not require training, as I believe that not all shelters would train their scorers. Using the tool will hopefully then allow shelters to differentiate

between cats that likely will become adopted fast, and those that might end up staying at shelters for prolonged periods and that might be in need of further resources to cope with the environment to become adopted. Although it is possible to question the usability of an assessment tool that takes only a few minutes to complete, it is unlikely that shelters would utilise a tool that would require a long time to complete.

9 Populärvetenskaplig sammanfattning

Tamkatten är det vanligaste sällskapsdjuret i Sverige, och även i stora delar av västvärlden. Tyvärr innebär denna popularitet att antalet oönskade katter ökar. Katter som har blivit övergivna av människor lämnas in på djurhem för omplacering eller för avlivning, och i vissa fall blir de en del av världens förvildade (ferala) tamkatter.

En vanlig anledning till avlivning eller att man lämnar katten till djurhem för omplacering är att katten har beteenden som för ägarna kan vara oönskade t.ex. urinmarkering eller aggression mot människor eller andra djur i hemmet. Dessa beteenden har genom tidigare studier visat sig ofta vara kopplade till att katterna bor i en otillräcklig eller olämplig miljö. En vanlig orsak till både urinmarkering och aggression är stress och rädsla som ofta beror på den sociala miljön. Tamkatten härstammar från den Afrikanska vildkatten, en ensamlevande opportunistisk jägare. Det finns fortfarande stora likheter i beteendet och behoven hos tamkatten jämfört med vildkatten. Detta innebär att till skillnad från t.ex. hunden som härstammar från en socialt levande art så är det inte självklart att katter trivs med att leva ihop med andra katter.

Syftet med denna avhandling var att närmare undersöka hur katter påverkas av livet i grupp och hur vi i så fall kan avgöra detta utan att störa eller negativt påverka katten. Studierna är utförda på djurhem för katter (katthem) eftersom tidigare forskning har visat att katthem kan vara en extrem miljö för många katter. Med extrem miljö menas t.ex. närvaro av okända katter, högt smittryck genom trängsel samt nya rutiner och en ny miljö jämfört med tidigare. Dessa faktorer har tidigare studier visat kunna orsaka stress och rädsla hos katter.

I den första studien undersöktes genom en enkät hur vanligt det är med grupphållning på svenska katthem. Frågorna berörde även vanliga problem som kan uppstå på katthem, så som förekomst av sjukdomar. Dessa problem

har i tidigare studier från andra länder varit vanliga. Det visade sig att majoriteten (82 %) av katthemmen hade någon form av grupphållning. Trots detta upplevde de flesta katthem få problem, t.ex. låg förekomst av sjukdomar, även infektionssjukdomar kopplade till stress, jämfört med resultat från tidigare studier från andra länder. Detta undersöktes vidare under den andra studien där besök utfördes på 11 katthem som deltagit i enkätstudien. Under studien samlades en kindsvabb och ögonsvabb in från katterna på katthemmen för att undersöka förekomst av vanliga sjukdomar kopplade till grupphållning och stress hos katter. Vi samlade även in salivprov och blodprov för att försöka avgöra om salivprov, som anses vara mindre stressande, kunde ersätta blodprov för mätningar av stresshormonet kortisol. Endast hos 5 katter (av 89) fann vi någon infektionssjukdom. Det var svårt att få tillräcklig mängd saliv vid salivprovtagningen och endast ett fåtal prover (11 av 89) innehöll tillräcklig mängd för att göra individuella analyser. Ett flertal prover visade sig även innehålla små mängder av blod. Spår av blod i saliven berodde sannolikt på att katterna hade problem med munhälsan, t.ex. inflammation av tandköttet. Detta i kombination med svårigheten att samla in tillräcklig mängd saliv påvisade svårigheterna med att använda saliv för utvinning av kortisol hos katt under liknande upplägg av studier. Därför valde vi att inte gå vidare med salivprovtagning i de senare studierna.

För att ta fram en ny metod för att avgöra hur katter trivs med att leva i grupp utarbetades ett beteendeprotokoll baserat på tidigare protokoll. Detta protokoll kan liknas vid en bedömningsmall med beskrivningar av katters beteende. Syftet med protokollet är att kunna avgöra om katten uppvisar tecken på stress eller inte. Under den tredje studien observerades katter som hölls i grupp respektive ensamma på ett katthem i USA, enligt det omarbetade protokollet. Syftet var att koppla katternas beteenden till den tid de blev kvar på katthemmet innan de adopterades. Flera av de beteenden som fanns med i protokollet observerades aldrig, framförallt hos de katter som hölls ensamma. För både katter i grupp och ensamma fann vi beteenden som kunde kopplas till både kortare och längre tid till adoption. Dessa beteenden användes sedan i den fjärde studien. Eftersom alla katter levde i grupp i den fjärde studien undersöktes endast stabiliteten för de beteenden som i tredje studien kopplade till tid till adoption hos katter i grupp. I den fjärde studien undersöktes om de beteenden som vi fann i studie 3 var stabila, det vill säga om de uppvisades ofta eller aldrig hos en och samma katt som hölls i en stabil miljö. Med stabil miljö menas en miljö utan förändringar i varken sammansättning av grupp eller den fysiska miljön. Detta studerades för att bedöma om beteendena är pålitliga mätvärden för att fastställa en påverkan av miljön eller inte. Flera

observationer på samma katter utfördes över flera dagar. På detta sätt kunde vi avgöra om beteenden var desamma hos en katt eller förändrades mellan dagar. Beteenden som förändrades mellan dagarna räknades inte som stabila. Av de beteenden som undersöktes var majoriteten (15 av 19) stabila hos minst 75 % av katterna. Skillnaden mellan katterna, med avseende på om ett beteende var stabilt eller ej, kan förklaras i form av individuella skillnader i hur katter hanterar sin miljö, så kallade *copingstrategier*. Hur detta påverkar en bedömning av stress hos katter behöver undersökas vidare.

Resultaten från studierna i denna avhandling tyder på att påverkan av att hålla katter i grupp inte behöver ha stora negativa konsekvenser för katterna, även under mer "extrema förhållanden" som på katthem. En viktig faktor verkar vara vilken typ av miljö som erbjuds i form av antal resurser (antal liggytor, gömslen, kattlådor m.m.) och hur katthemmet sköts gällande rutiner kring karantän och hur grupperna av katter sätts ihop.

Kopplingar mellan fysiologiska mätningar av stress, t.ex. nivåer av stresshormonet kortisol, och beteenden relaterade till stress har i tidigare vetenskapliga studier varit svåra att hitta på katt. På grund av detta, samt problematiken med salivprovtagning i den andra studien, valde vi istället att leta efter beteenden som kopplar till tiden på katthem fram till adoption. Vi hittade flera beteenden som kopplade till tid till adoption hos både katter i grupp och ensamma. Majoriteten av dessa beteenden verkar vara stabila över tid, vilket visade sig under upprepade mätningar hos katter i grupp i en koloni som hölls under stabila förhållanden.

Resultaten från studierna användes för att vidareutveckla ett beteendeprotokoll för att avgöra hur en katt sannolikt kommer att hantera vistelsen på ett katthem. Genom att titta på beteenden, och förstå hur de hänger ihop med tid fram till adoption, har vi även tagit fram ett förslag på ett verktyg som ger information om huruvida katten mår bra eller inte även i ett tidigt skede. Hur en katt hanterar miljön på ett djurhem bedöms genom sannolikheten att det kommer ta kort eller lång tid tills katten blir adopterad. Ett förslag på ett nytt protokoll, kallat *Cat Behaviour and Well-being tool* (CatBeWell) togs fram för grupphållna katter på katthem. Syftet med CatBeWell var att erbjuda en objektiv metod för att bedöma om en social grupp eller miljö är kattvänlig eller inte och om katterna riskerar att bli kvar en längre tid på katthemmet innan adoption.

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Appendix 1. The *extended Stress Assessment*

Date:_____ **Time:**_____ **Protocol (am or pm):**_____ **Cat name/ID#:**_____ **Cage ID#:**_____ **Room ID:**_____ **Scorer:**_____

Body laid out (on back/on side/on stomach) sitting standing moving crouched (all fours) shaking 'tense' 'stiff' flattened
Comments:_____

Belly exposed not exposed not visible slow/normal vent. fast vent.
Comments:_____

Legs fully extended/stretched out front legs laid out hind legs laid out standing, extended standing, bent bent near surface paws turned in
Comments:_____

Tail extended loosely wrapped round body up loosely downward twitching tense downward close to body
Comments:_____

Head laid down (on surface) chin upward on plane of body (somewhat crouched) lower than body (crouched) flattened near surface over body moving
Comments:_____

Eyes normal (open) ½ open pressed together closed slow blink wide open (partially dilated) fully open (dilated)
Comments:_____

Pupils normal partially dilated dilated fully (very) dilated
Comments:_____

Ears normal erect to front (forward) erect to back partially (slightly) flattened fully flattened fully flattened & back on head prickled
Comments:_____

Whiskers normal forward lateral back
Comments:_____

Vocalization chirp/'greet' none/quiet purring meow yowling growling plaintive meow
Comments:_____

Activity hide sleeping/resting awake alert/look around groom rub playing cramped sleeping trying to escape
actively exploring/prowling motionless alert allo-groom knead
Comments:_____

Other completely relaxed drool close, crouched look all out defence hair flattened 'aware' 'rage'
Comments:_____

Behaviours taken from the Cat-Stress-Score Kessler & Turner, 1997; the Global Assessment Score McCune, 1992 with additions from the wider cat literature including those behaviours indicative of more positive states.