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Kennelled dog welfare - effects of housing and husbandry

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Abstract

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Samantha A. Gaines

This thesis assesses and compares physiological and behavioural indicators of kennelled dog welfare, and explores their relationships with aspects of housing and husbandry.

The use of urinary cortisol to creatinine ratio (C/C) as an indicator of welfare was explored via responses to a psychological stressor, a standardised veterinary examination. The dogs did not respond uniformly to this stressor, but basal C/C levels of long-term kennelled dogs were significantly higher than those of dogs housed in domestic environments, suggesting that elevated levels of C/C are indicative of chronic stress and thus compromised welfare.

A study of two military working dog populations revealed that behaviour in kennels differed according to time of day and was also influenced by the presence of an observer. Hence, recording at one time of day or using a single observation technique could lead to incomplete or inaccurate welfare assessment.

Surveys of two military working dog populations revealed that the most critical factors for welfare differed between the two populations. However, for both populations, and possibly for kennelled dogs in general, levels of exercise, noise and predictability of routine appeared the most influential for welfare, whilst the relationship between environmental stimulation within the kennel and welfare appeared complex.

Duration of exercise and the availability of feeding enrichment were manipulated in populations of military working dogs. An increase in exercise from twenty to sixty minutes appeared to improve welfare, decreasing C/C levels and increasing resting behaviour. Providing long-term feeding enrichment did not measurably change welfare indicators but was a valued resource for the majority of subjects as indicated by their response to its removal. The provision of feeding enrichment had no effect upon perceived working ability, dog-human aggression, or health, and hence is viable in military establishments.

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Author's declaration

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original, except where indicated by special reference in the text, and no part of the dissertation has been submitted for any other academic award.

Any views expressed in the dissertation are those of the author.

SIGNED: ...[signature]... DATE: 31/3/08

Contents

List of tables and figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6.1

Chapter 6.2

Appendix 1

Appendix 4

Chapter 1

Introduction

1.1 Chapter outline

The domestic dog (*Canis familiaris*) is now very much integrated into modern society. There are approximately 6.8 million dogs in the UK (Pet Food Manufacturers' Association, www.pfma.org.uk, 2008), most are kept as companions, but many are utilised for traditional roles such as herding and guarding and increasing numbers are being used for more novel tasks, for example, hearing dogs for deaf people, detection dogs for bladder cancer. Within this thesis, I will be focusing upon dogs utilised for three tasks common within the UK Military; *arms and explosive search do*gs; trained to locate explosives and weapons (Rooney et al, 2007a), *PATrol Arm True (PATAT) dogs*; trained to guard areas, detect and indicate the presence of people in an area and to chase and apprehend people by biting the lower right arm but release upon command, and *police dogs*; trained to track and search for the presence of people, search within and for property, chase and apprehend criminals, obey a range of commands and demonstrate agility.

In light of the current climate of terrorist threat, the demand for dogs to fulfil these roles is great. With this comes an increased pressure to procure suitable dogs to train for these highly specialised tasks. Thus in recent years, search and police dogs have become a focus for scientific enquiry with a growing body of research looking not only at selection (Champness, 1996, Rooney and Bradshaw, 2004, Rooney et al, 2004, Slabbert and Oodendaal, 1999) but also training and general ability (Gazit and Terkel, 2003, Haverbeke et al, 2008a, Wells and Hepper, 2003, Williams and Johnston, 2002). However, one area which is yet to receive significant interest is the impact of factors such as kennelling, training and transportation upon the dogs' physical and psychological state, i.e. its welfare (1.3).

As recently demonstrated in a study conducted by myself and others, the welfare of military working dogs can be influenced by kennelling (not included in this thesis) (Rooney et al, 2007b). In this study we monitored the stress response (urinary cortisol, section 1.4.1.1) of 31 dogs in a domestic environment and followed their introduction into novel kennels at a military training establishment. Half of the dogs were habituated, whilst in the domestic environment, to confinement in a kennel. Following the transition, the levels of cortisol in all dogs were significantly higher compared to levels measured in the domestic environment showing that the transition was stressful. However, the levels of cortisol in previously habituated dogs were significantly lower than those dogs which were naive to the kennelled environment. This suggests that previous experience of a kennelled environment can mitigate the stress response, but because the levels were still significantly higher than when in the home, this suggests that there must be other factors, in addition to the kennel, per se, within the kennelled environment that induce stress responses. In addition, these stress responses can also have a significant impact on how effectively dogs carry out their function as those which experienced high levels of acute stress following their introduction into kennelling tended to show significantly lower search ability (Rooney et al, 2005). Therefore it is important, both from ethical and pragmatic viewpoints, to understand which factors of housing and husbandry within a kennelled environment influence welfare the most, and this is the focus of this thesis.

Within this chapter, the subject animal in question, the military working dog, is introduced (1.2). In section 1.3, the concept of welfare is discussed and a working definition of welfare appropriate for military working dogs is presented (1.3.3). Section 1.4 then discusses how welfare can be measured and assessed using both physiological and behavioural parameters. Finally, section 1.5 discusses factors of housing and husbandry which may affect welfare and, where appropriate, evidence from studies of rescue and laboratory dogs is described.

1.2 The Domestic Dog

1.2.1 Domestication and artificial selection of the dog

The results of a number of studies combining behaviour, vocalisations, morphology and molecular biology indicate that the principal and probably the only, ancestor of the dog is the wolf (*Canis lupus*) (Clutton-Brock, 1995, Parker et al, 2004). Whilst archaeological and cultural evidence indicates that the dog was first domesticated between 12 and 14 000 years ago, mitochondrial evidence suggests that domestication of the dog may have taken place as long as 100 000 years ago (Vila et al, 1997).

During domestication, biological and cultural processes resulted in some wolves becoming reproductively isolated from their native population and enfolded within the structure of the human community becoming objects of ownership. Through artificial and natural selection these tamed wolves began to look less like their native population. Early stages of domestication saw a general reduction in size of head, body and dentition (a characteristic feature of early stage domestication, Clutton-Brock, 1995) with artificial selection during the latter stages of domestication for particular desirable traits e.g. coat colour and leg length as well as different aspects of temperament and behaviour.

Artificial selection has resulted in over 400 different breeds of dog in the world today (Clutton-Brock, 1995), 209 of which are recognised by the UK Kennel Club (The Kennel Club, www.kennel-club.org.uk, 2008). Many are a result of selecting for a specific function e.g. suitability for varied terrains, or hunting, and dogs have arguably been more heavily selected for behavioural traits than any other domesticated species. Yet, due to social structure changes and industrialisation, today, the majority of these breeds function solely as companions to their human owners and are rarely used for their original purposes. However, there are a number of breeds which still have specific roles within society e.g. sheepdog breeds which herd livestock. In addition there are an increasing variety of roles which numerous breeds can accomplish e.g. assistance dogs used by the Guide Dogs for the Blind, Canine Partners for Independence and particularly relevant to this study, military working dogs; arms and explosive search dogs, police dogs and patrol dogs.

1.2.2 The Military Working Dog

The ability of dogs to detect a wide range of substances using olfactory cues has long been acknowledged with the use of dogs to detect chemicals (during hunting) dating back 12 000 years (Furton and Myers, 2001), but it is only in the latter half of the 20th century that this acute olfactory system has been fully exploited (Johnston et al, 1998). During the first and second World Wars, a diverse range of breeds was recruited as security, tracking, ambush patrol, ambulance and messenger dogs and following World War II, the German Shepherd Dog (GSD) was used extensively for explosives detection (Furton and Myers, 2001).

The prevalence of counter terrorism both in the UK and abroad continues to dictate the requirement for such dogs. At present, due to the characteristics required for search and detection disciplines (Rooney et al, 2004), gun dog breeds such as Labrador Retrievers and English Springer Spaniels are widely used, whilst shepherd breeds, traditionally selected for guarding behaviour, are still predominantly used for patrol and police work. Within the UK, the Defence Animal Centre (DAC, www.defenceanimalcentre.com) procures and trains all dogs required by the joint services; Royal Army Veterinary Corps and the Royal Air Force, as well as the Ministry of Defence Police and some private organisations. Currently, there are numerous sources from which these breeds are procured including rescue shelters and dealers who obtain dogs from various sources and then sell them to the DAC. However, the most predominant source is the general public, whereby a dog no longer wanted, or unable to be kept, is donated as a gift. This may be particularly problematic because upon arrival at the DAC all dogs will be housed in kennels. The majority of donated dogs have previously been housed in a domestic environment, are naïve to the kennelled environment and thus the transition into a kennelled environment can impact upon their welfare as discussed in section 1.5.1.

Following procurement and successful training, a minimum of three months, qualified military working dogs are then deployed either within the UK or overseas. The vast majority of dogs will continue to be housed in a kennelled environment, typically at a site housing a number of dogs, cared for by kennel assistants and their handlers until they are retired from service between eight and ten years of age.

In the following section the concept of welfare is introduced and a definition suitable for the subject is presented.

1.3 Animal Welfare

1.3.1 The development of animal welfare as a science

Current attitudes to animal welfare have been shaped through history, culture and society (Young, 2003) and have evolved from humans' concern or compassion for the well being of animals. The concept of animal welfare is apparent in early writings of religious text (Exodus 23: v12, Deuteronomy 25:v4 as cited in Duncan and Fraser, 1997) reporting criticism regarding the treatment of animals by humans, which evolved further in response to the slaughter of thousands of animals during the Roman era. These criticisms were often strongly disregarded, fuelling theories such as that of Descartes (1569-1650) (Young, 2003):

'Animals are only bodies without souls'

yet animal welfare did begin to be accepted into society, and hence contrasting theories developed such as that proposed by Bentham (1748-1832):

'The question is not, can they reason, nor can they talk, but can they suffer?'

Such theories, contrasting with those of Descartes, saw a change in the perception of animal welfare, leading, in 1826, to the implementation of the first ever international animal welfare law being introduced in the UK. However, although the concept of animal welfare became recognised, it was not until the 1960s that animal welfare science developed. This followed criticism of the treatment of farm animals and the recognition of abnormal behaviours in laboratory primates (Young, 2003).

Animal welfare may not have arisen as a purely scientific concept, but rather to express ethical concerns, by society, of how animals are treated. However, in light of this, there now exists a great debate as to how we actually define welfare in a scientific context and, in turn, how we use the definition to scientifically assess and measure animal welfare (Duncan and Fraser, 1997).

However, no matter how it is defined, the term welfare is used to describe a characteristic of all animals, not something that is given to only those held in captivity, and it is usually (and throughout this thesis) conceived as a continuum as opposed to a state, varying from very good to very poor (Broom, 2002).

1.3.2 Defining welfare

Many of the early definitions of welfare embraced a wide range of factors (Duncan, 1996) with many researchers attempting to define welfare in a single sentence, but in so doing the definition often became vague or contradictory, and hid the complexity of the issue in hand (Rushen and de Passille, 1992).

Animal welfare scientists have therefore pursued different aspects of the welfare debate to hopefully find a more accurate definition, which in turn will aid the assessment of animal welfare. Historically, there have been three discrete research areas used when defining and assessing welfare, which correspond to three ethical concerns regarding the quality of life of animals. These define welfare using a

- 'biological function'-based approach,
- 'feelings'-based, or subjective, approach, or
- in terms of 'natural living' (Fraser et al, 1997).

*1.3.2.1 **Defining welfare using a 'biological function' based approach***

Disease, injury and malnutrition and other disturbances to the biological functioning of animals have been a common element in ethical concerns over the treatment of animals (Fraser et al, 1997). In addition there is common belief, particularly adopted by animal producers and veterinary surgeons, that good health is a major determinant of welfare in animals (Hughes and Curtis, 1997). This has therefore led some scientists to base the concept of animal welfare on the biological functioning of animals or health-related aspects (e.g. Broom, 1991).

Using these concepts, good welfare will be indicated by, and can be assessed by high levels of growth, reproductive success, and normal functioning of physiological and behavioural processes, which are ultimately demonstrated by high rates of longevity and biological fitness (Duncan and Fraser, 1997). Hence a great emphasis is placed on physiological responses when assessing welfare using this approach (Barnett and Hemsworth, 1990).

Function based approaches have been adopted by a number of research groups (Duncan and Fraser, 1997) for several reasons. Firstly, they may be accepted by default, because the main alternative, subjective states (1.3.2.2) are very difficult to assess scientifically (Gonyou, 1993) and therefore can be considered to fall outside the realm of scientific enquiry (van Rooijen, 1981 as cited in Fraser et al, 1997). At this time, biological functioning offers an adequate and convenient means of obtaining relevant information. Secondly, there are those who simply attach no importance to how an animal feels (McGlone, 1993). To view welfare only as a perceived feeling of ill health is too simplistic and inappropriate and therefore McGlone believes it is futile to pursue welfare assessment using any approach other than that of functioning. Thirdly, there are those who encompass both physiological (biological functioning) and psychological aspects (feelings and subjective experiences) of welfare but believe that welfare is governed by a hierarchy of needs, in order of importance; life sustaining needs and comfort sustaining needs (Curtis, 1987 as cited in Duncan, 1996). Needs here are defined as those requirements for normal development and maintenance of good health which are distinguished from desires predominantly concerned with motivation and experience.

However the function based definition is problematic. Firstly, biological indices commonly used to assess welfare can also been seen to rise during activities such as eating and copulation (Toates as cited in Dawkins, 2003). Secondly the use of this definition alone can infer good welfare where there are in fact problems, for example, selection, over generations, has increased the egg output of hens (*Gallus domesticus*). If we use a functioning definition alone, the welfare of this animal appears good as the hen is functioning at a high level. However, as egg production increases, the amount of calcium required for the process increases, resulting in leg weakness, injury and possibly death. Thus one could argue that welfare is in fact very poor (Appleby, 1999). Finally, if the emphasis of welfare is based on an order of priority i.e. life sustaining needs are more important than comfort sustaining needs, how do we justify the decrease in disease achieved through individual housing and benefiting a small proportion of individuals, over the resulting social and behavioural deprivation which affects a much larger number of animals (Rushen and de Passille, 1992)?

1.3.2.2 Defining welfare using a 'feelings ' or subjective experiences based approach

This approach is based upon a further ethical and moral concern we have for animals (Duncan and Fraser, 1997). It is generally accepted that it is only the higher animals to which the concept of welfare can be fully applied (Broom, 2002) and it is only these higher animals which are believed to have the capability for subjective experience and thus be sentient beings (Duncan, 1996). Therefore, Duncan (1996) argues that sentience is implicitly what welfare is actually about, and as sentience is directly related to feelings, welfare should be defined in terms of feelings.

In this conception, it is conscious states (feelings) which define welfare; the absence of states of suffering and the presence of states of pleasure denote good welfare, which is therefore the satisfaction of wants and desires rather than the satisfaction of needs (Duncan, 1996). So regardless of whether pigs, (*Sus scrofa*) for example, need straw bedding to maintain thermal equilibrium, if they want straw and it is unavailable then their resulting welfare will be compromised (Rushen and de Passille, 1992).

However, this approach is often refuted, particularly by positivist thinkers (Fraser et al, 1997) as it is not an approach which is simple and open to empirical study; the processes cannot be observed directly as can biological functioning (Duncan and Fraser, 1997). Rather, the scientific enquiry of subjective feelings requires an approach in which one must develop an understanding of unobservable processes, requiring additional logical steps and assumptions, which are then open to questioning and revision (Duncan and Fraser, 1997). However, this concept of welfare should not be viewed as a defeat, but rather as a challenge to understand (Gonyou, 1993). Importantly, it is now commonly accepted that animals do have subjective experiences such as fear, pain and frustration and that an animal's actions, preferences, vocalisations and physiological changes can provide an understanding of these subjective experiences (Fraser and Broom, 1997).

It has been argued that a definition of welfare, using subjective states, cannot be applied to all situations. For example, Broom (1991) provides five examples of situations in which welfare can be poor in the absence of suffering. These include the administration of endogenous opiates to prevent pain perception and a period of sleep which may interrupt a period of suffering. So in the first example, because the state of the animal has been affected through injury, its welfare is poor. However using only a feelings based approach, one would conclude that the welfare is good as there is an absence of perceived suffering achieved through analgesia. Similarly, in the second example, the injury would still persist throughout the period of sleep and hence the animal's welfare is still poor but again a feelings based approach would conclude that welfare is good. In these examples, Duncan (1996) believes that it is not necessary to consider welfare; it is more accurate to say that when an animal is in a state of unconsciousness and hence in a non-sentient state, it (temporarily) has no welfare status.

1.3.2.3 Defining welfare in terms of 'natural living'

In addition to biological functioning and feelings based approaches, animal welfare definitions have also included natural living. The welfare of an animal depends upon being allowed to perform its natural behaviour and live a natural life both through development and using natural adaptations and capabilities (Fraser et al, 1997). It is thought that if an animal is not kept in an environment in which it can fulfill its full behavioural repertoire (Kiley-Worthington, 1989 as cited in Appleby, 1999) and perform most types of natural behaviour (Webster et al, 1986 as cited in Duncan and Fraser, 1997) then its welfare will be reduced. Additionally, the environment in which the animal is housed must feature elements of the natural environment such as fresh air and sunshine (Appleby, 1999).

There are however shortcomings with such an approach. Whilst allowing a lion (*Panthero leo*) to chase natural prey species may be seen to enhance the welfare of the lion, it does not enhance the welfare of the prey, even though fleeing from a predator is, in turn, its natural behaviour. Moreover, not all behaviour within a repertoire will necessarily enhance welfare, as many activities allow adaptation to adverse conditions e.g. panting in the heat. So, keeping animals in an environment which allows them to perform their full behavioural repertoire e.g. high ambient temperature to induce panting, could in fact induce suffering. Similarly, natural environments in themselves are also difficult to characterise. For instance, how do we characterise the natural environment of the domestic dog which has changed considerably over the past 12, 000 years (Appleby, 1999)?

I suggest that Rollins' (1993) proposal that each animal had an inherent genetically encoded nature termed 'telos' is more useful. We do not need to provide or rear animals in the complete natural environment in which they evolved, but instead to promote and improve welfare, each animal should be raised in such a way that respects that animal's nature e.g. it is in the nature of canaries to fly, pigs to root and cattle to ruminate. Evolution in higher animals has favoured not just a simple repertoire of actions which is performed with a characteristic frequency but a set of conditional rules so in certain circumstances the animal responds with certain types of behaviour. Hence, if pigs fail to wallow in a given environment that does not necessarily indicate that welfare is poor but if the pigs are completely prevented from wallowing when hot then their nature is not provided for and consequently welfare is compromised (Fraser et al, 1997).

1.3.2.4 Integrative definitions of welfare

Whilst there are a number of researchers who focus on only one aspect of welfare (1.3.2.1-1.3.2.3), there are many who pursue a more integrative approach. Broom (2002), originally focussing on health aspects only, but now proposes a dualistic definition of welfare as 'a state of the individual as regards its attempts to cope with its environment - includes feelings and health'. Dawkins (2004) similarly captures both the biological functioning and feelings aspects of welfare through the questions: 'Are the animals healthy?' and 'Do they have what they want?' The latter question also addresses the natural aspect of welfare.

The five freedoms developed by Webster and revised by the Farm Animal Welfare Council (1992) incorporate all three aspects (1.3.2.1 – 3) within their definition, which is now widely used (Appleby, 1999).

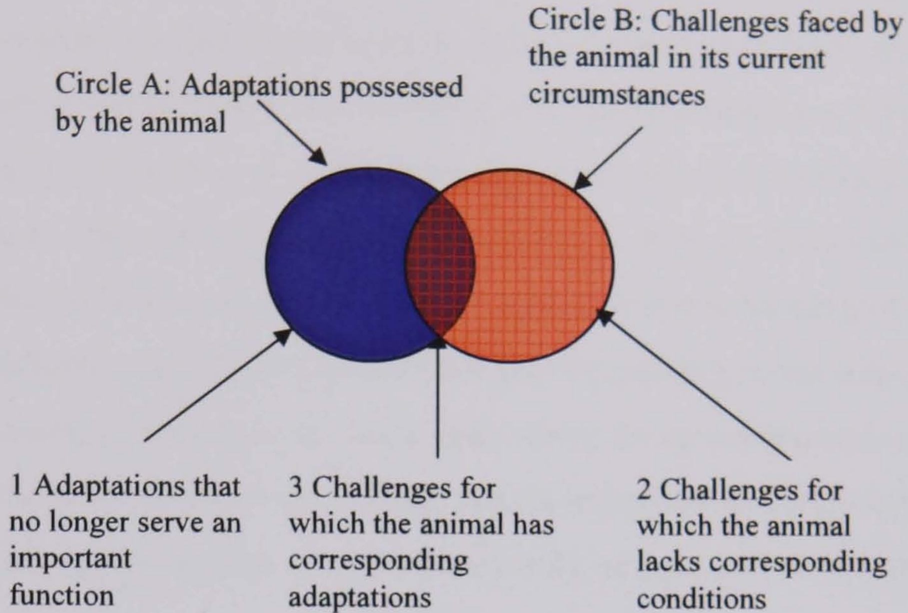
"Good welfare is achieved through five freedoms: freedom from hunger and thirst; freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and distress."

Independent of the definitions above, Fraser et al (1997) have designed a further integrative model which provides the guidance necessary for satisfactory animal welfare research. Their model is comprised of two circles A and B (Figure 1.1). Circle A represents a set of adaptations which are possessed by the animal, mainly resulting from evolutionary history, perhaps modified and augmented by genetic changes during domestication and via learning and development. Such adaptations include anatomical, physiological and behavioural changes. Further adaptations include subjective feelings e.g. cold and hunger which would motivate the animal to act in a particular way or stimulate further forms of learning. Circle B represents challenges faced by the animal in its current circumstances, including for example cold, exposure to pathogens and aggression from its pen mates.

If an animal's current circumstances correspond closely to the circumstances in which the genotype evolved and the individual developed then we expect close correspondence between the adaptations that the animal possesses and the challenge it faces. There would therefore be a large overlap in the two circles. However, for most captive animals there is an imperfect correspondence, leading to three distinct problems which taken together cover the major quality of life concerns that make up the subject matter of animal welfare. These three problems are labelled 1-3 on Figure 1.1.

Concerns regarding the subjective experience of animals will occur mainly in areas one and three. In area one, problems may arise when adaptations possessed by the animal no longer serve a purpose. For example preventing a strongly motivated behaviour, e.g. sucking in calves may not impair biological functioning but may result in negative subjective states. In area three, concerns about subjective experiences will occur only when the adaptations are inadequate for the degree of challenge e.g. a change in temperature may make an individual feel cold but biological functioning is also likely to be impaired. Concerns about biological functioning will also occur in area two because animals face challenges to which they lack corresponding adaptation. For example, air pollutants may impair biological functioning but subjective feelings may not be affected. Natural living concerns will occur in areas one and two where an animal's adaptations do not match the challenges it faces.

Figure 1.1 Conceptual model illustrating 3 problems encountered when adaptations possessed by an animal (Circle A) make an imperfect fit with the challenges it faces in the circumstances in which it is kept (Circle B) (Fraser et al, 1997).



1.3.3 A definition of welfare for military working dogs

Such differing views of animal welfare reflect the diverse concepts used by scientists to extrapolate their different value positions on to what constitutes a good or satisfactory quality of life for animals. It is apparent that the use of only one approach when assessing or measuring welfare is not satisfactory and thus a multi-dimensional approach may be the most satisfactory. This is what I have chosen to adopt for dogs. However, it may not be possible to incorporate natural living as it is arguably difficult to characterise the natural environment of the domestic dog. One such definition which encompasses biological functioning and subjective experiences is that of Broom (2002) stated earlier as '*the state of the individual as regards its attempts to cope with its environment*'. This state includes how much the animal is having to do to cope, the extent to which it is succeeding in or failing to cope, and its associated feelings (Broom, 1996). This is particularly relevant to this study since the majority of military working dogs are procured from domestic environments and placed into kennelled environments and their ability to cope with such a transition may clearly affect their welfare. Furthermore, Broom states that if the animal is failing to cope and hence the animal's welfare is poor, then the animal will be undergoing stress. Whilst there is no clear definition, stress is commonly defined as the biological response elicited when an individual perceives a threat to its homeostasis (Moberg, 2000). This response can be split into three general stages; recognition of the stressor, the biological defence of the stressor and the consequences of the stress response (Moberg, 1985). It is the consequences of this response that determine whether an animal is experiencing a brief episode in its life which will have no significant impact on its welfare or whether the stress response will have a deleterious effect on an individual's welfare. It is important to differentiate between these two outcomes. Whilst the former is referred to as stress, the latter state is referred to as distress. Stress and distress have often been used synonymously (e.g. Broom and Johnson, 1993) but Moberg (2000) believes that the term distress helps to differentiate between non-threatening stress responses, i.e. those which animals can adapt to or cope with, and a state where the stress response is severe and/or prolonged so has a deleterious effect on welfare. This is important as it highlights that an animal can be stressed but its welfare may not necessarily be poor and so it is important that this is acknowledged.

Broom's definition is useful, but does not directly address what the animals' 'want', which I believe an important part of welfare. An animal may want something which it is highly motivated to obtain, even if unable to have access to it. This can then in turn have negative effects on subjective states. This is encompassed by Dawkins' (2004) whereby welfare is defined as 'Are the animals healthy? Do they have what they want?'

Using both Broom's and Dawkin's definitions in conjunction, combines all the aspects of welfare most likely required when describing kennelled dog welfare:

'The welfare of an animal is affected by its interaction with the environment, how it attempts to cope with the environment, and the influence this has on biological functioning and subjective states'

This is the definition I assume throughout this thesis. Using this definition and the subject animal of interest i.e. military working dogs, if when moved from a domestic environment to a kennelled environment, it may be stressed temporarily but if it copes or adapts well and without detriment to health or subjective states, welfare will be good. However, if the dog is unable to cope because the stress response is severe and/or prolonged, it becomes ill, or experiences negative subjective states, then its welfare will be poor.

1.4 Indicators of stress and welfare

Having adopted a definition for military working dogs, it is now necessary to decide upon indicators which best reflect this. There are numerous indicators which have been used in a variety of species. I will now review the main ones and their past or potential use for dogs. As introduced in section 1.3.3, the stress response commences with a perceived potential threat to homeostasis. As a consequence, the central nervous system develops a biological response consisting of a combination of one, several or all of the following; the neuroendocrine response, the autonomic nervous system response, the immune response and the behavioural response (Moberg, 2000). These responses can provide potential methods for assessing stress and welfare state.

1.4.1 Physiological indicators of stress and welfare

Neuroendocrine, automic nervous system and immune responses include changes in the plasma concentrations of various hormones (Terlouw et al, 1997). These have direct effects on physiological systems providing a number of physiological indicators.

1.4.1.1 Glucocorticoids: Hypothalamic pituitary adrenal (HPA) system

In the majority of stress studies, the HPA system has been the primary neuroendocrine system monitored, with increases in Glucocorticoids (GCs) long associated with stress (Moberg, 2000). GCs are released primarily in response to activation of the HPA system following perception and evaluation of a stressor. When a stressor is perceived, corticotrophin-releasing hormone (CRH) is released by the hypothalamus. CRH then initiates the anterior pituitary to produce adrenocorticotrophic hormone (ACTH). This is carried in the blood to the adrenal cortex where GC's are released (Broom and Johnson, 1993). Their actions are essentially catabolic, stimulating the release of glucose, and are potentially harmful if elevated long-term, thus the response is controlled and regulated via a negative feedback loop within the HPA system (Janssens, 1994). Circulating glucocorticoids act directly on the pituitary to inhibit adrenocorticotrophic hormone (ACTH) secretion and on the hypothalamus to suppress corticotrophin releasing hormone (CRH) secretion.

It is important to note that, although widely used in the assessment of stress, GC levels can become elevated in situations which appear unrelated to stress. For example, increased GC levels can be induced by meals reflecting their involvement in homeostatic metabolic processes (Mormede et al 2007).. Furthermore, levels of GCs are also temporarily elevated during beneficial functional responses or those which are pleasurable such as during courtship, mating, active prey catching and social interaction (Broom, 2002).

In dogs, cortisol is the GC which has been used for welfare assessment. Cortisol levels can be measured in plasma (e.g. Hennessy, 1997; 2001), although compliance with the Animal Scientific Procedures Act (ASPA, 1986) is required and the amount of restraint required to take the sample is seen as a stressor in itself (Beerda et al, 1996) with an associated cortisol response seen between two and three minutes post handling (Kobelt et al, 2003, Tuber et al, 1996). Any handling effect associated with salivary cortisol collection is as yet unknown, although four minutes of restraint did not result in any associated cortisol response (Kobelt et al, 2003). Thus saliva collection is advantageous compared to plasma and has been widely used in dogs (Beerda et al, 1997, Coppola et al, 2006, Dreschel et al, 2005, Haubenhofer and Mrchengast, 2007 and Schalke et al, 2007). However, it may be of limited use in dogs of an unknown temperament which may become fearful or aggressive. Hence excretory products, faeces and urine, are a useful alternative. Whilst faeces have been used in dogs (Farca et al, 2006, Slotta-Bachmayr et al, 2007), the highest concentration of cortisol metabolites are excreted into the urine, sequestered over a two to three hour period (Schatz and Palme, 2001) thus urinary cortisol has been favoured and has been used as an indicator of acute stress in numerous studies (Beerda et al, 1999, Hiby et al, 2006, Stephens and Ledger, 2006) including one conducted by myself and others (Rooney et al, 2007b).

In that study we explored the validity of urinary cortisol as an indicator of stress by monitoring 31 Labrador Retrievers for seven days in a domestic environment and then for a further ten days once transferred into a military kennelled environment. We further manipulated the impact of this stressor by previously habituating half of the subjects to confinement in a kennel. We hypothesised that urinary cortisol levels would increase significantly in all dogs but would be mitigated by kennel habituation. Cortisol was observed to increase in all dogs but, as predicted, the increase was significantly greater in those dogs which had not been kennel habituated, thus leading to the conclusion that urinary cortisol is a valuable indicator of acute stress.

The previous studies all refer to the use of cortisol as an indicator of acute stress but during chronic stress where the duration of a stressor is long lasting or repeated at a regular/high frequency (Moberg, 2000) its value as an indicator is more problematic. When an animal is experiencing chronic stress and thus poor welfare, the functioning of the HPA system may undergo a number of changes leading to a sensitised adrenal cortex and when stimulated, a greater response in cortisol production is subsequently seen. So rather than measuring resting or basal levels which may not accurately reflect the dog's current stress status, when evaluating chronic stress, it may be more important to challenge the HPA system, achieved either by injecting ACTH (Raussi et al, 2003) or stressing the animals with an acute external stressor e.g. unfamiliar visual (toy car) and auditory stimuli (air blast) (dogs; Haverbeke et al, 2008b). However the sensitisation of the system is subject to debate and an increase in cortisol secretion may not be evident in all cases (Terlouw et al, 1997). In some cases where chronic stress is severe, the response to ACTH may become lowered leading to low circulating levels of cortisol and the response to a challenge being hypo responsive rather than hyper responsive (Vazquez et al, 2000 as cited in Gunnar and Cheatham, 2003).

In all the aforementioned studies of acute stress, cortisol excretion was determined by measurement of the urinary corticoid to creatinine ratio (C/C), a technique derived for the diagnosis of canine hyperadrenocorticism (Rijinberk et al, 1988). Creatinine is a by-product of muscle breakdown produced at a fairly steady rate in the dog, thus C/C ratios are measured to compensate for variation in body weight, urinary output, potential dilution by water in the collection vesicle and completeness of specimen collection (Crockett et al, 2000). However, the release of creatinine may be dependent upon activity; a slight decrease was observed following an hours treadmill exercise in beagles (Chanoit et al, 2002) whilst increases have been noted following exhaustive racing in sled dogs (Hammel et al, 1977) and racing in greyhounds (Snow et al, 1988). Therefore, although C/C ratios are used widely across a number of species e.g. cats (*Felis silvestris catus*) (Westropp et al, 2006), ferrets (*Mustela putorious furo*) (Schoemaker et al, 2004) and blue foxes (*Alopex lagopus*) (Korhonen et al, 2003), in dogs, the effects of intense exercise upon creatinine levels should be considered and, if necessary, controlled for.

It is important to acknowledge that urinary cortisol may be subject to diurnal effects as early morning levels have been shown to be significantly lower compared with those in the afternoon (Beerda et al, 1996) so peaks may be evident in an non-stress situation, but this diurnal effect is not observed in every case (Horvath et al, 2007, Koelvska et al, 2003).

1.4.1.2 Catecholamines: Sympathetic adrenal medulla (SAM system)

The release of the catecholamines; noradrenaline and adrenaline is dependent upon the autonomic nervous system (the involuntary nervous system) made up of two systems, the sympathetic and parasympathetic, acting in opposition. Sympathetic activation inhibits digestive function and stimulates cardiac output whilst parasympathetic activation does the reverse. Plasma noradrenaline is principally released by sympathetic nerve endings with a lesser amount released by the adrenal medulla whereas adrenaline is exclusively secreted by the adrenal medulla.

The relative activity of each of the systems is dependent upon the situation, the individual and the type of stressor to which the organism is exposed. The resulting balance of the two systems determines whether the heart rate increases or decreases in response to the stressor (Terlouw et al, 1997) and prepares the animal for activity. Therefore, when using the SAM system to measure stress and welfare, both catecholamine levels and heart rate can be used as physiological indices.

As seen with the collection of plasma cortisol, there are similar concerns regarding the use of plasma noradrenaline or adrenaline levels to measure stress and welfare, although plasma is widely used in several species e.g. cows (*Bos taurus*) (Lay et al, 1992), sheep (*Ovis aries*) (Parrot et al, 1994) and reindeer (*Rangifer tarandus tarandus*) (Sakkinen et al, 2004). Beerda et al (1996) used an insulin-induced hypoglycaemic model to stimulate the SAM system in dogs, thus providing a valid paradigm to correlate plasma catecholamine responses with those in urine. Unfortunately, their study failed to validate urinary catecholamine as a non-invasive alternative to acute catecholamine in plasma although, in a group of five dogs from the same study, a significant correlation was seen between insulin induced plasma adrenaline responses and post treatment adrenaline/creatinine ratios. However, recent research, using dogs, has demonstrated an increase in urinary epinephrine and norephinephrine associated with a hospital visit (Kook et al, 2007), suggesting that urinary catecholamines may be useful for the measurement of acute stress as they are in pigs (Hay et al, 2001) and Asian (*Elephas maximus*) and African (*Loxodonta africa*) elephants (Dehnhard, 2007). This still requires further validation.

1.4.1.3 Heart Rate

Heart rate can also be used to measure the activity of the sympathetic nervous system (1.4.1.2). In dairy cattle, heart rate has been observed to increase following isolation of an individual from the familiar herd (Hopster and Blokhuis, 1994). Similarly, Sakkinen et al (2004) saw an effect of manual blood sampling on the heart rate of reindeer. In dogs, heart rate has been used to measure the response to an electric shock collar (Schalke et al, 2007) and has been observed to elevate in anticipation or response to stressors e.g. loud noise (Engeland et al, 1990) and air/road transport (Bergeron et al, 2002). Problems originally associated with invasive devices have been resolved through use of telemetric devices e.g. Polar Sport tester (Vincent and Leahy, 1997) attached to straps or harnesses although substantial habituation may be required for such devices.

1.4.1.4 Prolactin

Prolactin, an anterior pituitary hormone involved in numerous roles but principally lactation, has been used to measure stress in a number of species. For example, when investigating the response of sheep, prolactin was seen to rise in response to transport simulation but not to the physical stress of standing in water (Parrott et al, 2004). Thus, the response may be stressor-specific, limiting its use as a stress measure. However, in an experiment on rats (*Rattus norvegicus*), Kant et al (1983) suggested that prolactin may be more useful than corticosterone (the principal GC in rodents) for assessing the intensity of a wide range of stressors. Whereas corticosterone was seen to be sensitive to only mild stressors, and even reached maximal levels following exposure to these, prolactin appeared to be more sensitive over a larger range of stressors, and not just those which were mild. However, the use of this measure in dogs is yet to receive investigation

1.4.1.5 Stress-induced hyperthermia

Stress-induced hyperthermia, a rise in body temperature, occurs in rats and humans in response to stressful situations (Terlouw et al, 1997) and has been observed in pigs following mixing (de Jong et al, 1999). However, as a reliable measure, it may be limited. When comparing the effects of manual blood sampling to remote blood sampling in reindeer, Sakkinen (2004) saw a significant increase in body temperature during manual sampling. However, the readings taken were just under the skin and thus unlikely to represent the core, and it is likely that the increases were related to the physical activity of the animal and the ambient temperature as opposed to a stress related change. Whilst this problem could be alleviated by measuring the core temperature, implantation of temperature monitors in the peritoneum of animals would be required. This is an invasive procedure, and therefore research of this kind would have to comply with the ASPA (1986). The use of temperature as a measure of welfare in dogs may be complicated and is possibly why there is not yet research in this area.

1.4.1.6 Immune function

Immunosuppression can lead to an increased susceptibility to both disease and infection, although the exact mechanisms underlying the immunosuppressive effects of stress are still not fully known (Terlouw et al, 1997). Activation of the HPA system in response to stressors is known to affect the immune system in two ways: reducing lymphatic tissue size and reducing the number of circulating lymphocytes. This is mediated by the binding of glucocorticoids to cytoplasmic receptors and the movement of the steroid receptor complex into the nuclei, altering enzyme activity or destroying the nucleus (Jarvis, *pers comm*).

For example, a five minute snare restraint in pigs reduced the circulating lymphocyte population by 15% (Dubreuil, 1990) whilst the mixing and transporting of pigs was seen to reduces the immune response to a mitogen challenge of phytohaemagglutinin (Ekkel et al, 1995). However the change in immune function does appear to be dependent upon the nature of the stressor. Minton and Blecha (1990 as cited in Coppinger et al, 1991) subjected lambs to both acute heat stress and a six hour period of restraint and isolation. This did not result in any measurable change in immune function but when Coppinger et al (1991) repeatedly applied the same stressors they saw a reduced lymphocyte response to an antigen challenge.

Whilst the methodology is apparently validated in a number of farm species, the use of immunocompetence as a measure of stress in dogs is yet to be validated (Beerda et al. 1999) although recent studies suggest it may be useful; dogs transported by both air and road revealed an increase in neutrophilia and lymphopaenia (Bergeron et al, 2002).

As immune suppression can lead to increased susceptibility to both disease and infection, the prevalence of disease or infection may be useful indicators of welfare. For example, diarrhoea can be a clinical sign of colitis or inflammatory bowel disease which is often caused by stress (Bush, 1995). Thus recording the occurrence of such symptoms may be useful for welfare assessment in the dog.

1.4.1.7 Blood plasma

Characteristics of blood plasma may be affected by stress. An experiment by Dubreuil (1990) stimulated the increase of plasma sodium, potassium, calcium, phosphorus, cholesterol and glucose and decreased plasma free fatty acids in pigs via restraint and elevation. Similar to previous indicators, compliance with the ASAP 1986 would be required.

1.4.1.8 Average daily weight gain

Average daily weight gain is frequently used by veterinary surgeons and farmers as an indicator of welfare in farm animals; good welfare will be indicated and can be assessed by high levels of growth (1.3.2.1). This measure may not be as relevant in the adult dog as weight is unlikely to change over such a short period of time. However it may be useful for longitudinal studies or during those whereby weight may be expected to change e.g. change in exercise, working pattern.

1.4.1.9 Summary of physiological indicators

Based upon the physiological indicators reviewed, cortisol (1.4.1.1) is likely to be the most useful as it has received the most investigation within the dog. Furthermore, the collection of urinary cortisol should mitigate any of the risks associated with saliva and does not require compliance with the ASPA (1986) unlike the collection of plasma. Although some training will be required, this should be minimal in comparison to that required for heart rate monitoring.

However, some of the dogs described in this thesis may experience chronic stress having being kennelled for long periods of time. As the HPA system may change in situations of chronic stress, it is important to determine whether basal levels of C/C or levels of C/C in response to a challenge are the most indicative of long term stress in dogs. Therefore a study was undertaken to investigate which measure should be taken to assess chronic stress. This is described in Chapter 2.

1.4.2 Behavioural indicators of stress and welfare

Whilst some physiological indicators appear to have great potential as indicators of welfare, e.g. cortisol (1.4.1.1), there are associated disadvantages with their use, most notably, their response to non-stress related and pleasurable situations, cost and potential variations with age, breed and sex. However, behaviour has been shown to be an easily observable manifestation of stress and thus welfare, providing information about the animal's needs, preferences and internal subjective states (Mench & Mason, 1999). Additionally, it is believed to be non-invasive and non-intrusive with many observations causing little disturbance to the subject (Dawkins, 2004).

In the following section, I introduce six different types of behaviour which are used commonly as indicators of poor welfare; displacement behaviour (1.4.2.1), learned helplessness (1.4.2.2), activity or hyperactivity (1.4.2.3), vacuum behaviours (1.4.2.4), stereotypies (1.4.2.5) and an absence of normal behaviours (1.4.2.6) (Friend, 1990). In addition to these categories, vocalisations (1.4.2.7) are discussed and an overview of behaviours used in past studies of dog welfare is given (1.4.2.8).

1.4.2.1 Displacement behaviours

This type of behaviours is characterised by the fact that they are both recognisably similar to, and derived from, the species' typical motor patterns, and are movements which appear to be totally irrelevant to both the behaviour preceding and succeeding them (Tinbergen, 1952 as cited in MacFarland, 1999). Such behaviours tend to occur in situations where an animal is motivated to perform two or more behaviours which are in conflict with one another e.g. displacement feeding by cocks during fighting and displacement grass pulling by herring gulls (*Larus argentatus*) during a territorial dispute (MacFarland, 1999). In domesticated species, laboratory rats have been shown to perform displacement grooming in response to 10 minutes of white noise stress (Windle et al, 1997). Dogs housed in relatively high austerity perform displacement behaviours e.g. drinking, when approached by a familiar person, presumably because they are in conflict as to whether to approach or retreat (Beerda et al, 2000).

1.4.2.2 Learned helplessness

Animals unable to escape from a frightening stimulus or those experiencing chronic unavoidable stress may develop a state of learned helplessness whereby the animal is no longer able to make the appropriate behavioural responses to its environment (Seligman, 1975 as cited in Friend, 1990). In such a state, the animal becomes apathetic and shows an overall decrease in responsiveness to its environment. The learned helplessness effect has been observed in rats and there is some evidence in mice (*Mus musculus*), cats, goldfish (*Carassius auratus*), pigeons (*Columba palambus*), chickens and humans (Job, 1987). There is the possibility that dogs kennelled over a number of years may also develop a state of learned helplessness (Wells et al, 2002a), and Taylor and Mills (2007) surmise that a lack of control over the kennelled environment may result in this state.

1.4.2.3 Activity

Activity, and specifically hyperactivity, has been used as a measure of welfare (Friend, 1990). Activity, the time spent moving or inactive, the time spent resting, are both common indicators in studies of kennelled dog welfare (Campbell et al, 1988, Clark et al, 1997, Graham et al, 2005a &b, Hubrecht, 1993a; 1995, Hubrecht et al, 1992, Hughes et al, 1989, Mertens and Unshelm, 1996, Wells and Hepper, 1992; 1998; 2000, Wells et al, 2002a & b). Findings include, for example, that dogs housed in the most austere conditions display high levels of locomotory activity, and following a mild disturbance, further increases are observed (Beerda et al, 2000).

1.4.2.4 Vacuum behaviours

Vacuum behaviours are those behaviours performed in the absence of suitable stimuli, and as a result, it is thought that the animals carrying out these behaviours are highly motivated to do so (Dawkins, 1988). Classic examples of vacuum behaviours include the attempts of a prenatal crated sow to build a nest in the absence of bedding, and chickens attempting to dust bathe in the absence of any substrate (Petherick and Rushen, 1997), non-nutritive teat sucking in calves (de Passille and Rushen, 1997) and vacuum chewing (mock leaf-feeding behaviour) by giraffes (*Giraffa camelopardalis*) in the absence of browse (Baxter and Plowman, 2001). The use of vacuum behaviours as a measure of welfare in dogs is not reported however.

1.4.2.5 Stereotypies

A stereotypy is defined as a behaviour pattern that is invariant and repetitive, with no obvious function or goal (Mason, 1991). These behaviours are commonly suggested to indicate welfare problems, as they develop in situations where an animal may be frustrated, stressed, fearful, restrained or receiving a lack of stimulation (Mason, 1991). In light of this, stereotypies are often used to assess welfare (Mason and Latham, 2004). For example, a scale developed by Broom and Johnson (1993) linked the frequency with which stereotypies are performed with welfare; welfare is defined as very good if an animal performs none or only an occasional stereotypy caused by a minor frustration, but is very poor if the animal performs stereotypies for 40%, or more, of the time. However, there is now a growing body of evidence which links stereotypic performance to relatively good (less poor) welfare, suggesting that stereotypies may not always be accurate indicators of poor welfare and suffering. Some stereotypers in an environment have been shown to have better welfare than non-stereotypers. Mason and Latham (2004) suggest that there are four processes which could explain the link between stereotypies and good welfare; including the performance of stereotypies as a means of 'do it yourself' enrichment, whereby these behaviours allow animals to express and perform natural behaviours. Secondly, stereotypies may be performed because of their 'mantra' effect i.e. repetitive actions serve to calm and change moods. Thirdly, stereotypies may become habitual, their performance no longer relates to the current state of welfare but instead control of the behaviour shifts into a form of central processing. Here the behaviour is performed with minimal cognitive processing and can become triggered by a wide range of cues. The final explanation is related to perseverative behaviour whereby stereotypies are symptoms of altered behavioural control. Animals produce responses which are inappropriate to cues or signals because the whole animal's behavioural repertoire has been altered. In addition, there are other situations, not linked to welfare, which can elicit stereotypies. For example stereotypies may be seen as a result of social facilitation in bank voles

(*Clethrionomys glareolus*; Cooper and Nicol, 1991). In Mason and Latham's review, it was concluded that stereotypies are valuable indices of welfare but care should be taken in their use and more importantly the motivational basis should be understood.

Stereotypies have been used in a number of dog welfare studies and are considered indicative of prolonged stressful situations (Beerda et al, 1997) i.e. during chronic stress. In kennelled dogs, classic examples include repetitive pacing (walking or trotting back and forth along a boundary line), circling (walking or trotting around pen), spinning (turning in a tight circle pivoted about hind legs), and wall bouncing (jumping at wall and rebounding); all are observed in dogs kept in restricted environments over a long period of time e.g. rescue shelters (Hubrecht et al, 1992). Similarly, they have been observed in military working dogs. Hiby (2005) observed 46% of dogs stereotyping whilst Denham (2007) observed 93%. In Denham's study, the incidence of stereotyping, both in response to, and in the absence of, arousing stimuli were observed in attempt to explore its motivational basis. The numbers of dogs stereotyping varied substantially according to the stimulus presented with form and duration of stereotypy varying also. For example, only 16% of dogs performed stereotypies when alone with all bar one circling whilst over 70% of dogs stereotyped when a care assistant walked past and of these over half performed a spin, bounce combination.

1.4.2.6 Absence of normal behaviours

When animals are undergoing stress or disturbance, it is not uncommon for some behaviour to be suppressed. Behaviour associated with play and exploration may well be affected by stress (Mench and Mason, 1997) as both of these are very sensitive indicators of an animal's internal state (Friend, 1990) and are thought to be luxuries which are inhibited during stressful situations (Mench and Mason, 1997). For example, in one study, sixteen laboratory cats (*Felis catus*) were first given a 10 day baseline period of standard predictable procedures. Half the group was then subjected to an unpredictable care routine for 21 days. This resulted in both play and active exploration being suppressed (Carlstead et al, 1993a). Similarly, the movement of captive leopard cats (*Felis bengalensis*) from one barren home environment to a novel barren environment also resulted in the suppression of exploratory behaviour (Carlstead et al, 1993b). Conversely, the presence or increase in play and exploratory behaviour may suggest an improvement in welfare. The level of play behaviour of pigs housed in enriched environments was greater than that of pigs housed in barren environments (Bolhuis et al, 2005) demonstrating the utility of play as a behaviour of both good and poor welfare.

1.4.2.7 Vocalisations

A behavioural category not included in Friend's list (1990) was vocalisation, most likely because until recently there has been very little literature regarding auditory communication in domestic animals (Watts and Stookey, 2000). However, vocalisations are often given in response to stressful situations and may well be of great significance in determining welfare. The use of vocalisations in the assessment of pain[1] is relatively common. For example communication by vocalisation is used as a means of assessing pain in response to tail docking and castration of lambs (Molony and Kent, 1997). Similarly vocalisations in response to the docking of three to four day old pups were recorded at the time of the procedure and at a number of intervals following the procedure until the puppy went to sleep (Noonan et al, 1996). The responses in this study did suggest that puppies do feel pain and the greater the intensity and duration of vocalisations during the procedure, the longer the recovery period and time taken to sleep. Barking as a means of communication is heavily relied upon in the domestic dog and has been used as measure of welfare in a number of studies (see below).

1.4.2.8 Behavioural indicators of stress and welfare in the dog

A number of studies have identified potential behavioural indictors of stress and welfare in dogs which in addition to previous behaviours (1.4.2.1 – 7) may be of use in this thesis. Indicators tend to have been split into two types; in response to an acute stressor where the stimulus is short lived (1.4.2.8a) or in response to a chronic stressor where the stimulus is prolonged or repeated at a regular frequency over an extended period of time (1.4.2.8b)

a) Behavioural indicators of acute stress and welfare

The administration of an acute acoustic stressor to a group of laboratory beagles resulted in the increased performance of tongue out, snout lick, paw lift, lowered body posture and body shake (Beerda et al, 1997), behaviours similarly observed in response to harsh training methods (Schwizgebel, 1982 as cited in Beerda et al, 1997). Additionally, the lowering of

[1] Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (International Association for the Study of Pain 1979).

body posture was observed consistently across a variety of noise intensities, suggesting that it may be the most reliable indicator of acute stress compared with other behaviours.

In section 1.4.1, I introduced a study conducted by myself and others (Rooney et al, 2007b) during which 31 Labrador Retrievers were transferred from a domestic environment into a military kennelled environment. In addition to urinary cortisol indicators, we also measured behaviour. Over a ten day sampling period, starting the day after the dogs arrived into military kennels, the behaviour of each dog was recorded for 30 minutes a day. Over the ten day period, some behaviours were noted to change consistently. For example vocalisations, paw lifting and panting decreased, whilst grooming increased. The changes in panting, paw lifting and grooming are likely to indicate an improvement in welfare, as all three measures have previously been linked to stress (panting; Beerda et al, 1997, Hiby, 2005, paw lifting; Hiby, 2005, grooming; Friend, 1990, Hiby, 2005). However the decrease in vocalisations, whilst potentially indicating an improvement in welfare, is more likely due to the carer not responding to the behaviour and hence not reinforcing it, leading to extinction.

b) Behavioural indicators of chronic stress in the dog

In addition to the performance of stereotypies (1.4.2.5), several other behaviours appear to be performed at increased frequencies during prolonged stressful situations; low postures, urinating, nosing, excessive auto-grooming, paw lifting, coprophagy (eating of own faeces), vocalising, locomotory activity (Beerda et al, 1999; 2000), inactivity (Hubrecht et al, 1992) and barrier manipulation (Hetts et al, 1992). Furthermore, dogs undergoing chronic stress show a range of behaviour in response to situations of mild disturbance (Beerda et al, 2000). For example, after the slamming of a door, dogs reacted with increased locomotor activity, circling and nosing, additionally displaying behaviours indicative of acute stress: body shaking, yawning, ambivalent postures and displacement behaviours.

1.4.2.9 Summary of behavioural indicators

I have reviewed a number of behavioural indicators and many appear easily recordable in the kennelled environment; activity, stereotypies, vocalisations, play, exploration. Past studies of dog welfare have also revealed other indicators; panting, grooming, coprophagy which may be of use. These established behaviours will be used to measure the welfare of kennelled dogs within this thesis but the behaviour of kennelled dogs may vary depending upon the time of day at which it is recorded and may also be affected if the animal is aware of the presence of an observer. Within a kennelled working dog environment, diurnal activity may be strongly influenced by husbandry events occurring at specific times during daylight hours, so it is likely that dog behaviour will change substantially depending upon the time of observation. Furthermore, many dogs greatly value human companionship, which in a kennelled environment is rare, and so may react to the presence of an observer. Hence Chapter 3 describes a study in which the behaviour of kennelled dogs was compared when sampled in the presence of a person and when filmed remotely and also when filmed at two different times of day, in order to determine how and when best to observe the behaviour of kennelled dogs.

1.4.3 Preference Testing

Preference testing is a key technique used in animal welfare as it provides a distinct method of getting animals to express, through behaviour, how important both environments and commodities are to them (Dawkins, 1998) and what they want (Dawkins, 2004). At this present time, most of the research in this area has been conducted using farm and laboratory animals (Kirkden and Pajor, 2006).

An animal's preference can be measured in two ways; either using single choice tests where it is offered choices between alternatives, or via operant conditioning where animals are trained to make a response such as pecking a key or pressing a lever to either gain access to or avoid certain resources or consequences (Kirkden and Pajor, 2006). Once a preference has been determined this method can then be used to ascertain what cost an animal will pay (either by key pecking or lever pressing) for a reward (e.g. access to pen mates or bedding) which measures the value the animal places on a particular resource. If the animal continues to pay and works harder even though the cost has increased then it can be said that the animal places high value on that particular resource. In this situation the animal is showing *inelastic* demand, typical for resources which are 'necessities'. If however the animal stops paying when the work load increases, the animal is showing *elastic* demand and this is typical of 'luxurious' resources (Dawkins, 1990). Elastic and inelastic demands can be represented diagrammatically as curves. Slopes with inelastic demands will have shallow gradients and elastic demands conversely have steep gradients. Analysis of these curves, as proposed by Dawkins (1988), then provides an objective measure of motivational strength and allows comparison between motivations for different activities or resources. This interpretation is however subject to some debate. Houston (1997) argues that a better measure of welfare would be to take the area under the curve, an approach taken by welfare economists. This is open to error as a slight difference in experimental procedure may affect the area and more so the gradient of the curve holds important biological information which would otherwise be lost (Dawkins, 1997). However, Houston's method is rarely used (Jensens et al, 2004).

In addition to mathematical issues there are a number of methodological issues surrounding the use of preference testing when generating demand functions. Jensens et al (2004) showed through a number of discrete experiments that the degree to which the target behaviour can be performed, the duration of access to perform the target behaviour and the social context may all affect elasticity. In addition when implementing operant conditioning, the response may be more difficult to train in some animals than in others, and those choices seen in the short term may not necessarily reflect those which would be made in the long term (Dawkins, 1988). This is a significant problem, particularly when trying to test behavioural priorities in experimental subjects and extrapolate the results to those animals kept commercially. Cooper (2004) has therefore discussed in depth some practical measures which will aid in increasing the external validity of results in captive farm animals. Recommendations include using experimental farm animals which have been reared under commercial conditions, subsequently housed in the test apparatus and then given free access to all resources typical of animals in commercial environments. However, there are problems with these types of long term closed economies, as the animal, if not controlled, will often spend much longer with a resource if it has had to work hard to get to it. This invalidates the calculation of demand elasticity (Jensens et al, 2004). To further increase validity, when training animals to given conditioned responses, naturalistic tasks should be used as they are likely to be easier to train e.g. weighted doors or narrow gaps. Similarly the cost should be as alike as possible to those types experienced in commercial husbandry systems.

It is obvious to see that the use of preference testing, like a number of other behavioural parameters, is not necessarily straightforward, although as a methodology it is highly beneficial when trying to understand subjective states and motivation. Therefore, these caveats call for great care when embarking upon such an experimental procedure. As of yet there are no published studies describing studies using domestic dogs.

1.4.4 Conclusion

This section has demonstrated the variety of physiological and behavioural parameters associated with the assessment of animal welfare and highlighted the importance of a multidisciplinary approach. For this reason, I have used a range of behavioural indicators in conjunction with urinary cortisol for the welfare assessment of subjects in this thesis. Preference testing was not used due to financial constraints and the time which would have been required to train operant conditioned tasks necessary for the use of such procedures.

1.5 Factors likely to affect welfare of Military Working Dogs within a kennelled environment

In section 1.2, the subject species was introduced and in the sections thereafter the concept of, and the measurement of, welfare was described (1.3 & 1.4). This section now links the two topics together by discussing the welfare of military working dogs. The first part (1.5.1) discusses the procurement of military working dogs and how the transition from a domestic environment to a kennelled environment can impact on their welfare. The second part (1.5.2) then looks specifically at the factors within the kennelled environment that may affect welfare. I then discuss whether welfare is compromised (1.5.3) and how welfare may affect the working ability of military working dogs (1.5.4).

1.5.1 Procurement of military working dogs and its impact on welfare

Currently the predominant sources of potential working dogs for the UK military are rescue shelters and donations from the general public (1.2.2). Most of these dogs will have been housed in a domestic environment at some point in their life with little, if any, experience of a kennelled environment. Within most domestic environments, there are many opportunities for dogs to explore, investigate and to interact socially with both humans and other species. In contrast, military working dogs during training, and the majority once operational, will be housed singly in kennels and will remain in kennels for a significant proportion of each day during their working life. Unlike most domestic environments, the kennelled environment limits both the time and control over exploration, investigation and interactions. Such an environment may not provide the facilities which dogs need to meet their behavioural needs and likewise may not correspond to the individual's expectations (Poole, 1992).

Thus the transition from a domesticated environment to a kennelled environment is likely to be stressful and our previous study (Rooney et al, 2007b) confirmed that this was the case. In this study, the mean level of urinary cortisol was significantly greater following the transition into a kennelled environment compared to when in the domestic environment. However, the levels of cortisol in dogs which had been gradually habituated to the kennel environment were significantly lower than dogs which had been reared solely in the home environment (1.1 and 1.4.1.1). This shows that previous experience of a kennel environment can mitigate the stress response, but because the levels were still significantly higher than when in the home, this suggests that there must be other factors, in addition to the kennel per se, within the kennelled environment that induce stress responses.

Furthermore, the group reared solely in the home still had significantly elevated levels of cortisol following ten days of kennelling when compared to levels in the home, and slightly elevated levels after 12 weeks of kennelling. This suggests that some dogs were unable to cope with the kennelled environment, most likely due to factors of housing and husbandry which those dogs found persistently stressful and were regularly exposed to and thus their welfare was compromised. The following section discusses factors of housing and husbandry which may influence a dog's ability to cope, and its welfare, within a kennelled environment.

1.5.2 Factors of housing and husbandry which may influence welfare

1.5.2.1 Space allowance

Within UK military kennels the space allowance currently ranges between 5.0m^2 and 6.5m^2 depending upon the type of kennel. This is greater than the space provided to the majority of singly housed dogs in research institutions (see Appendix 1 for an overview of minimum space allowance per dog (m^2) in research institutes) but not dissimilar to the space allowance provided to dogs housed in rescue shelters (5.0m^2 and 6.0m^2) although those both smaller and larger have been reported (Taylor and Mills, 2007).

Studies assessing the effects of space allowance have been criticised (Taylor and Mills, 2007) for a number of reasons including inadequate sample size, but it should also be noted that in some studies the range of welfare indicators have been limited and the difference between cage/pen sizes insufficient. For example, early work by Hughes et al (1989) used only activity as a measure of welfare, and as an increase in time spent active was not observed they concluded that the welfare of laboratory dogs was adequate in smaller laboratory cages. In addition, they only compared a small number of cage sizes which differed very little in space allowance to one another ($1m^2$ vs. $2m^2$). Nonetheless, more recent studies have shown that dogs housed in the smallest areas (cages) spent less time moving and more time manipulating barriers when compared to dogs in pens and runs, (Hetts et al, 1992). Furthermore, dogs housed in small enclosures have been shown to have a high prevalence of stereotypic or abnormal behaviours (Hubrecht et al, 1992) which may result in tail tip lesions as observed in Belgian Malinois, a common breed of military working dog within the US. Malinois, housed in small kennels, are reported to develop tail tip lesions or abrasions on the side of the tail from the constant beating of the tail as the dog's circles in the kennel and scrapes the tail on the wall (Jennings, 1991). In contrast, when dogs are housed in larger areas, a wider range of behaviour is observed. In a study by Hubrecht et al (1992) trotting and running was seen. Inadequate space within the kennel area may result in compromised welfare as both movement, and the repertoire of locomotion may be limited.

1.5.2.2 Kennel design and layout

Across laboratories, rescue shelters and military working dog kennels, there is great variability in design and layout of kennelled housing. This can greatly impact upon the welfare of dogs, and in particular the ability to make social contact with other dogs and humans. Whilst it is recommended that kennels used in laboratories and research institutions provide visual, auditory and olfactory contact with other dogs (Prescott et al, 2004), many kennels within the military working dog environment restrict or prevent visual contact (Figure 1.2). If unable to see out of the kennel, and thus make contact with other dogs, dogs may spend a lot of time standing on their hind legs, or develop stereotypical jumping behaviours as a result of frustrated attempts to see out of the kennel (Hubrecht, 1993a). In contrast, there may be some dogs which also become frustrated because they cannot exercise control over their social interactions by moving out of sight of each other (Bebak and Beck, 1993).

Figure 1.2 Design and layout of kennels and their effect upon visual contact

a) Visual contact with adjacent dogs restricted

b) Visual contact with adjacent dogs prevented

1.5.2.3 Provision of bedding

A lack of bedding and the consequence that the dog may become chronically cold, can adversely affect welfare (1.5.2.5). The provision and type of bedding varies considerably in kennelled environments and is often seasonally dependent. Nonetheless, in addition to warmth and comfort, bedding can enrich the environment. Straw can be olfactorily stimulating whilst fleece bedding can provide some outlet for chewing behaviours, although there are financial implications associated with its use.

A preliminary result from an experiment by Heath (as cited in Prescott et al, 2004) confirms that bedding provided to a laboratory dog is well utilised and soft shredded material seems to be favoured. Bedding may also provide health benefits as it offers protection to pressure points which may otherwise become hyperkeratotic or fluid filled as observed in military working dogs which lie for extended period on hard surfaces (Jennings, 1991). It may also decrease the incidence of self-mutilative behaviours which often result from repetitive licking of ulcerated areas (Prescott et al, 2004). The provision of bedding may well improve welfare; dogs may feel warmer but health may also be improved.

1.5.2.4 Noise

Kennel establishments are typically very noisy environments, with readings commonly in excess of 100dB and often reaching 125 dB (Sales et al, 1997). Whilst most noise is produced by the dogs themselves, other events including cleaning and outside noise sources contribute to the acoustic environment (Sales et al, 1997). Barking is commonly thought to result from territorial rivalry, with social facilitation resulting in barking spreading to other dogs (Fox, 1971 as cited in Sales et al, 1997). However the presence of humans will stimulate dogs to bark, and likewise anticipation of specific events (Gamble, 1982, Denham, 2007). The domestic dog is more sensitive to sound than man, detecting sounds ranging from 40Hz up to around 50KHz (Sales et al, 1997). Whilst there is little published information there is some evidence that noise may be a stressor to dogs (Treptow as cited in Gamble, 1982). Glycaemic increases in a litter of dogs were seen following exposure to 80dB over 5-10 minutes. In other species, noise is noted to cause damage and stress to humans, and can cause seizures in rats and mice. Several species become startled and their activity is reduced and levels exceeding 80dB can disturb hormonal, haematological and reproductive parameters whilst levels exceeding 95dB disrupts lipid metabolism and causes atherosclerosis in rats (Gamble, 1982).

Conversely, some acoustics are commonly believed to help dogs relax within a kennelled environment and this is reflected by many establishments having a radio present within the kennelled environment. In a recent study comparing five different types of auditory stimulation (human conversation, classical music, heavy metal music, pop music and control), dogs were observed to spend more time resting, less time standing and vocalising when classical music was played compared to any other type of auditory stimulation (Wells et al, 2002b). In contrast, heavy metal music was linked to a longer duration of barking compared to any other type of auditory stimulation. Classical music therefore appears to have beneficial effects upon the welfare of kennelled dogs. However, for reasons above, the level at which it is played may be of importance.

1.5.2.5 Temperature

If provided with an adequate source of both food and water, and given a suitable period of time to acclimate, dogs are extremely adaptable to wide ranging temperatures (Prescott et al, 2004). Nevertheless, many kennel designs have not been built with the consideration of extreme temperatures. The Animal Boarding Establishments Act (1963) and Joint Services Publication 315 (Military document providing guidance for kennelling) state that the temperature within the kennel area should never drop below 10°C. As many military kennels are not heated, it is not uncommon for temperatures to drop below zero in winter particularly those located in the northern UK. In other animals, chronic exposure to low temperature elicits responses similar to that of chronic stress. Ten variables were measured in young male rats at room temperature and after five days in the cold (5°C) (Strack unpublished data as cited in Dallman, 2001). During the cold period, body weight did not increase as it did at room temperature, even though food intake increased. However, even though food intake increased it was not at a maximal level and the animals showed a marked reduction in calorific efficiency. The cold conditions activated the HPA system to a slight degree, demonstrated by the elevated trough levels of morning plasma cortiscosterone, increased adrenal weight and decreased thymus weight. Combining all measures, the results demonstrated that the animals were stressed during the cold treatment. Dallman states however that the rats did cope well during the treatment even though they exhibited signs of pain, distress and suffering. The animals used both their physiological and behavioural repertoires to counteract the potentially harmful effects of the stress or challenge. However it is unlikely that this would have been sustained over a longer period so their welfare would most likely have become compromised.

In one study of potential military working dogs, cold temperatures were negatively linked to urinary cortisol levels, suggesting that dogs may be physiologically stressed in periods of cold weather (Hiby, 2005).

If we consider the other extreme and look at elevated temperatures, the Animal Boarding Establishment's Act (1963) and Joint Services Publication 315 states that if a temperature of 26°C is exceeded then some mechanical ventilation should be available. This is not as yet available in some of the military kennel types and consequently extreme high temperatures may influence welfare.

Exposure to a range of low and high temperatures and an inability to move to control or avoid this exposure may significantly affect the welfare of kennelled dogs.

1.5.2.6 Kennel husbandry

In the past, the design of many kennels and enclosures has solely concentrated on the ease of husbandry from a human perspective. Kennels are often very small necessitating little time and effort in cleaning. Consequently, if the dog is not moved during cleaning, it is either subjected to a number of aversive stimuli or if available, locked into a separate area contained within the kennel, both of which may be stressful. Furthermore, poorly planned husbandry regimes can often lead to dogs standing on wet floors for extended periods during the day. Constant moisture can be attributed to soft pads which become ulcerated, bleed, and ultimately result in lame dogs (Jennings, 1991).

Additionally, whilst the disinfecting of the kennel environment serves to prevent harbouring of bacteria and subsequent disease transmission, it may impact upon the behaviour of the dog towards others in its vicinity. The majority of military dogs are kennelled individually so identification of conspecifics is difficult. The marking frequency of resident dogs increases in response to the visual and olfactory stimuli of a new dog (Sommerville and Broom, 1998). However, these olfactory cues, used to aid in the identification of conspecifics and the maintenance of hierarchies, are removed daily by disinfection and ultimately this may affect a dog's behaviour, potentially causing frustration, and may detrimentally affect its ability to adapt to its environment. A similar effect has been observed in mice (*Mus musculus*; Gray and Hurst, 1995, Van Loo et al, 2000).

1.5.2.7 Exercise

When considering how best to improve the welfare of kennelled dogs, much emphasis is placed on the benefit of exercise (Wolfle, 1987). Yet there is little scientific evidence to underpin this. In fact, some of the scientific evidence would appear to refute any benefit of exercise. Hughes et al (1989) examined the effects of cage size on exercise and demonstrated that only a reduction in cage size, to below legal standards, stimulated exercise and movement. However as with space allowance (1.5.2.1), this research has focussed upon incremental increases in cage size as a means of encouraging exercise (Bebak and Beck, 1993, Hughes et al, 1989) rather than looking at exercise away from the home cage. Indeed, in research institutions it is currently recommended that exercise be carried out in a separate area away from the home kennel, thus providing the stimulation of a novel environment (Prescott et al, 2004). Hubrecht et al (1992) has shown that an opportunity to exercise in a large area increases the number of species-typical locomotory behaviours e.g. running and trotting and increases activity. Not only this, exercising in larger areas allows a dog to roam, investigate, explore and actively seek information about its surroundings, fulfilling behaviours inherent to its nature.

For laboratory housed dogs it is recommended that exercise for a minimum of 20 minutes per day will sustain and maintain high levels of welfare (Prescott et al, 2004). Prescott et al (2004) also recommend that humans and conspecifics (Figures 1.3) should be present during exercise as past research (Hughes et al, 1989) has demonstrated an increase in activity when this occurs. The amount and type of exercise provided may well influence kennelled dog welfare.

Figure 1.3 Exercise with conspecifics in a paddock

a) Group exercise b) Paired exercise

1.5.2.8 Inter-specific contact

As a result of domestication (1.2.1), dogs form strong social bonds to humans (Serpell, 1995) developing specific attachments (Hart, 1995) and strong affectional bonds (Palestrini et al, 2005) to individuals. Thus for a dog, one of the greatest stressors upon arrival in the kennelled environment is the permanent separation from their familiar social group, including owners and other household members. The provision of social interactions with humans has therefore received much investigation and there are numerous studies demonstrating the benefits of human contact on dog welfare. Regular human contact within the kennelled environment has been observed to reduce salivary cortisol levels (Coppola et al, 2006) whilst interactions such as stroking can increase plasma phenylethylamine (a neurotransmitter with amphetamine-like properties) concentrations (Odendaal and Lehmann (2000). In potentially stressful situations e.g. during venipuncture and exposure to psychogenic stressors, the presence of a human can mitigate the stress response (Hennessy et al, 1998; 2002). This effect appears greater than when in the presence of conspecifics e.g. dogs subjected to a novel environment in the presence of a human caretaker showed no stress response, whilst in the presence of kennel mates, dogs showed both an increase in activity and in glucocorticoid levels (Tuber et al, 1996).

However, it is important to consider that some dogs may have received little socialisation with humans during puppyhood and hence human social contact can in fact be aversive. These dogs are therefore likely to be very stressed in a kennelled environment as there may be numerous unfamiliar people which the dog may see outside the kennel on a daily basis (Wolfle, 1987). In addition encounters with people may not necessarily be positive e.g. husbandry activities where humans use a high pressure hose can be aversive to dogs. Such fear can be addressed through behavioural modification therapy based around positive reinforcement, ultimately enabling dogs to have socially enriched lives (Wolfle, 1987).

Furthermore within the kennelled environment there is little time for social interaction implemented into the daily care routine of dogs. For example during a study of four sites, Hubrecht et al (1992) observed that as little as 0.24-2.5% of the sampled time was available for dogs to interact with humans. Studies have shown that if the contact is increased, even as little as 30s per day, the relationship between carer and dog is improved and dogs are perceived to friendlier, more approachable and character assessments are more accurate (Hubrecht, 1993a).

The amount and type of contact with humans within the kennelled environment may well have a significant influence upon welfare for many dogs.

1.5.2.9 Intra-specific contact

Dogs as pack animals have, inherently, a great desire for social contact with other dogs although, as with humans, this can depend upon experiences throughout ontogeny. However, the majority of military working dogs are single housed with limited opportunity for any physical interaction with conspecifics.

Housing dogs individually leads to a restriction of their behavioural repertoire (Mertens and Unshelm, 1996) and is associated with an increased incidence of behavioural abnormalities (Hubrecht, 2002). In a study by Hetts et al (1992), laboratory dogs housed in a high degree of social isolation, without any visual or tactile contact, were seen to spend the most time moving, showed the greatest number of stereotypies, a high degree of barrier manipulation and spent the most time vocalising. However, when housed as pairs, the same dogs were seen to spend more time sleeping and showed a lesser tendency to vocalise; a behaviour also noted to decrease following the pair housing of dogs in rescue shelters (Mertens and Unshelm, 1996). Thus providing dogs with visual and physical contact with conspecifics may have a positive effect on their psychological well being. Indeed, when given the opportunity, dogs will observe other dogs and will act to maintain this contact (Wells and Hepper, 1998). Group housing can further enhance social contact between dogs, allowing them to engage in social behaviour and physical contact as well as increasing the novelty of an otherwise barren environment (Hubrecht et al 1992). In addition, the reported number of stereotypies is seen to decrease within a group environment. Additional behavioural changes with paired and group housing are documented by Hetts et al (1992). An increase in the olfactory activity of dogs is seen when group housed (Hubrecht et al, 1992) which may reflect the enrichment of the dog's sensory environment. It would appear that this is also important to dogs when singly housed, as many dogs in this environment are observed attempting to increase the available sensory input by pushing their muzzles against the front of the kennel whilst stood on their hind legs (Hubrecht et al, 1992).

The welfare of dogs may be improved if greater intra-specific contact is provided within the kennelled environment. Yet there may be dogs, inadequately socialised, which may find increased contact detrimental to welfare. Equally, for dogs which are adequately socialised, the ability to see a large number of dogs, over which control cannot be exercised, may be frustrating.

1.5.2.10 Cage Furniture

The majority of current kennel designs used to house military working dogs are very barren and offer little opportunity for dogs to carry out natural behaviour or gain information about their surroundings. This can be potentially stressful for dogs. However, within laboratory housing, platforms (Figure 1.4) are now a common feature. They were introduced to mimic lookout mounds used by wolves and to help frustrated attempts to see out of pens which might lead to stereotypies (Hubrecht, 1993a). Likewise, they provide a degree of environmental complexity and choice. In trials, platforms were used extensively by laboratory dogs; when provided over a two month period, 55% of the dog's daily budget was spent either playing or resting on them (Hubrecht, 1993a).

Figure 1.4 Sleeping platform

Furthermore, they were seen to have a marked effect on dog behaviour. Hubrecht (personal observation as cited in Prescott et al, 2004) reported that when given a clear view of the door to the room, dogs were much more relaxed when people passed along corridors or entered the room, and the level of arousal was reduced. Additionally, platforms increase the complexity of the pen, utilising the third dimension and are also likely to offer protection from the cold floor, providing a more comfortable and warmer area for sleep and rest.

1.5.2.11 Toys and chews

Many dogs kept in kennels are reported to chew either the kennel structure or items within the kennel such as bedding or furniture. This behaviour is often seen as a means of creating novelty, in response to an environment which provides little stimulation (Poole, 1992). Thus it could be considered as a form of 'do it yourself enrichment', but like stereotypies (1.4.2.5) this behaviour should be seen as an indicator of an unsuitable environment. Furthermore in military working dogs the chewing of metal pans, water dishes, fencing, the kennel etc can be attributed to traumatic dental disease (teeth fractures) (Jennings, 1991). Providing toys (Figure 1.5) can provide a more appropriate outlet for chewing behaviour, allowing the opportunity for an increased behavioural repertoire whilst additionally increasing the complexity of the environment. The effects of toys have received much investigation and these studies are described in detail in Chapter 6.1.

Figure 1.5 Dogs interacting with toys

a) Tetra grip toy

b) Football

However, within the military working dog population, there is still reluctance to provide dogs with stimulation such as toys and chews. It is widely believed that toys can make dogs possessive (further described in Chapter 6.2) and there are concerns regarding the ease of kennel cleaning. Yet in studies of laboratory dogs (Hubrecht, 1993a; 1995) the suspension of toys on chains avoided both monopolisation and soiling. Inter-individual preference (Prescott et al, 2004) and habituation is often stated as a likely problem although this was not seen in Hubrecht's (1993a) dogs, even after two months. What's more, it could be addressed by preference testing, rotating or changing the presentation of toys (Hubrecht, 1993b) or engaging humans in play sessions.

Additionally there is the belief that provision of toys within the military working dog kennel will decrease the dog's motivation to work (studied in Chapter 6.2). Yet based on anecdotal evidence, the use of Kongs™ and smoked bones given to search dogs within a kennelled environment did not result in any detrimental effects upon search ability.

1.5.2.12 Olfactory enrichment

As introduced in section 1.2.2, the olfactory ability of dogs is acute, thus it is feasible that the presence of different odours, within a kennelled environment, may have some influence upon their welfare. In a study conducted by Graham et al (2005a), five different types of olfactory stimulation were investigated (control, lavender, chamomile, rosemary and peppermint). Lavender and chamomile resulted in more resting, less moving and vocalising whilst rosemary and peppermint encouraged more standing, moving and vocalising. Thus lavender and chamomile appear to have beneficial effects upon the welfare of kennelled dogs promoting behaviours indicative of relaxation. In contrast the presence of other odours associated with cleaning or inadequate husbandry such as disinfectant or high levels of ammonia may be detrimental to welfare.

1.5.2.13 Visual enrichment

A recent study by Graham et al (2005b) investigated the influence of five different types of visual stimulation; control (no visual stimulation), blank, and moving images of conspecifics, humans and interspecifics upon the behaviour of kennelled dogs. Whilst some effects were seen; less movement and less vocalisation, leading the authors to conclude that behaviour is influenced by visual stimulation, the benefits were unlikely to be as great as those animals with well developed visual systems. The effects were not dissimilar to those described anecdotally when dogs are able to see livestock or other animals e.g. birds outside of the kennel; increased resting and decreased vocalising (Gray, *pers comm.*). However, it should be equally considered that the ability for some dogs to see something over which it has no control may be stressful.

1.5.2.14 Veterinary treatment

Following their arrival into a military environment, dogs are required to undergo a number of veterinary procedures, including vaccinations and hip x-rays. In pet dogs, owners often report that visiting veterinary establishments and the associated procedures are stressful for dogs. Research has shown that some dogs exhibit stress responses to these stimuli; blood pressure and heart rate were seen to increase in response to veterinary visits, suggesting a transient autonomic response to the stress of the veterinary clinic (Kallet et al, 1997). Van Vonderen et al (1998) investigated the urinary cortical response of dogs to three different treatments; a vaccination, an orthopaedic examination and hospitalisation. All three treatment groups responded with an increase in cortisol although there was significant variation between individual animals. Similarly, venipuncture produced a plasma cortisol response (Hennessy et al, 1998). It may therefore be beneficial to delay some of the diagnostic veterinary procedures for at least a couple of weeks as they may impose unnecessary stress upon the dog. These are factors of which the exposure, unlike many of the others discussed in this section, can actually be controlled and avoided.

1.5.2.15 Diet

Following transfer into the military environment, it is very rare for a new arrival to be fed the same diet as in its previous home or rescue shelter. This is for a number of reasons. Firstly the procurement personnel may not necessarily ask for the dog's current diet and so the food is not available when the dog arrives at the new establishment. Secondly it is very difficult for the establishment to cater for all diets and so all dogs are fed on one brand. This does however have implications for any dog which arrives. To prevent gastrointestinal disturbance, which may result in diarrhoea and/or vomiting, it is recommended that a new diet is gradually introduced. As this is not possible for the majority of military working dogs, it is likely that some dogs may experience gastric upsets for a period of time following arrival which may be stressful, and hence their welfare will be reduced.

1.5.2.16 Control and predictability

The controllability and predictability of an animal's environment may have a significant impact upon its welfare (Appleby and Waran, 1999). However, the kennelled environment offers little control or predictability for dogs within it. The lack of controllability and predictability within a kennel environment has been postulated as a cause of stress particularly for those dogs from a domestic environment where the ability to control and predict rewarding events would have been likely much higher (Hennessy et al, 1997; 1998).

In current kennel designs, the limited ability to socially interact and absence of toys give the dog very little opportunity to exercise control over its environment. Other mammals appear to suffer if they have no control over their environment. For example, when male monogamous tree shrews (*Tupaia belangeri*) were housed individually but with unrestricted visual access to one another, the subordinate exhibited elevated plasma corticosterone and catecholamines, rapidly lost weight and died (von Holst, 1986 as cited in Wiepkema and Koolhaas, 1993). If two tree shrews were housed together but were given the opportunity to hide, the ability of the subordinate to control his contact with the dominate shrew, by hiding, resulted in its survival. Hence hiding places or providing a choice of where to go within the kennel may be beneficial to kennelled dogs.

Within the kennel environment, aspects of husbandry regimes may differ on a daily basis with changes of staff almost certain between weekdays and weekends. Hennessy et al, 1998 suggests that dogs from domestic environments with a previously predictable routine are likely to find this stressful. In laboratory cats, the transition from a standard predictable routine to an unpredictable care routine resulted in behavioural changes indicative of poor welfare (Carlstead et al, 1993a). Similarly Weiss's study (1972) demonstrates with rats the importance of predictability. Even when receiving an electric shock, if the rat was able to predict the shock (via a light), the stress response seen was no greater than that of control rats which received no shock. However those rats which could not predict the forthcoming shock showed a significant stress response.

These three examples demonstrate the importance of both a predictable and a controllable environment. The persistent long term lack of, or a low level of, predictability and controllability can result in chronic stress symptoms (Wiepkema and Koolhaas, 1993) for kennelled dogs.

1.5.3 Welfare can be compromised in a kennelled environment

Within section 1.5.2, I have shown that specific aspects of the kennelled environment can influence much normal behaviour which many dogs are highly motivated to perform. For example, a lack of stimulation, restricted exercise and space allowance can influence the behavioural repertoire, and ability to explore and investigate, whilst limited intra and inter specific contact, poor kennel design and layout can affect social interactions. Using Fraser et al's model (1997) (1.3.2.4), the prevention of strongly motivated behaviour e.g. exploration and investigation, can result in negative subjective states and by my definition (1.3.3), compromised welfare. Additionally, I have highlighted challenges within the kennelled environment with which the dogs may be unable to cope or adapt to e.g. high levels of noise, hard surfaces and a change in diet. In these situations, biological functioning is most likely to be impaired. Some challenges, may correspond to adaptations possessed by dogs. For example in response to cold temperatures, many dogs grow a more dense coat, but for shorter haired breeds, such as German Short haired Pointers, this is not possible and so not only will the dogs feel cold but physiological systems are likely to be altered, thus subjective states and biological functioning will be impaired and welfare compromised.

Upon arrival into a kennelled environment, dogs will be stressed, but some dogs will cope with these limitations and restrictions, they will adapt to the environment, learn effective coping strategies and their welfare will no longer be compromised. In Rooney et al's study (2007b) the levels of urinary cortisol, on average, increased significantly upon arrival, but in some dogs, decreased over a period of ten days, returning to levels indiscriminate from that pre stress, and changes in behaviour suggested they had learnt effective coping strategies. For example, increases in the time spent in the sleeping compartment over the ten day period, suggest dogs had learnt that this was an effective way to keep warm. Dogs with experience of a kennelled environment may adapt even easier having previously learnt successful coping strategies (Hiby et al, 2006, Rooney et al, 2007b).

However, some dogs will not learn to cope or adapt, and chronic stress will ensue as indicated by the elevated levels of urinary cortisol of some dogs following twelve weeks of kennelling (Rooney et al, 2007b) (1.5.1), and their welfare will be compromised. The high incidence of abnormal behaviours, indicative of compromised welfare, observed in studies of long term kennelled dogs provides further evidence that some dogs do not adapt or cope. Between 46% (Hiby, 2005) and 93% (Denham, 2007) of dogs were observed to stereotype in a military establishment. Injuries resulting from the kennel environment; tail lesions, teeth fractures and sore elbows (Jennings, 1991) suggest that some kennel environments may be inadequate. It is therefore acceptable to assume that the welfare of many dogs in a kennelled environment can be compromised and this is worthy of enquiry.

1.5.4 Impact of poor welfare on the working ability of the military working dog

For ethical reasons, it is important to study, understand and improve the welfare of kennelled dogs but literature also suggests a link between welfare and performance.

Studies of a variety of species have shown that chronic stress within a captive environment can lead to impaired brain function. This can result in reduced learning of spatial tasks (Garner & Mason, 2002, Ohl & Fuchs, 1999) debilitated olfactory discrimination (Martin et al, 1999) and loss of memory (McEwen & Sapolsky, 1995, Mendl, 1999). In addition, chronic stress leads to decreased immunological function (1.4.1.6) and hence increased susceptibility to disease and infection (Clark et al, 1997, Moberg, 1985, Puppe et al, 1997). Such effects may impinge on the training and working ability of military working dogs; rejection rates may increase, productivity, training efficiency and operational effectiveness may decrease. In sum, the overall ability of a military working dog may be compromised.

Indeed trainee search dogs (Rooney et al, 2005) with high levels of acute stress showed significantly lower search ability. Likewise, stressed dogs are regularly shown to fail as guide dogs (Vincent and Leahy, 1997). Based on this evidence, improving the welfare of kennelled working dogs will not only ensure duty of care but the operational effectiveness, training efficiency and productivity is likely to also increase. Thus improvement to the welfare of working dogs is also very valuable for practical reasons.

1.5.5 Summary

Within this chapter I have highlighted a number of factors within housing and husbandry which are likely to affect the welfare of kennelled military working dogs and potentially also their working ability. However, the relative importance of each aspect of housing and husbandry and its influence upon welfare is not known. To improve the welfare of kennelled dogs and in particular military working dogs, it is important to understand which factors appear to influence welfare the most and whether changing or manipulating those factors do improve welfare and also the working ability of military working dogs.

1.6 Aims of this thesis

The general aim of this thesis is to understand how factors of housing and husbandry relate to the welfare of military working dogs, to identify those which appear the most influential and measure their effects upon welfare and working ability. Based upon previous evidence (1.5.3), the welfare of many military working dogs is assumed to be compromised by the kennel environment, and I hypothesise that numerous factors of housing and husbandry within the kennelled environment will influence welfare, but to varying extents. Changes in the most critical factors will improve welfare and working ability.

The first two chapters describe investigations into ways of measuring the welfare of military working dogs, using physiology and behaviour. As discussed in section 1.4.1.1, the HPA system may undergo changes in those animals undergoing chronic stress and thus absolute levels, as measured during acute stress, may not be reliable. Within this thesis, many subjects were kennelled long term and may have been subject to chronic stress (1.5.3). It was therefore important to determine how best to measure the cortisol levels of these dogs. Chapter 2 describes a study in which absolute resting levels of cortisol and the relative cortisol response to a challenge were compared between three different populations of dogs, each housed in different conditions. Chapter 3 addresses the influences of observer presence and diurnal variation upon kennelled dog behaviour and determines how best to sample behaviour for an accurate assessment of welfare. In Chapter 4, I use established indicators of welfare, and those developed in Chapters 2 and 3, to explore associations between the housing and husbandry of long term kennelled dogs and their welfare. Those factors which appear to be the most important for kennelled military working dog welfare are identified. Then in Chapters 5 and 6 I describe manipulations of two factors identified in Chapter 4, exercise regimes, and the provision of feeding enrichment and experimental studies of their effects upon working dog welfare.

Chapter 2

Urinary cortisol as a measure of welfare:

Variations in basal cortisol, and responses to an acute stressor, between populations of dogs with different housing and husbandry

2.1 Introduction

Within the introductory chapter of this thesis, I assumed that many of my subjects would be experiencing poor welfare as a result of the inability to cope with, or adapt to, persistent factors within their kennelled environment (1.5); e.g. separation from social attachment figures (1.5.2.8), unpredictability (1.5.2.16), lack of control over the environment (1.5.2.16), low temperatures (1.5.2.5) and limited opportunities for inter and intra-specific contact (1.5.2.8 and 1.5.2.9 respectively). Behaviours indicative of long term stress and compromised welfare e.g. stereotypies (1.4.2.5) have been observed in similar military working dog populations, supporting this assumption; 93% of dogs were observed to behave repetitively in response to at least one or more different stimuli e.g. feeding preparation, care assistant walking past the kennel, presented within the kennelled environment (Denham, 2007).

During chronic stress, cortisol levels have given conflicting information. Levels may be higher than when compared to normal or pre-stress levels; sows housed in crates show higher cortisol than those housed in straw pens (Cronin et al, 1991) and dogs housed in the most austere conditions have higher levels of cortisol than those in less austere conditions (Beerda et al, 2000). However, due to adaptation of the HPA system, levels of cortisol may also return to pre-stress levels; levels of cortisol in pigs, separated from their social group, returned to pre-stress levels even though other indicators of stress suggested that the subjects had not adapted to the stressor (Schrader and Ladewig, 1999). Consequently, using resting or basal levels of cortisol, as indicators of chronic stress, may be unreliable (Jarvis et al, 2006).

As introduced in 1.4.1.1, when undergoing chronic stress, the adrenal cortex response to adrenocorticotrophic hormone (ACTH) can change becoming hyper responsive to an acute stressful challenge resulting in a greater release of cortisol (Jarvis et al, 2006). Although, dogs housed in an environment both socially and spatially restricted, showed an attenuated cortisol response to ACTH compared to that measured whilst housed in spacious group housing (Beerda et al, 1999). Nonetheless, instead of measuring resting levels of cortisol, which as demonstrated above, are not necessarily reliable, many studies including some of dogs (Beerda et al, 1999, Garnier et al, 1990) have used secretagogues e.g. CRH or ACTH (see 1.4.1.1 for a description of their function within the HPA system) to challenge the HPA system. The corresponding release in cortisol is then compared either within subjects, to those levels measured pre-stress e.g. dogs (Beerda et al, 1999), or to a population perceived to be less stressed or un-stressed e.g. calves (Raussi et al. 2003), mule ducks (Guemene et al. 2006) and pigs (Jarvis et al, 2006) to determine the response of a chronically stressed animal. Whilst challenge tests may be useful to determine stress status, the invasive administration of secretagogues such as CRH and ACTH requires a home office licence and this would be difficult for the population of dogs which I studied. Thus the development of a non-invasive challenge test was required. A recent study conducted by Horvath et al (2007) used a strange human approaching threateningly, to elicit an HPA response in working police dogs. This social challenge proved to be effective, with overall post encounter salivary cortisol levels significantly higher than pre encounter levels. However, when the dogs were grouped according to the behavioural strategy adopted in response to the challenge, the change in cortisol was not always significant. For example, the cortisol response of fearful dogs whilst low was significant. The response of ambivalent dogs was significant and pronounced and the response of aggressive dogs did not change significantly. These results show that the response to a person may vary between individuals, most likely as a result of their experience with humans. Thus to be successful, it is imperative that the challenge is as universally stressful as possible.

It is generally assumed that dogs find visits to a veterinary surgeon stressful and empirical studies have shown that in many dogs the HPA system is stimulated, demonstrated by elevated cortisol levels post-visit (van Vonderen et al, 1998). Thus for this study, a routine veterinary examination was chosen, including the measurement of rectal temperature, as a challenge test, measuring urinary cortisol to creatinine ratios (C/C, 1.4.1.1) before and after administration of the stressor. Four sampling times were chosen; an early morning sample on the day of the veterinary examination, immediately post examination, two hours post examination and an early morning sampling the day after the veterinary examination. The time of the third sample; two hours post examination, was based on van Vonderen et al (1998) with an additional sample collected immediately post the examination. It is possible that some dogs may have responded to stressors prior to the examination such as transportation, or cues indicating that a visit to the veterinary surgeon was to occur. Collection of urine immediately post examination ensured that any cortisol released in response to these stressors was measured.

To investigate whether basal levels and the response to the examination was affected by housing and husbandry, I measured the C/C of three populations of dogs; army dogs kennelled for at least one year in a large multi-kennel complex, civilian police dogs kennelled in a home environment, and pet dogs housed in a domestic environment.

The relationship between cortisol and behaviours indicative of acute stress in dogs is unclear, with links between behaviour and physiology evident in some cases (Hiby et al, 2006, Horvath et al, 2007) but not others (Beerda et al, 1997). So, in this study, I explored the relationship between the cortisol response and a number of behavioural indicators. Behaviours indicative of fear and stress (1.4.2.8) e.g. lowered tail position, low ear positions, panting and lowered body posture were measured by an observer immediately before the dog went into the examination room. Veterinary surgeons were then asked to rate both the behavioural reactivity and the dog's level of fear and aggression during the examination.

Stereotypies are behaviours potentially indicative of chronic stress and suffering in many species (1.4.2.5) including dogs (Beerda et al, 1997) and may be performed both in the presence and absence of people. Therefore the army dog population were observed for the incidence of stereotyping both in the absence and presence of a person, to investigate whether basal levels of C/C or the physiological response to an acute stressor differed between dogs which stereotyped with those that did not.

Thus the overall aim of this study was to

- compare the basal levels of C/C, and those after a routine veterinary examination between three different populations of dogs,
- explore the relationship between behaviours indicative of short term and long term stress and urinary cortisol,
- determine which measure is most useful for assessing chronic stress, either absolute levels of cortisol or the response in cortisol to a perceived challenge.

2.2 Methods

2.2.1 Subjects

Three separate populations of male German Shepherd Dogs (GSDs) were used for the study. All subjects were at least 46 months of age, healthy and were not receiving any steroidal medication. The first population comprised 13 UK army PATAT (1.1) dogs housed singly at four different sites in Northern Ireland. The second comprised ten civilian police dogs (1.1) kennelled at their handlers' homes in the Bristol area. The final population comprised 11 pet dogs owned by the general public, all housed in home environments in the Bristol area. The 13 army dogs ranged in age from 51 to 100 months (mean= 71.7 ±14.2) and one was neutered. The pet dogs ranged in age from 46 to 131 months (mean= 86.5 ±22.7), four were neutered and the status of one dog was unknown. The police dogs ranged in age from 59 to 87 months (mean= 71.4 ±10.8) and all were entire.

Army and police dogs were selected based upon their availability during the study period. Pet dog owners were recruited via mailing all eligible owners from two veterinary practices in the Bristol area; Langford Small Animal Practice, Langford and Lucas Veterinary Surgery, Longwell Green.

Subjects within the army, pet and police populations were each studied for two consecutive days between November and December 2006, December 2006 and March 2007 and December 2006 and February 2007 respectively.

2.2.2 Housing and husbandry

2.2.2.1 Army dogs

The number of dogs housed at the four separate sites was two, four, four and three respectively. Each dog was housed singly in a kennel ranging from 8.6 to 20.5m^2, consisting of a sleeping compartment and outdoor run. High levels of activity may lead to increased levels of creatinine (1.4.1.1). Therefore, none of the dogs were worked or received any form of training during the study but underwent their normal husbandry and exercise regime; all dogs were exercised either on lead or off lead, by their own handler or a duty handler, two to three times per day each for between 20 and 30 minutes.

2.2.2.2 Police dogs

Each dog was housed in a kennel ranging from 2.6 to 5.6m^2 at the handler's home; one subject was pair housed with a non-subject dog and three were kennelled adjacent to another dog. One of the dogs was given continual access to the house and never kennelled, the remaining nine were kennelled for between eight and 22 hours per day. Exercise was provided by the handler for between 15 and 60 minutes, two to three times per day. Police dogs similarly were not worked and did not receive any form of training during the study.

2.2.2.3 Pet dogs

Dogs in this population were housed within the home environment and were cared for and exercised according to their normal routine, receiving between two and three walks per day each for between ten and 30 minutes.

2.2.3 Procedure

2.2.3.1 Pre veterinary examination

On day one of the study, between the hours of 05:30 and 09:00 hours, each dog was either exercised on the lead or taken out into their home garden so that the first naturally voided urine sample (2.2.4.1) could be collected (from hereon referred to as *Day 1 basal*).

Each dog was next taken to the veterinary surgeon by its handler or owner for a routine examination. Army dogs were walked to their usual examination room located within each individual dog site and examined by their regular veterinary surgeon between the hours of 13:40 and 14:25. At three of the sites each dog waited in turn outside the examination room for approximately two minutes. At the fourth site, dogs were walked straight from the kennel into the examination room. Of the pet dogs, all were taken to their usual veterinary practice but four were examined by an unfamiliar veterinary surgeon. Eight were transported via a vehicle whilst two were walked to one of the two veterinary practices and after waiting in the waiting room for between zero and 25 minutes (mean=8.2 ±7.8) were examined between the hours of 09:24 and 17:47. Police dogs were not examined by their usual veterinary surgeon but instead each dog was transported to the Langford Small Animal Practice and examined between the hours of 11:34 and 16:20. Eight of the ten dogs remained in a transit kennel within the handler's vehicle for between three and 20 minutes (mean= 11.4 ±5.9) before being examined. Due to an emergency, the other two dogs remained in their transit kennel for 80 and 92 minutes respectively.

2.2.3.2 Veterinary examination

As each dog was taken into the examination room its behaviour (2.2.4.2.1) was scored by one of three observers. Eighteen of the dogs were observed by the author (female, aged 29), 13 by a research assistant, trained by the author (male, aged 26) and four by an experienced behavioural scientist (female, aged 34)[1]. During the examination, dogs were weighed, had their eyes, ears, heart and lung function checked, and auscultation of the abdomen was performed. The examination ended with each dog's rectal temperature being measured, although one police dog was unable to have its temperature taken due to aggression caused by an inflamed and painful scrotum.

2.2.3.3 Post veterinary examination

Immediately following the veterinary examination, the veterinary surgeon rated the dog's behaviour during the examination (2.2.4.2.1), and the dog was taken outside and a urine sample collected by their owner or handler (from hereon referred to as *Immediately post examination*). Only one pet dog did not urinate at this time. Army dogs were then returned to their kennels, pet dogs to their homes and police dogs into their transit kennels for transportation home. Two hours after the veterinary examination, a further urine sample was collected (from hereon referred to as *Two hours post examination*).

A final urine sample was collected on day two, at approximately the same time as day one; between the hours of 05:30 and 10:00 (*Day 2 basal*).

2.2.3.4 Additional procedures in army dogs

On day one, two additional behavioural observations were taken in army dogs only. Between the hours of 12:00 and 13:25, prior to the veterinary examination, the kennelled behaviour of each army dog was recorded both remotely and directly (2.2.4.2.2); the behaviour of dogs

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[1] A research assistant had been employed to assist with this study and was responsible for observing each dog prior to the examination. However, due to difficulties in obtaining volunteers, the study exceeded the RA's contract and so the remaining dogs were observed by either the author or an experienced behavioural scientist. To mitigate any differences between the observer's ratings, the author provided training to both the RA and the experienced behavioural scientist.

differs in the presence of a person compared to when alone (Chapter 3). Hence dogs were firstly recorded in the presence of an observer for two minutes so that an assessment of the dog's response to a novel person (myself) could be made. A second observation was then made by remotely recording six minutes of behaviour using a video camera placed on a tripod.

2.2.4 Data collection and analysis

2.2.4.1 Sampling and analysis of urinary cortisol to creatinine ratios (C/C)

A mid-stream of naturally voided urine was collected in a disposable plastic tray by the dog's owner or handler. Approximately $10cm^3$ was decanted into a plastic vial and frozen immediately at -18°C.

Urine samples were then analysed by Cambridge Specialist Laboratories for cortisol content using a routine radioimmuno-assay (RIA), and for creatinine using reaction with picric acid and subsequent photo-chromatography to calculate C/C[2].

In addition to absolute levels of C/C, two variables were calculated describing the change in C/C; ***change in C/C immediately post examination compared to day 1 basal*** (Day 1 basal level subtracted from level immediately post examination) and ***change in C/C two hours post examination compared to day 1 basal*** (calculated as above).

2.2.4.2 Sampling and analysis of behaviour

2.2.4.2.1 Behaviour before and during the veterinary examination

Four behavioural variables (Table 2.1) were recorded using a tick sheet immediately before entry to the veterinary inspection room. These were used to construct an ***observer composite fear scale*** which described the number of behavioural indicators of fear. For example, if a dog's ears were flat against its head, with a low tail and body posture whilst panting then a score of four was recorded as it displayed four behavioural indicators of fear. As the number of dogs displaying three or four behavioural indicators of fear was low (n=3), the scale was converted into a three point scale; 0=no behaviours indicative of fear, 1=one behaviour indicative of fear, 2= two to four behaviours indicative of fear.

[2] Assays were run with quality controls and validated according to pre-determined acceptance criteria.

Table 2.1 Descriptions of the four behavioural variables measured before the veterinary examination

Variable	Definition	Scale	Score on scale if indicative of fear
Ear height	Position of ears	2=upright, 1=relaxed, 0=flat against head	0
Tail height	Position of tail	1=very low to 5=very high	Less than 3.0
Lowered body posture	Body position of dog	0=normal, 1=lowered	1
Presence of panting	Mouth open and tongue hanging out	0=absence, 1=presence	1

During the veterinary examination, the veterinary surgeon observed each dog for the presence of four other behaviours (Table 2.2) and also rated the dog's fear and aggression.

Table 2.2 Descriptions of the six behavioural variables recorded during the veterinary examination

Variable	Definition	Method of measurement
Struggle	Attempts to move away from veterinary surgeon and resists during examination	0=absence, 1=presence
Bark	Staccato vocalisation	0=absence, 1=presence
Growl	Low pitched grumbling vocalisations, lips pulled back from teeth	0=absence, 1=presence
Whine	High pitched extended vocalisation with mouth closed	0=absence, 1=presence
Aggression scale	Subjective rating of level of aggression	1=very low to 5=very high
Fear scale	Subjective rating of level of fear	1=very low to 5=very high

The four variables; struggle, bark, growl and whine measured by the veterinary surgeon were used to construct a ***veterinary surgeon composite reactivity scale*** which described how intensely each dog reacted to the examination

0= dog does not struggle or vocalise during examination

1=dog vocalises during examination

2=dog struggles during examination

3=dog struggles and vocalises during examination

The veterinary surgeon's subjective ratings of aggression and fear were kept as raw scores.

2.2.4.2.2 *Behavioural recordings of dogs in kennels*

To record the behaviour of dogs in the presence of people (directly), I stood 0.5m from the front of the kennel with a Sony Handycam vision CCD TRV78E/59E with an attached wide-angle lens Raynox DVR 5000 0.5x and recorded the dogs' behaviour for two minutes. At the end of the observation, I either moved to another subject or terminated observations. After all dogs had been recorded, the behaviour of each was then recorded in the absence of people using the same video camera (remotely). The camera was placed on a tripod and positioned to maximise the amount of kennel area visible. Six minutes were recorded but only the last five were analysed to minimise any effects of my departure upon the dog's behaviour. The camera was then either moved to another subject or removed. During the filming period, the compound in which the dogs were housed was locked, to stop anyone from approaching the dogs and affecting their behaviour.

Only one behaviour was transcribed from both the recordings; repetitive behaviour (stereotyping), defined as dog performs at least two consecutive:

- *bounces*; jumping at a wall and rebounding from it or repetitive jumping on one spot; either all four legs leaving the floor or with hind legs continuously in contact with floor and forelegs only leaving the floor,
- *spins;* turning in a tight circle pivoted about hind legs,
- *circles;* walking or trotting around pen,
- *paces*; walking or trotting back and forth along a boundary line.

Two presence/absence variables were then calculated; stereotyping when in the absence of a person and stereotyping when in the presence of a person.

2.2.5 Statistical analysis

2.2.5.1 Is there an effect of population and sampling time upon levels of C/C?

C/C levels were transformed using Log 10 and a nested analysis of variance (ANOVA) (subject dog nested within population; army, pet or police) was conducted to explore the effect of two factors; population and sampling time (day 1 basal, immediately post examination, two hours post examination and day 2 basal) upon C/C levels. Where effects and interactions between factors were evident, univariate analysis was used to explore the effect of each factor.

2.2.5.2 Does population have an effect upon the observer composite fear scale, veterinary surgeon composite reactivity scale or the veterinary surgeon subjective rating scales of fear or aggression?

The effect of population upon the observer composite fear scale, veterinary surgeon composite reactivity scale and both veterinary surgeon subjective rating scales were explored using Kruskal Wallis tests.

2.2.5.3 Does the behaviour during the veterinary examination relate to the response in C/C post examination?

The observer composite fear scale, veterinary surgeon composite reactivity scale and the veterinary surgeon subjective rating scales for fear and aggression were tested for association with both absolute levels and change in C/C measured immediately and two hours post examination using Spearman Rank Correlations. As the veterinary surgeon rating the behaviour of dogs in army dogs differed to the veterinary surgeon who rated pet and police dogs, correlations were tested twice, once using data from all three populations and once using pet and police dogs only.

2.2.5.4 Does the observer composite fear scale and veterinary surgeon composite reactivity scale relate to the veterinary surgeon subjective scale of fear and aggression?

Relationships between these variables were explored as above.

2.2.5.5 Do army dogs performing behaviours indicative of chronic stress i.e. stereotypies, differ in their basal levels of C/C and response to an acute stressor to those dogs not performing stereotypies?

A nested univariate ANOVA was conducted to investigate the effect of stereotyping when alone, stereotyping when in the presence of a person, sampling time (basal day 1, immediately post examination, two hours post examination and day 2 basal) and the interaction between all three upon levels of C/C (subject dog was nested within interaction between stereotyping when alone and when in the presence of a person).

Levels of C/C were not affected by sampling time, stereotyping when alone or stereotyping in the presence of a person but there was an interaction between sampling time, stereotyping when alone only and stereotyping when in the presence of a person only (2.3.6). To explore this relationship further the effect of stereotyping alone only and stereotyping in the presence of a person only upon the C/C measured at each sampling time was tested using univariate ANOVA. Although there was a tendency for stereotyping when alone only and when in the presence of a person only to have an effect upon C/C measured two hours after the examination (2.3.6.1), this relationship could not be explored any further. To do so would have required further categorising dogs; never stereotype, stereotype when alone only, stereotype when people are present only and stereotype both when alone and when people are present, and the sample sizes within each category were too small.

2.3 Results

2.3.1 Individual baseline C/C and response in C/C post examination

Figures 2.1 to 2.3 display the basal C/C and response in C/C post examination of individual dogs within each of the three populations. Within the army (Figure 2.1) and pet (Figure 2.2) population, a wide range of responses in C/C were detected, some individuals showed a marked increase immediately post veterinary examination, others showed a marked increase two hours post examination, several dogs showed a marked decrease, and for other dogs the change was negligible. Within the police population, none of the dogs showed a substantial increase in C/C; individuals showed only a marked decrease or a negligible response (Figure 2.3).

Figure 2.1 Basal C/C and response in C/C post examination of individual army dogs. An example of each response is presented on the graph.

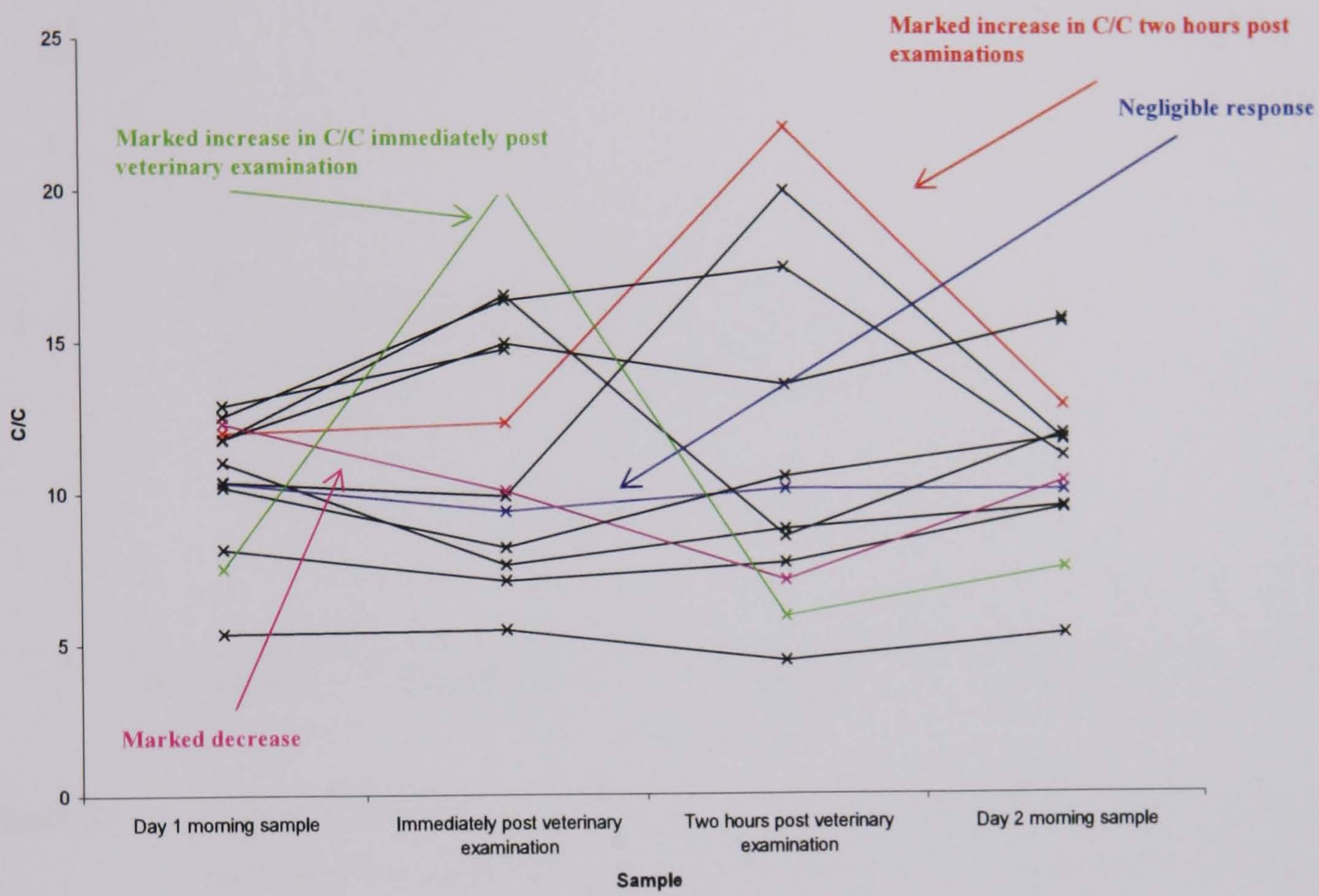


Figure 2.2 Basal C/C and response in C/C post examination of individual pet dogs. Coloured lines defined as Figure 2.1.

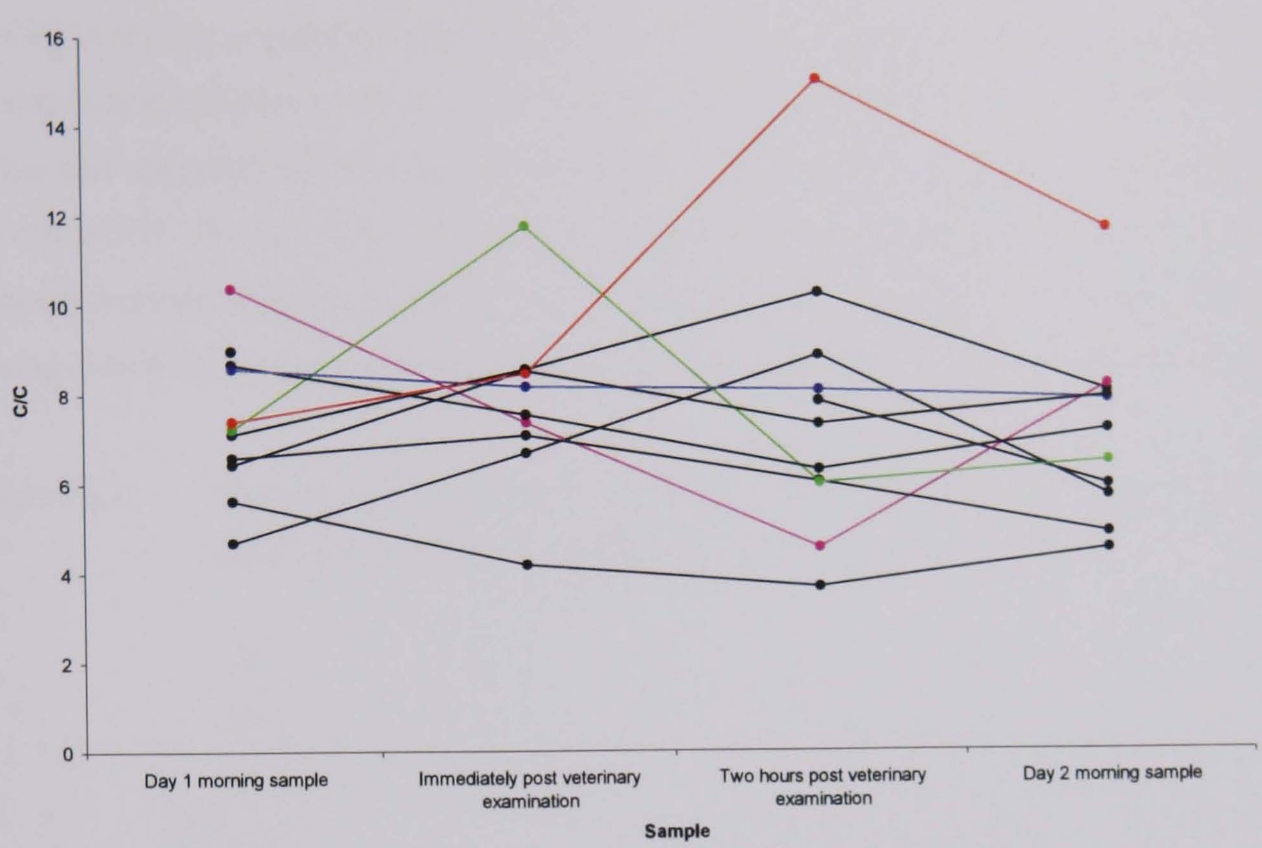
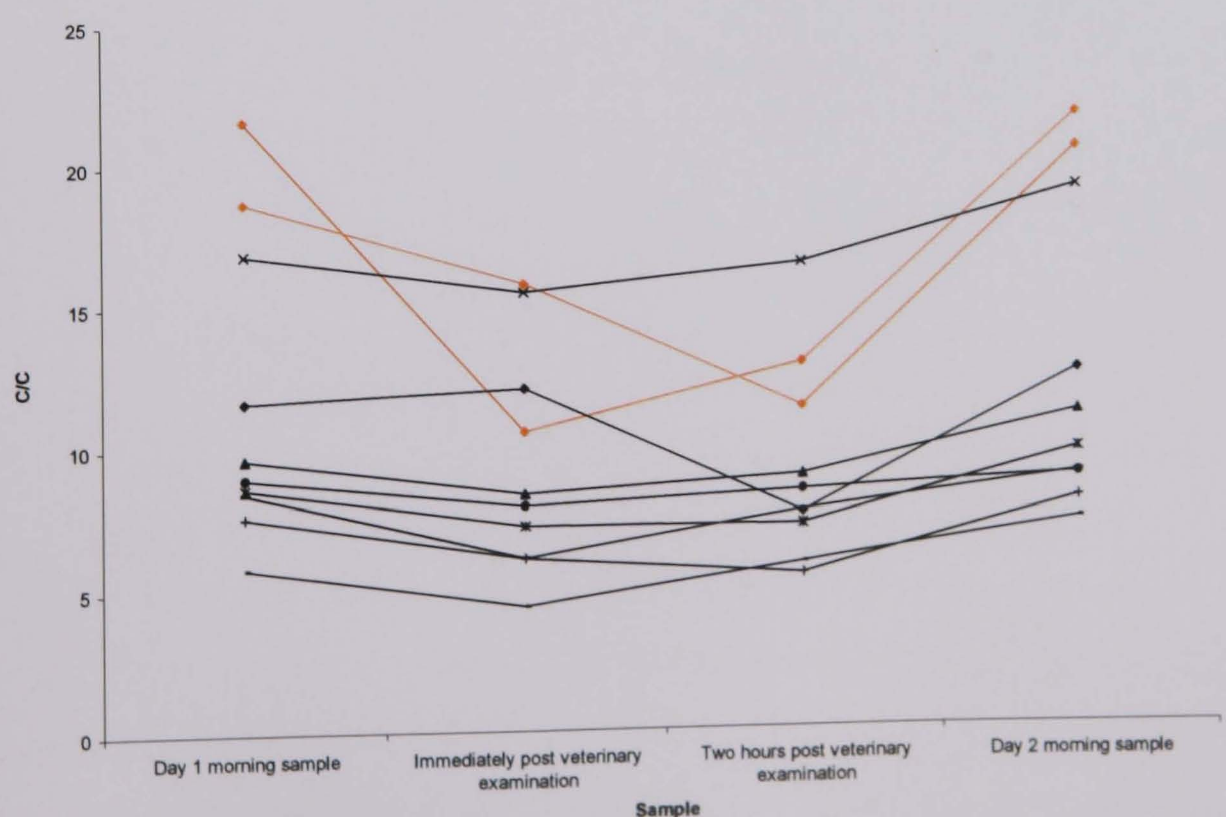


Figure 2.3 Basal C/C and response in C/C post examination of individual police dogs. Orange lines denote two dogs which remained in their transit kennel for 80 and 92 minutes before being examined (see section 2.2.3.1).



2.3.2 Is there an effect of population and sampling time upon levels of C/C?

Population had a significant effect upon levels of C/C ($F_{(2,31)}$=4.9, p=0.014) (Figure 2.4) with levels in the pet population significantly lower overall compared to army (7.3 vs. 10.5, Independent samples t-test; t=5.5 p<0.001) and police dogs (7.3 vs. 9.9 t=-4.0, p<0.001)). This was apparent at both basal samples (2.3.2.1). Sampling time had no overall effect upon levels of C/C ($F_{(3,91)}$= 1.3, p=0.27). There was however a significant interaction between population and sampling time ($F_{(6,91)}$=2.5, p=0.03) (Figure 2.5) and this was explored further, using ANOVA, by examining the effect of sampling time on each population individually.

Figure 2.4 The effect of dog population upon mean levels of C/C (±SE) ($F_{(2,31)}$=4.9, p=0.013), (Army N=13, Pets N=11, Police N=10).

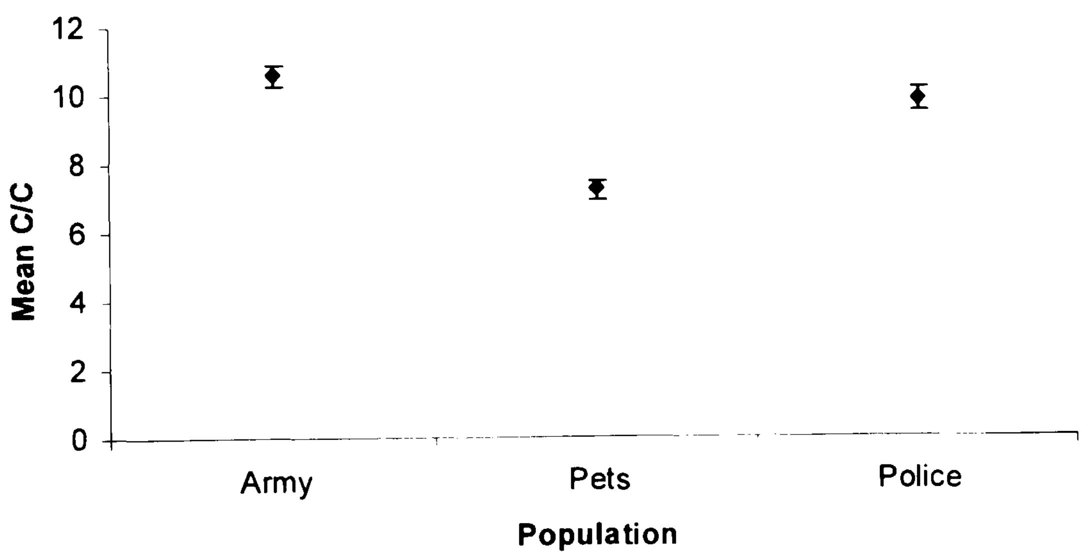
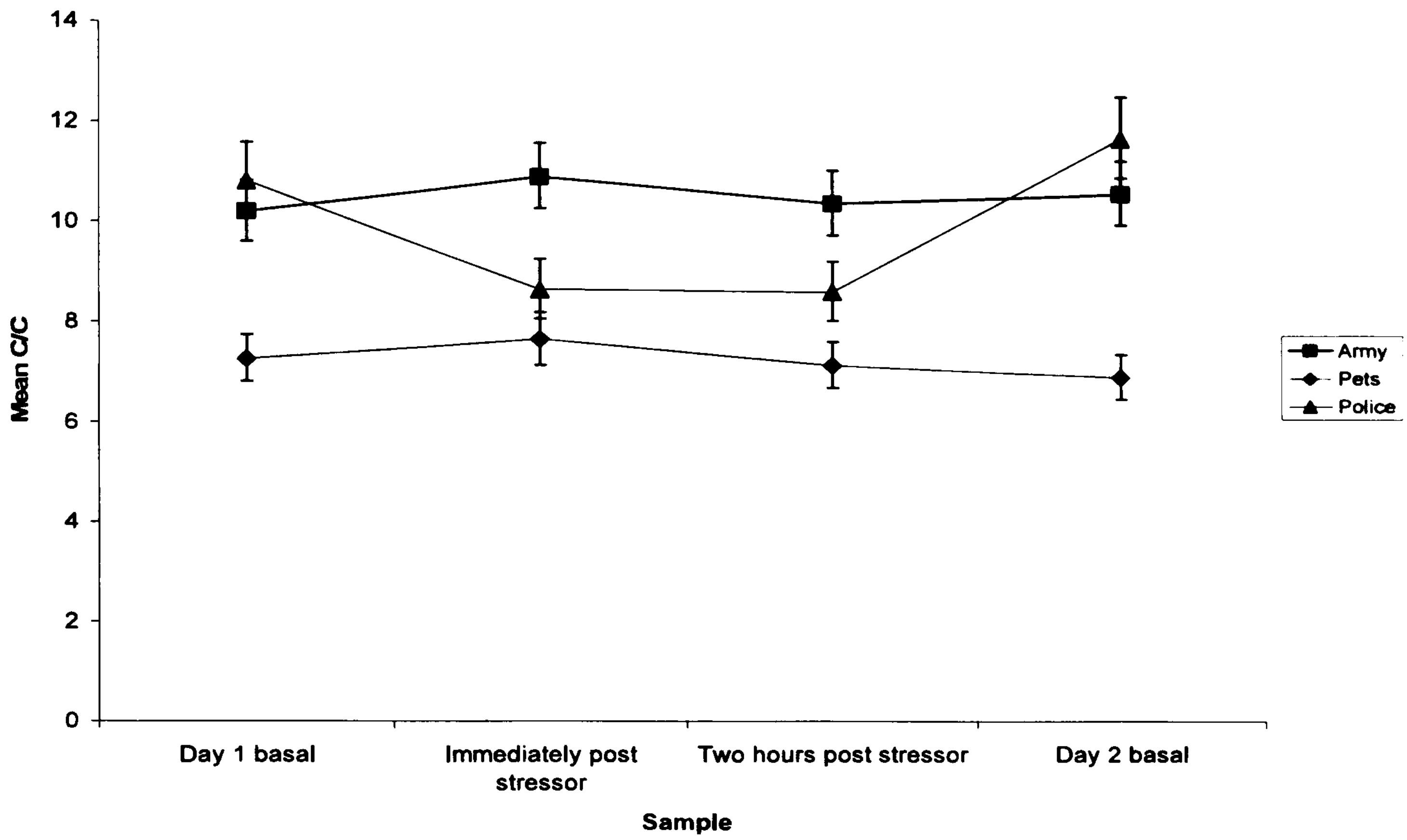


Figure 2.5 The interaction between dog population (Army N=13, Pets N=11, Police N=10) and sampling time. Mean levels of C/C (±SE) presented ($F_{(6, 91)}$=2.5, p=0.03).



2.3.2.1 The effect of population upon basal levels of C/C

A significant effect of population upon the mean levels of day 1 basal C/C was demonstrated; $F_{(2,29)}$=5.4, p=0.01. Levels of C/C in pet dogs were significantly lower than both army dogs (7.1 vs. 10.5, Univariate ANOVA $F_{(1,20)}$=12.7, p=0.002)) and police dogs (7.1 vs. 11.5, $F_{(1,17)}$=8.8, p=0.009)) (Figure 2.5). The same effect on mean levels of day 2 was also evident (($F_{(2)}$=8.1, p=0.001) (Figure 2.5)); levels of C/C were significantly lower in pet dogs than both army dogs (7.1 vs. 10.5, ($F_{(1,20)}$=10.0, p=0.005)) and police dogs (7.1 vs. 11.5, ($F_{(1,17)}$=11.2, p=0.004)).

2.3.2.2 Exploring each population individually, does sampling time have an effect upon mean levels of C/C?

There was no significant effect of sampling time upon C/C in the army ($F_{(3, 35)}$=0.20, p=0.89) or pet ($F_{(3, 29)}$=0.33, p=0.81) populations. However, a significant effect of sampling time was observed in the police population ($F_{(3,27)}$=13.7, p<0.001). Levels of C/C measured immediately post examination were significantly lower than both day one (8.7 vs. 10.8, Paired t-test t=3.5, p=0.007) and day two basal samples (8.7 vs. 11.8, t=-5.0, p=0.001) and levels of

C/C two hours post examination were also significantly lower than day one (8.7 vs. 10.8, t=3.5, p=0.007) and day two samples (8.7 vs. 11.8, t=-5.3, p<0.001). This analysis was repeated with the two dogs which waited for 80 and 92 minutes excluded, a significant effect of sampling time was still observed in the police population ($F_{(3,27)}$=11.1, p<0.001). Levels of C/C measured immediately post examination were significantly lower than both day one (7.9 vs. 9.3, Paired t-test t=4.1, p=0.005) and day two basal samples (7.9 vs. 10.2, t=-5.1, p=0.001) and levels of C/C two hours post examination were also significantly lower than day one (8.0 vs. 9.3, t=2.8, p=0.003) and day two samples (8.0 vs. 10.2, t=-4.8, p=0.002).

2.3.3 Did population have an effect upon the observer composite fear scale, veterinary surgeon composite reactivity scale or the veterinary surgeon subjective ratings; fear and aggression?

Population had no significant effect upon the observer composite fear scale (χ^2=1.9, p=0.38) or the veterinary surgeon subjective rating scale of fear (χ^2=1.3, p=0.52). However, both the veterinary surgeon composite reactivity scale and the veterinary surgeon subjective rating scale of aggression varied significantly between populations (χ^2=9.1, p=0.01 and χ^2=14.1, p=0.01 respectively). As the veterinary surgeon making the ratings in army dogs differed from that rating pet and police dogs, post hoc analysis using Mann Whitney U, was conducted to explore whether there was a significant difference in both the variables between the pet and police dogs only. Police dogs were significantly more reactive (U=19.0, p=0.05 3.0 (3.0, 3.0) vs. 0 (0, 2.5), (Figure 2.6)) and aggressive (U=7.0, p<0.001 (3.0 (2.0, 5.0) vs. 1.0 (1.0, 1.0), (Figure 2.7)) than pet dogs. The range of reactivity showed much greater variation in pet dogs compared with police dogs (Figure 2.6) whilst the range of aggression showed much greater variation in police dogs compared to pet dogs (Figure 2.7).

Figure 2.6 The difference in the veterinary surgeon composite reactivity scale between police and pet dogs (U=19.0, p=0.05, police population; 3.0 (3.0, 3.0) vs. pet population; 0 (0, 2.5)).[3]

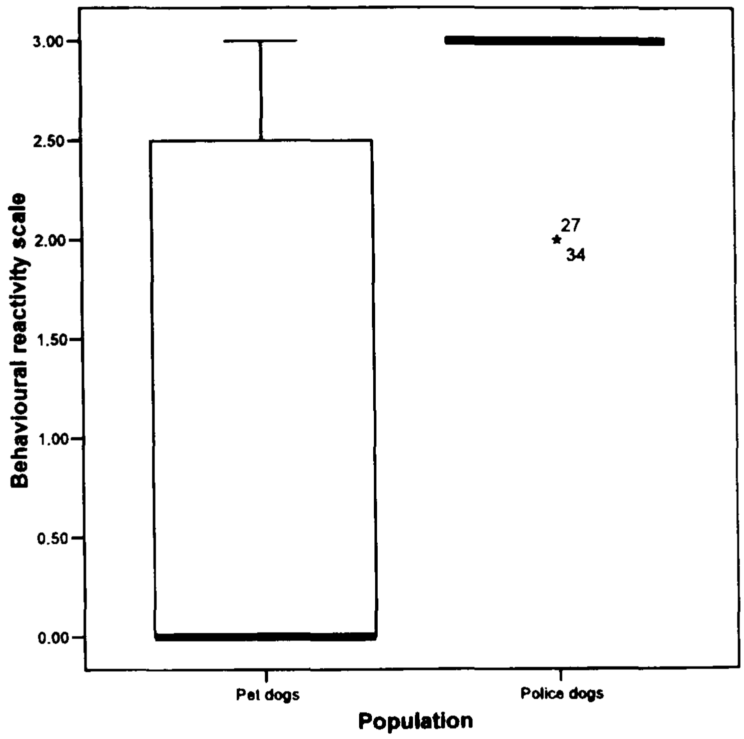
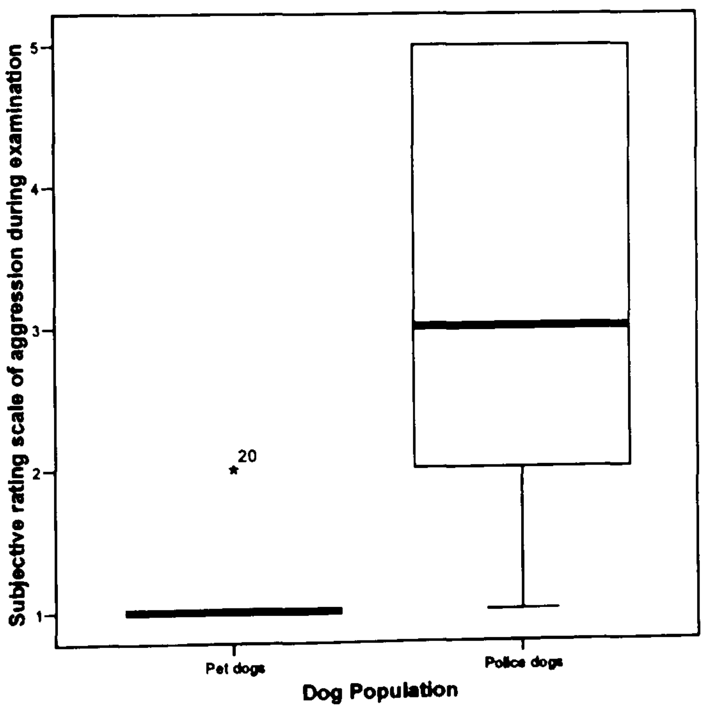


Figure 2.7 The difference in the veterinary surgeon subjective rating scale of aggression between police and pet dogs. (U=7.0, p<0.001, police population; 3.0 (2.0, 5.0) vs. pet population; 1.0 (1.0, 1.0)).



[3] Figure 2.6 and 2.7 are box plots. Thick horizontal lines represent the median value. The height of each box represents the interquartile range and contains the central 50% of cases. The whiskers show the smallest and largest value with outliers denoted by a circle if >1.5 box lengths from edge of box and asterisk if > 3 box lengths from edge of box.

2.3.4 Did the observer composite fear scale, veterinary surgeon composite reactivity scale or the veterinary surgeon subjective rating scales of fear and aggression relate to the response in C/C?

The observer composite fear scale and the veterinary surgeon's subjective rating of fear did not relate to either of the changes or any of the absolute measures of C/C (Rho<0.01, p>0.32). However, using data from all three populations, dogs with the greatest change in C/C (immediately post stressor minus day 1 basal) tended to be rated lower for aggression (Rho=-0.31, p=0.08) and were behaviourally less reactive (Rho=-0.42, p=0.014). These relationships became even more significant when only the data from pet and police dogs, rated by the same veterinary surgeon, were tested (Rho=-0.47, p=0.04 and Rho=-0.54, p=0.01 respectively).

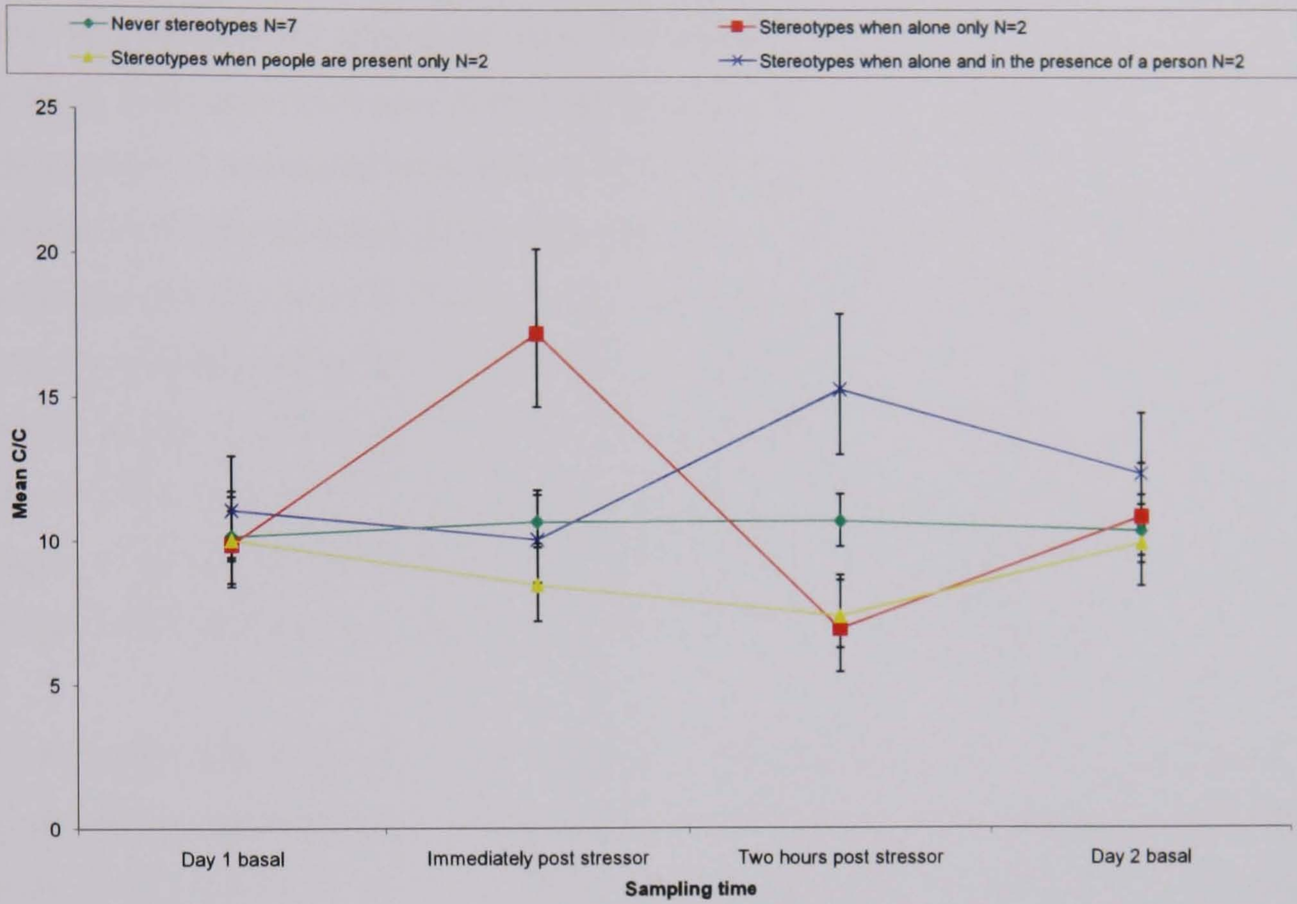
2.3.5 Did the observer composite fear scale and veterinary surgeon composite reactivity scale relate to the veterinary surgeon subjective scales of fear and aggression?

The observer composite fear scale did not correlate significantly to either of the veterinary surgeon's subjective scales (Rho<0.09, p>0.60). Whilst the veterinary surgeon composite reactivity scale did not relate to the veterinary surgeon subjective fear scale (Rho=0.15, p=0.41), using data from all three populations, dogs which were the most reactive during the examination were rated higher for aggression by the veterinary surgeon (Rho=0.42, p=0.01). This relationship reduced in significance when only populations two and three were tested (Rho=0.39, p=0.08).

2.3.6 Did army dogs performing behaviours indicative of chronic stress i.e. stereotypies, at different times, differ in their basal levels of C/C and response to an acute stressor?

Sampling time, stereotyping when alone only and stereotyping in the presence of a person did not have a significant effect upon C/C (F<0.75, p>0.53) but the interaction between the two stereotyping variables and sampling time was significant ($F_{(3,26)}$=3.7, p=0.025) (Figure 2.8). This relationship was explored further using ANOVA to explore the effect of stereotyping when alone only and stereotyping when in the presence of a person only upon levels of C/C at each sampling time (2.3.6.1).

Figure 2.8 The interaction between sampling time, stereotyping when alone and stereotyping when in the presence of a person. Mean levels of C/C (±SE) presented ($F_{(3, 26)}=3.7$, $p=0.025$).



2.3.6.1 Exploring each sampling time individually, does stereotyping when alone only and stereotyping in the presence of a person only have an effect upon C/C?

Exploring each of the sampling times individually, overall there were no significant effects of the stereotyping variables upon C/C ($F<0.04$, $p>0.14$). However, there was a tendency for an interaction between the two stereotyping variables upon levels of C/C, measured two hours post examination ($F_{(3, 26)}=0.096$). This relationship could not be explored further (2.2.5.5).

2.4 Discussion

2.4.1 How did individual dogs within the three populations respond to the examination?

In the current study, individual dogs were seen to vary in their response to the examination; in some, C/C increased, in others it decreased or showed very little change. These results contrast with those of van Vonderen et al (1998) where dogs showed only an increase or negligible change in response to a veterinary examination. However, a similar study, also using army dogs, was recently conducted following protocols based upon this study (Denham, 2007) and dogs responded in the same three ways. The variability in responsiveness between individuals is likely to be dependent upon their experience of the veterinary practice and their past experiences of associated procedures. Those dogs which did not respond may have received extensive socialisation throughout their early development, for example being taken to a veterinary practice and handled regularly, thus learning that both the practice and procedures can be rewarding experiences, and as a result such dogs do not become stressed or fearful during routine examinations. Those dogs which showed a marked increase in C/C may not have been so well socialised or may have experienced an event which has resulted in a negative association. Unfortunately within this study I was unable to quantify the socialisation which each individual dog had received and so could not explore this relationship.

It is possible that those dogs which showed a marked decrease in C/C may have found the examination, and associated human contact, rewarding. In a study of domestic cats (Carlstead et al, 1992) where each cat was subjected to a number of stressors opposite C/C responses, both increases and decreases, were measured. Behaviourally, those cats which showed a decrease in C/C were those which were the most tractable and affiliative. It is therefore suggested that they may have found the handling during blood sampling so rewarding that this outweighed any aversion to this sampling itself. Within this study, the level of friendliness towards people was not measured and so this relationship could not be explored any further but is worthy of future investigation.

Studying the peak C/C level of positive responders (Figures 2.1 to 2.2), it is evident that the time at which the peaks occurred differed. It takes between two to three hours for cortisol to be sequestered into the bladder (2.1), so it is probable that those dogs whose C/C levels peaked immediately post examination had responded to earlier cues or additional stressors such as transportation. Those dogs which peaked two hours post examination were unlikely to have received or responded to such cues but were instead responding to the procedure itself. Thus multiple sampling, as in this study, appears to assist in differentiating between these dogs. Had I not sampled immediately after the examination, it is possible that some kennelled dogs may have urinated in the period between the examination and the two hour sample. Those dogs which were responding to predictive cues and other stressors would not have been detected and analysis of the two hour sample would have shown very low levels of C/C suggesting this dog was not stressed. Repeated sampling is therefore recommended.

2.4.2 How did the three populations respond to the examination and did the response differ significantly between populations?

Overall, none of the populations responded to the challenge test as expected i.e. none showed a marked increase in C/C in response to the examination, even though a few individuals appear to have done so. In the army and pet populations, there was no significant increase between C/C measured on day one and the levels of C/C measured immediately after and two hours post the veterinary examination. In the police population, there was a significant change between levels of C/C measured on day one and those measured immediately and two hours post the examination, yet this was not in the anticipated direction and instead showed a marked decrease.

It is difficult to comment as to whether the current pet population, as a whole, responded in the same way as that studied by van Vonderen et al (1998). Their analysis was conducted on an individual level only and did not compare mean C/C levels before and after the examination. However, Denham (2007) did compare the mean levels of C/C before and after a veterinary examination, in a population of army dogs similar to those tested in the current study. In Denham's study (2007), C/C was significantly elevated following a veterinary examination compared to baseline whilst my study saw no such rise. As the breed, sex, age and the use of the dog was similar to that in my study, differences in examination procedures probably account for the elevated C/C. Whilst Denham followed most of my examination methodology, he performed, in addition, a procedure which may have been stressful. At the end of the examination the joints of each dog were manipulated. It is likely that joint manipulation was painful for some dogs, due to their age and breed, and this may therefore have lead to greater increases in C/C compared to my methodology when joint manipulation was not performed.

In the police population, there was a significant decrease between levels of C/C measured at baseline on day one and those measured immediately, and two hours, post the examination.

Although Figure 2.3 suggests that the two dogs which waited much longer to be examined may have significantly contributed to these relationships, even when these two dogs were removed, the relationships were still significant. However. it is difficult to interpret why the police population showed a marked decrease in C/C in response to the veterinary examination, I pose a number of plausible explanations;

1. It is common place for civilian police forces to procure potential police dogs as puppies, rearing them alongside their current working dogs. As a result. once adult and operational, most police dogs have formed strong attachment bonds to their handler. It is possible that due to this strong bond, when apart from its handler. a dog may experience stress. Nine out of the ten police dogs were kennelled overnight and it is therefore feasible that whilst in the kennel and apart from their handler, levels of C/C are elevated, reflected in high baseline levels, but once removed from the kennel and with their handler, these levels decrease, reflected in low C/C levels immediately and two hours post examination. So the decrease is C/C may not be related to the examination at all but to normal diurnal cycles induced by kennel or working patterns.
2. It is plausible that the basal levels measured in some of the dogs are not definitive baselines. The HPA system can be temporarily elevated during beneficial functional responses (1.4.1.1) thus cortisol may be released in response to 'positive' stress. It is therefore possible that the basal levels on day one and two are in response to the dog seeing its handler first thing in the morning. Thus the difference between basal samples and samples collected post veterinary examination are much greater than would be expected had the basal sample been taken at a time when the dog was not anticipating the arrival of the handler. This may offer a similar explanation for those individual dogs which also showed marked decreases in the army and pet dog population.
3. Police dogs are kennelled at their handler's home and as a result are transported regularly for both training and operational duties. In addition, police dogs spend much time in their transit kennels and many are likely to have formed positive associations with their transit kennel. Thus transportation is unlikely to activate the HPA system and may explain why the levels of C/C immediately post examination are significantly lower than when in their home kennel.

Even though, overall, the police dogs showed a significantly marked decrease, which differed to the overall negligible response shown by pet and army dogs, none of the populations responded as expected and thus none of the populations overall found the challenge stressful. The highly variable response to this examination within each population indicates that it is not a reliable way to determine or identify differences in the stress status of military GSDs. However, basal levels of C/C were significantly different in the three different populations which may be explained by differences in background stress status.

2.4.3 Did basal levels of C/C differ between populations?

Basal levels of C/C did differ significantly between the three populations; levels in pet dogs were significantly lower than in army and police dogs. These findings are consistent with Beerda et al (2000); basal urinary C/C levels were highest in dogs housed in a high degree of spatial and social restriction, and declined progressively to the lowest values in dogs housed in a domestic population provided with a social, spacious and enriched environment. From these results, Beerda et al (2000) suggests that elevated levels of basal C/C are indicative of chronic stress and that as conditions worsen, levels become progressively higher. Within my population, this interpretation supports the idea that kennelled dog welfare is poorer than that of pet dogs housed in a domestic environment.

However, one cannot automatically assume that the welfare of pet dogs is never poor. Whilst most pet dogs lead relatively contented lives in which they are housed in comfort, adequately fed and exercised, cared for when sick and generally well-looked after, there are many which suffer, at least to some extent due to inadequate, excessive or misguided care (Hubrecht, 1995). For example, separation anxiety and noise phobias are especially prevalent in pet dogs (Sherman and Mills, 2008). This is supported by a recent study undertaken by McGreevy and Masters (2008) which showed that 80% of owners reported some level of separation-related distress displayed by their dog when left alone. Whilst I did not collect data regarding the prevalence of behaviour problems in pet dogs, owners were asked to record any potentially stressful events such as thunderstorms and fireworks during the study. In addition, none of the subjects was undergoing consultation with a behavioural counsellor. The current study is supported by two others indicating that pet dog welfare is on average better than kennelled dog welfare, Beerda et al (2000) and also Hennessy et al (1997), who measured plasma cortisol levels of dogs at an animal shelter over a ten day period and in the absence of a basal control group, used dogs maintained as house pets as a comparison. In my study, the cortisol levels of pet dogs were significantly lower than those of the dogs in the shelter further supporting the interpretation that kennelled dog welfare is poorer than that of pet dogs.

One may hypothesise that police dogs would be less stressed as they are kennelled within a home environment however there was no significant difference in the levels of stress experienced by army and police dogs. However the housing conditions of the police dogs varied greatly in this study with some dogs kennelled for only nine hours per day whilst others were kennelled for 22 hours. This lack of standardisation in the police population, may account for the absence of a significant difference.

My findings, in combination with Beerda et al's (1999; 2000) suggest that basal levels of C/C can be indicative of chronic stress. Basal levels showed significant differences between populations. In contrast, changes in C/C in response to a stressor, showed no such significant differences. Thus throughout my PhD, basal C/C will be used as a measure of stress in conjunction with other behavioural and health parameters. However, all basal C/C results will be interpreted with caution as apparently normal levels of C/C do not necessarily negate chronic stress, since physiological adaptation and stressor specific responses can not be ruled out (2.1 and Beerda et al, 2000).

2.4.4 Does behaviour vary with C/C?

The observer composite fear scale derived from indicators of fear and stress exhibited immediately before a dog entered the examination room did not relate to absolute C/C or the change in C/C. Thus those dogs which were the most physiologically stressed by the examination did not necessarily perform behaviours indicative of stress before the examination. There may be a number of reasons for this lack of association. As discussed in Chapter 1, assessment of welfare is often difficult, requiring a multi dimensional approach but these difficulties are likely to be even greater in the domestic dog. Bradshaw (2005) proposes that if it is assumed that the capacity to experience suffering evolved as an adaptation that was useful to the dog's wild ancestors, then it is possible, that selective breeding for behavioural traits may have modified the original links between sensation, emotion and behaviour and possibly physiology. Thus, it is very difficult to assess when an individual is suffering (Barnard and Hurst, 1996, Bradshaw, 2005). It is also possible that links between behaviour and physiology may have been further modified by the behaviour of the owner or handler, reinforcing behaviours indicative either of fear or of a relaxed state. Dependent upon owner/handler behaviour, some dogs which are acutely stressed may be ignored if behaviours indicative of stress are performed so the dog learns only to perform behaviours indicative of a relaxed state. In contrast, other dogs which are not stressed may have learnt that only behaviours indicative of stress result in attention. Thus the true physiological state is masked.

It could also be argued that those behaviours measured prior to the examination did not accurately reflect the stress status of the subjects because at this point they had not been examined by the veterinary surgeon and only those indicators measured during the examination should be compared to the physiological response. However, even in those studies which have measured acute stress in dogs using both behaviour and physiology, little evidence for a relationship between physiology and behaviour has been found (Beerda et al, 1997; 1998).

The veterinary surgeon's subjective rating of fear was based upon the behaviour during the examination, but did not relate to the physiological response either. In a number of cases, the level of fear during the examination was rated as low but the increase in C/C was marked. Likewise, some dogs with no marked C/C response were given a high rating for level of fear. In this case, it could be argued that the lack of relationship was due to the ratings being based upon subjective measurements as opposed to objective measurements. In van Vonderen et al's study (1998), owners were asked to rate their dog's behaviour during the veterinary examination as; unchanged, positive or negative but the type of behaviour did not predict the C/C response. The lack of relationship was attributed to subjective judgements made by owners and thus it was hypothesised that a relationship would only be evident if the measures were based upon ethology. However, as described earlier, there may still be a lack of agreement, even if behaviour is scored more objectively.

During the examination, the veterinary surgeons scored ethological measures which were used to make a composite reactivity scale; dogs which struggled and vocalised were scored as more reactive than those which did neither. This reactivity scale was significantly related to aggression, and each variable was negatively linked to the change in C/C immediately post examination. Thus a lack of, or very small change in C/C, was more likely in dogs which were reactive and/or aggressive during the examination. I would suggest that the veterinary surgeon was rating reactive dogs as aggressive dogs and that the aggression manifested during the examination was unlikely to be a result of fear but instead was a learnt response. Police dogs are specifically trained to guard their handler and display aggression in situations where they are threatened and of the two populations compared, police dogs were more aggressive than pet dogs. This suggestion therefore seems plausible. Alternatively, the small change in C/C in some aggressive police dogs suggests that some dogs may be using aggression as an effective coping strategy but this would require further investigation.

In section 2.4.1, I discussed work conducted by Carlstead et al (1992), in which tractable and affiliative cats showed a decrease in C/C when handled, attributable to the human contact received during the procedures. In this current study, dogs which showed a marked change; an increase or decrease in C/C were rated as less aggressive towards the veterinary surgeon. It is plausible that those dogs which showed a marked decrease in C/C were in fact friendly and thus found the human contact throughout the examination rewarding resulting in decreased C/C. However this requires further investigation and possible temperament testing of subjects to accurately assess their affiliative behaviour with people and its relationship to C/C.

2.4.5 Do army dogs performing behaviours indicative of chronic stress i.e. stereotypies, differ in their basal levels of C/C and response to an acute stressor compared to non-performers?

Analysis showed that change in C/C was related to whether or not, and in which situation, a dog stereotyped. This effect seemed most pronounced within the sample collected two hours post examination. Unfortunately this effect could not be explored further due to sample size. However, overall the results suggest that the physiological response varies dependent upon whether dogs stereotype, and whether the behaviour is performed when in front of a person or alone. This is similar to the findings of Denham (2007) who also demonstrated that the physiological response to an examination differed dependent upon when a dog stereotyped. However, Denham (2007), unlike myself, used a wide range of stimuli, not just people, to elicit stereotypies. Based upon this current study, and using, where possible, Denham's findings (2007), I will now discuss some potential reasons why dogs with different stereotyping responses may respond in different physiological ways (Figure 2.8).

In general, those dogs which did not stereotype (N=7), did not show a pronounced physiological response either, suggesting that these dogs are generally unreactive or unresponsive to stimuli. It is plausible that these dogs may have adapted to a kennelled environment, so do not manifest behaviours indicative of chronic stress, and further have been socialised to the procedures such as those before and within the examination. It is also feasible that dogs which neither respond behaviourally nor physiologically are incapable of any response, being in a state of learned helplessness where by an animal becomes apathetic and shows an overall decrease in responsiveness in its environment (1.4.2.2 and Seligman, 1975 as cited in Friend, 1990). However, the results in the current study contrast with those of Denham (2007). Non-stereotyping dogs in Denham's study did exhibit a physiological response to the examination; with the most pronounced increase immediately post examination. These dogs also had the lowest levels of C/C leading him to conclude that these dogs were in fact the least stressed and responding in the expected way. Had the examination in the current study been consistently stressful, non-stereotyping dogs may have responded in the same way as Denham's. On the other hand, Denham observed dogs in response to multiple stimuli and as a result his group which did not stereotype comprised only two dogs and so caution should be heeded in drawing conclusions from this.

The small number of dogs observed to stereotype only in the presence of a person, appeared to show a decrease in C/C following the examination. As discussed in section 1.4.2.5, stereotypies can be indicative of stress suggesting that some dogs may become stressed when in the presence of a person. However, these behaviours may also be indicative of frustration. Inter-specific contact is limited within the kennel environment (1.5.2.8) and so dogs are often unable to control or make human contact. It is therefore feasible that those dogs which find human contact rewarding develop these behaviours as a result of frustration or to attract human attention and when contact is provided (e.g. in a veterinary examination), they show a decrease in C/C levels.

Dogs which stereotyped when alone tended to excrete increased levels of C/C immediately following the examination whilst similar dogs in Denham's study showed a marked decrease. Further, in the current study, the C/C of those dogs which stereotyped in both the presence and absence of a person increased, two hours following the examination. It is difficult to propose reasons as to why these relationships tend to be present, particularly as the numbers of dogs in all cases are small. However the mechanisms underlying stereotypy performance are complex (1.4.2.5) and identifying the cause of stereotypies is difficult. I would therefore suggest that further work in this area is warranted.

2.4.6 Can a consistently stressful challenge be developed?

In light of these results it is apparent that a routine veterinary examination was not a successful challenge largely because the response was variable between individuals. Within the army and pet populations, some individuals responded to the examination and its associated cues, as demonstrated by the markedly increased levels of C/C both immediately and two hours post examination, but on a population level, this was not a significant effect. It is possible that the examination within this study was not uniformly stressful due to individual differences in experience, but it is also plausible that the stressor was not long enough in duration to elicit a response. In van Vonderen et al's study (1998), the response in C/C to three separate procedures was measured; a visit to a veterinary practice, an orthopaedic examination in a referral clinic and hospitalisation for 34 hours. Whilst a number of individuals responded to each procedure, the largest number of responders was seen as a result of hospitalisation, suggesting this was either the most severe stressor or that the duration of the stressor was long enough to elicit an HPA response in many individuals. Hospitalisation is likely to be severely stressful as it is encompasses a number of social and environmental stressors, separation from social attachment figures (1.5.2.8), unpredictability (1.5.2.16), lack of control over the environment (1.5.2.16) and limited opportunities for social contact with both humans (1.5.2.8) and dogs (1.5.2.9), similar to those also present when dogs are first introduced to kennels, a procedure known to significantly activate the HPA system (1.4.1.1 and Rooney et al. 2007b).

Nonetheless, the most successful challenge is likely to be that which is independent of past experience and equally stressful to all subjects such as HPA secretagogues administration or, as suggested by Hiby (2005), physical stress. As adrenal glands respond to exercise, Hiby proposed that dogs be exercised at a standard heart rate for a set amount of time, C/C being measured before and after.

2.5 Conclusion

The non-invasive challenge developed within this study did not successfully activate the HPA system of all dogs. Some individuals responded to the challenge, but with both increases and decreases in C/C, so the response differs which, I suggest, may be dependent upon past experience.

The time at which the response occurred varied between individuals demonstrating the effects of other stressors such as transportation and other possible cues and anticipation. This highlights the need for regular sampling to ensure C/C excretion is accurately represented.

Basal levels of C/C differed significantly between populations with levels of C/C lowest in pet dogs. Assuming the latter are less likely to be chronically stressed, this suggests that elevated levels of C/C are indicative of chronic stress. These results are consistent with the conclusions of Beerda et al (1999; 2000), so throughout this thesis I will interpret levels of C/C accordingly. The possibility of physiological adaptation and stressor specific responses will however be considered.

Neither subjective nor objective measures of fear or stress related significantly to physiological responses. Thus, behaviour did not predict the physiological response of dogs to a potentially stressful event. Behavioural measures of reactivity were related to ratings of aggression both of which were negatively linked to changes in C/C: those dogs which showed small changes in C/C were the most aggressive toward the veterinary surgeon suggesting that they were either not stressed by the procedure or that aggression helped them cope with the situation. Previous research using cats suggested that those animals which showed marked decreases in C/C may find the examination rewarding due to the resulting human contact. On the basis of findings in cats, I suggest that this may also be true of dogs. However, further research and the use of temperament testing is required before this can be determined.

The C/C response to the examination of army dogs appears to differ depending upon if and when the dog stereotypes. Sample sizes were however too small to determine definitive relationships.

A successful challenge needs to be one which is independent of past experience or based upon the administration of secretagogues; ACTH or CRH. A Home Office Licence would be required.

Chapter 3

Kennelled dog behaviour:

Exploring differences due to time of day and observation technique

3.1 Introduction

In Chapter two, I explored a physiological indicator of welfare but since I am measuring welfare using a multi disciplinary approach, I also need to develop the best way to measure behaviour. Behaviour is an easily observable manifestation of welfare status, providing information about the animal's needs, preferences and internal states (Mench and Mason, 1997). Behavioural observations are non-invasive and non-intrusive and it is widely assumed that they cause minimal disturbance to the subject; hence they have a major role in assessing welfare (Dawkins, 2004).

However, the time of day at which behavioural observations are made may affect the behaviours observed, even in domesticated animals. For example, rats are generally more active in the morning than in the afternoon (Saibaba et al, 1995), and cockerels (*Gallus gallus*) engage in more preening behaviour during the afternoon than in the morning or evening (Daniyan et al, 2001). Observations taken at only one point during the day are therefore unlikely to reflect the full repertoire of behaviour, either qualitatively nor quantitatively.

The observation technique employed may also affect the behaviour measured. If the animal is aware of the observing person, the frequency, form or duration of behaviours may change (Caine, 1990) confounding or biasing hypothesis testing (Macfarlane and King, 2002). Observations by an unconcealed person have been used across a wide range of domestic species to measure behaviour, e.g. pigs (Mason et al, 2003), cats (Ottway and Hawkins, 2003), wild guinea pigs (*Cavia aperea pamparum*) (Guichon and Cassini, 1998) and horses (*Equus caballus*) (Mills and Davenport, 2002). Yet in a recent review of 15 studies where direct observations were used to record behaviour, evidence for an effect of observer presence was apparent in over half of the papers (Wade et al, 2005). For example, in the presence of a person, defensive behaviour patterns of cats were more common than when the observer was absent (Nott and Bradshaw, 1994).

Studies of welfare of kennelled dogs, often use behavioural observations (Beerda et al, 1999; 2000, Campbell et al, 1988, Graham et al, 2005a & b, Hetts et al, 1992, Hiby et al, 2006, Hubrecht, 1993; 1995, Hubrecht et al, 1992, Lore and Eisenberg, 1986, Mertens and Unshlem, 1996, Rooney et al, 2007b, Wells, 2004b, Wells and Hepper, 1992; 1998; 2000; 2001 and

Wells et al, 2002a & b) yet neither the effects of diurnal variation, nor observation technique have been investigated systematically for this species.

Husbandry of kennelled working dogs, such as feeding, exercise and cleaning, mainly occurs at specific times during daylight hours. Some studies have recognised the possibility of temporal effects, and measurements have either been carried out at the same time each day (e.g. Graham et al, 2005a & b, Wells and Hepper, 1998, 2000) when exposure to extraneous events can be avoided, or observations have been recorded across the day (e.g. Bebak and Beck, 1993, Hubrecht, 1993) to average out any temporal bias. The optimal method has yet to be determined.

In addition, dog behaviour is highly likely to be affected by the presence of an observer. Human companionship is very important to dogs (Tuber et al, 1996), yet social contact can be limited within the kennelled environment. For example, Hubrecht et al (1992) observed that for laboratory dogs, human contact occupied as little as 0.24-2.52% of the time observed. Therefore, dogs may react very differently when in the presence of a person observing their behaviour than when being filmed remotely. Dependent upon their past experience, dogs may attempt to increase sensory input from, or to be nearer to, the observer e.g. by standing on hind legs (Hubrecht et al, 1992) or may, if unsocialised, display fearful or apprehensive behaviours such as barking, lip licking or paw lifting (Beerda et al, 1997).

This effect can be reduced by hiding the observer (Beerda et al, 1999, Wells and Hepper, 1992,), allowing habituation (Mertens and Unshelm, 1996) or approaching in a manner which is unlikely to disturb the dog (Wells and Hepper, 1992). However, of the studies which have recorded behaviour in the presence of an observer, (Graham et al, 2005a & b, Mertens and Unshlem, 1996, Wells, 2004b, Wells and Hepper, 1992, 1998, 2000, 2002b, Wells et al, 2002) only one has quantified the effect upon behaviour (Hubrecht et al, 1992); laboratory dogs were less active and spent more time standing when in the presence as compared to the absence of a person.

Within this study I have investigated whether the behaviour recorded in two populations of kennelled dogs differed dependent upon time of day and the observational technique used.

Within each population I sampled behaviour recorded remotely by videotape and recorded directly by an observer (myself). I also taped at two different times of day: mid-day and evening. I then compared, between populations, the number of dogs performing each behaviour in each observation. This determined whether differences between observations could be analysed using combined data; from both populations, or whether the populations should be analysed separately. I was then able to compare differences in behaviour between each pair of observations, determining whether differences were applicable to the two populations and therefore perhaps might apply to all kennelled dogs, or were population specific and hence likely to be influenced by differences in husbandry regimes and background levels of human contact.

3.2 Methods

3.2.1 Subjects

Two populations, owned by two different agencies, were used for the study. Dogs were randomly selected from eight sites within each population, such that no more than nine dogs were sampled within each site. Any dog reported to be overtly aggressive was not chosen due to safety concerns.

3.2.1.1 Population one

Population one (N=45) was housed within England and Scotland, and predominantly comprised police dogs (1.1) which were worked both during the day and night. The subjects were housed at eight different sites; 3, 6, 5, 4, 6, 9, 6 and 6 respectively and each site was visited between April and July 2004. The subjects comprised 42 German Shepherd Dogs (GSD), two Belgian Malinois and one Belgian Malinois x GSD cross breed. A total of 43 dogs were male, 36 entire and the remainder neutered. Both females were neutered. At the time of the study, the dogs ranged in age from 27 to 124 months of age (mean =68 ±4), and had been housed at their current establishment for between 1 and 73 months (mean=25 ±3).

3.2.1.2 Population two

Population two (N=47) was housed within England, Scotland and Northern Ireland and predominantly comprised Patrol Arm True (PATAT) dogs (1.1) which worked during the night only. The subjects were also housed at eight different sites; 5, 4, 6, 4, 7, 6, 7 and 8 respectively and each site was visited between April and August 2005. The subjects comprised 43 GSDs, three Belgian Malinois and one Belgian Malinois cross breed; 45 were male of which 36 were entire with the remainder neutered. Both females were neutered. At the time of the study, dogs ranged from 24 to 104 months of age (mean =60.1 ±3.0), and had been housed at their current establishment for between 2 and 57 months (mean=26.5 ±2.4).

3.2.2 Housing and husbandry

3.2.2.1 Population one

All dogs were housed singly in kennels which comprised a sleeping compartment and outdoor run. The kennel area ranged from 5.8m^2 to 12.3m^2 (mean= 7.0m^2). Kennel assistants were responsible for the kennel cleaning, feeding and daytime exercise of all dogs at each of the sites, except for one where daily husbandry was provided by the dog handler who was working during that day. Exercise was either provided via a leashed walk or within an enclosed compound where the dog could run around. Dogs were fed either once or twice a day and exercised for between 0 and 30 minutes per day.

3.2.2.2 Population two

All dogs were housed singly in kennels which comprised a sleeping compartment and outdoor run. The kennel area ranged from 15.9m^2 to 19.7m^2 (mean= 18.6m^2). In population two, kennel assistants were responsible for the kennel cleaning and feeding, either once or twice a day. Exercise periods, in which the dog was loose run, ranged from 0 to 30 minutes per day, and was provided by the dog's own handler.

Throughout the study period, none of the dogs were worked but all underwent their normal husbandry and exercise regime to ensure that all were similarly rested and in their kennels at the time of filming.

3.2.3 Sampling and analysis of observations

For each subject, three observations were made, all on the same day; one in the presence of a person and two recorded remotely.

3.2.3.1 Observations recorded in the presence of a person (direct)

To minimise extraneous disturbance, dogs were observed between the hours of 09:30 and 11:15, and during the kennel assistant's morning break. I stood 0.5m in front of the outdoor area of the kennel for two minutes and used a Psion™ handheld event recorder to record the occurrence of twenty two behaviours (derived from those used by Hiby et al, 2006; Table 3.1). At the end of the observation, I either moved to another subject or terminated observations. A two minute observation period was chosen since pilot studies indicated that if longer samples were taken, some but not all dogs began to habituate to the observer, thereby increasing potential inter-individual variation.

3.2.3.2 Observations recorded remotely

The timing of both remote observations was chosen to minimise any human disturbance and thus two observations, one between the hours of 12:00 and 13:30 during the care staff's lunch break (midday) and one between 16:00 and 18:40, after all care staff had left (evening), were recorded using a Sony Handycam vision (2.2.4.2.2). The camera was placed on a tripod and positioned to maximise the amount of kennel area visible. Six minutes were recorded but only the last five were analysed to minimise the effects of the departure of the experimenter upon the dog's behaviour. The camera was then either moved to another subject or taping was terminated. During the filming period, the compound in which the dogs were housed was locked, to stop anyone from approaching the dogs and affecting their behaviour.

The same 22 behaviours recorded during the direct observation (Table 3.1) were analysed from the video recordings of midday and evening behaviour using the Psion handheld event recorder. The behaviours from both direct and remote observations were downloaded using 'The Observer' v.5 software package (Noldus Information Technology).

Behaviours performed by less than 10% of the subjects overall were discarded in order to eliminate any effect of bias in favour of recording rare behaviour patterns in the five minute as compared to the two minute samples. The 17 remaining behaviours from all three observations were converted into rates per minute.

Table 3.1 Descriptions of behaviours measured during the three observations. Those behaviours performed by greater than 90% of the overall population and thus analysed further are denoted in bold text.

Behaviour	Definition	Method of measurement (f=frequency, s=durations in seconds)
Bark	See section 2.2.4.2.1 Table 2.2	f
Howl	High pitched, loud vocalisation, with mouth open and head extended	f
Growl	See section 2.2.4.2.1 Table 2.2	f
Paw lift	Single fore limb raised and held above ground	f
Area transitions	Number of passages between any of the 5 areas of kennel (front left, front right, back left, back right and inside)	f
Drink	Ingest water	f
Lick lips	Tongue extruded from mouth and run over lips	f
Yawn	Jaws open wide without vocalising	f
Stretch	In standing position, full extension of either hind limbs or fore limbs whilst body pulls against extension	f
Sniff	Nose held close to or in contact with ground or kennel environment, inhalation occurs simultaneously	f
Stereotype	See section 2.2.4.2.2	s
Pant	See section 2.2.4.2.1 Table 2.1	s
Groom	Behaviours directed to own body including licking, stretching and scratching	s
Lie down	a) Back legs bent, front legs straight out in front on floor b) laid on side with body curled up c) laid flat on one side	s
Stand	All legs straight, head up or down	s
Sit	Hind quarters in contact with floor, fore limbs in air or in contact with kennel side/mesh	s
Stand on hind legs	Front legs in air or in contact with kennel side/mesh	s
Located in sleeping compartment	In sleeping compartment of kennel	s
Gaze at exit	Dog's head and eyes orientated in direction of exit	s
Investigate surroundings	Nose in contact with kennel environment e.g. door or ledges	s
Investigate object	Nose in contact with object in kennel environment e.g. toy, bowl	s
Play with object	Dog manipulating object either with mouth or paws	s

3.2.4 Statistical analysis

To control for any potential diurnal effects, the direct observation was compared with the remote observation which, in time, was the closest, i.e. midday. Pairs of observations (remote midday vs. remote evening and direct vs. midday) were compared in the same way described below, the remote midday vs. remote evening comparison is used as an example.

3.2.4.1 Comparing population profiles of behaviour

To decide whether populations should be analysed together or separately, each behavioural variable, for each population, was coded as follows: number of dogs performing the behaviour in remote midday observation only, number of dogs performing the behaviour in remote evening observation only, and number of dogs performing the behaviour in both observations. The two data sets, one from each population, describing the number of dogs performing each behaviour and in each combination of the observations, were compared using Chi square tests, or, if the number of dogs performing the behaviour, in any observation, was less than five, Fisher's Exact test was used.

If the two population profiles for a behavioural variable did not differ significantly from one another then the data from the populations was combined. If population profiles of the variable did differ, then the data was not combined and instead that behavioural variable was analysed separately in each population.

3.2.4.2 Comparing pairs of observations (remote midday vs. direct and remote midday vs. remote evening).

Frequencies and durations of variables recorded during the direct observation and remotely at midday and remotely during the midday observation and in the evening were compared for differences in magnitude using Wilcoxon signed rank tests, and consistency within individual dogs was examined with Spearman rank correlations.

3.3 Results

3.3.1 Comparing behaviour observed remotely at two different times of day

When comparing population profiles between midday and evening, two of the 17 behavioural variables, were found to differ; pant, and stand (Table 3.2). For these behaviours, populations were not combined but instead, each population was analysed separately.

Table 3.2 Percentages of dogs performing each behaviour pattern, comparing the two populations, at the remote midday and evening observations. Test statistic is either Chi Square, or Fisher's Exact Test, denoted by an asterisk (*), if less than 5 dogs performed a behaviour in any of the categories. †Performed by too few dogs to test. Significant differences between populations denoted in bold text.

	Percentage of dogs performing behaviour in remote midday observation only		**Percentage of dogs performing behaviour in remote evening observation only**		**Percentage of dogs performing behaviour in both observations**		**Test statistic**	**P value**
	POP 1	POP 2	POP 1	POP 2	POP 1	POP 2		
Behaviour								
Bark	8.9	12.8	0	4.3	0	2.1	1.7	0.42
Howl	13.3	6.4	0	2.1	0	0	1.7	0.2
Growl	2.2	2.1	0	0	0	0	†	
Paw lift	2.2	2.1	0	0	0	0	†	
Area transitions	40.0	21.3	4.4	6.4	17.8	36.2	5.7	0.06
Lick lips	26.7	29.8	11.1	17.0	11.1	19.1	0.47	0.79
Yawn	8.9	23.4	4.4	8.5	6.7	8.5	0.58	0.75
Stereotype	8.5	12.8	2.2	6.4	0	0	*	1.0
Pant	**24.4**	**12.8**	**0**	**8.5**	**0**	**14.9**	**11.7**	**0.003**
Groom	8.9	19.1	13.3	17.0	4.4	2.1	1.4	0.50
Lie down	8.9	6.4	8.9	4.3	77.8	85.1	1.1	5.8
Stand	**2.2**	**12.8**	**31.1**	**6.4**	**64.4**	**78.7**	**11.6**	**0.003**
Sit	28.9	25.5	4.4	8.5	4.4	6.4	0.80	0.67
Stands on hind legs	4.4	6.4	0	2.3	0	0		1.0
Located in sleeping compartment	11.1	6.4	17.8	14.9	33.3	17.0	0.55	0.76
Gaze at exit	15.6	38.3	4.4	12.7	8.9	31.9	0.28	0.87
Investigate surroundings	6.7	21.3	0	14.9	0	2.1	2.1	0.34

When comparing the 15 behaviours which did not differ in population profile, eight showed significant differences between the times of sampling and a further two tended to show significant differences (Table 3.3). At midday, dogs spent more time sitting, gazing at the exit and moving around the kennel. They barked, howled and yawned more and tended to stereotype and stand on their hind legs for longer. During the evening they spent more time lying down and in their sleeping compartment. Dogs were consistent in their performance of five behaviours (($Rho>0.21$, $p<0.05$), Table 3.3): time located in their sleeping compartment, lying down, gazing at the exit, licking their lips and yawning (Table 3.3), but for all other behaviour patterns, correlations were below 0.2 (less than 5% of variance explained), indicating little or no within-dog consistency between different times of day.

Table 3.3 Comparison between remote midday and evening observations for variables where the population profile did not differ, and whether individuals behaved consistently between these two observations (last two columns). Mean rate per minute (see Table 3.1), Wilcoxon signed rank test, z value and p value presented. Consistency between samples tested by Spearman rho.

Behaviour	**Mean for remote midday observation**	**Mean for remote evening observation**	**Wilcoxon signed rank test statistic (z)**	**P value**	**Spearman Rank Correlation Coefficient (Rho)**	**P value**
Stereotype	0.91	0.36	-1.8	0.07	-0.074	0.48
Lie down	42.3	49.1	-2.3	0.02	0.33	0.002
Sit	5.1	0.6	-3.8	<0.001	0.09	0.37
Stands on hind legs	0.02	0	-1.8	0.08	-0.03	0.81
Located in sleeping compartment	14.7	20.8	-2.7	0.007	0.60	<0.001
Investigate surroundings	0.12	0.12	-0.49	0.63	-0.03	0.78
Bark	0.45	0.10	-2.4	0.01	0.15	0.17
Gaze at exit	7.2	3.8	-3.1	0.002	0.37	<0.001
Howl	0.19	0.01	-2.3	0.02	-0.03	0.74
Area transitions	1.2	0.67	-3.9	<0.001	0.13	0.23
Lick lips	0.35	0.32	-1.1	0.29	0.21	0.05
Yawn	0.09	0.04	-2.7	0.007	0.36	<0.001
Groom	1.1	1.7	-0.4	0.66	-0.03	0.81

When comparing the two behaviours which differed in population profile, pant and stand, dogs in population one only panted during the remote midday observation (4.0 vs. 0, z=-3.9, p=0.002) but did not differ in the time spent standing between the remote midday and remote evening observations (12.4 vs. 8.9, z=-1.2, p=0.2). Dogs in population two did not differ in the time spent panting (6.6 vs. 7.9, z=-2.4, p=0.81) or the time spent standing (13.0 vs. 12.0, z=-0.1, p=0.48). In both populations dogs were moderately consistent in their performance of standing (Pop 1: Rho=0.41, p=0.005; Pop 2: Rho=0.44, p=0.002) whilst in population two, dogs were moderately consistent in their performance of panting (Rho=0.44, p=0.002).

3.3.2 Comparing behaviours observed using direct and remote observation techniques

Of the 17 behavioural variables, when comparing population profiles, four differed in their occurrence between populations; lick lips, stand, located in sleeping compartment and investigate surroundings (Table 3.4). These behaviours were not combined, but instead, each population was analysed separately.

Table 3.4 Percentages of dogs performing each behaviour pattern, comparing the two populations at the direct and remote midday observations. Test statistic is either Chi Square, or Fisher's Exact Test, denoted by an asterisk (*), if less than 5 dogs performed a behaviour in any of the categories. †Performed by too few dogs to test. Significant differences between populations denoted in bold text.

	Percentage of dogs performing behaviour in direct observation only		**Percentage of dogs performing behaviour in remote midday observation only**		**Percentage of dogs performing behaviour in both observations**		**Test statistic**	**P value**
	POP 1	POP 2	POP 1	POP 2	POP 1	POP 2		
Behaviour								
Bark	42.2	51.1	8.9	4.3	0	10.6	5.2	0.08
Howl	22.2	2.1	11.1	4.3	2.2	2.1	2.3	0.32
Growl	13.3	8.5	2.2	2.1	0	0	*	1.0
Paw lift	0	17.0	2.2	2.1	0	0	*	0.2
Area transitions	22.2	17.0	31.1	25.5	24.4	31.9	0.99	0.61
Lick lips	**22.2**	**12.8**	**26.7**	**14.9**	**11.1**	**34.0**	**8.0**	**0.02**
Yawn	4.4	6.4	13.3	29.8	0	2.1	0.6	0.74
Stereotype	6.4	17.0	6.7	6.4	2.2	6.4	1.1	0.59
Pant	2.2	6.4	20.0	21.3	4.4	8.5	0.88	0.64
Groom	8.9	0	13.3	19.1	0	0	*	0.09
Lie down	0	0	57.8	66.0	31.1	25.5	*	0.64
Stand	**31.1**	**6.4**	**4.4**	**2.1**	**62.2**	**89.4**	**0.2**	**0.006**
Sit	20.0	17.0	20.0	14.9	31.1	17.0	0.57	0.75
Stands on hind legs	8.9	10.6	2.2	2.2	13.3	4.3	0.16	0.92
Located in sleeping compartment	**8.9**	**2.1**	**24.4**	**23.4**	**20.0**	**0**	**7.7**	**0.02**
Gaze at exit	2.2	0	22.2	70.0	0	0	5.8	0.06
Investigate surroundings	**6.7**	**0**	**6.7**	**23.4**	**0**	**0**	*	**0.03**

When comparing the 13 behaviours which did not differ in population profile, eight showed significant differences with observation method whilst two more showed a tendency (at 10%) (Table 3.5). In the presence of the observer, dogs spent more time standing on their hind legs, barking, growling and paw lifting, and tended to sit more. In the absence of the observer. dogs spent more time panting, grooming, lying down, and gazing at the exit, and tended to spend more time yawning. Dogs were significantly consistent (Rho>0.21, p<0.05) in their performance of the following behaviours; stereotyping, panting, lying down, standing on hind legs and tended to be consistent when sitting (Rho=0.20, p=0.06), though in only one of these (standing on hind legs) did the correlation account for more than 10% of the variance. For all other patterns measured, less than 5% of the variance could be attributed to consistency between the observations

Table 3.5 Comparison between direct and remote midday observations for variables where the population profile did not differ, and whether individuals behaved consistently between these two observations (last two columns). Mean rate per minute (Table 3.1), Wilcoxon signed rank test, z value and p value presented. Consistency between samples tested by Spearman rho.

Behaviour	**Mean for direct observation**	**Mean for remote midday observation**	**Wilcoxon signed rank test statistic (z)**	**P value**	**Spearman Rank correlation coefficient (Rho)**	**P value**
Stereotype	2	0.9	-1.3	0.19	0.24	0.02
Pant	1.4	5.4	-3.1	0.002	0.26	0.01
Lie down	13.7	42.3	-6.7	<0.001	0.21	0.04
Sit	8.3	5.1	-1.7	0.09	0.20	0.06
Stands on hind legs	0.39	0.02	-3.0	0.003	0.34	0.001
Bark	15.7	0.45	-6.0	<0.001	-0.03	0.78
Gaze at exit	0.49	7.2	-5.3	<0.001	-0.03	0.98
Growl	0.22	0.01	-2.8	0.005	-0.05	0.62
Howl	0.67	0.19	-1.1	0.28	0.07	0.52
Area transitions	1.6	1.2	-1.1	0.28	-0.04	0.73
Yawn	0.04	0.09	-1.7	0.097	-0.05	0.63
Paw lift	0.15	0.01	-2.5	0.01	-0.05	0.66
Groom	0.25	1.1	-2.1	0.04	-0.10	0.36

When comparing the four behaviours which differed in population profile; stand, located in sleeping compartment, investigate surroundings and lick lips, differences between observations were evident for all four behaviours, but not in both populations. Dogs spent more time standing in the presence of an observer than when filmed remotely in both populations (Pop 1: 38.3 vs. 12.4, z=-4.6, p<0.001; Pop 2: 37.1 vs. 13.0, z=-4.6, p<0.001). Dogs in population two spent significantly more time in their sleeping compartment when filmed remotely (8.6 vs. 0.09, z=-2.9, p=0.04) whilst the same result was evident as a tendency in population one (21.1 vs. 11.1, z=-1.8, p=0.08). In population two, dogs spent significantly more time exploring their surroundings when filmed remotely compared to when observed directly (0.21 vs. 0, z=-2.9, p=0.03). This was not observed in population one. In both populations, the number of lip licks did not differ between observations (z<-0.81, p>0.13). None of the correlations between individuals in the two observations were significant (Rho<0.27, p>0.10) although dogs tended to be consistent in their performance of standing in population one (Rho=0.27, p=0.06). However this did not account for more than 10% of the variance indicating little or no consistency between different observation methods.

3.4 Discussion

3.4.1 Time of sampling

3.4.1.1 Does behaviour differ when observed at two different times of day?

This study has shown that time of observation strongly influences the behaviour of kennelled dogs. In general, during the midday observation, levels of barking, howling, standing on hind legs, gazing at exit, moving around the kennel, stereotyping, yawning and sitting were all greater than those observed in the evening whilst the time spent lying down and in the sleeping compartment were greatest in the evening. It is possible that these differences are a reflection of the dogs' diurnal rhythm, since they are more likely to rest at night. However the midday observation was taken during the kennel assistant's lunch break so it is equally plausible that these variations are linked to the timing of husbandry procedures. For example, some of the dogs had not been exercised at the point of filming, neither had they been fed. Therefore these behaviours may be indicative of anticipation, stress or frustration. Yawning can be a behavioural response to acute stress (Beerda et al, 1997) whilst stereotypical behaviour can indicate frustration manifested as circling or pacing (Mason, 1991). Dogs associate people with husbandry events and thus these dogs may be seeking contact with people as reflected by the increased levels of vocalisations; barking and howling can be a means of seeking contact with humans or other dogs (Bradshaw and Nott, 1995). Furthermore, standing on hind legs would allow the dogs to increase their sensory input and increase their opportunities for visual contact with people (Hubrecht, 1993a), whilst gazing in the general direction from which a person would appear in the kennel area would additionally increase the opportunity for visual contact. Higher levels of moving around the kennel and sitting suggest that the dogs were less relaxed at lunchtime. However, during the evening, once the kennel assistants have departed and all husbandry events have been completed, dogs are able to predict a decrease in stimulation, and a reduction in diversity of repertoire is observed, as dogs spend significantly more time in their sleeping compartments. An influence of husbandry regimes upon behaviour has been noted in fish (Almazan-Rueda et al, 2004) and laboratory rats (Saibaba et al, 1995) and the findings from this study suggest that husbandry also influences the diurnal rhythm of kennelled working dogs.

3.4.1.2 Are differences between midday and evening observations general to different populations of kennelled working dogs?

Some differences attributable to time of sampling do not appear to be general to kennelled working dogs; the population profiles for panting, which can be indicative of acute stress (Beerda et al, 1998) differed between the two populations. In population one, dogs panted more during the midday observation than during the evening but this relationship was not evident in population two. I suggest that this disparity is due to the subtle differences in working patterns and husbandry regimes. Dogs in population one work during the day and night whilst population two work during the night only. Hence it is feasible that dogs in population one may be awaiting the arrival of their handler, and as a result display indicators of stress. Furthermore, husbandry, and specifically exercise, within population one is provided by kennel assistants and so is much more predictable than exercise provision in population two which is provided by the handler. Dogs may therefore be anticipating exercise yet to occur in the afternoon and display acute stress responses as a result.

3.4.1.3 Are individuals consistent in their tendency to perform behaviour at two different times of day?

When comparing behaviour measured at midday with that during the evening, only three behaviours appeared to be unaffected by time of sampling; investigating surroundings, grooming and the number of lip licks. The population means for these behaviours were very similar in both observations, but when examining individual dogs, there was only consistency in the performance of lip licking between observations. This suggests that the tendency to perform this behaviour is a consistent aspect of a dog's temperament. However, individuals were inconsistent in the time spent grooming or exploring the kennel environment. Hence observing dogs at only one time of day may lead to incomplete or inaccurate welfare assessments of individuals, and underestimates of the numbers of dogs performing some welfare indicators throughout the day. This is further highlighted if the performance of stereotypic behaviour is considered. Whilst the mechanisms underlying the performance of stereotypies are complex (1.4.2.5, Mason and Latham, 2004), they are commonly used to indicate welfare problems (Mason, 1991) and thus are often used to assess welfare (Mason and Latham, 2004). However, in this study, even though there was no discernable average difference between the populations (at 5%), individuals were not consistent in their performance between the two observations. For example, a total of 14 dogs were observed to stereotype, four during the evening and ten at midday, but none stereotyped in both observations. Performing only one of the observations, and particularly just the evening observation, would have lead to a substantial underestimate of the number of dogs stereotyping. Thus sampling at several points during the day would be necessary if all the individuals performing stereotypic behaviour were to be identified.

3.4.2 Observation techniques

3.4.2.1 Does behaviour differ when observed directly and remotely?

In addition to the time of day, the presence of a person also strongly influenced the behaviour of these dogs; they barked, growled and paw lifted more, but also spent more time sitting and standing on their hind legs. When filmed remotely in the absence of a person, dogs spent a higher proportion of time panting, grooming and lying down, gazing at the exit and yawning. Vocalisations are performed in a wide range of contexts (Bradshaw and Nott, 1995) but because the observer was novel it is likely that both barking and growling were performed to threaten or warn the person. These vocalisations were often observed whilst the dog was standing on its back legs, enabling the dogs to increase the intensity of any threats or warning by decreasing the distance between itself and the observer, but standing on hind legs is also commonly seen in other single-housed dogs even in the absence of people (Hubrecht et al, 1992) and has been interpreted as a means of increasing sensory input about their surroundings, so in this study it may have been performed to increase sensory information about the observer.

Paw lifting is not completely understood, although it is often interpreted as an attention seeking behaviour or an indication to play (Prescott et al, 2004). However, it is hypothesised to be performed when chronic stress is experienced and welfare is compromised (Beerda et al, 1999). The performance of this behaviour is most likely influenced both by the dog's current level of human contact and previous experience with humans and hence the meaning of the behaviour can only be deduced by considering other behaviours performed concurrently.

In the absence of the observer, dogs spent more time gazing at the exit. They may have been seeking visual contact with people within the environment or, for those dogs which are less human-orientated, gazing at the exit could provide some form of visual stimulation outside of the kennel. However, since gazing at the exit was also performed at high levels during the midday observation, by comparison with the evening, it most likely indicates anticipation of human contact. Lying down or resting and grooming was more often performed when the dog was alone as compared to when a person was present, probably due to the decreased stimulation. Panting and yawning can both be indicative of acute stress (Beerda et al, 1997; 1998; 2000) although yawning may also indicate more general anxiety (Prescott et al, 2004). These behaviours may indicate that some dogs were becoming distressed in the continued absence of a person, particularly those which are very human orientated.

It should be noted that the remote and direct observations were also taken at two different times of day and so I cannot conclude that the differences in behaviour discussed above are solely attributable to the difference in observation techniques. Whilst it was not possible in this study, I would suggest, that this study be repeated but controlling for time of day.

3.4.2.2 Are differences between direct and remote observations consistent between populations of kennelled working dogs?

Although the performance profiles differed, dogs in both populations were still observed to spend more time standing and less time in their sleeping compartment when an observer was present, suggesting that the influence of an observer upon these two behaviours is general to all dogs. In previous studies, time spent standing was greater in the presence of an observer (Hubrecht et al, 1992), most probably because an observer provides a stimulus and inhibits resting. This may also account for time spent in the sleeping compartment being significantly greater when an observer is not present. The population profile for time spent investigating surroundings also differed between populations; the presence of an observer only influenced the behaviour of population two, in which dogs spent more time investigating their surroundings when alone compared to when in the presence of the observer, most probably because an observer disrupts the performance of this behaviour. Subtle differences in human contact and working patterns may account for the presence of this relationship in population two only. Exploring is a behaviour which may be affected by stress (Mench and Mason, 1997) suggesting that population two may be less stressed than one. However investigating this further this was beyond the scope of this study.

3.4.2.3 Are individuals consistent in their tendency to perform behaviour when a person is present and when alone?

When comparing behaviour recorded remotely with that recorded directly by an observer, only moving around the kennel was performed at similar levels; although, levels of stereotyping and howling were not substantially different. However, individuals were not consistent between observations in their performance of two of these behaviours, howling and moving around the kennel. Howling can be performed by individuals as a means of seeking contact with humans or other dogs (Bradshaw and Nott, 1995) whilst moving around the kennel may indicate a non-relaxed state in some dogs. This shows that individual dogs seem to vary in their expression of these behaviours throughout the day. As I have concluded with time of day, observing individuals, using only one observation method, will lead to an inaccurate and incomplete welfare assessment. This problem is further highlighted if I consider paw-lifting. Not only was there a discernable population difference, but inconsistency within individuals between observations was also found. Using only one observation method may thus result in incomplete welfare assessments of individuals and also populations.

3.4.3 Are there any behaviours which are not influenced by time of day or observation method?

Throughout this chapter, I have focussed upon those behaviours which differed significantly between each pair of observations. However, when comparing the observations, it is plausible that some behaviours will be less susceptible to observation time and technique. If uninfluenced by people and/or diurnal effects, levels of performance will not differ between observations, hence the choice of observation time or technique may be insignificant. However, there are very few behaviours where this was the case. When comparing the midday and evening behaviours, in all dogs, only three behaviours were performed at similar levels: the time spent investigating surroundings, grooming and the number of lip licks, whilst in population two, observation method had no discernible effect upon the time spent both panting and standing. When comparing behaviour recorded remotely with that recorded directly, all behaviours differed to some degree with only the number of area transitions nearing similar levels in both observations. These results demonstrate that it is in fact paramount to consider sampling technique, as behaviour is influenced to some degree by the presence of a person, and time of day likewise has a strong influence.

3.5 Conclusion

In general, the behaviour of both populations was influenced by time of sampling and technique in the same way. Those few behaviours where the population profiles did vary are, I suggest, a consequence of differences in housing or husbandry regimes specific to that population. Thus the influence of time of sampling and technique described here are likely to be representative of kennelled working dogs in general, and may also generalise to all kennelled dogs, although the details might be expected to vary from breed to breed. Moreover, even when similar levels of a particular behaviour were recorded at two different times or using two different techniques, there was generally little consistency in which individuals were performing that behaviour. This will have a profound effect on the accuracy of information about the welfare status of individual dogs, even if little difference is discernable at the population level.

I suggest that for accurate welfare assessment of kennelled dogs, observations should occur both in the presence and absence of an observer and at various times of day. Use of a single technique or recording at one time of day could lead to incomplete or inaccurate welfare assessment. Thus future studies within this thesis will implement these findings and always sample both in the presence and absence of people and at multiple times of day. Chapters 4 and 6.1 recorded the behaviour of dogs at different times of day and in the presence and absence of people. Chapter 5 recorded the behaviour of dogs at multiple times throughout the day.

Chapter 4

Exploring relationships between welfare and the housing and husbandry of military working dogs

4.1 Introduction

It is widely accepted that factors of housing and husbandry can influence the welfare of animals kept for food production (e.g. ruminants, Mounier et al, 2007; rabbits, (*Oryctolagus cuniculus*) Trocino and Xiccato, 2006 and pigs, Meunier-Salaun et al, 2007), experimental purposes (e.g. rodents, Balcombe, 2006, Sherwin, 2004, and non-human primates, Olsson and Westlund, 2007) and companion animals in shelters awaiting re-homing (e.g. cats, Hawkins, 2005). For example, space allowance influences both health of cattle and the behaviour of cats; the smaller the pen space allowance, the greater the occurrence of tail tip damage in cattle (Schrader et al, 2001) and the higher the stress level, as measured using behaviour, in cats (Kessler and Turner, 1999). Husbandry procedures, such as premature separation from conspecifics, can lead to increased stereotypic bar chewing in gerbils (*Meriones unguiculatus*) (Waiblinger and Konig, 2004), whilst handling of laboratory rats can disrupt social behaviour (Burman and Mendl, 2004). The opportunity for social contact (pair/trio housing) in previously isolated male olive baboons (*Papio Anubis*) significantly reduces the incidence of abnormal behaviour (Bourgeois and Brent, 2005) whilst the type of contact with stock people significantly affects the behaviour of livestock (Hemsworth, 2007); use of hand, moderate loud vocalisations and the use of a stick are associated with more stepping and kicking of cows, unwanted behaviour, and a lower milk yield (Waiblinger et al, 2002).

The effect of some of these factors upon the welfare of dogs has received investigation (introduced in section 1.5). For example, when investigating kennel size, laboratory dogs housed in small enclosures show a higher prevalence of stereotypic behaviours (Hubrecht et al, 1992) whilst the availability of intra-specific contact, both visual and physical, has significant effects upon behaviour; dogs housed in isolation generally showed a restricted behavioural repertoire (Mertens and Unshelm, 1996) and a greater incidence of behavioural abnormalities e.g. stereotypies (Hubrecht et al, 1992). Furthermore, dogs which receive positive inter-specific interaction are physiologically less stressed that those which do not (Coppola et al, 2006).

However, much of the research investigating the effect of factors upon dog welfare has concentrated on those housed either in laboratories or in re-homing shelters. Only one study has investigated the effect of factors upon the welfare of military working dogs and this was limited to investigating the effect of environmental enrichment upon behaviour and physiology (Hiby, 2005). Yet, as introduced in section 1.2.2 and 1.5.1, the majority of military working dogs are kennelled throughout their working life and this environment presents numerous factors, in addition to the availability of environmental enrichment, which may affect stress levels. I have identified ten factors associated with a kennelled environment which I believe may influence the welfare of military working dogs which vary sufficiently to be measurable and I now describe each. These factors were introduced in section 1.5, so within this chapter, only their mechanisms as stressors are discussed.

a Space allowance

Dogs are, by their nature, a roaming species; when feral, their home range is large and much time is spent active (Boitani et al, 1995). Yet, in many sites, the space provided within a kennel is relatively small, as it is intended only to be an area for rest as opposed to an area for living. The consequent restriction of movement, and lack of ability to perform species-typical behaviours, could be stressful (Prescott et al, 2004).

b Temperature

Whilst many kennels used to house military working dogs are heated, some are not. During winter, these dogs may be exposed to low temperatures. In other species, chronic exposure to low temperature elicits responses similar to that of chronic stress (1.5.2.5). In a similar study of military working dogs, low temperatures were related to an increase in C/C (Hiby, 2005) suggesting dogs may also be stressed when exposed to cold temperatures.

c Noise

Kennel establishments are typically very noisy environments with readings commonly exceeding 100dB and often reaching 125 dB (Sales et al, 1997). Detrimental effects of noise upon a number of different species have been noted (1.5.2.4) and it is therefore likely that there will be similar effects in dogs e.g. changes in physiological and behavioural parameters during periods of increased noise.

d Husbandry procedures

Husbandry procedures may be stressful for kennelled dogs. For example, during cleaning, if not removed from the kennel, the dog may be subjected to a number of aversive stimuli i.e. powerful jets of water and high levels of noise resulting from water Alternatively, they may be locked within the sleeping compartment of the kennel which may be stressful as the dog will be unable to control the stimuli.

e Inter-specific contact

Dogs have been selected for their propensity to bond with humans. Thus, many find social interaction rewarding and even stress-relieving; interactions such as petting (Hennessy et al, 1998), playing (Coppola et al, 2006) and mere human presence (Tuber et al, 1996) result in lower physiological stress levels. What is more, positive interaction between humans and dogs e.g. talking softly to, touching and stroking, leads to increased levels of phenylethylamine in the dog (a neurotransmitter causing feelings of elation, euphoria and exhilaration) (Odendall and Lehmann, 2000). However, the amount of positive contact provided by kennel assistants is likely to be varied, dependent upon the number of dogs for which each have responsibility.

f Intra-specific contact

Dogs are social animals (1.5.2.9) with an inherent desire, if socialised adequately, for social contact with conspecifics. Within the military kennelled environment, physical contact with other dogs is limited, as the majority of dogs are singly housed. This may be frustrating for some dogs, as they often have visual, olfactory and auditory contact with other dogs, but cannot control this contact themselves. Additionally, any attempts to make physical contact tend to result in negative consequences. For example, when leaving the kennel, many dogs will try to make contact with neighbouring kennelled dogs as they pass each another. Often behaviour performed as a means of soliciting interaction e.g. vocalising, is interpreted as aggression and thus dogs are rapidly moved away which may lead to increased levels of frustration. As a consequence, those dogs which either are on a through route and are passed by many dogs and those that pass a large number of dogs may well be more stressed than those which view or pass only small numbers.

g Environmental stimulation within the kennel

The majority of current kennel designs used to house military working dogs are featureless and many dogs are reported to chew either the kennel structure or items within the kennel such as bedding or furniture. This behaviour is often seen as a means of creating novelty, in response to an environment which provides little stimulation (Poole, 1992). Whilst the provision of toys and bones may help to provide a more appropriate outlet for this behaviour and in turn improve the welfare of some individuals (Hiby, 2005), there is often reluctance to provide enrichment as it is believed that items within the kennel may reduce motivation to work or lead to increased aggression (see also Chapter 6.2).

h External environmental stimuli

Environmental stimulation may also be present outside of the kennel. The ability to see livestock or other animals e.g. birds, is anecdotally reported to be beneficial to dogs with increased resting and decreased vocalising in those dogs where such visual contact is provided (Gray, *pers comm.*). However, for some dogs, the ability to see something over which it has no control may be stressful. Music is often played in kennel environments as it is believed dogs may benefit from auditory stimulation. Nonetheless, the benefit is dependent upon the type of music played. A study conducted by Wells et al (2002b) showed that classical music resulted in behaviours indicative of relaxation whilst rock music was linked to dogs spending more time barking.

i Exercise

Exercise, in the form of walks, provides a number of benefits for dogs, allowing behaviours including roaming, investigating and exploring to be performed. In addition, a wider range of movement can be displayed. However, the type, frequency and duration of exercise provided within a kennelled environment depend greatly upon the available resources. For example, exercising a dog off the lead or with other dogs is only possible if secure, large open spaces are available. The frequency and duration will depend upon the number of dogs each kennel assistant has to exercise, and the time available.

j Predictability of routine

Within the kennel environment, the ability for dogs to predict forthcoming events can be limited. For example, husbandry regimes may differ on a daily basis due to staff changeovers and will almost certainly change between weekday and weekend. Studies of laboratory cats and rats have demonstrated that unpredictability can have deleterious effects upon welfare (1.5.2.16) and Hennessy et al (1997; 1998) have suggested that unpredictability within the kennelled environment is a source of stress and fear.

Factors a-j are likely to influence the welfare of military working dogs. Therefore the purpose of the study described in this chapter was to explore the relationships between these factors and potential indicators of welfare in a population of military working dogs, and evaluate the relative importance of each factor upon welfare. Whilst my initial intention was to use military working dogs from only one agency, due to the small number of available dogs, military working dogs from two agencies were studied (from hereon referred to as populations one and two). However, both populations comprised the same breeds, and dogs were utilised for the same purposes. Furthermore, the way in which the dogs were housed and cared for appeared superficially similar. The two populations were housed across a number of different sites representing a wide range of housing and husbandry regimes. All sites were visited and information was gathered on the husbandry and behaviour of the dogs via a questionnaire and by interviewing kennel assistants. Details of housing and husbandry were recorded. Based on findings of Chapter's 2 and 3, baseline physiological measures of stress were taken and the behaviour of the dogs was recorded in the presence and absence of a person. However, as this study was exploratory, I also gathered information about each dog's health as it has been shown that compromised welfare can detrimentally affect the immune system leading to increased susceptibility to disease and infection (1.4.1.6). The information gathered was then used to derive potential indicators of welfare and factors describing aspects of housing and husbandry which might affect welfare.

The aim of the study was to combine the data from both populations; factors and indicators of welfare, so that general relationships between housing, husbandry and welfare could be explored. However, analysis of the factors revealed that, although the two populations appeared superficially similar, the way in which the dogs were housed and cared for were in fact significantly different (4.3). It was therefore not feasible to combine the data from both populations, but instead each population had to be analysed separately. For each population, the housing, husbandry and welfare at each site was compared and significant relationships between factors and indicators analysed (4.4). The similarities and differences in relationships between the two populations were then explored, identifying relationships either specific to one population only or general to both, and therefore potentially common to military working dogs in general (4.5).

4.2 Methods

4.2.1 Subjects

Two populations were used for the study. The first population (n=104), predominantly comprised police dogs (1.1) which were worked both during the day and night and were housed at seven different sites within England and Scotland. The median number of dogs at each site was 15, ranging from 5 to 31. The second population (n=114), were mainly PATrol Arm True (PATAT) (1.1) which worked during the night only, and were housed at eight different sites within England, Scotland and Northern Ireland. The median number of dogs at each site was 14.5, ranging from 7 to 28.

To compare the welfare status of dogs at different sites, I measured some indicators of welfare in all dogs, but some indicators were more time demanding and needed dogs to be rested to avoid confounding effects. For example, high levels of activity have been linked to elevated levels of creatinine (1.4.1.1). Thus a sub-sample of dogs was selected for detailed measures and from hereon such dogs are referred to as sample dogs. Sample dogs were chosen only if they were not working during the data collection period but those deemed overtly aggressive were omitted as this had implications for urine sampling. Similarly, any dogs requiring steroidal medication during the study were eliminated from the sample. The sample dogs have been previously described in the study in Chapter 3. However, one site in population one, which housed three dogs, was used to develop methods for the current study and so is excluded in the study sample. The demographics therefore differ from those in Chapter 3 and are described here.

4.2.1.1 Population one

The sample of dogs comprised 40 German Shepherd Dogs (GSD), one Belgian Malinois and one Belgian Malinois x GSD cross breed. A total of 40 dogs were male and of the males, 34 were entire with the remainder neutered. Both females were neutered. At the time of the study, dogs ranged in age from 27 to 124 months of age (mean =69 ±24.7). Dogs had been housed at their current establishment for between 1 and 73 months (mean=24.7 ±18.7). The number of dogs at each of the seven sites was 6, 5, 4, 6, 9, 6 and 6 respectively.

4.2.1.2 Population two

The sample comprised 43 GSDs, three Belgian Malinois and one Belgian Malinois cross breed; 45 were male and of the males, 36 were entire with the remainder neutered. Both females were neutered. At the time of the study, dogs ranged from 24 to 104 months of age (mean =60.1 ±3.0). Dogs had been housed at their current establishment for between 2 and 57 months (mean=26.5 ±2.4). The number of dogs at each of the eight sites was 5, 4, 6, 4, 7, 6, 7 and 8.

The housing and husbandry regimes used in populations one and two are as described in section 3.2.2.

4.2.2 Procedure

Data was collected over a three day period. On day one, at 14:00 hours, thermometers (4.2.3.2.5) were placed within a kennel and then, between 14:00 and 16:00, I delivered two questionnaires (4.2.3.1.1 and 4.2.3.2.1) to the kennel assistant with primary responsibility for the sample dogs. At 16:00, the behaviour of all dogs, in response to me, was observed for 30s (*screening observation*, 4.2.3.1.2a). This was conducted at the start of the study to ensure that familiarity with me was standard across all dogs.

Between 05:30 and 09:00 hours on day two, the first naturally voided urine sample (4.2.3.1.3) from each sample dog was collected by a kennel assistant. During the kennel assistant's mid morning break, between 09:30 and 11:30, the behaviour of each sample dog in the presence of me was observed for two minutes (*direct observation*, 4.2.3.1.2b). Between the hours of 12:00 and 13:30, (the kennel assistant's lunch-break), the behaviour of each sample dog was recorded remotely for six minutes using a video camera placed on a tripod (*remote midday observation*, 4.2.3.1.2b). A further six minute remote recording of each sample dog was taken at the end of the day, once the kennel assistant had left (between the hours of 16:00 and 18:40) (*remote evening observation*, 4.2.3.1.2b). In addition, throughout the second day, I collected information about the kennel building/compound and observed husbandry procedures (4.2.3.2).

A second urine sample was collected on the morning of day three from each sample dog. Also during the morning details of disease and illness were extracted from the site's health records (4.2.3.1.4). At 12:00 hours, thermometers were removed and I left the site.

4.2.3 Data collection and analysis

4.2.3.1 Indicators of welfare

4.2.3.1.1 Delivery and analysis of questionnaires

During the questionnaire (presented as Appendix 2), I asked the kennel assistant whether any of the sample dogs displayed stereotypical behaviour (2.2.4.2.2) or were coprophagic (1.4.2.8b), and from this information two presence/absence variables describing stereotyping and coprophagy were generated; *reported to stereotype* and *reported to be coprophagic*. The incidence of illness in sample dogs in the past month was also reported by the kennel assistant and a presence/absence variable generated; *reported illness in the past month.*

4.2.3.1.2 Sampling and analysis of kennelled behaviour

a) Screening observation

For the first 20 seconds (phase one), I stood in front of each dog, 0.5m away from the kennel looking towards the floor. During this time, I recorded two behaviours, barking and stereotyping, as frequencies on a tick sheet. Postures and positions which the dogs displayed for the majority of the 20 seconds within the kennel were noted. During the final 10 seconds (phase two), I attempted to make visual contact with the dog. The dog's reaction to this was noted along with its tail and ear position, posture and vocalisations. Stereotyping was converted into a presence/absence variable as was barking. However, barking was measured in both phases and so the presence /absence variable described whether the dog had barked during either phase. From hereon this observation will be referred to as the screening observation. Ten behaviours were recorded (see Table 4.1).

Table 4.1 Descriptions and methods of measurement and phase of measurement of behaviours observed in the screening observation.

Variable	Definition	Method of measurement	Phase; 1, 2 or both
Bark	See section 2.2.4.2.1, Table 2.2	0/1	Both
Stereotype	See section 2.2.4.2.2	0/1	1
Tendency to be at back of the kennel	Position of dog, within kennel, for majority of Phase 1	0=front, 1=back	1
Rest	Either, hind quarters and rump in contact with floor, fore limbs straight or in one of three down positions; a) back legs bent, front legs straight out in front on floor b) lying with body curled up c) lying flat on one side for majority of Phase 1	0=moving or standing, 1=sitting or laid down	1
Move	Either walking or stereotyping for majority of Phase 1	0=sitting, laid down or standing, 1=moving	1
Growl	See section 2.2.4.2.1, Table 2.2	0=no, 1=yes	2
Tail height	See section 2.2.4.2.1, Table 2.1for majority of Phase 2	See Chapter 2, Table 2.1	2
Ear height	See section 2.2.4.2.1, Table 2.1for majority of Phase 2	See Chapter 2, Table 2.1	2
Posture	Position of dog for majority of Phase 2	0=sitting or laid down, 1=standing, 2=moving	2
Tendency to look away	Dog's head and eyes orientated away from the observer during Phase 2	0=does not look away, 1=looks away intermittently, 2=looks away from observer (me)	2

b) Direct and remote observations

Twenty two behaviours were recorded from the videos in both the direct and remote observations. The method used to collect the 22 behaviours is described in section 3.2.3 and descriptions of the behaviours are detailed in Table 3.1.

Taking each population in turn, those behaviours which were performed in the direct and remote observations, by less than 10% of the dogs, were discarded. In population one, activity measured in the remote evening observation was rare and therefore all variables from the remote evening observation, with the exception of stereotypies, were discarded. As stereotyping is potentially an important indicator of stress, it was not discarded, but stereotyping in both the remote midday and evening observations were combined to give a single variable of whether each dog stereotyped when alone. The remaining behavioural variables were converted into rates per minute and those which were irregularly distributed were recoded as shown in Table 4.2.

Table 4.2 Behaviours measured in the direct and remote observations. The observation, population measured in, variable type and units are stated.

Variable	Observation	Population; 1, 2 or both	Variable type	Units
Bark	Remote midday	Both	Scale	0=0, 1<50, 2>50
	Direct	Both	Scale	0=0, 1<50, 2>50
Growl	Direct	Both	0/1	
Paw lift	Direct	2	0/1	
Area transitions	Direct	Both	Scale	0=0, 1<5, 2>5
	Remote midday	Both	Scale	0=0, 1<5, 2>5
	Remote evening	2	Scale	0=0, 1<5, 2>5
Lick lips	Direct	Both	Scale	0=0, 1<1, 2>1
	Remote midday	Both	Scale	0=0, 1<1, 2>1
	Remote evening	2	Scale	0=0, 1<1, 2>1
Yawn	Remote midday	2	0/1	
	Remote evening	2	0/1	
Stereotype	Direct	Both	0/1	
	Remote midday	Both	0/1	
	Remote evening	Both	0/1	
Pant	Direct,	Both	Duration	Seconds
	Remote midday	Both	Duration	Seconds
	Remote evening	2		
Groom	Remote midday	2	Duration	Seconds
	Remote evening	2	Duration	Seconds
Lie down	Direct	Both	Duration	Seconds
	Remote midday	Both	Duration	Seconds
	Remote evening	2	Duration	Seconds

Stand	Direct	Both	Duration	Seconds
	Remote midday	Both	Duration	Seconds
	Remote evening	2	Duration	Seconds
Sit	Direct	Both	Duration	Seconds
	Remote midday	Both	Duration	Seconds
	Remote evening	2	Duration	
Stand on hind legs	Direct	Both	0/1	
Located in sleeping compartment	Direct	1	0/1	0<30, 1>30
	Remote midday	Both	0/1	0<30, 1>30
	Remote evening	2	0/1	0<30, 1>30
Investigate surroundings	Remote midday	2	0/1	
	Remote evening	2	0/1	
Gaze at exit	Remote midday	Both	Duration	Seconds
	Remote evening	2	Duration	Seconds

4.2.3.1.3 Sampling and analysis of urinary cortisol to creatinine ratios (C/C)

Urine samples were collected and stored as described in section 2.2.4.1. Cortisol and creatinine were analysed and C/C calculated as described in section 2.2.4.1.

4.2.3.1.4 Sampling and analysis of health

Following procurement into the military and throughout each dog's working life, all veterinary treatment is recorded in a document (referred to as a health record) which is taken with the dog wherever it goes. This document was accessed for each sample dog and any visits to the veterinary surgeon for diarrhoea, sores resulting from excessive auto grooming, tail damage, foot problems and idiopathic skin complaints were recorded, as these were symptoms that could possibly be stress related or a result of stress. From this information, in population one, a presence/absence variable describing whether sample dogs had visited the veterinary surgeon in the past six months was generated (Table 4.3). The same information was gathered for all dogs but the presence/absence variable described whether dogs had visited the veterinary surgeon in the past year. In population two, a similar variable for sample dogs was generated but this described whether the dogs had visited the veterinary surgeon since arriving at its current station (Table 4.3). There is disparity between variables collected in population one and two because, having visited population two, I decided that a variable recording incidence of disease in sample dogs since arriving at the current site was more useful as some dogs had arrived within the last six months and hence looking at the last six months may reflect the last establishment as well as the current. However, I did not have the equivalent data for population one. All variables were included because the relationships between factors and indicators of welfare in the two populations were analysed separately.

4.2.3.1.5 *Overview of welfare indicators*

Table 4.3 provides an overview of the potential welfare indicators used in the current study. The source of the measure, population and dogs in which measured are given.

Table 4.3 Indicators of welfare used in analysis: the source of the variable, population in which measured and whether the data was collected from sample dogs only or from all dogs.

Variable	Source	Population; 1, 2 or both	Sample dogs=0, All dogs =1
Bark	Screening	Both	1
	Remote midday	Both	0
	Direct	Both	0
Growl	Direct	Both	0
	Screening	Both	1
Paw lift	Direct	2	0
Area transitions	Direct	Both	0
	Remote midday	Both	0
	Remote evening	2	0
Lick lips	Direct	Both	0
	Remote midday	Both	0
	Remote evening	2	0
Yawn	Remote midday	2	0
	Remote evening	2	0
Stereotype	Questionnaire	2	0
	Screening	Both	1
	Direct	Both	0
	Remote midday	Both	0
	Remote evening	Both	0
Pant	Direct,	Both	0
	Remote midday	Both	0
	Remote evening	2	
Groom	Remote midday	2	0
	Remote evening	2	0
Lie down	Direct	Both	0
	Remote midday	Both	0
	Remote evening	2	0
Stand	Direct	Both	0
	Remote midday	Both	0
	Remote evening	2	0
Sit	Direct	Both	0
	Remote midday	Both	0
	Remote evening	2	0
Stand on hind legs	Direct	Both	0
Located in sleeping compartment	Direct	1	0
	Remote midday	Both	0
	Remote evening	2	0
Investigate surroundings	Remote midday	2	0
	Remote evening	2	
Gaze at exit	Remote midday	Both	0
	Remote evening	2	0

Dog visited vet since arrival at current station	Health records	2	0
Dog visited vet in past six months	Health records	1	0
Dog visited vet in past year	Health records	1	1
Reported illness in past month	Questionnaire	Both	0
Urinary C/C	Urine samples	Both	0
Reported to be coprophagic	Questionnaire	Both	0
Resting	Screening	Both	1
Moving	Screening	Both	1
Body Posture	Screening	Both	1
Tail position	Screening	Both	1
Ear position	Screening	Both	1
Tendency to look away	Screening	Both	1

4.2.3.2 Measures of housing and husbandry

4.2.3.2.1 Delivery and analysis of questionnaires

Using a second questionnaire (presented as Appendix 3), I asked the kennel assistant questions relevant to the general husbandry of all dogs on the site, including details of their feeding, exercise, grooming routines and enrichment. In addition the questionnaire described in section 4.2.3.1.1 (Appendix 2) posed questions about individual sample dogs including the availability of enrichment e.g. toys and bones.

The information relevant to housing and husbandry derived from the two questionnaires were;

- whether or not the kennel was heated (0/1);
- usual location of dog during cleaning (sleeping compartment=1; elsewhere=0);
- amount of contact, overall, per day with kennel assistant (minutes);
- minimum number of people which dogs were reported to see each day whilst in the kennel block;
- whether kennel assistant played with dogs during exercise periods (yes/no);
- number of times per day which dog was able to make physical contact with kennel assistant;
- reported duration of exercise on lead per day (minutes);
- difference between minimum and maximum hours of operational work per week;
- usual hours worked by kennel staff;
- time of day when feeding usually occurred;
- person responsible for feeding;
- frequency of kennel cleaning;
- usual duration of exercise (minutes);

- usual frequency of exercise;
- type of exercise usually provided;
- usual time between periods of exercise (hours);
- whether each dog was given access to toys (continuously/sometimes/never);
- whether each dog was given access to bones (continuously/sometimes/never);
- music played in kennel (0/1);
- each dog exercised in paddock adjacent to another dog (0/1).

4.2.3.2.2 Collection and analysis of housing measures

All kennels within each site were essentially identical (with one exception, in population two, there were two kennel types at one of the sites), so a representative kennel at each site was drawn and measured, and details taken regarding the kennel design e.g. flooring, presence of bedding and platforms. Schematics and measurements of the kennel layout were made with notes regarding the entrance and exit points and any visual contact the dog had with other dogs and the external environment. The measures derived from these detail, for each dog, were;

- space allowance (m^2);
- number of dogs each dog can make visual contact with from its kennel;
- maximum number of dogs an individual dog passes en route to exercise;
- distance (m) to nearest kennel where visual contact with another dog is possible;
- whether dog can see external environmental stimuli e.g. livestock (0/1).

4.2.3.2.3 Observation and analysis of husbandry routine

The general routine of the daily kennel assistants with the sample dogs was observed, and the frequency and duration of exercise (minutes) on and off lead was recorded.

4.2.3.2.4 Recording and analysis of noise levels

Noise levels were recorded using an integrated sound level meter (Dosemeter Castle Associates Ltd). The meter was calibrated before each recording. A total of five recordings were made; one during each of the first daily exercise periods, mid morning break, midday, feeding time and following departure of all personnel at the end of the day. For all recordings, I stood in the centre of the kennel building, but out of sight of the dogs, and recorded the maximum peak over a 20s duration. From these recordings two measures were derived: maximum and mean noise level (dBA) recorded during the five key activities.

4.2.3.2.5 Recording and analysis of temperature

Two thermometers (Kestrel® 4000 Pocket™ Weather Tracker™) were placed in one kennel, one in the sleeping compartment and one in the outdoor run. The thermometers record continuously so the minimum and maximum temperature was taken from each of the thermometers at 12 noon on day two and day three of the study. All readings were then averaged over the two days to provide the average temperature in the kennel (combining readings from the sleeping compartment and the outdoor run).

4.2.3.2.6 The derivation of variables (including composites) to quantify factors of housing and husbandry which may affect welfare

Using the measures of housing and husbandry described above (4.2.3.2.1-5), eleven independent variables describing factors of housing and husbandry which might affect welfare were generated. These corresponded to all those factors likely to affect welfare (4.1) and in many cases, composite scales were generated.

a) Space allowance

This variable described the total floor space in square meters available to each dog and was generated using the actual measurements.

b) Temperature scale

This variable described the average temperature in the kennel over the two day period.

c) Heating

This variable described whether a heat source was present in the kennel area or not as reported by the kennel assistant (0=no heating, 1=heating present).

d) Noise scale

This scale described the amount of noise disturbance measured during five key activities; feeding, exercise, morning break, lunch period, following departure and was composed of two variables.

i. Mean noise level (dBA) recorded across all five activities (AVNSE: <60=0, 61-69=0.5, >70=1);
ii. Maximum noise level recorded across all five activities (MAXNSE: <90=0, 91-99=0.33, 100-109.9=0.66, >110=1).

These variables were combined via the following equation:

NOISE SCALE= (AVNSE+MAXNSE)/2.

e) Location during cleaning

This variable described the location of dogs during cleaning as reported by the kennel assistant (0=dog locked in sleeping compartment, 1=dog not locked in sleeping compartment).

f) Inter-specific contact scale

This scale described the amount of contact each dog had with people on a daily basis, as reported by the kennel assistant during the questionnaires and was composed of five variables.

i. Reported amount of contact per day with kennel assistant (minutes) (MINSDOGCON: <24=0, 24-35=0.33, 36-47=0.66, >48=1);
ii. Reported minimum number of people each dog has visual contact with per day in the kennel block (VISPEO: 1-2=0, 3-4=0.33, 5-6=0.66, 7-8=1);
iii. Kennel assistants report that they play with dogs during exercise (PLAYEX: 0=no, 1=yes);
iv. Reported number of times per day each dog was able to make physical contact with kennel assistant averaged over a week (ACCSTF: <3=0, 3-4.99=0.5, >5=1);
v. Reported duration of exercise on lead (minutes) (REPDURLD: <15=0, 15-30=0.33, 31-45=0.66, >45=1).

These variables were combined via the following equation:

INTER-SPECIFIC CONTACT SCALE=
(MINSDOGCON+VISPEO+PLAYEX+ACCSTF+PERDURLD)/5

g) Intra-specific contact scale

This scale described the amount of contact each individual dog had with other dogs housed at the station both whilst in the kennel and when exercised and was composed of four variables.

i. Observed number of dogs each individual dog can make visual contact with from kennel (VISCONTDG: 0=0, 1=0.2, 2=0.4, 3=0.6, 4=0.8, >5=1);
ii. Observed maximum number of dogs an individual passes en route to exercise (DOGPASS: 0=0, 1-3=0.33, 4-6=0.66, >7=1);
iii. The distance (metres) to the nearest kennel where visual contact with another dog is possible (MINDISTKEN: >1.5=0, 1.01-1.50=0.33, 0.5-1.0=0.66, <0.5=1);
iv. Dog is reported to be exercised in a paddock adjacent to another paddock also with a dog exercising (DOGPAD: 0=no, 1=yes).

These variables were combined via the following equation:
INTRA-SPECIFIC CONTACT SCALE =
(VISCONTDG+DOGPASS+MINDISTKEN+DOGPAD)/4

h) Environmental stimulation inside the kennel scale

This scale described the reported amount of environmental stimulation inside the kennel available to each individual dog and was composed of two variables.

i. Access to toys (TOYACC: continuously=1, sometimes=0.5, never=0);
ii. Access to bones (BONEACC: continuously=1, sometimes=0.5, never=0).

These variables were combined via the following equation:
ENVIRONMENTAL STIMULATION INSIDE THE KENNEL= (TOYACC+BONEACC)/2

i) Environmental stimulation outside the kennel scale

This scale described the observed amount of environmental stimulus outside the kennel available for each individual dog and was composed of two variables.

i. Music played in kennel area (MUSDAY: none=0, some days=0.5, every day=1);
ii. Visibility from kennel of environmental stimuli e.g. live building, fields (ENVSTIM: none=0, 1=presence of environmental stimuli).

These variables were combined via the following equation:

ENVIRONMENTAL STIMULATION OUTSIDE KENNEL SCALE= (MUSDAY+ENVSTIM)/2

j) Exercise scale

This scale described the reported and observed amount of exercise each dog received and the reported length of time between periods of exercise and was composed of three variables.

i. Reported duration of exercise (minutes) off lead (REPOFLD: 0=0, 1-30=0.33, 31-60=0.66, >60=1);
ii. Time between periods of exercise (hours) (BETWALK: <12=1, 12-24=0.5, >25=0);
iii. Observed duration of exercise (minutes) both on the lead and off the lead (OBSDUREX: <30=0, 30-60=0.5, >60=1).

These variables were combined via the following equation:

EXERCISE SCALE= (REPOFLD+BETWALK+OBSDUREX)/3

k) Predictability of routine scale

This scale described how predictable both the husbandry and operational routine was for the individual dog, as reported by the kennel assistant during the questionnaires and was composed of five variables.

i. Difference between the reported maximum and minimum number of hours of operational work by each dog per week (DIFOPHRS: 0=1, <24=0.66, <48=0.33, >48=0);
ii. Predictability of kennel staff presence (STFPRED: present weekdays and weekends worked either seven half days or seven full days=1, present weekdays and weekends and worked full days but leave Friday afternoon=0.75, present Monday to Friday half days not work weekends=0.5, present Monday to Friday full days but leave Friday afternoon, not work weekends=0.25, no kennel staff, handlers responsible for husbandry=0);
iii. Predictability of exercise (EXPRED: same frequency and duration of exercise daily=1, same frequency of exercise but duration and type differ=0.66, frequency and duration differ but dogs exercised daily=0.33, not always exercised daily=0);

iv. Predictability of feeding (FDPRED: same time every day by same person=1, same time every day but different person=0);

v. Predictability of cleaning (CLNPRED: kennel swept and hosed at same time each day=1, kennel swept daily but hosed intermittently throughout the week=0).

These variables were combined via the following equation:

PREDICTABILITY OF ROUTINE SCALE=

(DFOPHRS+STFPRED+EXPRED+FDPRED+CLNPRED)/5

4.3 Do factors of housing and husbandry differ between the two populations?

In this section, I explored whether or not the eleven factors of housing and husbandry differed between the two populations as this would determine whether the two data sets could be combined or whether each had to be analysed separately.

4.3.1 Statistical testing

Before testing for differences between the two populations, I explored each factor in turn to identify whether the factor varied most by site or within site. Measures for three of the factors; noise, space allowance and temperature, were consistent within site but varied between sites. Thus each site was given a single score for each of the factors measured, and independent sample t tests were used to test if there was a significant difference in the mean score for population one and two. For the remaining factors, which were not consistent within site but varied between individual dogs, each dog was given a score for each of the factors measured. For factors which consisted of continuous data; independent sample t tests were used to see if there was a significant difference in the mean factor score between populations one and two. For factors which were scored as presence/absence, Fisher's exact tests were used to see if there was a significant difference between the two populations.

4.3.2 Results

Of the eleven factors explored, seven differed significantly between the two populations ($p<0.05$) (Table 4.4). In general, dogs in population two had significantly larger kennels, more predictable routines, and more environmental stimulation available inside their kennels compared to dogs in population one, whereas dogs in population one had a significantly larger proportion of heated kennels, received a higher frequency and duration of exercise and a greater amount of contact with other dogs. At the time of data collection, temperatures in the sites of population two were higher than in the sites of population one, reflecting the different times of year during which the studies were conducted.

Table 4.4 Factors of housing and husbandry in two populations of military working dogs. Factors in bold type are those where a significant difference between the mean levels of each population was evident. For factors which were continuous variables, mean and standard deviation are presented. For presence/absence factors, the proportion of dogs with that factor is given. Where significant differences were found between the two populations, the test statistic and level of significance are presented. [a] Independent t-test used to compare means, [b] Fisher's Exact test used to compare means, test statistic not available. Levels of significance; $p<0.05$=*, $p<0.01$=**, $p<0.001$=***. Where either sites or individual dogs have scores between 0 and 1, the mean for each population can be calculated but the scores are relative and are only used for comparison between the two populations.

Factor	Mean and standard deviation		Test statistic	Significance level
	One	Two		
Space allowance (m^2)	7.3±2.5	18.6±1.2	[a]t=-11.6, df=14	***
Temperature (°C)	7.3±2.5	18.2±2.6	[a]t=-3.6, df=14	**
Presence of heating (0/1)	0.33	0.13	[b]	*
Noise (0-1)	0.62±0.29	0.47±0.29		ns
In sleeping compartment during cleaning (0/1)	0.32	0.19		ns
Inter-specific contact (0-1)	0.43±0.24	0.39±0.21		ns
Intra-specific contact (0-1)	0.46±0.13	0.27±0.22	[a]t=5.2, df=76.3	***
Environmental stimulation inside the kennel (0-1)	0.17±0.21	0.34±0.18	[a]t=-4.0, df=87	***
Environmental stimulation outside the kennel (0-1)	0.22±0.23	0.21±0.23		ns
Exercise (0-1)	0.40±0.31	0.21±0.18	[a]t=-6.5, df=87	***
Predictability of routine (0-1)	0.55±0.12	0.73±0.13	[a]t=-6.5, df=86.3	***

4.3.3 Conclusion

Although the two populations appeared superficially similar at the outset of the study, upon analysis, there were a large number of significant differences between the regimes used to house and care for the dogs. Whilst it would have been advantageous to combine both these populations allowing the application of more powerful statistical techniques, the differences in housing and husbandry suggested there could be large differences in the relationships between welfare, housing and husbandry of the two dog populations, and that it would be difficult to attribute these differences to any single factor. It was thus inappropriate to analyse these populations as a single sample. Instead, the relationships have been analysed within each population separately, and then compared. This should allow the identification of relationships common to both populations, which may then be generalised to all similar kennelled dogs, but will also identify factors specific to each population which appear to influence welfare.

4.4 Are factors of housing and husbandry systematically associated with specific indicators of welfare?

The following section describes how the relationships between welfare, housing and husbandry were tested in each population and the findings from this analysis are presented.

4.4.1 Statistical analysis

Multivariate statistics could not be applied to the whole dataset because of the relatively small number of dogs within each population compared to the number of independent and dependent variables. Instead, taking each population in turn, factors (independent variables) were firstly inspected to see if they varied within site. If there was no variation within site i.e. the measure was consistent within the site then each site was characterised by that single value which was then used in future analysis. If variables did vary within site, future analysis was carried out using individual dogs. Thus to enable testing between factors which varied by site and indicators of welfare, means per site for each welfare measure had to be calculated. For those factors which did not vary by site, associations with welfare indicators were tested using data for individual dogs. The method by which associations between factors and welfare indicators were tested varied dependent upon the combination of variables (Table 4.5).

Table 4.5 Statistical tests used to explore the relationships between factors and indicators of welfare.

Variable types	Statistical test
0/1 vs. 0/1	Chi square
continuous/ordinal vs. continuous	Spearman Rank Correlation
0/1 vs. ordinal	Mann Whitney U

In population two, there were two kennel types at one of the sites. Upon inspection, the measures for each of four factors; space allowance, temperature, heating, and noise (4.2.3.2.6), differed between the two kennel types, and thus this one site had to be treated as two sites, comprising 3 and 3 subjects. Hence, overall and for these four factors specifically, means of eight sites were used instead of seven. For the remaining variables, where there was no difference in the two kennel types, the site was considered as one and thus overall means of seven sites were used for analysis.

4.4.2 Results

In this section the relationships between factors and indicators of welfare for population one are stated first (4.4.2.1), followed by those found in population two (4.4.2.2).

4.4.2.1 Population one

In population one all possible relationships between each of the eleven factors and the 36 indicators of welfare were tested, in total, 396. Since the study had been designed to generate hypotheses for testing in subsequent studies (Chapters 5-6) and for comparison with population two, no correction factors for multiple testing were applied to P-values at this point. Twenty four relationships, significant at 5% or less were found, and a further 16 relationships, significant between 5 and 10% were found. All relationships at 10% or less are presented.

a) Relationships between welfare indicators and factors which varied by site but not within site.

At sites with more kennel space, dogs were more likely to spend more time lying down (Rho=0.74, p=0.02) and tended to bark less (Rho=-0.66, p=0.08) during the remote midday observation. During the screening observation, dogs were more likely to look away from the experimenter (Rho=0.75, p=0.03) whilst during the direct observation they tended to move around less (Rho=-0.65, p=0.08).

At sites where higher temperatures were recorded, more dogs were likely to have been ill in the past month (Rho=0.81, p=0.03) and tended to have lower C/C (Rho=-0.71, p=0.07).

At sites where kennels were heated, dogs were more likely to move around the kennel less (U=1.5, p=0.05, median = 0.17 (lower quartile = 0.08, upper quartile = 0.42) vs. 0.88 (0.67, 1.0)), tended to bark and gaze at the exit less (U=1.5, p=0.06, 0 (0, 0) vs. 0.17 (0.11, 0.17), U=1.5, p=0.06, 0 (0, 0) vs. 0.33 (0.25, 0.67)) respectively and were more likely to lie down during the remote midday observation (U=0, p=0.02, 60 (51.8, 60) vs. 35.1 (31.8, 37.0)). During the screening observation, at sites where kennels were heated, dogs were more likely to look away from myself (U=1.0, p=0.05, 0.8 (0.73, 1.1) vs. 0.4 (0, 0.67)) whilst during the direct observation tended to move less (U=2.0, p=0.09, 0.3 (0.3, 0.4) vs. 0.5 (0.5, 0.56)).

At sites where higher levels of noise were measured, dogs were likely to spend more time lying down during the remote midday observation (Rho=0.71, p=0.03) and were more likely to look away from the experimenter during the screening observation (Rho=0.70, p=0.05).

At sites where during the cleaning routine, dogs were locked in the sleeping compartment, more dogs were likely to bark in the screening observation than those that which were not (U=0, p=0.05, 0.71 (0.71, 0.72) vs. 0.64 (0.33, 0.61)) and dogs were more likely to have higher C/C than those which were not (U=0, p=0.05, 7.0 (6.6, 7.7) vs. 6.6 (6.3, 6.3)).

At sites where higher levels of inter-specific contact (Rho=0.9, p=0.006) and a more predictable routine (Rho=0.97, p<0.001) was provided, more dogs were coprophagic.

At sites where there was more environmental stimulation outside their kennel, dogs were more likely to howl during the direct observation (Rho=0.36, p=0.02).

At sites where levels of exercise were higher, dogs tended to rest more (Rho=0.74, p=0.06), stereotype less (Rho=-0.69, p=0.09) and bark less in the screening observation (Rho=-0.70, p=0.08), whilst there was a tendency for fewer dogs to be taken to the veterinary surgeon in the past year (Rho=-0.69, p=0.09). During the remote midday observation, dogs tended to bark less (Rho=-0.70, p=0.08) if levels of exercise were higher.

At sites where routine were more predictable, dogs tended to spend more time moving around (R=0.7, p=0.08), were more likely to bark (Rho=0.77, p=0.04) and tended to spend less time lying down (Rho=-0.74, p=0.06) when observed in the remote midday observation.

b) Relationships between welfare indicators and factors which varied within, as well as between, sites.

Dogs with a higher intra-specific contact score, during the screening observation were more likely to bark (U=133, p=0.011, 0.51 (0.41, 0.58) vs. 0.4 (0.27, 0.5)) and tended to stereotype (U=142, p=0.065, 0.52 (0.44, 0.52) vs. 0.45 (0.33, 0.52)). During the remote midday observation, dogs with more intra-specific contact tended to howl (U=61, p=0.06, 0.56 (0.52, 0.59) vs. 0.48 (0.33, 0.52)), whilst during the direct observation they tended to spend more time in their sleeping compartment (U=87.0, p=0.07, 0.58 (0.48, 0.59) vs. 0.48 (0.33, 0.52)). Also a higher intra-specific contact score was linked to being more likely to be reported ill in the past month (U=117.5, p=0.006, 0.55 (0.43, 0.59) vs. 0.44 (0.33, 0.50)).

Dogs with more environmental stimulation within the kennel were more likely to move during the screening observation (U=18.0, p=0.03, 1.0 (0.75, 1.0) vs. 0 (0, 0.5)) and during the direct observation (Rho= 0.31, p=0.04) and were less likely to lie down (Rho=-0.34, p=0.04). During the remote midday observation, dogs with more environmental stimulation were more likely to move around, (Rho=0.44, p=0.004), gaze at the exit (Rho=0.33, p=0.03), stand (Rho=0.38, p=0.01), lick their lips (Rho=0.38, p=0.01) but less likely to lie down (Rho=-0.34, p=0.02).

4.4.2.2 Population two

Associations between welfare indicators and both heating and location during cleaning could not be tested because there were too few dogs within the population with heated kennels and the variation in location during cleaning was insufficient. All possible relationships between each of the remaining nine factors and 50 indicators of welfare were tested; a total of 450. Twenty two relationships, significant at 5% or less and one, significant between 5 and 10% were found.

a) Relationships between welfare indicators and factors which varied by site but not within site.

At sites with more kennel space, dogs were less likely to yawn (Rho=-0.72, p=0.04) in the remote evening observation, have higher C/C (Rho=0.71, p=0.05), and rest less (Rho=-0.71, p=0.05) during the screening observation.

No significant relationships were found between any indicators of welfare and temperature.

At sites where higher levels of noise were measured, dogs were more likely, during the remote midday observation, to spend less time barking (Rho=-0.76, p=0.03), less time moving around (Rho=-0.74, p=0.04), less time standing still (Rho=-0.75, p=0.03) and during the remote evening observation, were more likely to spend time investigating the surroundings (Rho=0.93, p=0.001). Also, at sites with higher levels of noise, dogs tended to lie down more during the remote midday observation (Rho=0.63, p=0.10).

b) Relationships between welfare indicators and factors which varied within, as well as between, sites.

Those dogs with higher levels of inter-specific contact were less likely to have been referred to the vet since arriving at the current station (U=123.0, p=0.03, 0.5 (0.33, 0.7) vs. 0.33 (0.33, 0.4)) and laid down more during the remote midday observation (Rho=0.32, p=0.03).

Dogs with higher levels of intra-specific contact were less likely to spend time moving around their kennel (Rho=-0.35, p=0.02), spend time standing (Rho=-0.37, p=0.01) and yawn (U=57.0, p=0.005, 0.04 (0, 0.18) vs. 0.27 (0.12, 0.48)) but more likely to spend time lying down (Rho= 0.38, p=0.009) in the remote evening observation. During the remote midday observation, dogs with higher levels of intra-specific contact were more likely to look towards the exit (Rho=0.35, p=0.02) and during the screening observation tended to look away from myself (Rho=0.35, p=0.02). Dogs with higher levels of intra-specific contact were more likely to be coprophagic (U=88.5, p=0.03, 0.42 (0.27, 0.57) vs. 0.24 (0.08, 0.33)).

Dogs with more environmental stimulation in their kennel were more likely to have been referred to the vet since arriving at the site (U=120, p=0.02, 0.5 (0.25, 0.5) vs. 0.25 (0.25, 0.25)) and tended to paw lift during the direct observation (U=92.0, p=0.05, 1.0 (0.75, 1.0) vs. 0.5 (0.5, 1.0)).

Dogs with higher levels of environmental stimulation outside their kennel, (e.g. the playing of music or visibility of livestock), were more likely to spend time standing during the remote midday observation (Rho=0.30, p=0.03) and spend more than 30s in their sleeping compartment during the remote evening observation (U=70.5, p=0.003, 1.0 (1.0, 1.0) vs. 0 (0, 0.5)).

Dogs which were exercised the most frequently and for the longest durations, were less likely to bark during the direct observation (Rho=-0.29, p=0.05).

Dogs with a high level of predictability within their routine were more likely to spend more time panting during the remote midday observation (Rho=0.29, p=0.05).

4.4.3 Discussion

In this section, I take each factor in turn and discuss the relationships with welfare for each population.

4.4.3.1 Space allowance

a) Population one

Sites with larger kennels were associated with dogs, on average, spending more time lying down. Hite et al (1977) similarly found that dogs in large cages spent longer lying down, but Campbell et al (1988) and Hetts et al (1992) did not. However, in none of these studies was the space allowance comparable to the space allowance in this current study and therefore similar findings might not be expected.

In the presence of me, dogs housed in sites with larger kennels were more likely to look away. The relationship between increased space and the tendency to look away during the screening observation is difficult to explain. It could be argued that some dogs feel threatened in a large space and so do not make eye contact but it could also be that dogs in small kennels are more likely to be defensive or behave in a threatening way and so stare at the experimenter.

Overall, these current findings suggest that larger kennels are beneficial to dogs as they allow dogs to lie down for longer and hence rest.

b) Population two

Sites with larger kennels were associated with increased levels of C/C, dogs performing fewer yawns and less resting. As discussed in section 2.4.3, high levels of C/C appear to be indicative of chronic stress so this suggests that those dogs housed in the largest kennels were more stressed, as indicated by elevated C/C, than those in smaller kennels. Yawning has been linked to poor welfare in the past (Beerda et al, 1998) as well as to anxiety (Prescott et al, 2004). So, although C/C suggests welfare may be poor in larger kennels, the relationship with yawning suggests otherwise. I would suggest that further research investigating space allowance and C/C is warranted.

During the screening observation it is possible that some of the dogs were fearful or less relaxed in the presence of the experimenter. As a result of this fear, dogs may try to distance themselves from the experimenter which is probably easier in a larger kennel and so appear to rest less.

4.4.3.2 Temperature

a) Population one

At the time of recording, colder sites were associated with fewer reported incidences of disease in the month prior to the visit. Generally, it would be assumed that lower temperatures lead to increased incidence of disease however this result may be affected by the month in which data collection took place and had the recording taken place in winter, the result may have been different.

b) Population two

No significant relationships were found between any indicators of welfare and temperature, possibly because extremes of temperature were not evident during the study.

4.4.3.3 Heating

a) Population one

It is claimed that dogs are extremely adaptable in their temperature requirements, provided there are adequate sources of food and water and a suitable acclimatisation time (Prescott et al, 2004), yet many working dog kennels are not built with the consideration of extreme climes such as those experienced in the North of England and Scotland. In this study, sites with heated kennels were more likely to be associated with restful behaviours; decreased activity and more time lying down. In the screening observation, dogs were less likely to look at me. It is difficult to determine why the presence of heating may be linked to behaviour in the presence of a person as it is unlikely to affect the relationship between the dog and a human but the tendency to look away from the experimenter was also linked with space allowance suggesting there may be interplay between all these variables which is difficult to tease apart. Overall though, heating within a kennelled environment appears to be beneficial.

b) Population two

The lack of variation in heating in population two meant that relationships between welfare indicators and the presence of heating could not be tested (4.4.2.2).

4.4.3.4 Noise

a) Population one

Sites with the highest noise levels across the day were associated with dogs, on average, spending more time lying down during midday; a quieter time of day. I would suggest that dogs are unable to rest during periods of high activity and therefore take the opportunity to rest at quieter times of the day. For example, during periods of feeding and exercise, on average, levels were 95 and 92 dB respectively compared to 52dB recorded at midday. Also, at sites with the highest levels of noise, dogs, tended not to make eye contact with me. Whilst this welfare measure has been linked to a number of other factors, which have been difficult to explain, in this situation I would suggest that the dogs may be looking at the other dogs in the immediate area in response to elevated levels of barking which contribute to the higher levels. Alternatively they may be fearful and stressed due to the loud noise and so are less likely to show positive relaxed social behaviour.

b) Population two

At those sites where noise levels were high across the day, dogs, on average, spent less time moving around, less time standing and barked less at midday. I suggest these relationships can be explained in a similar way to the trends in population one; at noisy sites, restful behaviours may only be possible at quieter times of day. Furthermore, investigating surroundings was observed more during the evening observation at those sites which were noisiest. This may be an attempt by the dog to increase olfactory information which is only possible at quieter times of day but this relationship requires further investigation to fully understand whether this is the case.

4.4.3.5 Location during cleaning

a) Population one

On average, dogs which were locked in the back of their sleeping compartments during cleaning, barked more when a novel person (me) was stood in front of them and had elevated levels of C/C. Barking may be performed in a defensive context (Bradshaw and Nott, 1995) and high levels of C/C indicate both acute and chronic stress (see Rooney et al, 2007b and Chapter 2.2) so I conclude that this practice would seem to be aversive.

b) Population two

The lack of variation in location during cleaning in population two meant that relationships between welfare indicators and the presence of heating could not be tested (4.4.2.2).

4.4.3.6 Inter-specific contact

a) Population one

Sites which provided high levels of inter-specific contact with dogs, generally had more dogs which were reported to perform coprophagia. The cause of coprophagia is not completely understood but it has been linked to chronic stress in dogs (Beerda et al, 1999) particularly those which have been institutionalised or kennelled (Mugford, 1995) and to social stress in lowland gorillas (*Gorilla gorilla*) (Faraldo and Taylor, 2003). As human companionship is important to the domestic dog (Tuber et al, 1996), it is unlikely that high levels of inter-specific contact cause coprophagia in dogs. Instead, I suggest that it is a result of kennel assistants spending longer with the dogs and hence being better able to report this condition in the dogs. It is acknowledged that increased levels of contact results in carers being able to complete better character assessments of dogs within their care (Hubrecht, 1993a) and this result appears to support this.

b) Population two

Individual dogs with higher levels of inter-specific contact were less likely to have been referred to the vet since arriving at the current station and laid down more during the remote midday observation. As discussed above, kennel assistants who spend longer with dogs are better able to complete character assessments (Hubrecht, 1993a) and in population one appeared better able to recognise coprophagic dogs. If kennel assistants are in regular contact with dogs then they are more likely to recognise when dogs are ill and so may be able to intervene and treat the dog before veterinary care is required. Lying down at midday suggests that increased contact is beneficial to welfare as resting behaviours have been indicative of good welfare in other populations of dogs (Beerda et al, 1998, Graham et al, 2005a).

4.4.3.7 Intra-specific contact

a) Population one

Individuals with higher levels of intra-specific contact tended to bark in response to a novel person (me) outside their kennel, and were likely to have been ill in the month preceding the study. Considering that many dogs were kennelled in close proximity to other dogs, the high levels of barking were probably a result of social facilitation. Illness may have been due to the close contact of dogs facilitating disease transmission and kennel assistants handling large numbers of dogs.

b) Population two

Dogs that had high levels of intra-specific contact were less active and laid down more suggestive of resting. Although resting may indicate both good (Beerda et al, 1997, Graham et al, 2005a) and poor welfare (Meers et al, 2004), these results could be taken in support of

Wells and Hepper (1998) who suggest that higher levels of dog contact may be beneficial to kennelled dogs. In addition, dogs with high levels of intra-specific contact spent more time looking at the exit and when I was present, did not make visual contact. Spending time looking at the exit initially suggested that some dogs were expressing human directed separation-related behaviour. However, it is feasible that these dogs were actually looking at dogs across from their kennel as given the opportunity many dogs will make visual contact with other dogs (Wells and Hepper, 1998). This may further explain why some dogs did not make visual contact with the observer, as they were potentially seeking visual contact with other dogs. Alternatively they could be more dog-orientated so are less responsive to people. However, without experimental manipulation, it is not possible to determine true cause and effect.

Dogs with high levels of intra-specific contact were likely to yawn less and more likely to perform coprophagy. Yawning has been linked to states of poor welfare (Beerda et al, 1998) suggesting that high levels of dog contact may be beneficial to welfare. Although, this conflicts with the relationship seen with increased coprophagy, as this behaviour has been reported as an indicator of long term stress particularly in kennelled dogs (Beerda et al, 1999, Mugford, 1995). However, coprophagy is not well understood; it can signal a nutritional imbalance or as a means of supplementing energy to an inadequate diet (Mugford, 1995) or can be due to harsh treatment used during toilet training (Rooney et al submitted) and possibly in this current situation could be a socially transmitted behaviour as it is generally performed when dogs are in close proximity or contact with other dogs. I suggest that further research is required to fully understand why this behaviour is performed.

It is difficult, based upon these findings alone, to determine whether intra-specific contact is beneficial or detrimental to welfare. However previous research (1.5.2.9) has suggested that intra-specific contact is beneficial and increased resting and less yawning would suggest that is so.

4.4.3.8 Environmental stimulation within the kennel

a) Population one

Individual dogs with higher levels of environmental stimulation within their kennel were, on average, more restless than those dogs which had less environmental stimulation within their kennel, spending more time moving around when in the presence of a person and when alone and lying down less, both in the presence of a person and when alone. Furthermore dogs which had higher levels of environmental stimulation were more likely to gaze at the exit and lick their lips when alone. Whilst it could be argued that the provision of environmental stimulation results in restless behaviour and other behaviours potentially indicative of poor welfare, I would argue that this is not the case. It is possible that many of these dogs have been given toys or chews (resulting in a higher score for environmental stimulation) because kennel assistants and handlers have previously observed these behaviours, interpreted them as stress-related and hence provided enrichment. Thus the cause and effect may not be as one would initially assume and these relationships cannot be interpreted as causation, but instead associations. Overall, these results suggest that the enrichment provided to the dogs is not successful in ameliorating stress or improving welfare. The provision of toys and bones to military working dogs has already been show to be ineffective in improving the welfare of *all* kennelled dogs although the welfare of some individuals was improved (Hiby, 2005). It is not possible to say whether this was also true of individuals within this population.

b) Population two

Dogs with the highest level of environmental stimulation within their kennel were more likely to have been referred to the vet since arriving at the station and tended to paw lift during the direct observation. Paw lifting is not completely understood; although it is often interpreted as an attention seeking behaviour or an indication to play (Prescott et al, 2004); it can be performed in other contexts where chronic stress is experienced and welfare is compromised (Beerda et al, 1999, Rooney et al submitted). Perhaps dogs with a tendency to perform these behaviours are those which have been deliberately provided with toys or bones. However it is apparent from this relationship that the provision of toys and bones is not sufficient to change this behaviour further supporting findings in population one and those of Hiby (2005). In studies of dogs housed in re-homing shelters, similar findings were found (Wells, 2004b, Wells and Hepper, 1992; 2000). I would also suggest that paw lifting may be performed as a means to solicit play with the observer due to the presence of toys within the kennel and so may not be interpreted as indicative of compromised welfare at all.

4.4.3.9 Environmental stimulation outside the kennel

a) Population one

Dogs with the highest levels of environmental stimulation were more likely to howl during the direct observation. Howling is a vocalisation performed by individuals as a means of seeking contact with humans and other dogs (Bradshaw and Nott, 1995). It is therefore possible that dogs which have visual contact with people in the kennel environment (thus scoring high for environmental stimulation outside the kennel) howl in an attempt to make contact and the presence of a person during the direct observation elicits this same behaviour.

b) Population two

Dogs with the highest levels of environmental stimulation outside their kennel tended to stand more during the remote midday observation and spent more than half of the observed time in their sleeping compartment during the remote evening observation. I suggest that within the kennel environment, general activity and music is likely to be greater during the remote midday observation as this is when the kennel assistants are present. So, dogs are more likely to stand at the front of their kennel reacting to this stimulation, whilst during the remote evening observation, this stimulation would have decreased and so dogs would be more likely to be in their sleeping compartment resting. However, without fully understanding the cause and effect of this relationship, it is difficult to say whether the presence of environmental stimulation is beneficial or detrimental to welfare. The stimulation may well be distracting; preventing the dog from lying down. However the dog may benefit from the stimulation and stands to gain further stimulation which would otherwise be difficult if laid down. Hence further research is required in this area.

4.4.3.10 Exercise

a) Population one

No significant relationships at 5% were found for population one.

b) Population two

Dogs which were exercised the most frequently and for the longest durations, tended to bark less when observed by a person (direct observation). The exact intention of a dog's bark is not always clear (Bradshaw and Nott, 1995) and it may serve multiple purposes, but vocalisations can be performed in situations of frustration suggesting that a lack of exercise may be frustrating for some dogs.

4.4.3.11 Predictability of routine

a) Population one

Sites that provided highly predictable routines were associated with high incidences of coprophagia. In chimpanzees (*Pan troglodytes*), a more predictable feeding schedule was also linked to increased levels of coprophagy (Bloomsmith and Lambeth, 1995); however the effect was somewhat weak. Rigid scheduling or high predictability of certain animal care events can influence the expression of abnormal behaviours e.g. African elephants (Wilson et al, 2004) and in particular stereotypies e.g. American Black Bear (*Ursus americanus*) (Carlstead et al, 1991) so it is possible that at sites where events are highly predictable, coprophagia is triggered. However, across the literature as a whole, results contrast depending upon both the species and which aspect of the routine is predicted, suggesting that this is an area which is not yet fully understood. In this study, I would suggest that this result is related to how predictability is defined. A high predictability score would mean that care staff are present seven days a week and hence spend a lot of time with the dogs. This directly links to the association above; kennel assistants spend longer with the dogs, are more likely to see it and are therefore better able to report the condition.

High levels of predictability on average were linked to higher levels of barking suggesting dogs may be able to predict forthcoming events, and ahead of these events, may experience stress or frustration. Further research is therefore required to determine the optimal level of predictability.

b) Population two

Dogs with a highly predictable routine, spent more time panting during the remote midday observation. Panting has been observed and reported to occur in situations of acute stress (Beerda et al, 1997, Voith et al, 1987) which suggests that those dogs which are able to predict events likely to occur in the future, but not exhibit control over them, may find this acutely stressful. However this result is only marginally significant (p=0.05) and contrasts with studies on dogs (Coppola et al, 2006) and other species e.g. laboratory cats (Carlstead et al, 1993a) where a predictable routine was found to be beneficial to welfare. It may be that in the most predictable routines dogs are more affected by small changes to their routine (such as those inevitable during data collection) hence they show signs indicative of stress. This further emphasises the requirement for further research, as stated in the previous section, to determine the optimal level of predictability which to maximise welfare.

4.4.4 Summary

The relationships between housing, husbandry and welfare differ greatly between the two populations, with only two factors; noise disturbance and exercise, showing any similarity in their relationship with indicators of welfare. The following section attempts to account for these differences describing how the relationships seen in both populations compare and contrast with one another.

4.5 Comparing and contrasting the relationships between housing, husbandry and welfare between the two populations

In the following section, I describe how the relationships seen in both populations compare and contrast with one another (presented in Table 4.6). I take each potential factor in turn and summarise the welfare indicators it links to in population one and two. In order to understand why the populations differed graphical representations are included (Figures 4.1 to 4.9). Within the discussion section, potential explanations for the differences are posed. I first consider relationships for those factors which differed significantly between the two populations (4.5.1) and then in the second section (4.5.2), the relationships for factors where significant differences were not evident are described.

Table 4.6 Significant relationships between factors and welfare indicators in populations one and two (p< 0.10†, *p<0.05* *, *p<0.01* **, *p<0.001* ***). Factors in italics are those which differed significantly between the two populations. Welfare indicators in bold text are those which, in both populations, tended to relate to the same factor. ↑: tendency for the welfare measure to increase in relation to increasing levels of the factor, ↓: tendency for the welfare measure to decrease in relation to increasing levels of the factor. ^{s} behaviour observed during the screening observation, ^{d} the direct observation, ^{r1} the remote midday observation and ^{r2} remote evening observation.

Factor	Welfare measure	
	Population one	Population Two
Space allowance	↑ Lying down *^{r1} ↓ Barking †^{r1} ↓ Area transitions† ^{d} ↑ Tendency to look away* ^{s}	↓ Yawning *^{r2} ↑ C/C * ↓ Resting *^{s}
Noise	↑ **Lying down** *^{r1} ↑ Tendency to look away *^{s}	↑ **Lying down** †^{r1} ↑ Investigating surroundings **^{r2} ↓ Area transitions *^{r1} ↓ Standing *^{r1} ↓ Barking *^{r1}
Inter-specific contact	↑ Coprophagy **	↓ Referrals to vet * ↑ Lying down *^{r1}
Intra-specific contact	↑ Barking *^{s} ↑ Reported illness in past month ** ↑Stereotyping †^{s} ↑Howling†^{r1} ↑ Time spent in sleeping compartment†^{d}	↓ Area transitions **^{r2} ↑ Lying down **^{r2} ↓ Standing *^{r2} ↑ Gazing at exit *^{r1} ↑ Tendency to look away *^{s} ↓ Yawning **^{r2} ↑ Coprophagy *
Environmental stimulation inside kennel	↑ Moving *^{s} ↑ Area transitions *^{d}, **^{r1} ↓ Lying down *^{d}, *^{r1} ↑ Lip licking *^{r1} ↑ Gazing at exit *^{r1} ↑ Standing^{r1}*	↑ Referrals to vet * ↑ Paw lifting *^{d}
Environmental stimulation outside of the kennel	↑ Howling *^{d}	↑ Standing *^{r1} ↑ Time spent in sleeping compartment**^{r2}
Exercise	↓ **Barking** †^{s}, †^{r1} ↑Resting †^{s} ↓Stereotyping †^{s}	**Barking***^{d}

	↓Visited vet in past year $\dagger^{s}$	
Predictability within routine	↑ Coprophagy *** ↑ Area transitions $\dagger^{r1}$ ↓ Lying down $\dagger^{r1}$ ↑ Barking $*^{r1}$	↑ Panting $*^{r1}$

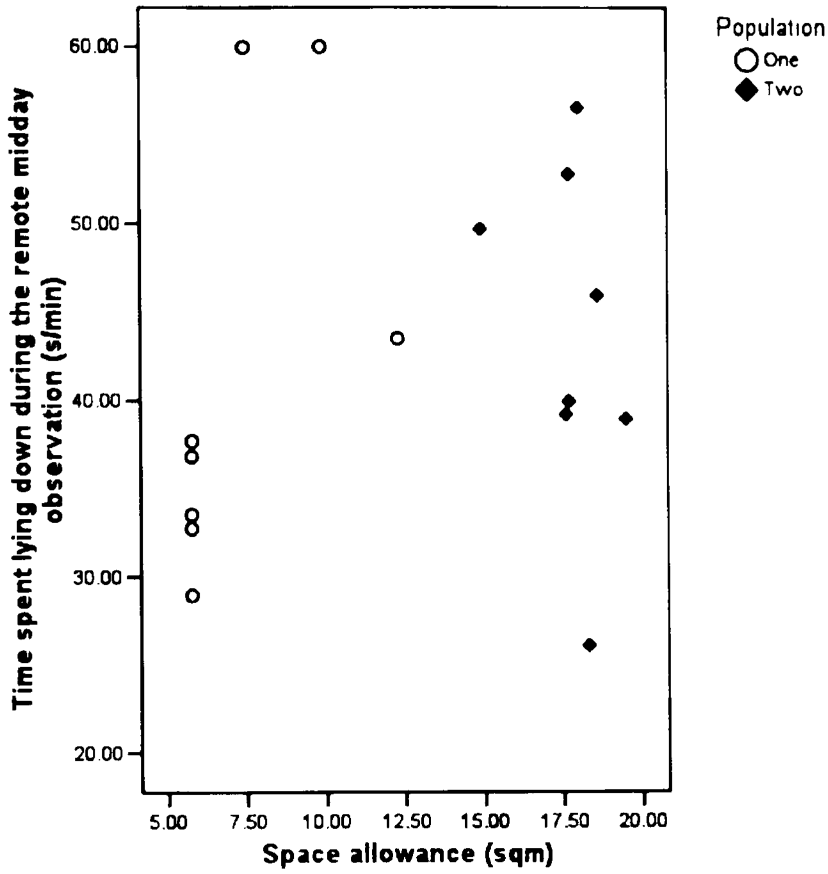
4.5.1 Relationships where factors which might affect welfare differed significantly between the two populations

4.5.1.1 Space allowance

The space allowance within population two was significantly larger than for those in population one (Table 4.4) and for that reason the same relationships seen within population one may not be expected to be evident within population two. In population one, space allowance was related to the time spent lying down in the remote midday observation (Figure 4.1) and tended to be related to barking during the remote midday observation, moving during the direct observation and a tendency to look away from myself during the screening observation (Table 4.5).In population two, space allowance was significantly related to yawning, levels of C/C (Figure 4.2) and resting (Table 4.6).

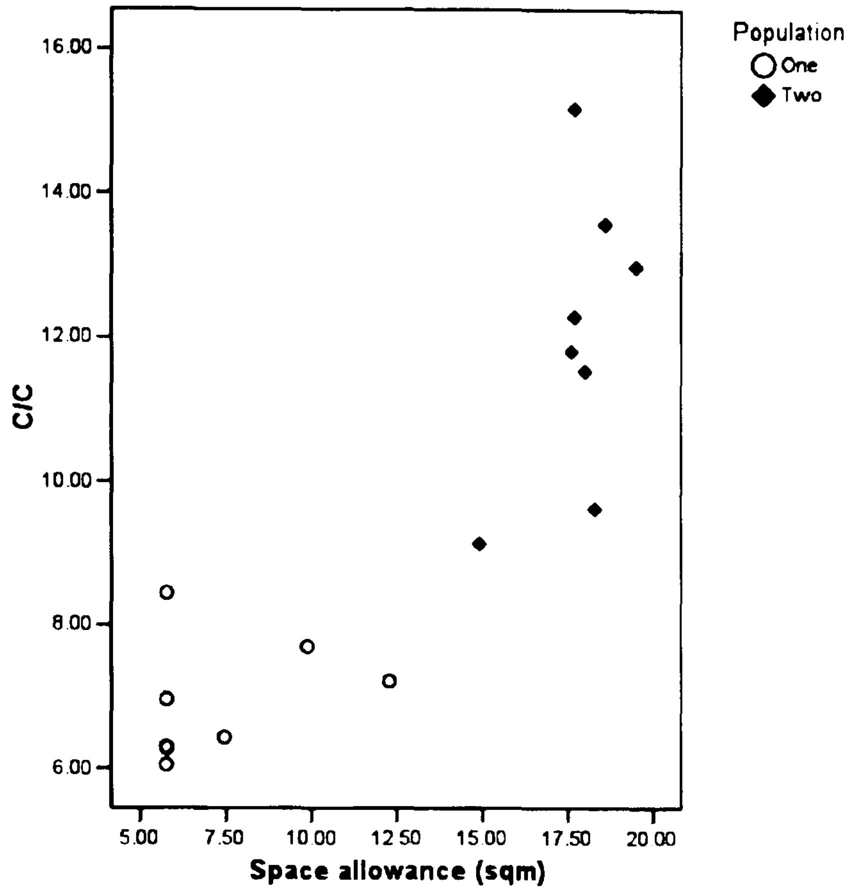
The relationship between space allowance and lying down was not evident in population two possibly because among dogs housed in larger kennels the proportion of time spent lying down is primarily affected by other aspects of husbandry e.g. inter and intra-specific contact (Table 4.6).

Figure 4.1. Relationship between space allowance (m^2) and time spent lying down during the remote midday observation (s/min). (Population one: Rho=0.74, p=0.02, population two: Rho=0.16, p=0.71).



Within population two, smaller kennels were also significantly associated (p=0.05) with decreased levels of C/C (Table 4.6 and Figure 4.2). However, no relationship between kennel size and C/C was seen in the dogs of population one, most likely because the kennels used to house population one's dogs were significantly smaller, ranging from 5.8 to 12.3 m^2 in area, whilst those in population two were 15.9 to 19.7m^2. Thus again, the same relationship between space allowance and levels of C/C would not be expected.

Figure 4.2. Relationship between space allowance (m^2) and C/C. (Population one: Rho=0.29, p=0.4, population two: Rho=0.71, p=0.05).

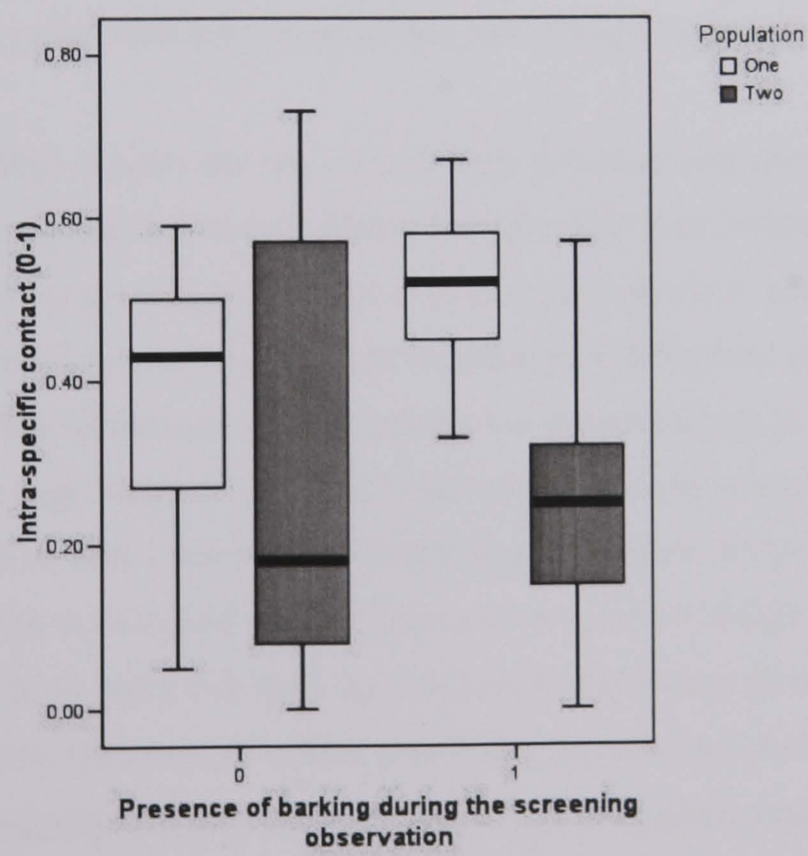


Based upon these findings and interpreting C/C as described in Chapter 2, one could suppose that dogs in smaller kennels are less physiologically stressed than those dogs in larger kennels. However as discussed earlier in this chapter (4.4.3.1), it is unclear how a larger kennel would induce more stress in a dog than a small kennel. In fact, I would predict that larger areas are more likely to reduce stress as they allow dogs to fulfil natural roaming, investigation and exploration. This result therefore appears complex suggesting there may be other factors influencing this relationship which were not measured within this study. I would therefore suggest that a study where dogs are housed in different sizes of kennel but cared for under standard conditions should be conducted. It is also possible that the HPA axis in those dogs housed in the smaller kennels had become less sensitive, the chronic stress of close confinement resulting in lower basal levels (1.4.1.1); further investigation of the long-term-effects of kennelling on cortisol levels would be required to resolve this.

4.5.1.2 Intra-specific contact

The level of contact with other dogs differed significantly between the two populations, the level of intra-specific contact was higher in population one than two (Table 4.4). At high levels of contact, (population one), this variable was linked to increased barking (Figure 4.3), incidence of illness (including diarrhoea, foot problems or tail damage) in the past month and tended to be linked to increased stereotyping, howling and time spent in the sleeping compartment (Table 4.6). In the population with lower average levels of intra-specific contact (population two), when levels of intra-specific contact increased, dogs were less likely to move around the kennels, stand and yawn but were more likely to lie down, look towards the exit, look away from the observer during the screening observation and perform coprophagy (Table 4.6).

Figure 4.3. Relationship between levels of intra-specific contact and whether the dog barked during the screening observation or not. (Population one: U=133, p=0.01 (0.51 (0.41, 0.58) vs. 0.4 (0.33, 0.50), population two: U=265, p=0.81).



These relationships suggest that in the population with the higher levels of intra-specific contact, when contact is increased, the effects tend to be upon behaviours which may be socially facilitated (barking, stereotyping and howling), and upon illness, which may be transmittable between dogs. In population two, where overall levels of intra-specific contact are lower, when contact is increased similar effects are not seen, its predominant effect is on behaviours indicative of rest; decreased area transitions, decreased standing and increased lying down and potentially of attempts by dogs to make contact with neighbours; looking away from myself and towards the exit (4.4.3.7). I suggest that these effects upon resting are not seen in population one's dogs because, resting in that population, is predominantly affected by noise.

4.5.1.3 Environmental stimulation inside the kennel

Dogs in population two were provided with significantly more environmental stimulation inside their kennels than dogs in population one (Table 4.4). This may reflect differences in opinion regarding the provision of environmental enrichment, as some handlers are concerned that enrichment may adversely influence working ability or aggression levels (Chapter 6.2). In population one, more stimulation was linked to increased moving, area transitions, lip licking, time spent looking towards the exit and decreased time spent lying down (Table 4.6). In population two, more environmental stimulation within the kennel was associated with increased referrals to the veterinary surgeon and paw lifting (Table 4.6).

All these relationships suggest that dogs which were provided with stimulation inside the kennel had poorer welfare than those without. I suggest that these relationships are evident because the decision to provide enrichment is based upon indicators which the dog displays (4.4.3.8). Yet the basis on which the decision is made may differ between the two populations and is likely to reflect differences between subjective interpretations of stress or poor welfare. In population two, dogs which are referred to the vet and paw lift are provided with toys and bones suggesting staff may interpret poor health as poor welfare, where as dogs in population one which show high activity and rest less (move more, perform a high number of area transitions and lie down less), lick their lips and look towards the exit are more likely to be given the stimulation. However, in neither population does the enrichment used seem to have been sufficient to significantly improve the welfare of the recipient dogs. An alternative form of enrichment; feeding devices, may prove more beneficial and this is investigated in Chapter 6.1.

4.5.1.4 Exercise

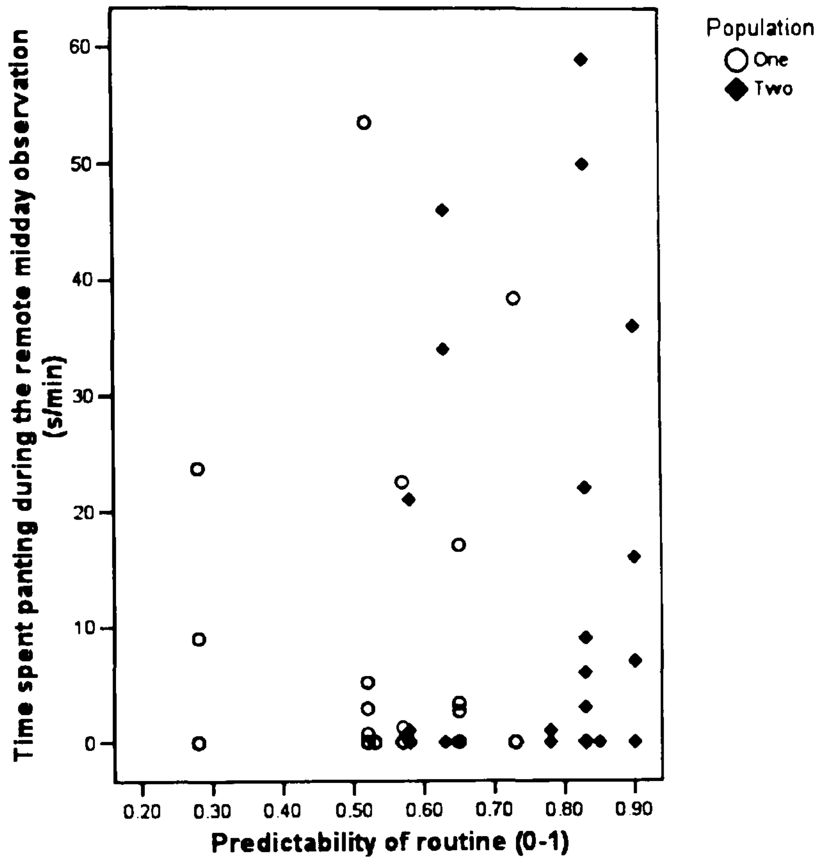
In spite of the significantly greater levels of exercise in population one (Table 4.4), exercise levels were inversely linked to barking in both populations (Table 4.6); decreased barking tended to be associated with increased levels of exercise. However, in population two, exercise was significantly related to barking during the direct observation whilst in population one, exercise was significantly related to barking observed during the screening and remote midday observation. There is obviously disparity between the two populations, dogs in population one with lower levels of exercise bark both in the presence of a person and when alone whilst dogs in population two bark only in the presence of a person. Barking can be indicative of frustration, so these results suggests that dogs, which receive low levels of exercise, may be frustrated in the presence of people with whom they associate exercise with. In population one, it is possible that some dogs could see people outside of the kennel area during the remote midday observation and so still exhibited the same behaviour.

4.5.1.5 Predictability of routine

The routine of population two was significantly more predictable than that of population one (Table 4.4) and was linked to duration of panting during the remote midday observation (Figure 4.4 and Table 4.6). Within population one, significant positive relationships were seen between predictability and both presence of coprophagy and the frequency of barking. There was also a tendency for predictability to be positively linked with frequency of area transitions and negatively linked with time spent lying down (Table 4.6).

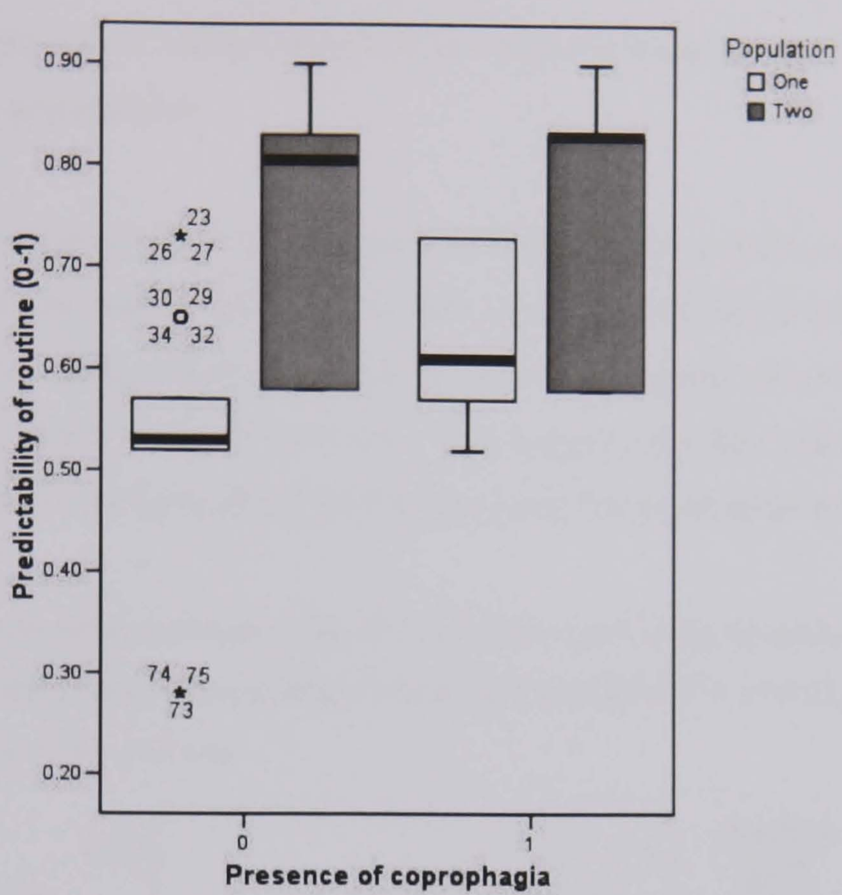
Even though the overall levels of predictability in population one were lower, the factors were linked to behaviours indicative of stress e.g. coprophagy and barking, probably due to frustration and restlessness. This suggests that for both populations, when predictability is increased, behaviours indicative of stress are exhibited although how this stress manifests differs between the two populations. Yet, previous studies, particularly in companion animals, suggest that predictability is beneficial to welfare (Carlstead et al, 1993a, Coppola et al, 2006) and should not be stressful. Instead it could be that once predictability has reached a level at which dogs can clearly anticipate their daily routine, any difference from normal (such as during data collection) is a cause of acute stress and reveals itself as stress related behaviours such as panting (Beerda et al, 1997; 1998), increased vocalisation or restlessness. As it is difficult to determine true cause and effect, I suggest that this is an area worthy of further research.

Figure 4.4. Relationship between predictability of routine (0-1) and duration of panting during the remote midday observation (s/min). (Population one: Rho=-0.09, p=0.56, population two: Rho=0.29, p=0.05).



Within population one, predictability of routine was related to coprophagy which was not seen in population two (Figure 4.5). This difference may be because predictability of routine and inter-specific contact were correlated (Rho=0.71, p<0.001) and hence once predictability decreases below a certain threshold the contact with kennel assistants will decrease also. Thus encounters with dogs by kennel assistants will be irregular and their likelihood of noticing and accurately reporting coprophagy will be lower. In population two, the predictability of routine was higher so the likelihood of noticing and accurately reporting coprophagy would be much higher and thus a relationship would not be predicted.

Figure 4.5. Relationship between predictability of routine (0-1) and the number of dogs reported to show coprophagy. (Population one: U=85.5, p=0.03, population two: U=163.5, p=0.83).



4.5.1.6 Summary

This section (4.5.1) has compared and contrasted those relationships where factors which might affect welfare differed significantly between the two populations. Through discussion of each of the factors it is apparent that there are two main reasons as to why there are disparities between how welfare relates to housing and husbandry in the two populations. Firstly, in a number of cases, relationships in the populations are driven by either low or high levels within a factor and because the factors differ so greatly from one another it is often the case that the range in one population does not overlap with the other population e.g. space allowance (4.5.1.1). Hence the relationship is not evident in both populations, only in the one where the factor being considered is limiting for welfare. Secondly in many of the relationships the behaviour which is related to the factor in one population is affected by other factors in the other population, so the expression of that behaviour in the other population will be constrained.

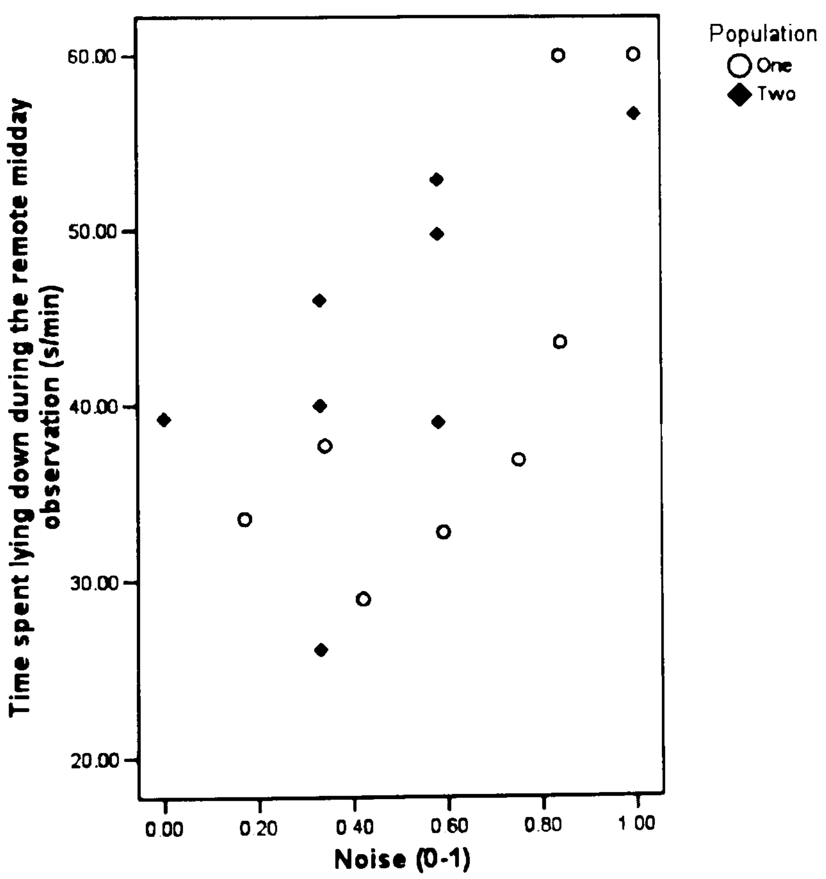
The following section (4.5.2) compares and contrasts relationships where factors did not differ significantly between the two populations.

4.5.2 Relationships where factors which might affect welfare did not differ significantly between the two populations

4.5.2.1 Noise

The levels of noise did not differ significantly between the two populations (Table 4.4). Within population one, high levels of noise were generally associated with dogs lying down more in the remote midday observation and the same relationship was also seen as a tendency in population two (Table 4.6 and Figure 4.6). This suggests that this is likely a robust result; increased noise levels are generally linked to increased resting at quiet times.

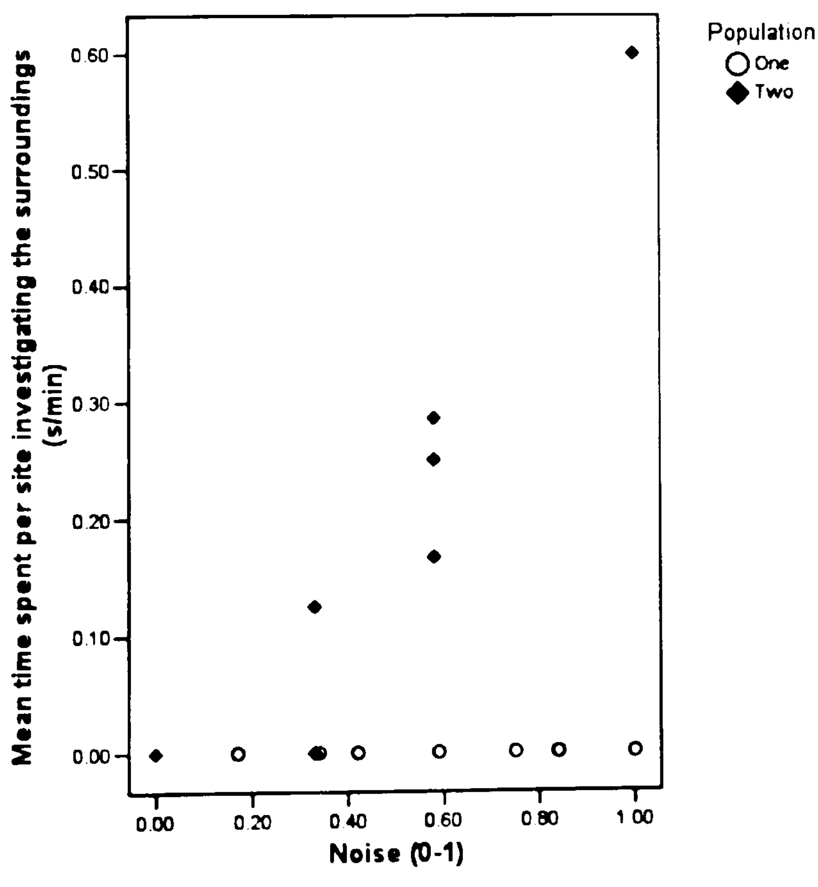
Figure 4.6. Relationship between noise (0-1) and time spent lying down during the remote midday observation (s/min). (Population one: Rho=0.7, p=0.03, population two: Rho=0.63, p=0.10).



In addition, in population two, high noise levels were associated with less barking, less time moving around, less time standing still during the remote midday observation and more time investigating the surroundings during the remote evening observation (Table 4.6).

Even though the noise levels do not differ significantly between populations, there are still differences in associations between the two populations. It could be possible that a particular factor greatly limits the performance of a particular behaviour and consequently that behaviour appears to be unaffected by other factors. For example the investigation of surroundings may be inhibited by small kennel sizes and it would therefore be expected that those dogs housed in small kennels such as those in population one did not exhibit this behaviour. During the remote evening observation, dogs in population one do not spend any time investigating their surroundings. This may be the reason why there is only a relationship between noise and investigating surroundings in population two, which are housed in larger kennels and so able to exhibit this behaviour (Figure 4.7).

Figure 4.7. Relationship between noise (0-1) and time spent investigating the surroundings during the remote evening observation (s/min). (Population one: Rho=0.2, p=0.46, Population two: Rho=0.93, p=0.001).



Although the indicators of welfare are different, in both populations dogs are exhibiting behaviours possibly indicative of rest at quieter times of day e.g. lying down and less time standing still (Beerda et al, 1998, Graham et al, 2005a) suggesting that high levels of noise are problematic as dogs cannot rest during the remainder of the day.

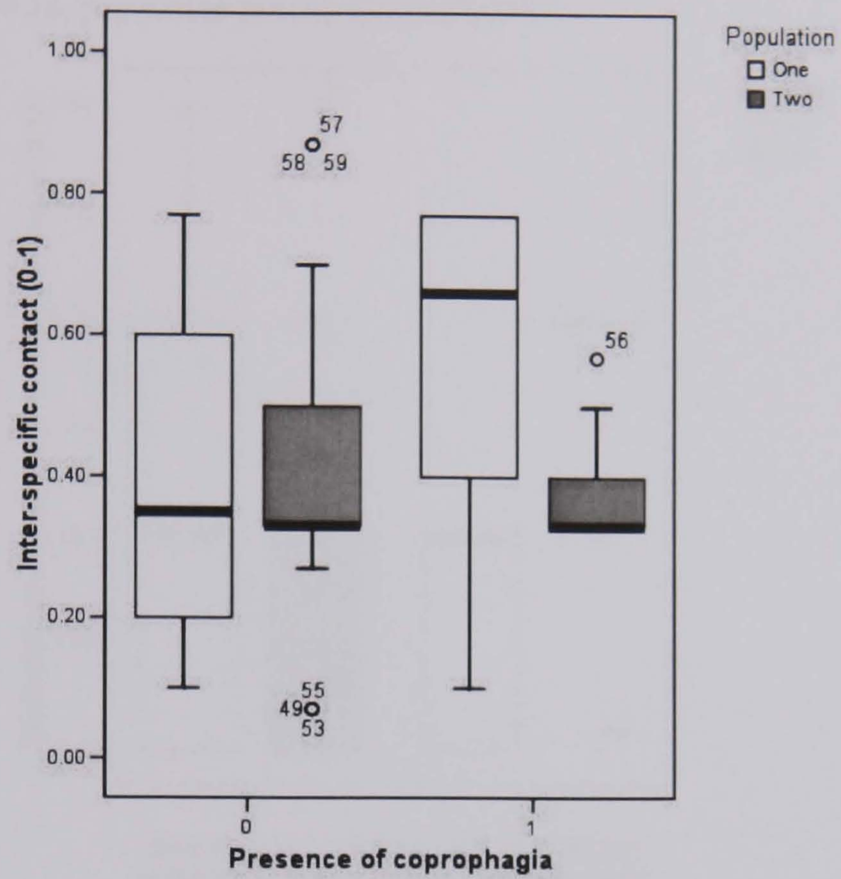
4.5.2.2 Inter-specific contact

Within population two, the time spent lying down during the remote midday observation and whether the dog had been referred to the veterinary surgeon were significantly related to the level of inter-specific contact which the dog received (Table 4.6). Within population one, even though the mean inter-specific contact did not differ significantly from that in population two (Table 4.4), these relationships did not exist and only coprophagy related to levels of inter-specific contact.

The absence of a relationship between lying down and inter-specific contact may be predicted in population one, since I propose that the small kennel sizes are the most critical factor to affect lying down, whilst in larger kennels other factors such as inter-specific contact are important.

Similarly, when studying the relationship between coprophagia and the level of inter-specific contact, there is a tendency for more dogs within population one with higher levels of interaction to be reported to show coprophagy (Table 4.6). However, this relationship does not exist within population two (Figure 4.8) even though the level of inter-specific contact does not differ significantly between populations. Predictability of routine and inter-specific contact are highly correlated (Spearman Rank Correlation; Rho=0.71, $p<0.001$). It is therefore expected that because in population one a relationship was seen between predictability of routine and coprophagy, a relationship between inter-specific contact and coprophagy would also be seen and this is true. So as routines become more predictable, kennel assistants spend more time with the dogs and thus encounters are more regular and the likelihood of noticing and accurately reporting coprophagy will be higher.

Figure 4.8. Relationship between inter-specific contact (0-1) and the number of dogs reported to show coprophagia. (Population one: U=91.5, p=0.04, population two: U=238, p=0.44).

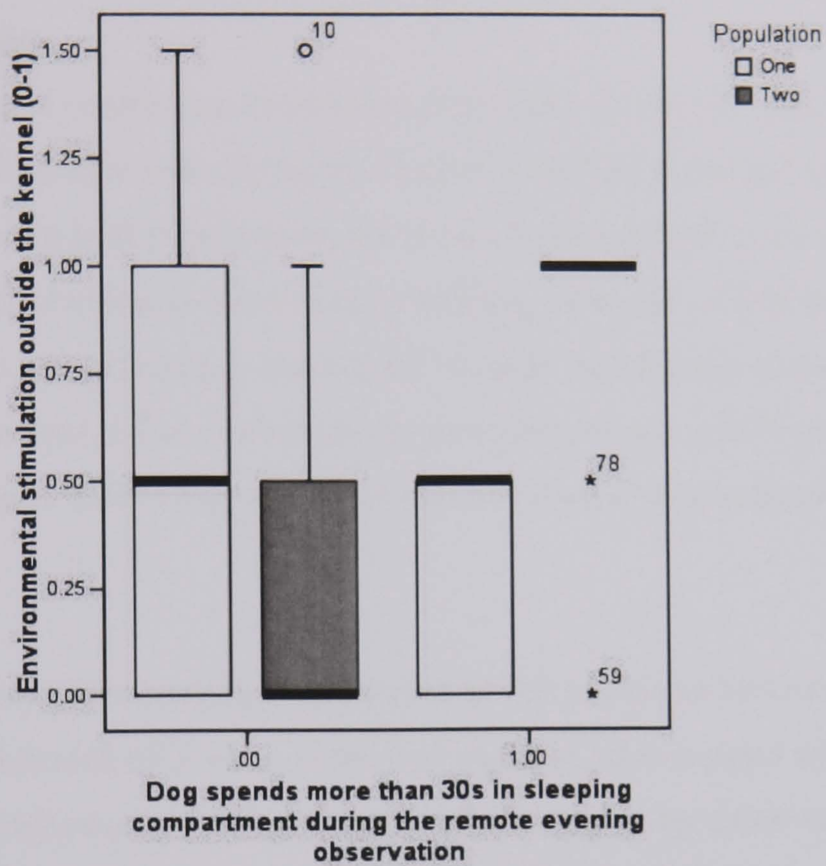


4.5.2.3 Environmental stimulation outside the kennel

The availability of environmental stimulation outside the kennel did not differ between the two populations (Table 4.4). However the relationships between this factor and indicators of welfare did still differ (Table 4.6).

In population one, dogs with more environmental stimulation outside their kennel generally howled more during the direct observation (Table 4.6). No relationship between stimulation and howling was apparent in dogs in population two, simply because no dog in population two howled during the direct observation. In population two, dogs with environmental stimulation outside their kennel tended to spend more than half of the observation time in their sleeping compartment during the remote evening observation whilst there was no such relationship in population one (Table 4.6 and Figure 4.9).

Figure 4.9. Relationship between the level of environmental stimulation present outside of the kennel (0-1) and whether the dog spends more than half its time during the remote evening observation in the sleeping compartment or not (Population one: U=159, p=0.16, population two: U=70.5, p=0.003).



It is possible that another explanation exists as to why some dogs in population two spend more time in their sleeping compartment. Dogs may retreat to their sleeping compartments if fearful (Gaines and Rooney, unpublished data) and it may be possible that for some dogs, the presence of environmental stimulation outside of their kennel elicits fear responses. Alternatively, some dogs may have learnt that spending time in the sleeping compartment results in less disturbance from activities outside.

4.5.2.4 Summary

Even though these three factors did not differ significantly between the two populations, there are still disparities between the two populations in the relationships between housing, husbandry and welfare. It is apparent that factors can interact in affecting the expression of behaviours e.g. investigating surroundings in small kennels. Alternatively other motivations or learnt behaviour could be influencing the expression of behaviours e.g. fear may be the cause of some dogs in population two spending more time in their sleeping compartments when stimulated by the environment outside of their kennel or dogs may have learnt that being in the sleeping compartment minimising disturbance.

4.6 Conclusions

When comparing and contrasting the relationships between the two populations it was evident that very few relationships were the same. Only two factors, noise and exercise, influenced the same behaviour in both populations; the level of noise related to the time spent lying down and the level of exercise was linked to barking, although in both cases the relationships were significant in one population and tended towards significance in the other. One factor, predictability of routine did not influence the same behaviours in both populations but in general the indicators with which the factor was associated were indicative of stress and frustration.

The large differences between populations seen in this study can however be explained by the major variations and lack of overlap in the way the dogs were housed and cared for. In a number of cases, indicators of welfare appeared to be driven by either very low or very high levels of housing or husbandry factors and these critical levels were found in only one of the two populations studied. Additionally, in many of the relationships, a welfare measure which was related to one factor in one population was strongly affected by other factors in the other population, masking the effects of the first factor.

Thus these two studies have shown that the factors which were critical to dog welfare differed between populations. With a small number of populations, it was only possible to identify these factors through examining and measuring individual populations. This study has therefore, not produced any definitive answers, but has proved a very valuable exercise for identifying trends worthy of future experiments. Surveying a large number of populations may more readily identify factors pertinent to most working dogs and may be useful in the future although it would be more labour intensive. I would suggest however, that it is apparent from these two studies that exercise and noise may be important factors and that other factors e.g. environmental stimulation within the kennel and predictability of routine, would benefit from additional research.

The remainder of this thesis therefore focuses upon factors which may be important for the improvement of military working dog welfare. Whilst levels of noise and predictability of routine appear to be critical for dog welfare, following this study, I was restricted to the number of factors which I could logistically and financially investigate. Furthermore, to investigate the influence of noise upon welfare, I would have had to measure the welfare of dogs under a number of conditions each differing in noise level. Considering that all of the research within this thesis has utilised military working dogs at operational sites, this would have been extremely difficult as I would have been unable to influence extraneous noise and such a study is perhaps better within a more controlled population such as laboratory dogs. However there were other factors, also critical, which were easier to manipulate within operational sites.

Exercise appeared to be critical in the welfare of military working dogs with low levels relating to indicators of frustration (4.5.1.4). This suggests that an increase in exercise provision may be beneficial. However it is not known how exercise should be provided. Thus Chapter 5 describes a study undertaken to investigate the effect of two types of exercise, differing in frequency and duration, upon the behaviour, physiology, working ability and health parameters of dogs procured as potential military working dogs.

Results from both populations also suggested that the current type of enrichment i.e. toys and bones, is not sufficient to improve the welfare of all military working dogs and the relationships between enrichment and welfare appear complex (4.5.1.3). Thus Chapter 6.1 describes a study conducted to investigate whether the welfare of *all* military working dogs can be improved by providing a food enrichment device, and whether working ability is detrimentally affected (Chapter 6.2), an opinion which appears to be a substantial barrier to the widespread use of such enrichment devices for military working dogs.

Chapter 5

Improving the welfare of military working dogs:

The effect of two exercise regimes upon indicators of welfare in potential military working dogs

5.1 Introduction

When discussing the welfare of kennelled dogs, great emphasis is often placed upon the benefit of exercise (Wolfle, 1987), which is believed to provide physical and mental stimulation (Prescott et al, 2004), most likely by analogy with wolves and feral dogs, which spend much time active and have large home ranges (Boitani et al, 1995). Yet the number of studies which have investigated the effect of exercise upon welfare is limited, and of those, very few have demonstrated any benefit. For example, Clark et al (1991) evaluated the effect of six different types of enclosure (A - an outdoor housing area with a 6.1 x 9.1m pen, B - an outdoor kennel with a 1.8 x 6.1 m run, C - an indoor 1.2 x 3.66 run, D - a 0.9 x 1.2 x 0.84m conventional laboratory cage, E - as D but with 30 minutes/weekday treadmill exercise and F - a 0.71 x 0.86 x 0.69 cage) upon exercise fitness, measured using exercising heart rate, muscle succinate dehydrohgenase activity and plasma cortisol. Neither the cage size nor an exercise training programme had any substantial effect upon the measures of physical fitness. A further two studies (Campbell et al, 1988, Clark et al, 1997) compared physiology and also behaviour of laboratory beagles given the opportunity to exercise, either individually or with a conspecific, with individuals which did not exercise. Neither group differed in levels of plasma cortisol or immune function, and likewise, when behaviour was observed, little difference between exercised and non-exercised dogs was evident (Campbell et al, 1988). Some limited effects were observed in Clark et al's study (1997); for example, barking occurred more frequently in exercised dogs compared to non-exercised dogs.

It could be argued that in all three studies the lack of substantial effects was a result of how exercise was provided. In Campbell et al's study (1988) exercise was provided either by increasing cage size or within an empty room, Clark et al (1991) provided exercise via large pens, runs or on a treadmill, whilst Clark et al (1997) provided exercise only within an empty room. In contrast, studies which have examined dogs exercised away from their home cage, suggest there may be more significant effects upon behaviour. For example, Meers et al (2004) compared the behaviour of nine beagles when walked once daily for 15 minutes to behaviour when no exercise was provided. During the walking programme, dogs were more "extrovert", active and attentive within their kennel environment, they spent more time standing, exploring, moving around the kennel, as well as displaying allogrooming and autogrooming, behaviours indicative of good welfare. However, when this program was ceased, the dogs became apathetic, suggesting that walking programmes are beneficial, but interruptions or cessation may cause stress.

Although studies investigating the effect of exercise are limited, results from Meers et al's study (2004) suggest that exercise away from the kennel environment may be more beneficial than that provided in an empty room or a cage of increased size (Campbell et al, 1988, Clark et al, 1991, 1997). In support of this, within Chapter 4, I found that dogs which received the most frequent and longest walks tended to bark and stereotype less and rest more (Table 4.6). However, in a kennelled environment the time that kennel staff can devote to each dog is often restricted, which can impact upon the frequency and duration of exercise provided. Under such restrictions, kennel staff will often decide how best to provide daily exercise within the time available, either providing one long walk each day or more frequent walks of shorter duration. However, how best to provide exercise has never been empirically investigated. One might predict that short regular periods of exercise might help to break up the day as dogs are removed from the kennel regularly and so may be more mentally stimulated; less frequent but longer periods of exercise will be both less disturbing for other dogs in neighbouring kennels, and more tiring for the individual, and so promote restful behaviour. Investigating the effects of two different regimes such as these may have practical value, ensuring that the kennel staff make best use of the time available and provide exercise in a way which optimises the dogs' welfare. Within the kennelled environment, there may also be instances where a change in regime is unavoidable e.g. due to kennel staff shortages or at the weekend compared to weekdays. Meers et al (2004) suggested that a change to a regime, even one implemented for a short period of time, may cause stress. If this is the case, it may be important to avoid changes and implement procedures which ensure this. This is also investigated here.

In this study, I first measured the welfare of dogs when given the usual routine exercise (as employed by the kennel establishment) twice daily, on lead, each for ten minutes. The amount of lead exercise was then increased to sixty minutes. Whilst sixty minutes of exercise is unlikely to be provided routinely within a military kennelled environment, I used this duration to maximise the chances of seeing an effect given the relatively small sample size available. The sixty minutes of exercise was provided in two different ways; regime one - six short walks and regime two - one single long walk. Dogs were exercised for five days under each regime. At the end of each regime, dogs returned back to their normal exercise routine for two days. Indicators of welfare were assessed to evaluate the effect of additional exercise and to compare differences between the two regimes. The effect of interruption of exercise was also assessed in the two interval days using the same indicators. The effect of exercise upon working ability could not be assessed in this study as subjects were newly procured un-trained military working dogs.

The aim of this study was to investigate

- whether sixty minutes additional exercise was beneficial to welfare;
- whether welfare differed depending upon whether the exercise was provided as one or several sessions;
- whether an interruption to an exercise routine caused physiological stress or an increase in behaviours indicative of frustration.

5.2 Methods

5.2.1 Subjects

Two groups of dogs, A (N=8) and B (N=7), procured by the Defence Animal Centre (DAC, 1.2.2) as potential arms and explosive search dogs (1.1) were used in this study. To balance each group for breed and age, group A, procured between August and September 2006, originally consisted of three Labrador Retrievers, one Labrador Retriever cross, and four English Springer Spaniels (ESS), all entire males and aged between 13 and 29 months (mean=16.3 ±6.0). Group B, procured between September and October 2006, consisted of three Labrador Retrievers and four ESS, all entire males and aged between ten and 21 months (mean=14.9 ±3.3). All dogs were procured at least ten days before the study started as previous research conducted by myself and others (Rooney et al, 2007b) indicated that a period of at least ten days was required for physiological adaptation to the kennelled environment.

Due to kennel cough, four dogs from group A could not be studied, and thus the subjects were reduced to one Labrador Retriever, one Labrador Retriever cross and two ESS aged between 13 and 22 months (mean=15.5 ±4.4) procured between 17 and 25 days (mean=19.5 ±3.7) before the study, three from rescue shelters and one donated by a member of the general public. In group B, one Labrador Retriever could not be studied due to an ongoing injury, and no more Labrador Retrievers could be procured to balance this group. Thus, two Labrador Retrievers originally intended for group A were included in group B. The resulting demographics were four Labrador Retrievers and four ESS aged between 10 and 21 months (mean=14.4 ±3.1), procured between 19 and 36 days (mean=26.4 ±6.3) before the study started, four donated by members of the general public, two from rescue shelters and two from dealers. One dog in group B became ill on the first day of the first regime and was also removed from the study.

5.2.2 Housing and husbandry

Upon arrival at the DAC, dogs procured from rescue shelters were placed in an isolation block for a period of five days to prevent the cross-transfer of contagions. All other dogs were housed singly, in a separate kennel section, amongst current resident dogs. Four days before the study started, each dog was vaccinated and anaesthetised for a hip x-ray. It was then transferred into a separate research section (Figure 5.1a) consisting of 12 standard wooden kennels (Figure 5.1b). Each kennel comprised an outdoor area (2.6m × 1.7m) and an enclosed sleeping compartment with a raised floor (1m × 1.7m) accessible through a small hatchway (50cm × 44cm). Each dog was provided with a piece of fleece bedding (Vet-bed™), placed within the sleeping compartment, and a chew toy (Nylabone®). Within the research section, each dog was placed in a kennel which allowed visual contact with at least two neighbouring dogs from its outdoor area but not visual contact with any other form of environmental stimulation outside of the research section e.g. personnel or livestock. Dogs were exercised according to the treatments described in section 5.2.4.2. Cleaning of the kennels took place once daily between 07:30 and 08:15am. Dogs were fed once daily at 16:30.

Figure 5.1 Kennel accommodation used for subjects

a) Layout of kennels

b) One individual kennel

5.2.3 Experimental design

Groups A and B were studied in succession, during September and October 2006 respectively. A cross-over design was used whereby each dog was exercised under each regime, in turn, over a period of 15 days. Order effects were balanced between the two groups by presenting the two regimes in both possible orders; group A was exercised using the short then long regime, whilst group B was exercised using the opposite regime order, long then short. Baseline indicators were collected over one day followed by five treatment days of exercise regime one, two non-treatment days (from hereon referred to as rest days), then five treatment days of exercise regime two and two rest days. Indicators of welfare were taken at baseline and at intervals throughout treatment and rest days.

5.2.4 Procedure

5.2.4.1 Baseline

Each dog was taken for a short lead walk by me between the hours of 07:15 and 08:15. This short lead walk was standardised for all dogs and provided every day throughout the study to allow urination and defaecation. At 08:00, recording of kennelled behaviour (5.2.5.1) began and continued for 24 hours. To mimic usual exercise routines, between 11:00 and 12:00 hours, each dog was lead walked for ten minutes either by me or by an assistant (male, aged 26) and then again between 14:30 and 15:30 hours. These lead walks were approximately one kilometre (km) in length and within the DAC grounds. At 16.30, each dog was fed and then thirty minutes later exercised off lead for approximately three minutes in a paddock to allow urination and defaecation. Urine samples were collected the following morning (first day of regime one) between the hours of 07:15 and 08:15 for the reasons discussed in section 5.2.5.2.

5.2.4.2 Treatment days

On each treatment day each dog was lead walked (as described above) between the hours of 07:15 and 08:15 and then further exercised between the hours of 09:30 and 12:30, and 13:30 and 16:30 either by me or by the assistant; the number of walks received during this time (one or six) depending upon the regime. Kennelled behaviour was recorded on the first and last regime day, starting at 08:00 and continuing for 24 hours. Urine samples (5.2.5.2) were collected on the morning after the first and last regime days. At the end of each treatment day, each dog was fed and exercised off-lead as described in section 5.4.2.1.

Regime 1

Regime 1 consisted of six short walks along the same set route as described in 5.2.4.1. When receiving short regular walks, each dog was taken out three times in the morning and three times in the afternoon. For standardisation across the two groups, each period of exercise always started at thirty minutes past the hour. Within each exercise period, dogs were always exercised in the same order i.e. dog 1 was always exercised first, and dog 4 in group A and 7 in group B exercised last. Each dog was alternated between the two walkers across the six walks each day.

Regime 2

The walk was approximately six km in length along a route through fields within the DAC. For Group A, two dogs were walked at 09:30 and two at 13:30; for Group B, two were walked at 09:30, two at 10:30, two at 13:30 and one at 14:30. As in regime 1, dogs were always exercised in the same order each day. During this regime, the contact with me and the assistant could not be balanced within the day thus the person exercising each dog alternated between days.

5.2.4.3 Rest days

The procedure for both rest days was the same as baseline. Urine samples on the morning of the first and second rest day were collected to measure the effects of the last regime day and the first rest day respectively.

An overview of indicators collected during the study is presented in Table 5.1.

Table 5.1 Indicators of welfare collected during study

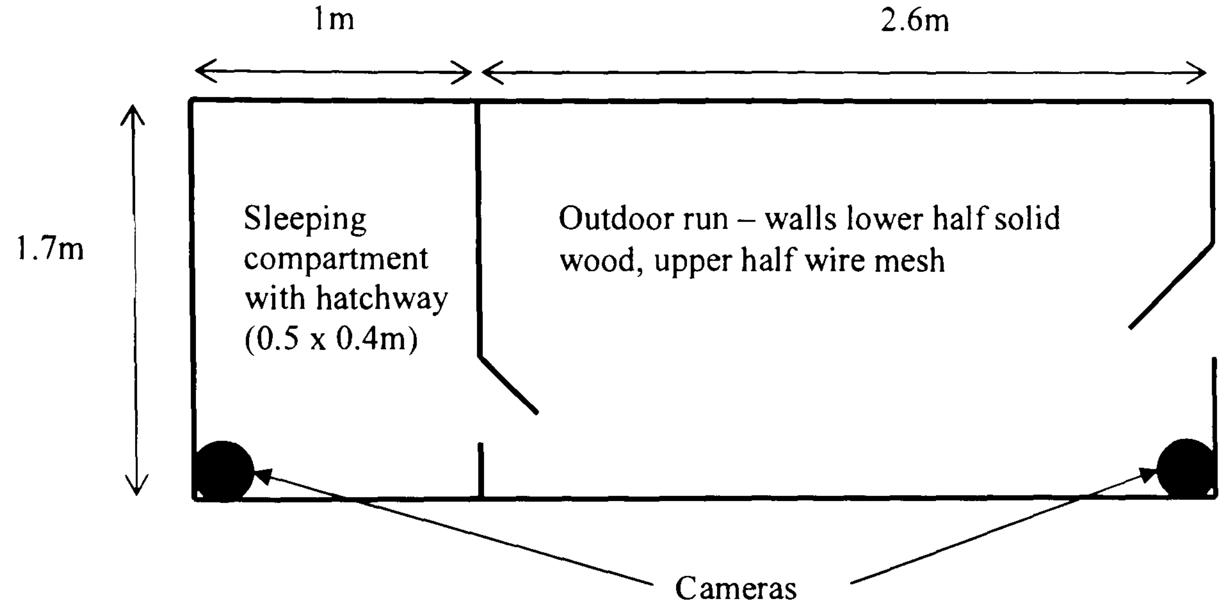
Day	Indicators taken
1 Baseline	24 hours kennelled behaviour video recording
2 Start of first regime	24 hours kennelled behaviour video recording, urine sample representative of baseline
3	Urine sample representative of first day of first regime
4	
5	
6 End of first regime	24 hours kennelled behaviour video recording
7 Rest day	24 hours kennelled behaviour video recording, urine sample representative of last day of first regime
8 Rest day	24 hours kennelled behaviour video recording, urine sample representative of first rest day post first regime
9 Start of second regime	24 hours kennelled behaviour video recording, urine sample representative of second rest day
10	Urine sample representative of first day of second regime
11	
12	
13 End of second regime	24 hours kennelled behaviour video recording
14 Rest day	24 hours kennelled behaviour video recording urine sample representative of last day of second regime
15 Rest day	24 hours kennelled behaviour video recording, urine sample representative of first rest day post second regime

5.2.5 Data collection and analysis

5.2.5.1 Sampling and analysis of kennelled behaviour

The kennelled behaviour of each dog was recorded using two waterproof cameras with light emitting diode (LED) illumination for night recording (RF Concepts, model 2020, lens 3.6mm), one placed in the sleeping compartment and one in the outdoor run of the kennel (Figure 5.2). Each camera was linked to a 16 channel multiplexer and a time lapse videotape recorder. The video recorder was started at 08:00am and continued recording for 24 hours.

Figure 5.2 Dimensions of the kennel and position of the cameras. The cameras were connected to a 16 channel multiplexer and time lapse video recorder.



From each 24 hour recording, two ten hour periods were analysed; daytime (09:30 to 19:30) and night time (20:30 to 06:30), using "The Observer" version five software package (Noldus Information Technology). The period between 06:30 and 09:30, when behaviour was disrupted by routine husbandry, was not recorded. Daytime observations were divided into one minute sample intervals for instantaneous[1] and one-zero[2] sampling (Martin and Bateson, 1993). The behaviours chosen for sampling (Table 5.4) were those which had previously been linked to exercise e.g. activity (Meers et al, 2004) and resting (Chapter 4, Table 4.6). Unfortunately, vocalisations, which have also been linked to exercise (Clark et al 1997; section 4.5.1.4), could not be recorded due to technical problems. At each sample point, six presence/absence variables describing activity state and position within kennel were recorded using instantaneous sampling. Analysis of stereotypical behavior (Appendix 4) revealed that whilst instantaneous sampling would approximate the record as obtained via one-zero sampling, one-zero sampling provided the most information. Hence, stereotyping was recorded using one-zero sampling i.e. on the instant of each sample point, the presence of stereotyping during the preceding sample interval was recorded.

[1] Instantaneous sampling – on the instant of each sample point the observer records whether or not the behaviour has occurred.
[2] One-zero sampling – on the instant of each sample point, the observer records whether or not the behaviour has occurred during the preceding sample interval

As activity state was predicted to be less variable during the night time, this period was sampled less frequently, divided instead into ten minute sample intervals. Instantaneous and zero-one sampling was used in the exact same way, recording presence-absence at each sample point for six of the behaviours and the presence of stereotyping in the preceding minute.

Table 5.2 Variables describing activity state and position within kennel

Variables	Definition
Lie down, Sit, Stand	See section 3.2.3.2, Table 3.1.
Stereotype	See section 2.2.4.2.2
Other active behaviour	Dog is performing one of the following behaviours; • *standing on hind legs*; see section 3.2.3.2, Table 3.1, • *walk*; three paws in contact with ground at any time whilst moving, • *trot*; two diagonal paws in contact with ground at any one time whilst moving, • *shuffle*; dog walks either forwards or backwards but no more than four steps are taken on any one occasion
Located in sleeping compartment	Dog in inside area of kennel
Absent/not visible	Dog either out of kennel on walk or behaviour could not be determined due to light spots or camera obscurities.

Taking the daytime and night-time observations in turn, each behaviour or kennel position was expressed as a proportion of all sample points for which the dog was visible, calculated as follows; *number of sample points on which the behaviour/kennel position occurred/ (maximum number of samples - number of samples where dog was either absent or not visible).* Only one dog stereotyped during the night-time observation so this variable was discarded.

5.2.5.2 Sampling and analysis of urinary cortisol to creatinine ratios (C/C)

Studies of dogs (Schatz and Palme, 2001) show that cortisol takes approximately 3 h to reach maximum concentration in the urine, and studies of pigs (Hay et al. 2000) suggest that an early morning sample represents a pool of cortisol, proportional to the production rate over 24 hours, and so is likely to reflect the excretion of cortisol in response to the previous day's exercise. For that reason, urine samples were always collected the morning after the day of interest. Urine samples were collected, stored and analysed as described in 2.2.4.1. Urine samples representative of the second rest day post second regime could not be collected from group A as the dogs were transferred to a different area in preparation for group B and so C/C levels representative of the second rest day post second regime were not used in any analysis.

5.2.6 Statistical analysis

The unexpectedly small number of subjects which completed the trial, and the poor balance between the groups, precluded the examination of all factors simultaneously by GLM, as had originally been planned. Therefore, all variables, whether analysed using ANOVA, Friedman's or Wilcoxon signed rank tests, were arranged chronologically, to examine the effects of additional exercise and its withdrawal, irrespective of regime, and also any differences between the two groups of dogs.

5.2.6.1 Investigating the effect of day, group and dog upon indicators of welfare

Nested ANOVAs were used to investigate the effects of day, group, dog and the interaction between day and group upon behaviour and C/C. The spread of the data was examined and transformations were employed to improve normality. Six variables were improved by transformation (Table 5.3).

Table 5.3 Variables investigated using nested ANOVAs and associated transformations

Variable	**Transformation**
Daytime proportion of visible time lying down	Arcsine-square root
Daytime proportion of visible time standing	Arcsine-square root
Daytime proportion of visible time performing other active behaviour	Arcsine-square root
Daytime proportion of visible time sitting	Arcsine-square root
Daytime proportion of visible time located in sleeping compartment	Arcsine-square root
C/C	Log_{10}

Two fixed factors; day and group, were included in the model, with dog as a random factor. Post hoc analysis using Dunnet's t-tests was used to further explore significant differences between days; baseline was used as the control and each day included in the model was compared to it.

Six variables could not be normalised by transformation (daytime proportion of visible time stereotyping and night-time proportion of visible time lying down, standing, sitting, performing other active behaviour and located in the sleeping compartment) so the effects of day were tested using Friedman tests. If a significant effect was found, further analysis was conducted using Wilcoxon signed rank tests comparing the first and last days of each regime and both second rest days following each regime with one another.

5.2.6.2 Do indicators of welfare show temporal variation?

Inspection of the daytime data (5.3.1.1) suggested that there were consistent changes over the study period. To test for this, regression analysis was performed with dummy variables incorporated to extract between-dog variation, on the following variables; C/C, and proportion of visible time lying down, standing, performing other active behaviour and located in sleeping compartment. The proportion of visible time sitting was not analysed as there was no significant effect of day.

5.2.6.3 Investigating differences between groups

Mann Whitney U tests were used to explore whether the two groups differed in the median number of days each dog had spent in DAC kennels prior to the study commencing.

5.2.6.4 Are changes in behaviour and physiology, over the course of the study, related?

Analysis revealed that four daytime behavioural variables; lie down, stand, other active behaviour and located in sleeping compartment, and C/C changed significantly over the course of the study period (5.3.1). To test whether the changes in behaviour were related to the change in physiology over the study period, five new variables were generated describing the change in behaviour and C/C over the study period. For each behaviour and C/C, levels measured at baseline were subtracted from levels measured on the first rest day post second regime for each dog. (The first rest day was used, as urine samples representative of the second rest day post second regime had not been collected (5.2.5.2)). Each behavioural variable describing the change over the study period was tested for correlation with the change in C/C using Spearman Rank correlations.

5.2.6.5 Does a decrease in exercise affect welfare?

The effects on behaviour of decreasing exercise from 60 minutes, to 20 minutes at the end, were tested. The difference between daytime behaviour performed on the last day of the second regime where 60 minutes of exercise was provided was compared with daytime behaviour performed on the second rest day where only 20 minutes of exercise was provided, using paired t-tests, for the proportion of time visible lying down, standing, other active, sitting and in sleeping compartment. Proportion of time visible stereotyping could not be transformed effectively due to a high number of zeros, so Wilcoxon signed rank tests were used to compare the two days.

The effect of decreased exercise upon level of C/C could not be measured, as urine samples representative of the second rest day post second regime had not been collected (see section 5.2.5.2).

5.3 Results

5.3.1 Is there an effect of day or an interaction between day and group upon behaviour and levels of C/C?

5.3.1.1 Daytime behaviour

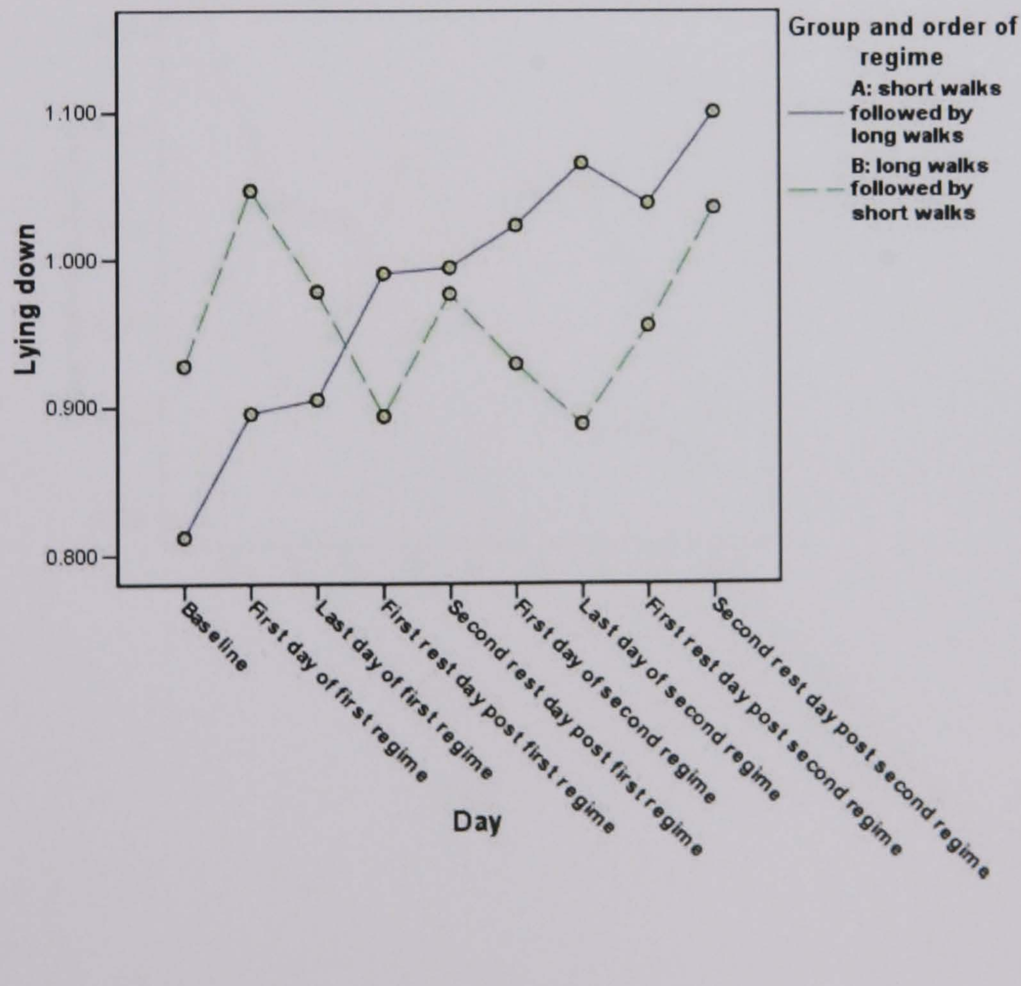
Effects of day, and the interaction between group and day, were evident for four of the five behavioural variables (see Table 5.4 and Figure 5.3). The two Groups did not differ significantly for any of the behavioural variables.

Table 5.4 The effect of day and interaction between group and day for each daytime behaviour pattern. (Degrees of freedom = 8,72)

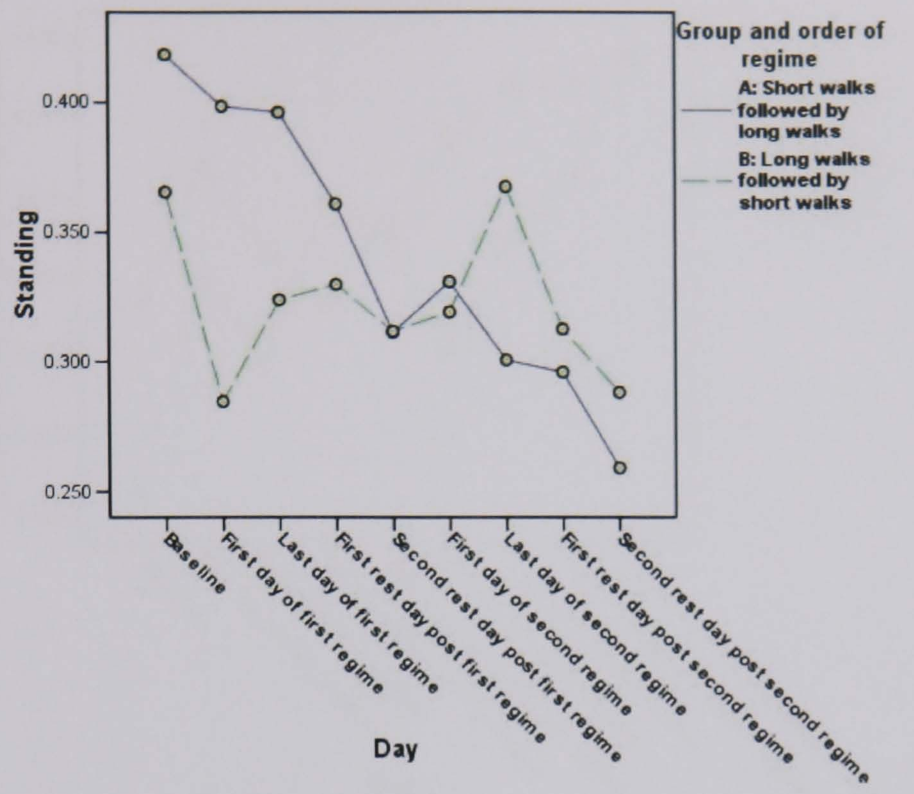
	Day		Group*day	
	F ratio	P value	F ratio	P value
Daytime proportion of visible time lying down	4.5	<0.001	5.0	<0.001
Daytime proportion of visible time standing	4.7	<0.001	3.1	0.004
Daytime proportion of visible time performing other active behaviour	5.4	<0.001	6.1	<0.001
Daytime proportion of visible time sitting	0.60	0.78	0.51	0.85
Daytime proportion of visible time located in sleeping compartment	8.9	<0.001	4.2	<0.001

Figure 5.3 Proportion of visible time and performing four behaviour patterns that varied significantly by day, plotted separately for each of the groups (A: short walks followed by long walks, N=4, B: long walks followed by short walks, N=7): a) lying down, b) standing, c) performing other active behaviour and d) located in sleeping compartment

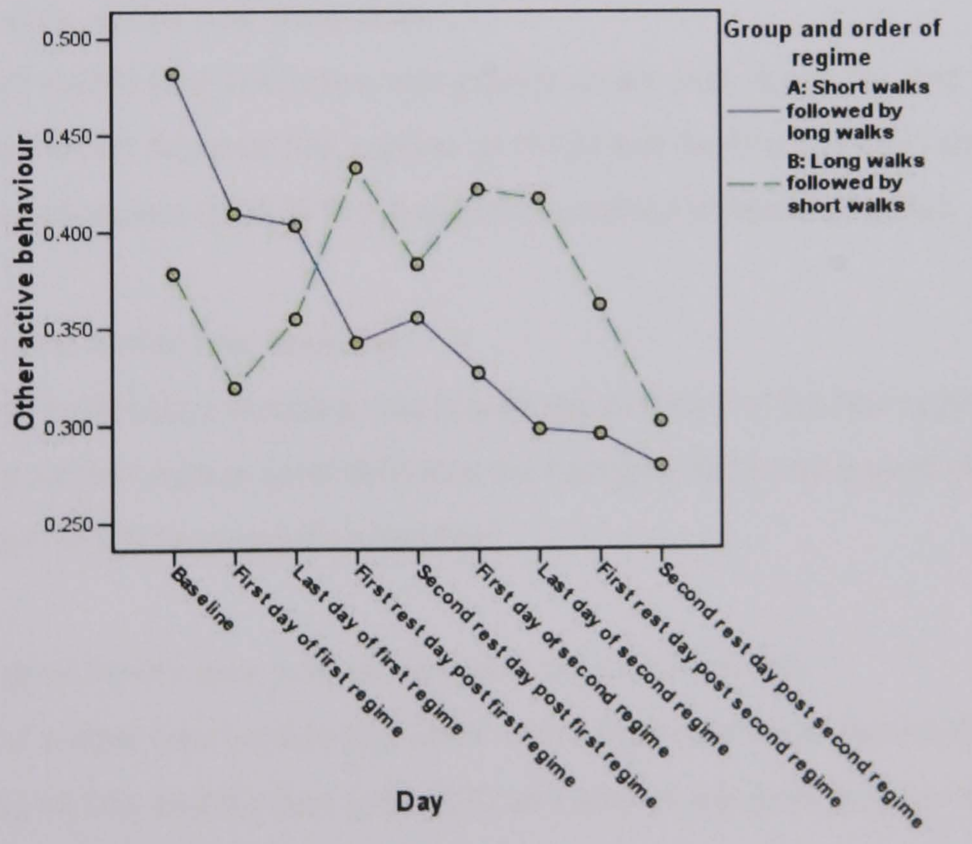
a)



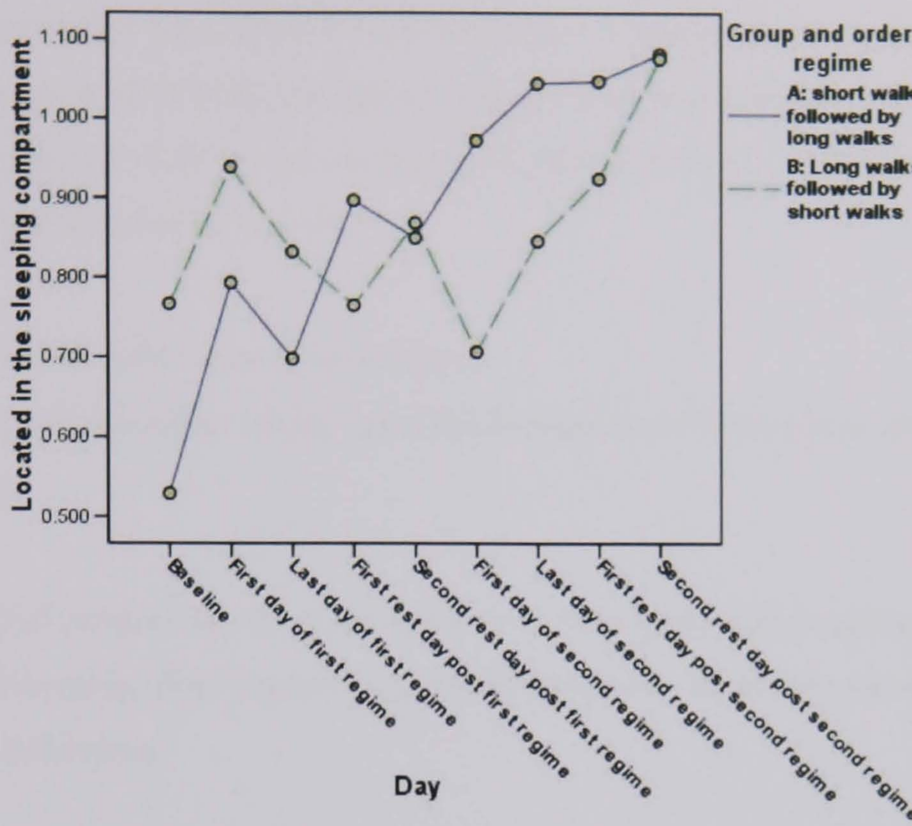
b)


Group and order of regime
A: Short walks followed by long walks
B: Long walks followed by short walks
Standing
0.400
0.350
0.300
0.250
Baseline
First day of first regime
Last day of first regime
First rest day post first regime
Second rest day post first regime
First day of second regime
Last day of second regime
First rest day post second regime
Second rest day post second regime
Day

c)


Group and order of regime
A: Short walks followed by long walks
B: Long walks followed by short walks
Other active behaviour
0.500
0.450
0.400
0.350
0.300
0.250
Baseline
First day of first regime
Last day of first regime
First rest day post first regime
Second rest day post first regime
First day of second regime
Last day of second regime
First rest day post second regime
Second rest day post second regime
Day

d)



a) Effect of day upon visible time lying down

The proportion of visible time laid down was greater on the first day of the first regime (p=0.02), the second rest day post first regime (p=0.03) and the first (p=0.03) and second rest days post the second regime (p<0.001) compared to baseline (Dunnet's t-tests).

b) Effect of day upon visible time standing

The proportion of visible time standing was less on the first day of the first regime (p=0.05), second rest day post first regime (p=0.007) and the first (p=0.003) and second rest days post second regime (p=<0.001) compared to baseline.

c) Effect of day upon visible time performing other active behaviours

The proportion of visible time performing other active behaviours was less on the first day of the first regime (p=0.04), and the first (p=0.007) and second rest days post second regime (p<0.001) compared to baseline.

d) Effect of day upon visible time located in the sleeping compartment

The proportion of visible time located in the sleeping compartment was greater on the first day of the first regime (p=0.004), the second rest day post first regime (p=0.01), the last day of the second regime (p=0.001), and the first (p<0.001) and second rest days post second regime (p<0.001) compared to baseline.

e) Effect of day upon visible time stereotyping

There was no significant effect of day upon the proportion of visible time stereotyping (χ^2=6.1, p=0.64).

Overall, as the trial progressed, the dogs spent more time lying and sleeping, and less time standing and performing other behaviour patterns. However, there was no significant change in stereotypical behaviour.

Of the four variables tested for consistency in change using linear regression, two were significant; standing and located in the sleeping compartment. A significant negative linear regression was found for day number and the proportion of visible time standing (*Beta*=-0.13, p=0.03) and a significant positive linear relationship was also found with proportion of visible time located in the sleeping compartment (*Beta*=0.23, p=0.001). So over time, dogs decreased the time they spent standing and increased their time in the sleeping compartment.

Comparing the two groups, dogs in group B, which tended to have spent more days in kennels than A (U=4.4, p=0.07, 23 (23, 25.5) vs. 18 (17.5, 21.5)), did not appear to respond in the same way to exercise as group A, until their second regime started (Figure 5.3). This could be interpreted either as an unintended difference between the groups at baseline, such that the exercise affected the groups differently, or that the long regime only influenced welfare if it followed the short regime (as in group A) but not if it was the first treatment received (as in Group B). Given the small number of subjects, it is not possible to distinguish between these two possibilities from the data available.

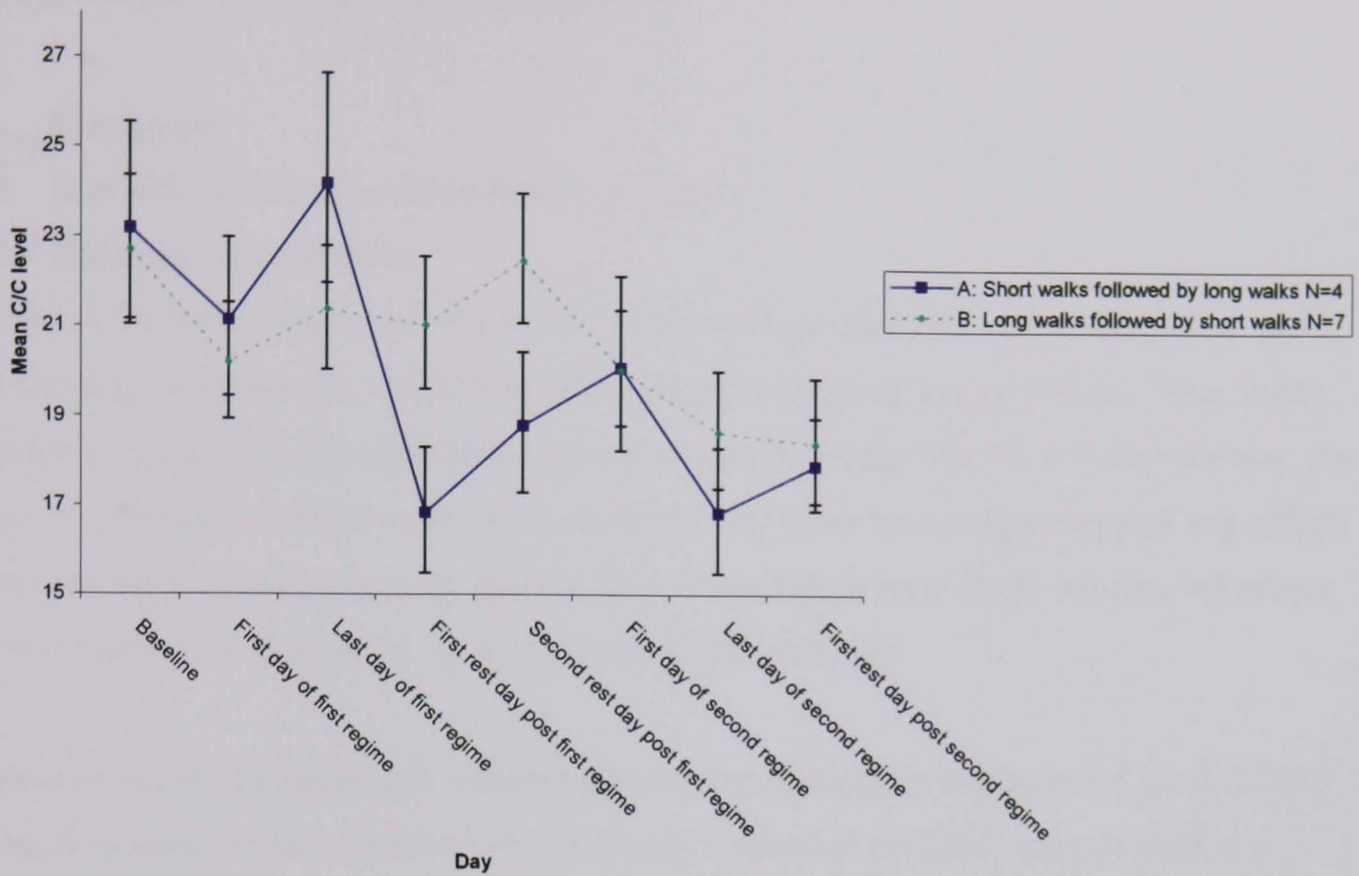
5.3.1.2 Night-time behaviour

Of the five behaviours, only two tended to be affected by day number; the proportion of visible time standing and sitting (χ^2=13.9, p=0.09 and χ^2=13.8, p=0.09 respectively) suggesting that behaviour performed during the night-time did not change significantly over the course of the study.

5.3.1.3 Levels of C/C

There was a tendency for C/C to differ between days ($F_{(7, 54)}$=2.1, p=0.06), although neither group ($F_{(1, 9.3)}$=0.33, p=0.58) nor the interaction between group and day significantly affected C/C ($F_{(7, 54)}$=0.86, p=0.55). As expected, individual dogs varied significantly in their average levels of C/C ($F_{(9,53)}$=6.5, p<0.001). Mean levels of C/C were significantly lower on the last day of the second regime (17.2 nmol/l:nmol/l ± 3.3, p=0.05) and tended to be lower on the first rest day post second regime of the study (17.1 nmol/l:nmol/l ± 2.0, p=0.06) than at baseline (21.9 nmol/l:nmol/l ± 5.9), suggesting, that over the course of the study, levels were decreasing (Figure 5.4). A significant negative linear regression between day number and C/C confirmed this (Beta=-0.25, p=0.004).

Figure 5.4 Changes in levels of C/C over the study plotted separately for each group.



5.3.2 Are changes in behaviour and physiology, over the course of the study, related?

For individual dogs, changes in the behaviours lie down, stand, other active behaviour and located in sleeping compartment, over the course of the study period were not significantly correlated to their change in C/C (Rho<-0.43, p>0.29).

5.3.3 Does a decrease in exercise affect welfare?

Of the six variables tested, three were significantly affected by the decrease in exercise and one showed a tendency. The mean proportion of visible time lying down was greater when 20 minutes of exercise was provided compared to 60 minutes (0.76 vs. 0.66, t=-3.1, p=0.01) whilst the mean proportion of visible time standing and performing other active behaviour was lower when 20 minutes of exercise was provided compared to 60 minutes (0.08 vs. 0.11, t=3.0, p=0.02 and 0.08 vs. 0.13, t=3.6, p=0.005 respectively). The mean proportion of time spent in the sleeping compartment tended to be greater when 20 minutes of exercise was provided compared to 60 minutes (0.78 vs.0.63, t=-3.4, p=0.07). There was no effect upon stereotyping (z=-0.17, 0.87) or sitting (t=-0.04, p=0.97). Overall, a decrease in exercise resulted in more time spent lying down and in the sleeping compartment and less time standing and performing other active behaviour.

5.4 Discussion

5.4.1 Is additional exercise beneficial to welfare?

5.4.1.1 Behavioural indicators

Overall, as the study progressed, during the daytime, dogs spent more time lying down and in their sleeping compartment, whilst less time was spent standing and performing other active behaviours, suggesting that additional exercise promoted restful behaviour during the day. No effects of additional exercise were found for night time behaviour, suggesting that any effects of exercise likely manifest only in daytime behaviour. This is most likely because behaviour is less variable during the night, so is less susceptible to change.

Whether or not restful behaviour, observed during the daytime, is indicative of good welfare is subject to some debate. Beerda et al (1997) and Graham et al (2005) suggest resting is indicative of enhanced welfare. Beerda et al (1997) observed less time lying down following the administration of several aversive stimuli including sound blasts, short electric shocks and a falling bag, whilst Graham et al (2005) observed dogs spending more time lying down in the presence of lavender and chamomile, odours which are thought to reduce anxiety and encourage well-being. However, Meers et al (2004) suggest that resting is indicative of poor welfare, whilst spending less time lying down and more time standing is indicative of good welfare. In their study, dogs provided with additional exercise spent more time standing and, when not exercised, spent more time lying down, which the authors posed was indicative of apathy. In rescue shelters, dogs which have spent more than five years in kennels tend to show more sedentary behaviour, spending much of their time resting (Wells et al, 2002). Whilst it is feasible that the welfare of Wells et al's dogs was very good, i.e. all dogs had successfully adapted to the kennel environment, it is just as likely, as suggested by the authors, that such states are indicative of apathy or boredom, perhaps even reflecting a state of learned helplessness (1.4.2.2). Therefore the increased levels of lying down in Meers et al's study (2004) may not be solely attributable to the cessation of exercise but a manifestation of the advanced age of the subjects and the number of years they had spent in kennels. Unfortunately Meers et al did not report behaviour prior to exercise so it is difficult to state categorically whether this is the case. Based upon the evidence of Beerda et al (1997) and Graham et al (2005), I interpret the restful behaviours, spending time lying down and in the sleeping compartment, as indicative of good welfare, suggesting that additional exercise provided over the study period improved welfare, although it could also be argued that the dogs were simply resting because of fatigue induced by exercise (see below).

It is also feasible that exercise also improved welfare indirectly, as these dogs were exercised in an area which was highly stimulating. Although routes within each regime were repeated without variation, the areas through which these routes crossed were widely used by DAC personnel both for dog exercise and training. In addition, horses were present in many of the fields. Thus the opportunity to explore, investigate and gather olfactory information was significantly greater than within the kennelled environment. These behaviours are known to be of importance to dogs (1.5.2.7) and allowing their expression is likely to enhance subjective states and by definition (1.3.3), welfare.

In this study, during exercise, dogs were on a lead and thus close contact with the walker was maintained throughout. Inter-specific contact is valued by dogs (1.5.2.8) and therefore it is also likely that the increased human contact, which results from additional exercise, contributes to the changes in behaviour, indicative of improving welfare. However, it is unlikely that the increased levels of contact are solely accountable for the behavioural changes, as past research shows that increasing the level of human contact does not change the overall behaviour of kennelled dogs; the behavioural time budget of dogs before and after a two month programme of increased human contact (30 seconds intense handling per day) did not differ significantly (Hubrecht, 1993a). I would therefore suggest that there is interplay between exercise and human contact which warrants further research.

Additionally, the changes in behaviour may not be solely due to increased exercise. In a study conducted by myself and others (Rooney et al, 2007b), 31 dogs were introduced into a novel kennel environment. Behaviour was recorded for ten consecutive days after transfer to the kennel and the time spent lying down and in the sleeping compartment was observed to increase over time. So whilst in the current study, the behavioural changes may be a result of improved welfare due to the treatment, welfare may also have improved naturally over time.

due to adaptation, even if additional exercise had not been provided. Repeating this study with a control group, which was exercised according to the DAC's usual exercise routine (ten minutes twice daily), and a treatment group which received 40 minutes additional exercise would allow me to determine whether behavioural changes resulted from adaptation or were attributable to the treatment. In the current study, this was not possible due to the limited availability of dogs. Alternatively, the study period could have been extended and basal indicators collected and analysed over several days, to ensure that initial adaptive responses to kennelling had occurred and behaviour was consistent prior to any treatments being implemented. This would have also been difficult in the current population as the time available for studying the subjects, before training commenced, was limited. Finally, the study could have been conducted using dogs similar to those studied in Chapter 4, all of which had been kennelled at their current establishment for at least one month (4.2.1) and having been transferred from DAC, kennelled in total for at least four months (1.2.2) so initial adaption to kennelling would have occurred. However, dogs in Chapter 4 were operational and so would never have been available for the period of time required for such a study. Nonetheless, the dogs in this current study had been kennelled on average for 23 days prior to the study commencing (range 17 to 36 days) so I believe it is probable that in general, dogs had adapted behaviourally to the kennelled environment and that the changes during the study indicate an improvement in welfare due to increased exercise.

The fatiguing effect of physical exertion should also be considered as this may have contributed to the increased duration of lying down and in the sleeping compartment. However, because the amount of exercise remains the same throughout the study, I would have expected the time spent lying down to plateau rather than continue to decrease over the study period. For this reason, I do not believe that fatigue is the only cause of the behavioural changes observed throughout the study.

When the two groups were compared it was apparent that dogs in group B did not respond in the same way to exercise as dogs in group A, until their second regime started. This could be interpreted either as an unintended difference between the groups at baseline, such that the exercise affected the groups differently, or that the long regime was only effective if it followed the short regime (as in group A) but not if it was the first set of walks received (as in group B). It is plausible that additional exercise affected the groups differently as differences between the groups at baseline were evident. Group B tended to have spent longer in kennels prior to the study commencing than those dogs in group A. Behaviour at baseline suggested that dogs in group B may have been more relaxed in kennels, already spending more time lying down and less time standing. As dogs adapt to kennelling, the time spent lying down increases whilst the time spent standing decreases (Rooney et al, 2007b). Thus these findings suggest that dogs in group B may have been better adapted to the kennelled environment, having learnt ways to cope with both the environment and the routine which preceded this study, and therefore were resistant or slow to respond to changes such as those of additional exercise. Group A dogs, which had spent fewer days in kennels, may have been less well adapted and hence less resistant to changes in routine, explaining why they responded immediately to changes such as additional exercise.

Due to the differences between groups in their response to exercise, and the unequal and small sample sizes, it was not possible to determine the effects of the two different regimes as was originally intended and so I have not been able to establish which regime is the most beneficial to welfare. As introduced in 5.1, investigating the effects of different regimes may have practical value, ensuring that the kennel staff make best use of the time available and provide exercise in a way which optimises the dog's welfare and so I still recommend that this be undertaken.

5.4.1.2 Levels of C/C

Unlike Clark et al's study (1991, 5.1) which found no effect of exercise upon levels of C/C throughout this current study, levels of C/C decreased significantly and approached levels not dissimilar to those of Labrador Retrievers measured in their pre-kennel home ((17.2 nmol/l:nmol/l ± 3.3 vs. 14.5 nmol/l:nmol/l (Rooney et al 2007b)). As was the case with behavioural indicators, it could be argued that this decrease is independent of additional exercise as levels of C/C are known to decrease during initial adaptation to the kennelled environment (1.4.1.1, Rooney et al, 2007b). However, the basal C/C of dogs in this current study (21.9 nmol/l:nmol/l ± 5.9) were similar to that of dogs measured by Rooney et al (2007b) following a period of ten days in kennels, suggesting that physiologically, they had already adapted substantially to the environment at the start of the study. This is further supported by previous research which indicates that plasma cortisol levels begin to rise on the second day of kennelling, peak between three and four days, decrease steadily until day nine and then begin to plateau after day ten (Hennessey et al, 1997). In this current study, all dogs had been kennelled for a minimum of 17 days (mean = 23 ±5.4). Hence, it is highly probable that the stress responses of most dogs will have adapted to the environment. In light of this, the decrease which is seen throughout this current study could feasibly be attributed to the additional exercise. To establish whether this is definitive, further research using a control group (as described for behaviour) is required.

The decrease in C/C levels could be also be a response to the indirect effects of human contact. In Coppola et al's study (2006), dogs admitted to a rescue shelter received contact with a person the day after arrival. During this session, dogs were played with, groomed, petted and obedience trained for 45 minutes. On the following day, the levels of salivary cortisol in those dogs were significantly lower than dogs which had received no human contact, suggesting that human contact lowers cortisol. These effects were short lasting as the contact with the person was only provided once. However, had the treatment continued, C/C levels might have continued to decrease. Whilst it is difficult to determine whether exercise or inter-specific contact reduced C/C based on this study alone, the overall effect of lower C/C levels suggests improved welfare. Similar to behaviour, there is a possible interplay between exercise and human contact which should be investigated further.

Interestingly, even though levels of C/C did not differ significantly between the groups, nor was there a significant interaction between group and day, overall differences between the two groups, following the first regime, did appear evident. In general, the C/C in group A appeared to decrease more quickly than in group B, which parallels the slower behavioural response in Group B and therefore suggests that both behaviour and cortisol had been affected by the same underlying mechanism(s). As for behaviour, the C/C results suggest either that the two groups had responded differently to the exercise, or that the effect upon welfare was dependent upon which regime came first.

Research has been suggested that creatinine may increase following exercise (1.4.1.1) and thus C/C may not be a suitable measure of welfare, even though effects on creatinine are generally only seen when exercise is exhausting or of high intensity (1.4.1.1). In the current study, if exercise did affect creatinine then changes in C/C would be expected as soon as exercise was increased. This did not appear to be the case as levels of C/C did not change, compared with baseline, until the end of the second regime. This suggests that overall the increase from twenty minutes to sixty minutes has no discernable effect upon creatinine, and therefore C/C can be used as a welfare measure in exercise studies of dogs.

5.4.2 Are changes in behaviour and physiology, over the course of the study, related?

At the level of the individual dog, the change in behaviour over the course of this study was not related to the change in levels of C/C. Similarly, in a study conducted by myself and others (Rooney et al, 2007b, 1.4.1.1), no significant relationships between behaviour and C/C were found. The lack of correlation in Rooney et al's study (2007b) was striking as the population was homogenous; all male, entire Labrador Retrievers of a similar age, and the response in C/C was as expected. In the discussion of that study we posed that even in a population where breed and sex does not differ, there can be individual coping strategies, due to genetic variation and subtle differences during ontogeny which have resulted in diverse temperament and personality differences. These differences then result in individuality of response to stressors which does not correlate to the expected physiological response (Rooney et al, 2007b). In this current study, I suggest that not only were there likely to be individual differences between the dogs, and this was demonstrated throughout the analysis, but in addition there were several breeds (5.2.1) in the sample, which are likely to show differences in behaviour which may further contribute to the lack of association. These findings support my initial recommendation that a multi dimensional approach to welfare assessment be taken, as individuals are likely to differ greatly in the way they manifest stress and thus welfare (1.4.4).

5.4.3 Does a decrease in exercise affect welfare?

In the current study, the change from sixty minutes of exercise to a regime where only twenty minutes of exercise was provided did affect behaviour, but not in the direction expected. The time spent lying down and in the sleeping compartment increased whilst the time spent standing and performing other active behaviours decreased. These changes are the same as those which occurred over the study period and for this reason, I believe that they are in fact carry-over effects from the day before when additional exercise was still provided and are not in response to a decrease in exercise. This is further supported by the non-significant difference in stereotyping between the two days. Had a change in routine led to frustration, then one would predict an increase in this behaviour on the rest day. This is not to say that a decrease in exercise is not stressful, maybe the expression of this frustration was delayed and had behavioural sampling continued over a number of days then a change in behaviour might have been observed. Changes in routine do have significant effects upon welfare. In laboratory cats, the transition from one routine to another, differing in unpredictability, resulted in behavioural changes indicative of poor welfare (Carlstead et al, 1993). Unfortunately within this study, it was not possible to test whether a decrease in exercise affected levels of C/C due to incomplete samples. Figure 5.4 does suggest that there are fluctuations in mean C/C levels over the course of the study which may be a response to decreasing exercise. I would suggest that this area is worthy of additional research.

5.5 Conclusion

Increasing exercise over a period of fifteen days coincided with increased restful behaviours; lying down and time spent located in the sleeping compartment. These effects upon behaviour were only observed during the daytime suggesting that night time behaviour is less sensitive to changes in exercise.

Levels of C/C decreased over the period during which exercise was increased suggesting that welfare was improved.

Whilst it can be argued that the changes in behaviour and physiology, indicative of improved welfare, were attributable to adaptation, based on evidence from other studies, it is highly probable that the dogs in this current study had already adapted to the kennel environment and thus the changes in behaviour and physiology observed were an effect of treatment. However, to establish this definitively, a further study should be conducted with a control group, exercised according to the DAC's usual exercise routine (ten minutes twice daily), and a treatment group which received 40 minutes additional exercise with welfare indicators taken in both.

Differences in welfare between the two exercise regimes could not be determined due to unexpected differences between the groups, and the small samples of dogs available. I suggest that the effect of different regimes could be investigated further

Interrupting the regimes had negligible immediate effects upon behaviour or physiology but this is most likely due to carry over effects of the previous regimes and the fact that exercise continues albeit at a lower level.

Overall, this study has shown that increasing exercise levels may benefit the welfare of military working dogs.

Chapter 6.1

Improving the welfare of military working dogs:

Investigating the effect of feeding enrichment upon military working dog welfare

6.1.1 Introduction

The majority of kennels used to house military working dogs are relatively barren, offering little opportunity for dogs to carry out natural behaviour such as exploring and investigating their surroundings. These environments can lead to compromised welfare, as discussed in section 1.5; one consequence of this is that many dogs chew either the kennel structure or items within the kennel such as bedding or furniture (1.5.2.11). Such destructive behaviour is often interpreted as an expression of frustration, in response to an environment which provides insufficient stimulation (Poole, 1992) and thus research has been conducted in an attempt to understand how best to provide stimulation or enrichment within the kennelled environment and thereby improve welfare (for a review see Wells, 2004a).

In past experiments, different methods have been used to enrich the kennelled environment (Wells, 2004a). The provision of toys, and their effects upon welfare, have received much investigation within laboratory (DeLuca and Kranda, 1992, Hubrecht, 1993a; 1995) and rescue dog populations (Wells, 2004b, Wells and Hepper, 2000), most likely because such items are relatively cheap, need little time for investment by human carers and as such, easiest to utilise.

In studies of laboratory dogs, following the provision of Rawhide[1], Gumabone[2] chews and reinforced plastic piping, 24% of dogs' time was spent interacting with the toys, even after they had been present for two months. Furthermore, the behavioural repertoire which dogs could choose to perform was greater, thus demonstrating benefits for welfare (Hubrecht, 1993a). These findings are concordant with other studies of laboratory dogs (DeLuca and Kranda, 1992, Hubrecht, 1995) but contrast with those from rescue populations where the introduction of a variety of toys often elicits little interest and thus few significant effects upon welfare (Wells, 2004b, Wells and Hepper, 1992; 2000). Similarly, in military working dogs, Hiby (2005) found that the provision of toys did not significantly improve the welfare of all dogs, and findings in section 4.4.2 support this result, although the relationships between the provision of toys and welfare appear complex. However, studies of both rescue and laboratory dogs have shown a preference for enrichment items which can be chewed. In a

[1] Centaur House, Torbay Road, Castle Carey, Somerset, UK (Hubrecht, 1993a)
[2] Nylabone®, PO Box 15, Waterlooville, PO7 6BQ, UK (Hubrecht, 1993a)

study of rescue dogs (Wells, 2004b), the time spent interacting with five different toys was compared; squeaky ball, non-squeaky ball, tug toy, boomer ball (a large ball specifically designed for chasing, The Company of Animals, UK) and Nylabone® chew. Dogs showed the most interest in Nylabone® chews, and similar toys (e.g. Gumabone chews) have also been favoured by laboratory dogs (Hubrecht, 1993a). This preference can most probably be attributed to the similarities in taste to, and the association with, food (Hubrecht, 1993a) suggesting that methods of enrichment which encourage feeding and chewing could be highly valued by dogs and thus beneficial to welfare. Indeed, in a recent study of canids (maned wolf, *Chrysocyon brachyrus*; Cummings et al 2007), the time spent exploring was significantly greater when dead mice were hidden around the enclosure compared to when a boomer ball was provided suggesting that the ability to forage for food may be a more effective enrichment strategy than introducing novel objects.

The effect of enrichment items which encourage feeding has not been evaluated in laboratory or rescue dogs but has received some attention in military working dog populations (Hiby, 2005). Two populations of military working dogs, gundog breeds and German Shepherd Dogs (GSD), were each provided daily with a Kong™, a hollow rubber cone shaped device, containing food that could be extracted by the dog. Behavioural and physiological indicators of welfare were assessed during a phase of provision and subsequent deprivation. In neither population did the device have a measurable effect upon welfare indicators even though many dogs in both populations showed positive anticipatory behaviour prior to its provision. However, in the GSD population, there was a trend towards decreased stereotyping during the period of enrichment. Further, those dogs which used their device the most showed an increase in cortisol levels when enrichment was discontinued, and a decrease when it was re-introduced, suggesting that the welfare of specific individuals was enhanced during enrichment provision. It was suggested by Hiby (2005) that the lack of significant effect may have been due to the relatively short period over which these devices were provided and greater effects upon behaviour and physiology might result, should a longer period of provision be employed. Therefore, in this study, I investigate feeding enrichment over a longer time scale.

To investigate the effects of long term feeding enrichment upon the welfare of military working dogs, basal indicators of welfare were taken in a population of working police dogs which were subsequently provided with a Kong, filled with gravy soaked biscuit, five times a week, over a period of four months. At the end of this period, the same behavioural, physiological and health indicators were taken to assess welfare levels during enrichment. Dogs were then deprived of their enrichment and the same indicators were taken to assess the level of stress induced by deprivation as an indication of how highly they valued the enrichment. Unlike Hiby's study (2005), I was unable to investigate the effect of re-introducing enrichment because the time during which the dogs were available was too short. In addition to enriched dogs, a control population was similarly assessed to ensure that any changes which were evident could not be attributed to natural changes with time.

The aim of this study was to investigate the effect of long-term feeding enrichment upon the behaviour, physiology and health of military working dogs, and the effect of subsequent discontinuation of enrichment upon their behaviour and physiology.

6.1.2 Methods

6.1.2.1 Subjects

The population (N=61) comprised 58 German GSDs, two Belgian Malinois and one Belgian Malinois cross, housed at four different sites (England N=1, Northern Ireland N=1 and Scotland N=2). The median number of dogs housed at each site was 14 and ranged from 11 to 17. There were 49 male and 12 female dogs aged between 24 and 112 months (mean =62.4m ±23.0).

As in Chapter 4, I measured several indicators of welfare in all 61 dogs but some measures were more time demanding and needed dogs to be rested to avoid confounding effects. Thus a sample of dogs was selected for these measures and from hereon such dogs are referred to as sample dogs. Dogs were chosen using the same criteria as described in section 4.2.1.

The sample (N=28) comprised 25 GSDs, two Belgian Malinois and one Belgian Malinois cross; 26 were male of which two were neutered. Both females were neutered. Three of the dogs were used as both site mascots and for public relations activities, the remainder were

PATrol Arm True (PATAT) dogs (1.1). At the time of the study, dogs were aged between 24 and 104 months (mean =62.9 ± 21.0) and all had been housed at their current site for between 4 and 56 months (mean =31.6 ±17.3). The number of dogs varied at each site and was 6, 7, 7 and 8 respectively.

Each site was visited on two occasions; once between May and August 2005, and once between February and April 2006. At each site, at least 220 days elapsed between visits.

6.1.2.2 Housing and husbandry

The total living area for sample dogs ranged from 18.3 to 19.7 m^2 (mean =18.9 ± 0.6). In one site the living area was split by a dividing wall into an exercise run (14.1m^2) and a sleeping compartment (4.2m^2). In the other three sites each dog had a wooden kennel (1.1m^2) in the centre of the living area. None of the kennels was heated and each dog was provided with a piece of fleece bedding (Vet-bed™).

All kennels were cleaned out daily in the morning and all dogs were fed once daily in the morning. The amount of exercise provided to PATAT dogs ranged from 0 to 30 minutes per day, depending upon the availability of the dog's handler, who was responsible for its exercise, whilst dogs used as site mascots or for public relations activities were exercised by kennel assistants for between 10 and 25 minutes per day.

6.1.2.3 Procedure

6.1.2.3.1 Measuring basal welfare (Measurement phase one)

Measurement phase one, during which basal indicators of welfare were collected, comprised three days. On day one (between the hours of 14:00 and 16:00) every dog housed at the site was observed in the presence of a person outside its kennel (myself) for 30 seconds (*screening observation baseline*, section 6.1.2.4.1a).

Between the hours of 07:30 and 09:00 on day two, each sample dog was lead-walked by a kennel assistant while urine samples (section 6.1.2.4.2) were collected. The kennel assistant then began their usual daily routine; feeding followed by kennel cleaning.

During the kennel assistant's lunch-break (between the hours of 12:00 and 13:30), I remotely recorded six minutes of behaviour from each of the sample dogs using a video camera placed on a tripod (*remote midday baseline*, section 6.1.2.4.1b). I then left the kennel area and the kennel assistant resumed the daily routine. A further six minute remote recording was taken of each sample dog at the end of the day, once the kennel assistant had left (between the hours of 16:00 and 18:40) (*remote evening baseline,* section 6.1.2.4.1b).

On day three, a second early morning urine sample was taken by the kennel assistant. Following urine collection, the health records (section 6.1.2.4.4) of every dog were inspected and I extracted visits to the veterinary surgeon for potentially stress-related symptoms during the preceding four months; diarrhoea, self biting, tail damage, foot problems and skin complaints. I then left the site.

Sixty days after the baseline data had been collected each dog was assigned to one of the two treatment groups; enriched or control (Table 6.1.1), and both enrichment devices and detailed guidelines (Appendix 5) for their use were sent to each site.

Table 6.1.1 Distribution of dogs between the two treatment groups and across the four sites. Dogs within site 1 were not balanced as a dog initially intended as a control was mistakenly included in the enriched group.

	Total number of dogs (N=61)		**Number of sample dogs (N=28)**	
Site	**Enriched**	**Control**	**Enriched**	**Control**
1	8	6	4	2
2	9	9	4	3
3	5	6	4	3
4	9	9	4	4

6.1.2.3.2 Provision of enrichment

The provision of enrichment started between 63 and 67 days (65 ±1.8) after the baseline phase. Each enriched dog was provided with a feeding device; a Kong (a hollow rubber shaped toy; Kong Company, Colorado, USA; Figure 6.1.1) stuffed with gravy soaked biscuits (part of the dog's daily feed). Hiby's (2005) previous study had used Kongs lined with meat paste but this was not financially viable for this study. Furthermore, a small pilot study had suggested that the choice of food had little effect on the time spent interacting with a Kong. The control dogs received no enrichment but were given a biscuit at the same time of day as the enriched group received their enrichment device, to balance the amount of contact with husbandry staff.

Feeding devices were provided by the kennel assistant every weekday evening between the hours of 16:00 and 17:00. Unfortunately it was not possible to segregate the enriched and control groups within each of the sites, so control dogs were able to see the enrichment being provided to the enriched dogs and it is possible that their behaviour may also have been influenced by the provision of enrichment. Devices remained in the dog's kennel overnight and were removed each morning for cleaning and re-filling. The total number of days over which enrichment was provided ranged from 105 to 125 days (average number of days per dog=116.3 ± 8.2).

Figure 6.1.1 Food filled Kongs used as enrichment devices.

6.1.2.3.3 Measuring the effect of enrichment and deprivation upon welfare (Measurement phase two)

On the final days of the period of enrichment, I returned to each section for a period of four days to assess both the effect of enrichment and subsequent deprivation upon welfare (Measurement phase two). At this point of the study, the number of sample dogs had decreased from 28 to 21 (enriched dogs = 13, control dogs = 8) (Table 6.1.2) due to death, illness or movement to another site.

Table 6.1.2 Distribution of sample dogs between the two treatment groups and across the four sites at the end of the period of enrichment (Measurement phase two).

	Sample dogs	
Site	**Enriched**	**Control**
1	3	2
2	3	1
3	3	3
4	4	2

To assess the effect of enrichment, indicators were taken between 14:00 hours on day one and 12:00 hours on day three. To assess the effect of deprivation, indicators were taken between 16:00 hours on day three and 16:30 hours on day four.

During the four days, the same behavioural and physiological indicators collected during Measurement phase one were taken from enriched dogs and control dogs (6.1.2.3.1). Additional measures to assess the anticipation of enrichment, time spent with the enrichment device, the dog's behaviour during deprivation of enrichment, and health during the four months of enrichment, were also collected. Additional behavioural observations of control dogs were collected to determine whether any changes seen in the enriched dogs were temporal effects or could be attributable to the enrichment. These additional measures are described below.

a) Measuring the effect of enrichment- Enrichment phase

On day one of Measurement phase two, a video camera was placed in front of each control dog's kennel so that the anticipatory behaviour of control dogs towards a biscuit could be assessed (6.1.2.4.1c). Each video camera was started and once I had left the kennel area, behaviour was recorded for one minute. Following one minute, the kennel assistant went into the kennel area and gave each of the control dogs a biscuit. Enriched dogs were also given a Kong at this time but their behaviour could not be assessed at this time due to a shortage of video cameras. Hence on the second day at 16:00 hours, the anticipatory behaviour of enriched dogs was recorded for one minute, following which the kennel assistant provided each dog with a food stuffed Kong and each control dog with a biscuit. Cameras were left recording for a further 60 minutes so that the time spent interacting with the enrichment could be measured (section 6.1.2.4.1c).

Health records were inspected to extract whether any of the subject dogs had been taken to the veterinarian with potential stress-related symptoms during the four month period of enrichment.

b) Measuring the effect of deprivation – Deprivation phase

On day three, at 16:00 hours, I placed a video camera in front of each enriched dog's kennel (*remote evening deprivation,* 6.1.2.4.1b) started recording then left the kennel area. One minute later, the kennel assistant, rather than giving the enriched dogs their enrichment device, provided both enriched dogs and control dogs with biscuits. Cameras were left recording for a further six minutes. Unfortunately due to camera shortage, it was not possible to record the behaviour of the control dogs concurrently. On day four, during the evening, enrichment devices were re-introduced to the enriched dogs and a biscuit was given to the control dogs. A six minute observation of control dogs was then made providing an observation which could be compared with the remote evening deprivation observation of enriched dogs filmed the day before (*remote evening control deprivation,* section 6.1.2.4.1b).

Figure 6.1.2 provides an overview of the study.

Figure 6.1.2 Timeline of Study.

14:00 Day 1	Start collecting basal indicators of welfare	Measurement phase one	
12:00 Day 3	Finish collecting basal indicators of welfare		
63-67d post measurement phase one	Dogs assigned to two groups: enriched and control Provision of enrichments starts		
	ENRICHMENT		
105-125d post provision Day 1 14:00	Start measuring effects of enrichment	Measurement phase two	*Enrichment phase*
Day 3 12:00	Finish measuring effects of enrichment		
Day 3 16:00	Start measuring effects of deprivation		*Deprivation phase*
Day 4 16:30	Finish measuring effects of deprivation		

6.1.2.4 Data collection and analysis

6.1.2.4.1 Sampling and analysis of kennelled behaviour

Behavioural variables recorded both in the screening observation and remote observation were based upon those which appeared from previous studies to be indicative of a dog's welfare (Chapter 4, Table 4.6). Stereotypical behaviours were recorded in both types of observations as they are commonly suggested to indicate welfare problems (Mason 1991) and thus are often used to assess welfare (Mason and Latham 2004, 1.4.2.5).

a) Screening observation; recording the behaviour of all dogs in the presence of a person

Five behaviours (Table 6.1.3) were recorded from every dog housed at each of the four sites during the screening observation. The methodology used to collect and analyse the five behavioural variables is described in section 4.2.3.1.2b.

Table 6.1.3 Variables measured in the screening observation.

Variable	Description	Method of measurement
Bark	Section 2.2.4.2.1 Table 2.2	Presence/absence
Stereotype	Section 2.2.4.2.2	Presence/absence
Rest	Section 4.2.3.1.2b Table 4.2	Presence/absence
Move	Section 4.2.3.1.2b Table 4.2	Presence/absence
Tendency to look away	Section 4.2.3.1.2b Table 4.2	0=does not look away, 1=looks away intermittently, 2=looks away from observer

Three new variables; ***change in behaviour upon enrichment compared to baseline***, ***change in behaviour upon deprivation compared to enrichment*** and ***change in behaviour upon deprivation compared to baseline*** were calculated for each of the four presence/absence variables (Table 6.1.3) to show how each dog's behaviour had changed upon both enrichment and deprivation. For example, calculating *change in barking behaviour upon enrichment compared to baseline*; a score of +1 was given if the dog barked upon enrichment but did not bark during baseline. A score of 0 was given if the dog did not change its barking behaviour between the two phases i.e. either barked in both phases or did not bark in either phase. A score of -1 was given if the dog did not bark upon enrichment but did bark during baseline. Similar calculations were made for the other three presence/absence variables but Tendency to look away remained as raw data.

b) Remote observation; recording the behaviour of dogs in the absence of a person

Eleven behaviours (Table 6.1.4) were recorded from the sample dogs during the remote midday and evening observations. The methodology used to collect and analyse the eleven behavioural variables is described in section 3.2.3.2.

Table 6.1.4 Methods of measurement of the eleven variables recorded during the remote observations.

Variable	Description	Method of measurement
Bark	Section 2.2.4.2.1, Table 2.2	Frequency
Area transitions	Section 3.2.3.2, Table 3.1	Frequency
Lick lips	Section 3.2.3.2, Table 3.1	Frequency
Yawn	Section 3.2.3.2, Table 3.1	Frequency
Stereotype	Section 2.2.4.2.2	Duration (s)
Pant	Section 2.2.4.2.1, Table 2.1	Duration (s)
Lie down	Section 3.2.3.2, Table 3.1	Duration (s)
Stand	Section 3.2.3.2, Table 3.1	Duration (s)
Gaze at exit	Section 3.2.3.2, Table 3.1	Duration (s)
Groom	Section 3.2.3.2, Table 3.1	Duration (s)
Play	Section 3.2.3.2, Table 3.1	Duration (s)

Two of the variables; lick lips and yawn were combined to form a new variable; *frustration*, as both behaviours may indicate frustration (Beerda et al, 1997, Voith et al, 1987). The relationship between standing and lying down was explored using Spearman Rank Correlations in both remote observations and found to be negatively correlated (Rho>-0.66, p< 0.01), thus lying down was discarded for future analysis. The variables standing and gaze at exit were performed by more than 50% of the sample dogs and were transformed using arc sine- square root since this improved their distributions for parametric analysis. All the other variables were performed by less than 50% of the sample dogs so the actual variables were not used, but three new variables were calculated per behaviour based on the change between phases. I calculated; ***change in behaviour upon enrichment compared to baseline, change in behaviour upon deprivation compared to enrichment*** and ***change in behaviour upon deprivation compared to baseline***. However, for these variables, scores were given dependent upon whether the behaviour increased, decreased or did not change between phases; +1 = an increase in behaviour, 0 = no change in behaviour, -1 = a decrease in behaviour.

c) Recording of anticipatory behaviour prior to enrichment and time spent interacting with enrichment

Due to a recording failure, only eight of the nine enriched dogs had data available to measure the anticipation of and interaction with enrichment. Anticipatory behaviour of enriched and control dogs was measured using four variables during the minute prior to the provision, and four immediately before the provision of enrichment (Table 6.1.5). There was very little variation in the four variables measured immediately prior to enrichment, labelled b in Table 6.1.5, hence these are discussed no further.

Table 6.1.5 Behavioural variables measured during the one minute prior to the provision of enrichment (a) and immediately prior to enrichment (b).

Variable	Description	Method of measurement	Phase
Vocalise	Dog barks (Section 2.2.4.2.1 Table 2.2), howls (Section 3.2.3.2, Table 3.1) or whines (Section 2.2.4.2.1, Table 2.2)	Frequency	a
Stereotype	Section 2.2.4.2.2	Duration (s)	a
Gaze at exit	Section 3.2.3.2, Table 3.1	Duration (s)	a
Pant	Section 2.2.4.2.1 Table 2.2	Duration (s)	a
Posture	Body position of dog	0=lying down, 1=sit, 2=stand, 3=standing on hind legs	b
Position	Place within kennel where dog is located	0=front of kennel, 1=back of kennel, 2=sleeping compartment	b
Orientation of gaze	Direction in which dog is looking	0=away from kennel assistant, 1=towards kennel assistant	b
Vocalise	Dog barks (Section 2.2.4.2.1 Table 2.2), howls (Section 3.2.3.2, Table 3.1) or whines (Section 2.2.4.2.1, Table 2.2)	Presence/absence	b

The total time spent interacting with the device was measured by summing the time spent performing each of the three variables described in Table 6.1.6.

Table 6.1.6 Definition of three behavioural variables used to calculate the total time (s) each dog spent interacting with the enrichment device.

Variable	Definition
Manipulate device	Dog interacts with enrichment device by licking, biting or chewing
Follow device	Dog drops Kong and moved behind it, orientated towards it at a distance of no greater than 0.2m, picking up Kong once reached
Ingest food from device	Dog eats food or licks floor where food has been dropped from Kong

One further variable was recorded; the last contact time with the Kong. This was defined as the point at which the dog did not return to the device within the 60 minute observation.

The time spent interacting and last contact time with the device could only be measured from four dogs as the other four took their devices into the sleeping compartment and thus were not visible. Therefore, due to the small sample size, it was not possible to conduct any further analysis using this data. Instead the interaction with the Kong is discussed qualitatively later.

6.1.2.4.2 Sampling and analysis of urinary cortisol to creatinine ratios (C/C)

Urine samples were collected, stored and analysed from sample dogs as described in section 2.2.4.1.

6.1.2.4.3 Sampling and analysis of behaviour reported by kennel assistants

At the end of the enrichment period, kennel assistants were asked to record whether Kongs were empty or not when collected each morning and whether they had noted any changes in the behaviour of enriched dogs during the period of enrichment. The results are discussed qualitatively (6.1.3.4).

6.1.2.4.4 Sampling and analysis of health

Incidences or symptoms of potential stress-related symptoms were recorded as presence or absence, and two further presence or absence variables were calculated from this data to indicate whether the dog had been taken to the veterinarian as a result of the illness or symptom *before* and *during* the provision of enrichment. A final variable; ***'change in health upon enrichment'***, was then calculated using a three point scale, to show whether the dog's health had got better, worse or was the same; *presence/absence of veterinarian visit during baseline phase minus presence/absence of veterinarian visit during the enrichment phase.*

6.1.2.4.5 Summary of data collected

Table 6.1.7 summarises the data collected throughout the study, the phases during which it was collected, whether all or sample dogs were used and which treatment group the data was collected from.

Table 6.1.7 Summary of the subjects, treatment group and phase from which data was collected. *Dogs:* A=all dogs, B= sample dogs only, *Treatment Group:* E=enriched only, C=control only, Z=enriched and control

	Phase		
Data	**Baseline**	**Enrichment**	**Deprivation**
Urinary cortisol	B, Z	B, Z	B, Z
Behaviour - screening observation	A, Z	A, Z	A, Z
Behaviour - remote midday	B, Z	B, Z	B, Z
Behaviour - remote evening	B, Z		B, Z
Anticipation of enrichment		B, Z	
Interaction with enrichment		B, E	
Behaviour reported by kennel staff		A, E	
Health	A, Z	A, Z	

6.1.2.5 Statistical analysis

There was a change in husbandry procedure at one of the sites (Table 6.1.2, Number 4), over the enrichment phase. Feeding frequency was increased from once to twice daily, with the second meal provided at the same time as their enrichment. Therefore, it was not possible to analyse the effects of enrichment upon behaviour at this site, nor to look at the anticipation and interaction with the feeding device. Hence, behavioural analysis was restricted to data from dogs housed at only three sites. It is also likely that the splitting of one meal per day into two smaller meals could reduce the incidence of diarrhoea or other GI problems, so this site was excluded when exploring the effects of enrichment upon health. The effects upon cortisol were however analysed using all four sites, as dietary changes that occur on a daily basis are unlikely to have a major impact on the basal or stress-induced levels of corticosteroids (Lane, 2006). This suggests that effects upon urinary cortisol would also be limited.

Anticipation and usage of enrichment was analysed first, as significant differences between enriched and control dogs would have suggested that the relationship between anticipation, usage and changes in behaviour and physiology would be worthy of exploration.

6.1.2.5.1 Anticipation of enrichment:

a) Did anticipatory behaviour of enriched dogs differ to that of control dogs?

The anticipatory behaviour performed during the minute preceding enrichment of enriched dogs was compared to that of control dogs using Mann Whitney U tests.

6.1.2.5.2 Behaviour:

a) How did enrichment and deprivation affect the behaviour of dogs in the presence of a person?

The four screening observation variables describing the change in behaviour upon enrichment, and eight describing the change in behaviour upon deprivation (6.1.2.4.1a), were analysed using a Chi squared test to determine whether there was a significant difference in the behavioural change of enriched compared to control dogs.

Testing enriched and control dogs separately, differences between phases in the tendency to look away from the observer (6.1.2.4.1a) were analysed using Wilcoxon signed rank tests, comparing enrichment to baseline and deprivation to baseline. Mann Whitney U tests were used to examine differences between enriched and control dogs in each of the three phases; baseline, enrichment and deprivation.

b) How did enrichment and deprivation affect the behaviour of dogs when alone at midday and in the evening?

Transformed variables; standing and gaze at exit (6.1.2.4.1b), were analysed using nested analysis of variance (ANOVA), with subject dog nested within treatment group, to explore the effect of experimental phase; baseline, enrichment and deprivation and treatment group; enriched and control upon behaviour. Where significant interaction effects were found, differences between phases were compared using paired t-tests and differences between treatment groups were compared using independent t-tests.

To determine whether there was a significant difference between enriched dogs and control dogs in behavioural change, the seven variables describing the change in behaviour upon enrichment (6.1.2.4.1b) and 14 variables describing the change in behaviour upon deprivation (6.1.2.4.1b) were analysed using Chi squared and Fisher's Exact tests

Evening behaviour recorded during the enriched phase, where the Kong was present, was not compared to evening behaviour at baseline or during subsequent deprivation because behaviour recorded was not comparable. During the enriched phase, dogs could perform behaviours which were not possible in the baseline and deprivation phases due to the lack of enrichment.

c) Were individual dogs consistent in their behaviour between observations?

The data was examined using Spearman Rank Correlations to see if individual dogs behaved consistently between the baseline, enrichment and deprivation observations, when observed in the presence of a person and when alone.

6.1.2.5.3 Levels of C/C:

a) Was there a significant difference in C/C levels between day two and day three of both baseline and enrichment phases?

C/C data from day two and three of both baseline and enrichment phases were transformed using log10, and means (paired t-test) and variances (Independent t-test) were compared to see if C/C levels differed significantly between the two days. If mean C/C levels did not differ and variances were equal, mean levels were calculated.

b) Did phase, site or treatment group affect C/C?

A nested analysis of variance (ANOVA), with subject dog nested within treatment group, was conducted to explore the effect of experimental phase: baseline, enrichment and deprivation; site: 1 to 4 and treatment group: enriched and control upon C/C levels. As the levels of C/C did not differ between days two and three (see section 6.1.3.3), mean levels of C/C were used.

6.1.2.5.4 Was there a significant difference in the health of enriched and control dogs following a period of enrichment?

A chi squared test was used to examine whether the change in health following enrichment differed significantly from that of control dogs.

6.1.3 Results

Relationships for which P was less than 5% are discussed and those at less than 10% are discussed as tendencies.

6.1.3.1 Anticipation of and time spent with enrichment

There were no significant differences between the anticipation of enriched and control dogs in any of the four variables measured during the one minute prior to the provision of enrichment (Table 6.1.8).

Table 6.1.8 Differences between enriched and control dogs in anticipation of enrichment measured during the one minute prior to the provision of enrichment. Test statistic value: Mann Whitney U.

Variable	Test statistic value	P value
Vocalise	20.5	0.62
Stereotype	20.0	0.58
Gaze at exit	15.5	0.27
Pant	23.5	0.94

Of the dogs which could be observed, the time spent interacting with the Kong ranged from 8.2 to 13.0 mins (mean = 10.9± 3.0) out of a possible maximum 60 mins and the time at which the last contact with the Kong was made, ranged from 13.6 to 17.5 mins (mean =15.2± 2.0) out of a possible maximum 60 mins.

As anticipation did not differ between enriched and control dogs, the relationships between anticipation, and changes in behaviour and cortisol were not explored. Relationships between usage, behaviour and cortisol could not be explored due to small sample sizes.

6.1.3.2 Behaviour

a) How did enrichment and deprivation affect the behaviour of dog in response to a person?

Of the four behavioural variables (Table 6.1.3) describing the change in behaviour upon enrichment, none differed significantly between enriched and control dogs. Of the eight behavioural variables describing the change in behaviour upon deprivation, one tended to differ; change in barking behaviour upon deprivation compared to enrichment (Fisher's exact test, $p=0.08$). Of the control dogs, none barked solely in the deprivation phase, 50% did not change their behaviour i.e. either barked in both phases or barked in neither and 50% barked in the enrichment phase but not the deprivation phase. Of the 19 enriched dogs, 5.3% barked upon deprivation but not in the enrichment phase, 73.7% did not change their behaviour and 21.1% barked in the enrichment phase but not the deprivation phase. Hence more control dogs changed their behaviour than enriched dogs.

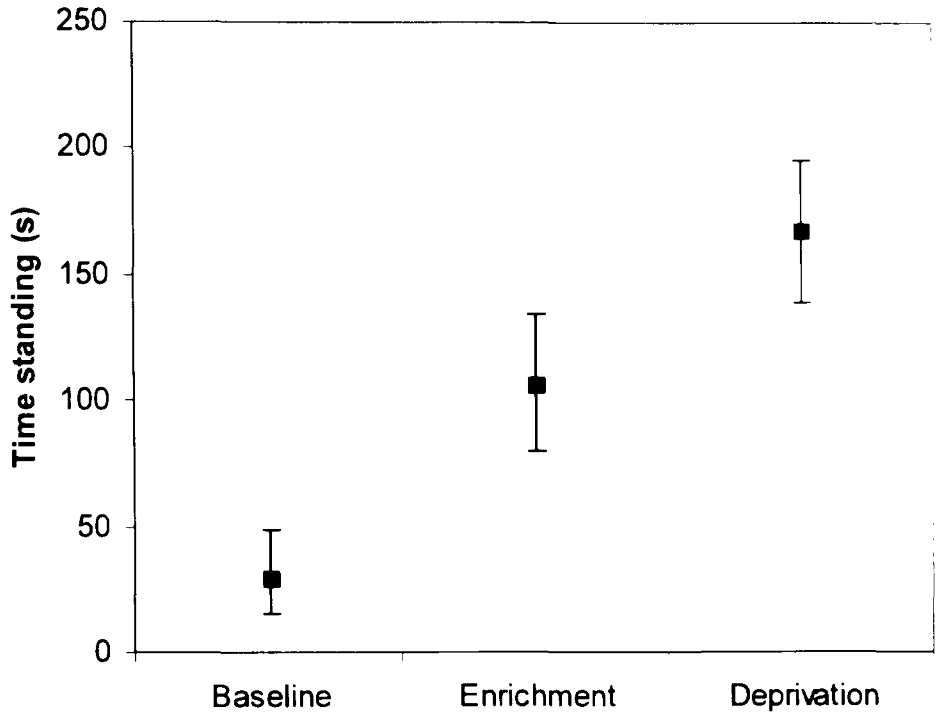
A further behavioural variable tended towards significance. During the enriched screening observation more control dogs tended to look away from the observer compared to the baseline phase, where dogs tended to make eye contact ($z = -1.9$, $p=0.05$, mean=1.1 vs. 0.5). In the deprivation phase, more control dogs tended to look away from the observer compared to the enrichment phase where dogs tended to make intermittent eye contact ($z = -1.9$, $p=0.05$, mean=1.5 vs. 1.1[3]). This relationship was not seen in enriched dogs.

b) How did enrichment and deprivation affect the behaviour of dogs when alone at midday?

The time spent standing during the remote midday observation was significantly affected by phase ($F_{(2,24)}=7.0$, $p=0.004$) (Figure 6.1.3). At deprivation, it was significantly higher than at baseline (166.3 vs. 29.7; $F_{(1,12)}=9.3$, $p=0.01$) and tended to be higher than during enrichment (166.3 vs. 107; $F_{(1,12)}=3.9$, $p=0.07$). During enrichment, it was significantly higher than during baseline (107 vs. 29.7; $F_{(1,12)}=5.2$, $p=0.05$).

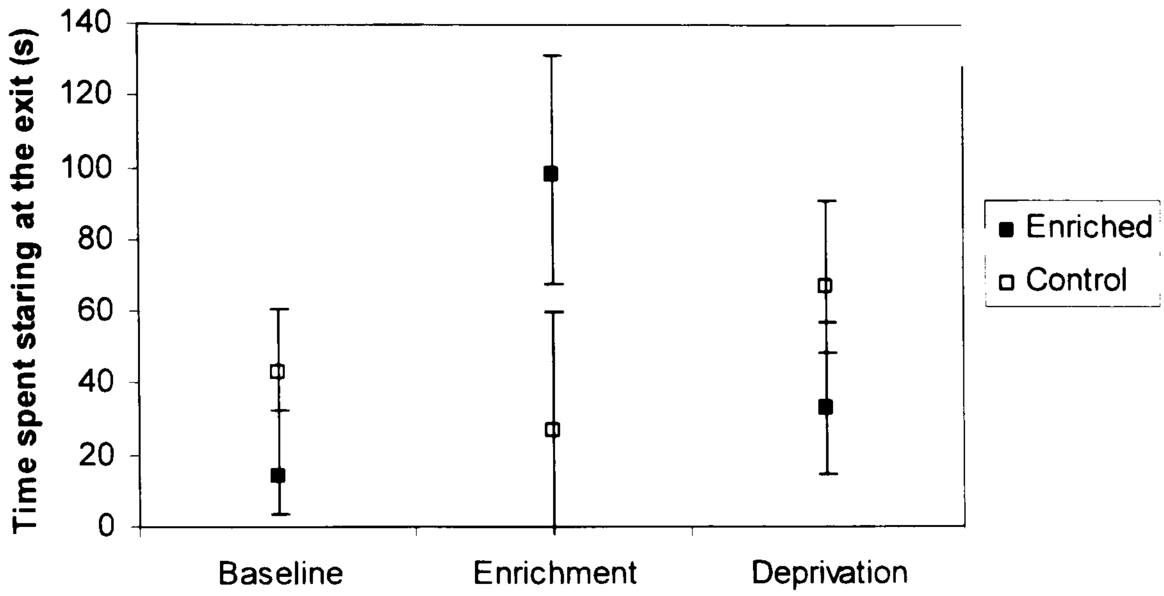
[3] Median, 25th and 75th percentile all equalled zero so means are provided

Figure 6.1.3 The effect of phase upon time standing in the remote midday observation (mean ±SE) ($F_{(2,24)}$=7.0, p=0.004).



There was a tendency for an interaction between treatment group and phase upon the time spent gazing at the exit during the remote midday observation ($F_{(2,24)}$=2.6, p=0.10) (Figure 6.1.4). In enriched dogs, it was greater during enrichment compared to baseline (98.5 vs. 14.3 t=-2.7, p=0.03) but there was no significant difference between enrichment and deprivation (t=0.96, p=0.37). In control dogs there was no significant difference in this variable between any two phases (t<0.57, p>0.60). There was no significant difference between the two groups in any of the three phases although during enrichment there was a tendency for enriched dogs to gaze longer than control dogs (98.5 vs. 27.1, t=-1.7, p=0.10).

Figure 6.1.4 The interaction between phase and treatment group upon the time gazing at the exit during the remote midday observation (mean ±SE) ($F_{(2,24)}$=5.2, p=0.04).

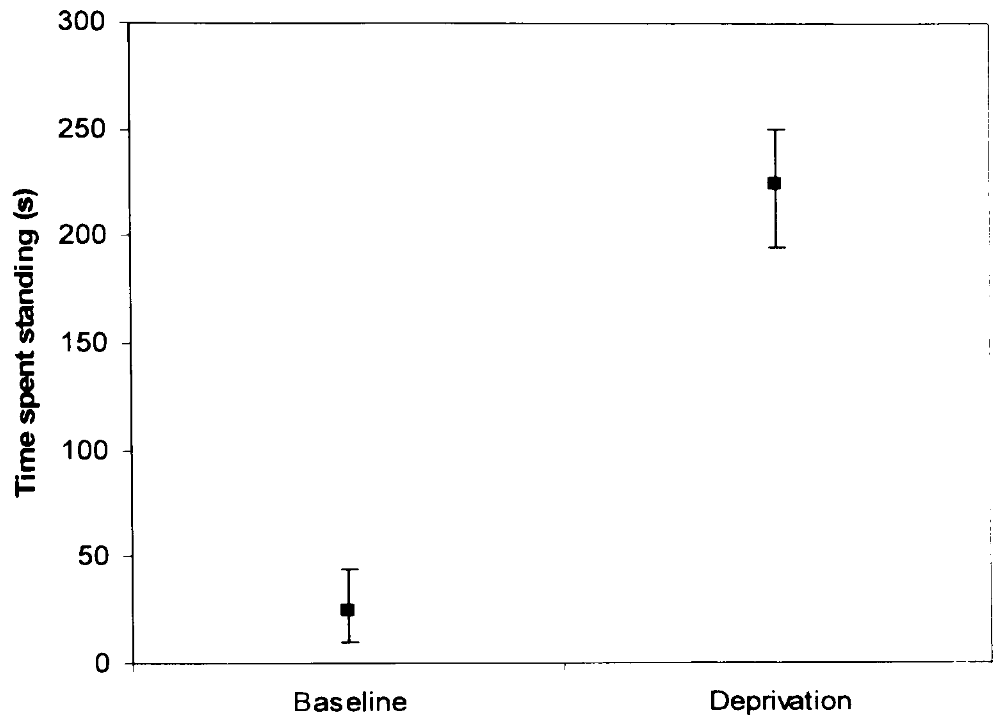


Upon subsequent deprivation of enrichment, there was a tendency for control dogs to increase the time spent grooming during the remote midday observation, (Fisher's Exact Test, p=0.085). Of the control dogs, half increased the time spent grooming and half did not change. Of the enriched dogs, 12.5% increased the time spent grooming, 62.5% did not change and 25% decreased.

c) How did enrichment and deprivation affect the behaviour of dog when alone in the evening?

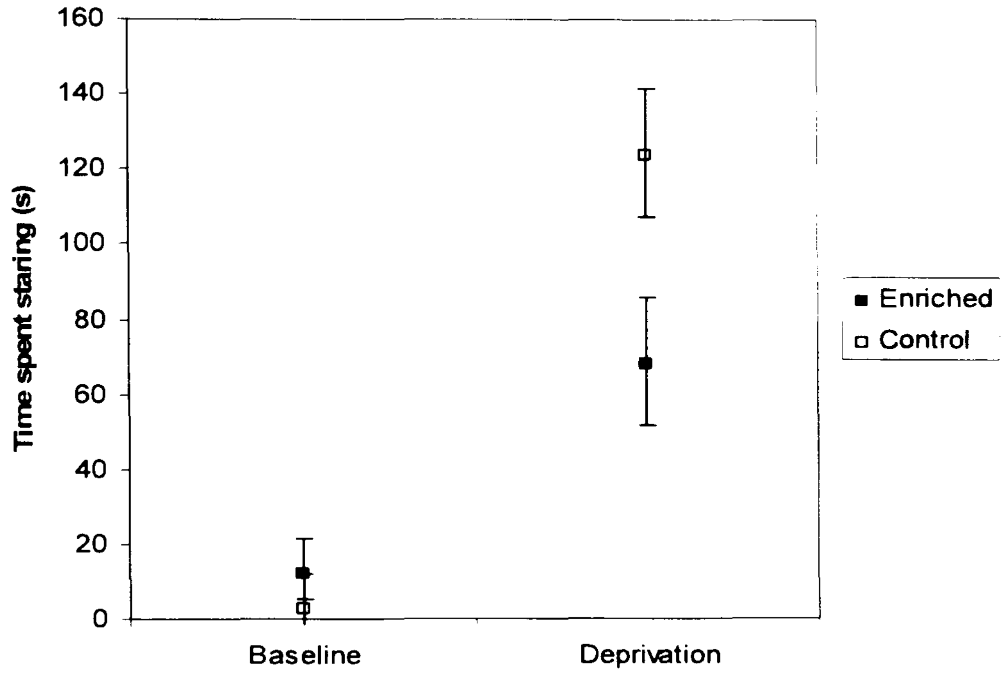
The time spent standing during the remote evening observation was significantly higher during deprivation than baseline ($F_{(1, 13)}$=20.6, p=0.01) (212.4 vs. 24.5s) (Figure 6.1.5).

Figure 6.1.5 The effect of phase upon time standing (mean ±SE) ($F_{(1,13)}$=20.6, p=0.01).



The time spent gazing at the exit during the remote evening observation was significantly higher during deprivation than at baseline ($F_{(1,13)}$=33.7, p<0.01) (87.4 vs. 7.6s). There was a tendency for an interaction between phase and treatment group ($F_{(1,13)}$=3.6, p=0.08) (Figure 6.1.6). Enriched dogs and control dogs both spent more time gazing at the exit during deprivation compared to baseline (enriched dogs: baseline vs. deprivation = 11.8 vs. 70s, t=-2.7, p=0.03; control dogs: baseline vs. deprivation = 3.0 vs. 118.6s, t=-0.71, p=0.01). There was no significant difference between the two groups in either phase (t<1.5, p>0.16).

Figure 6.1.6 The interaction between phase and treatment group upon time spent gazing at exit (mean ±SE) ($F_{(1,13)}$=3.6, p=0.08).



During the evening, when comparing behaviour upon subsequent deprivation with that during baseline, there was a tendency for enriched dogs to increase the time spent stereotyping (Fisher's Exact Test, p=0.10). When the nine dogs were deprived of enrichment, 44.4% increased the amount of time spent stereotyping whilst 55.6% did not change in the amount of time spent stereotyping. None of the control dogs changed in the amount of time spent stereotyping.

This increase in stereotypical behaviour appeared to be sustained over to the following day. When comparing stereotypical behaviour performed during the enrichment remote midday observation to that during deprivation, there was still a tendency for enriched dogs to stereotype (Fisher's Exact Test; p=0.085). Of the 8 enriched dogs (1 dog was not in its kennel during the remote midday observation of the enrichment phase), 50% increased the time spent stereotyping upon deprivation whilst 50% did not change. None of the control dogs changed the amount of time spent stereotyping.

d) Were individual dogs consistent in their exhibition of behaviours across both the screening and remote midday observations; baseline vs. enrichment and enrichment vs. deprivation?
Comparing baseline and enrichment remote midday observations, enriched dogs were consistent in their performance of two behaviours; standing (Rho=0.79, p=0.02) and barking (Rho= 1.0, p=0.01). In the same observation, control dogs were consistent in their performance of one behaviour; gazing at the exit (Rho=0.83, p=0.04).

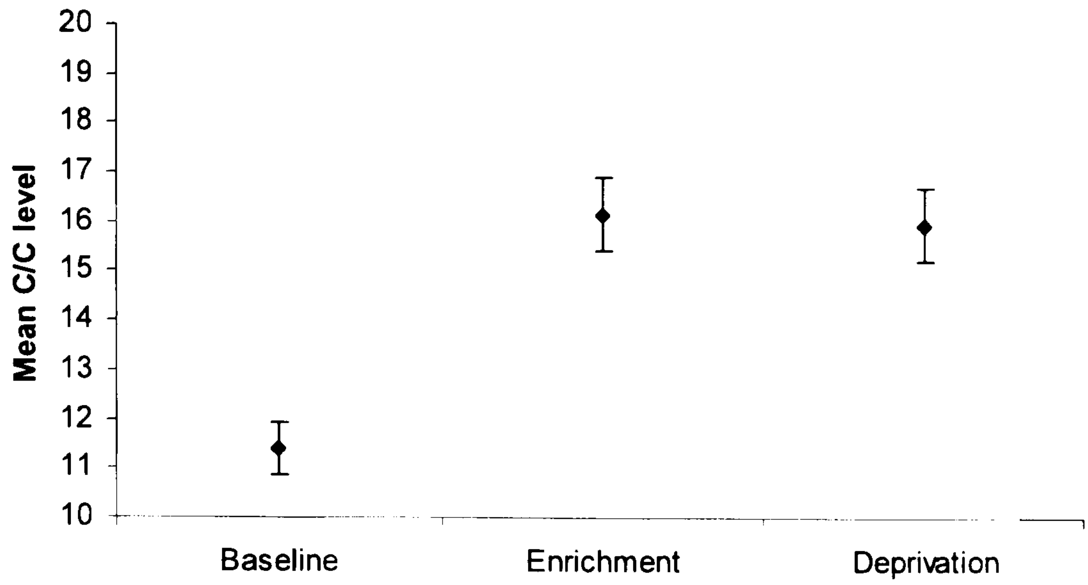
Comparing enrichment and deprivation remote midday observations, enriched dogs were consistent in their performance of three measured behaviours; panting (Rho=1.0, p=0.01), grooming (Rho=0.84, p=0.009) and area transitions around the kennel (Rho=0.8, p=0.03). They also tended to be consistent in their performance of stereotyping (Rho=0.63, p=0.09). Control dogs tended to be consistent in the performance of one behaviour; area transitions (Rho=0.76, p=0.08).

6.1.3.3 Levels of C/C

a) Was there a significant difference in levels of C/C between day two and day three of both baseline and enrichment phases?
As the means did not differ significantly (baseline: t=-1.8, p=0.09, enriched: t=-0.73, p=0.48) and equal variances could be assumed between days for both phases (baseline: F=0.49, p=0.49, enriched: F=0.20, p=0.66), mean values for day two and three of the baseline and enriched phases were generated for each individual dog for subsequent analysis.

b) Did phase, site or treatment group affect C/C?
A significant effect of the three phases was demonstrated ($F_{(2, 35)}$ =14.5, p<0.01). Mean C/C levels during both the enrichment and deprivation phase were significantly higher than during the baseline phase (Figure 6.1.7), (baseline vs. enrichment; 11.4 vs. 16.2, t=-4.5, p<0.001, baseline vs. deprivation; 11.4 vs. 16.0, t=-5.1, p=<0.001). There was a tendency for site to have an effect upon C/C ($F_{(3, 13.5)}$ =2.6, p=0.10). This relationship appeared to be dependent upon very high C/C levels of one individual dog at site number 1 and was no longer significant once this dog was removed from the analysis. Treatment group ($F_{(1, 13.8)}$=0.06, p=0.81) had no significant effect upon C/C levels.

Figure 6.1.7 Mean C/C levels (±SE) of all sample dogs across three phases; baseline, enrichment and deprivation.



6.1.3.4 Behaviour reported by kennel assistants

Kennel staff reported that all Kongs were always empty when they were removed from the kennel in the morning. Only one dog was reported to have shown a significant change in its behaviour which could possibly be attributed to the provision of enrichment. This dog, prior to the provision of enrichment would show overt aggression towards the kennel assistant every time its food bowl was placed in the kennel. However, following the provision of enrichment this behaviour stopped.

6.1.3.5 Health

No significant difference in visits to the vet, and thus health, was found between enriched dogs and control dogs (U=128.0, p=0.41).

6.1.4 Discussion

6.1.4.1 Anticipation of and time spent with enrichment

In this study, there was no measurable difference in anticipatory behaviour between the enriched and control group. Hiby (2005) suggested that quantifying anticipation might be a useful measure of how much an individual *values* enrichment. Thus, based on these findings, one may conclude that the control group valued the biscuit as much as the enriched group valued their feeding device. This raises the question as to whether the device itself is valued or some other factor associated with its delivery; perhaps the additional contact from the kennel assistant which delivery of a biscuit or device both provides. If this were the case then we might expect interaction with the devices to be limited and to have diminished over time. Yet, each of the enriched dogs in this study was observed and reported to interact with their enrichment every time it was delivered, even after four months of daily provision. Hence, the novelty of these devices seems to have been unimportant, which contrasts with other studies which found that toys are initially attractive due to their novelty value, which declines rapidly (Wells, 2004a) and that dogs become habituated to their presence (Wells, 2004b).

6.1.4.2 The effect of enrichment and its subsequent deprivation upon behaviour

6.1.4.2.1 The effect of enrichment upon behaviour

The behaviour of enriched dogs, when observed during the enrichment phase and in response to me, did not differ significantly from baseline. Thus feeding enrichment did not affect the behaviour of enriched dogs in response to me. However in control dogs, when observed during the enrichment phase, the tendency to look away from me was greater than during baseline. Making eye contact with a person can indicate confidence or an attempt to solicit social interactions whilst a tendency to look away can indicate fear. Behaviours indicative of fear were not measured in this study, so it is difficult to determine whether this is definitive. Alternatively, it is possible that as I was at no point a successful source of interaction they ignored my presence and no longer attempted to make visual contact with me. Whilst difficult to interpret, this relationship suggests that there are behavioural differences in response to people which may be influenced by enrichment.

When observed at midday during the enrichment phase, enriched dogs spent significantly longer gazing at the exit, compared to the baseline phase, and also tended to spend significantly longer gazing than control dogs. Results from Chapter 3 suggest that gazing at the exit is performed in response to anticipation of events yet to occur, such as exercise or feeding, as their performance is decreased at the end of the day when these events have been completed. Therefore, it is possible that the enriched dogs were anticipating the arrival of their enrichment devices, even though this observation was conducted four hours before the provision. The dog's cognitive ability to perceive time is not well understood, but it may be that dogs can anticipate events even when they are a substantial way ahead. If this was not possible, and the behaviour was just an effect of time of day, then we would also expect to observe a significant change in the time spent gazing by the control dogs during the midday observation. As the time spent gazing tended to be greater in enriched dogs compared to control dogs, I suggest that the enriched dogs could have been anticipating the arrival of enrichment well in advance of its provision.

When observed at midday during the enrichment phase, enriched and control dogs both spent more time standing than compared to baseline. An increase in the time spent standing may also indicate anticipation of the arrival of enrichment, yet this behaviour was also observed in the control group, suggesting that this was not an effect of enrichment but a diurnal change. During this study, due to the kennel layout, both groups were in visual contact with one another. It is therefore possible that the anticipatory behaviour of enriched dogs led to a facilitative effect and thus a similar increase in standing in the control dogs, likely due to the responsive and reactive nature of the breeds used. It could also be argued that the control dogs might have been anticipating the arrival of the kennel staff that provided them with a biscuit at the same time as the enriched dogs were given their feeding devices. However, if this were the case, then a significant increase in the time spent gazing at the exit in the control dogs would be predicted, and this was not observed.

Although some changes in behaviour were observed, overall there were no discernable changes in any behaviour indicative of improved welfare; e.g. grooming, playing or resting. Studies of rescue dogs have similarly observed little effect upon behaviour when various toys have been introduced (Wells and Hepper, 1992; 2000, Wells, 2004b). Wells (2004b) suggests that the lack of effect is because the rescue shelter itself is already so arousing and thus the presence of a toy has little significance to a dog, and rather than making the environment more enriched it renders the environment somewhat less impoverished. Likewise in the military working dog environment, dogs will be susceptible to a broad range of stimulating events but may experience additional stressors compared to many rescue dogs, as every military working dog in this study was socially isolated and had limited opportunities to interact with conspecifics.

However, unlike toys, feeding enrichment was expected to have some measurable effect upon the welfare of military working dogs, as a trend towards a decrease in stereotyping was observed in GSDs during a five day period of enrichment using the same device (Hiby, 2005). It was further hypothesized by Hiby that should a longer period of provision be employed then greater effects might result. However, results from this study suggest that this is not the case. Instead I propose a number of reasons which may explain why feeding enrichment has had little influence upon the behaviour and physiology of military working dogs.

Firstly, findings presented in Chapter 4 suggest that within individual populations there are particular factors which are critical to welfare e.g. inter-specific contact or space allowance, so unless those which are the most critical are also addressed it is unlikely that the provision of enrichment will enhance welfare to a discernible extent, and if there were any welfare benefits of the feeding devices, these may have been masked by such factors.

Secondly, it is important to remember that the dogs used in this study were operational and thus many factors which might influence their welfare were beyond my control. For example, it was not uncommon for a subject to undergo a change in handler or have no handler for part of the study, as a number of handlers were deployed to undertake four month operational tours. Circumstances such as these can cause additional stress to the animal as they may have formed a close attachment to their handler and thus become distressed in their absence or may suffer from lack of stimulation which is normally provided during training or normal work routines. This may lead to increases in errors, masking any effects of enrichment.

6.1.4.2.2 *The effect of subsequent deprivation upon behaviour*

Immediately following the deprivation of enrichment, when filmed alone in the evening, enriched dogs tended to spend more time stereotyping than control dogs. Similarly, when observed at lunchtime, the following day, enriched dogs tended to spend more time stereotyping compared to that spent during the enrichment phase, and also compared with the levels among control dogs. The levels of stereotyping in individual dogs were consistent between the remote evening and midday observations, with no significant decrease in the levels even 20 hours later, suggesting that some dogs may have found the deprivation stressful or frustrating, as stereotypies are often performed in response to stressful situations or when frustrated (Mason, 1991). Mice reared in enriched housing and then transferred to standard housing, where enrichment was no longer provided, also exhibited high levels of stereotyping (Latham and Mason, 2006) suggesting that animals may stereotype when enrichment to which they have become accustomed is removed.

The absence of a significant change in stereotyping of the control dogs suggests that the increase in stereotyping in some enriched dogs could be an effect of deprivation. I therefore suggest that the deprivation of enrichment may be a frustrating event for dogs and that the enrichment may have been highly valued to have such an effect upon behaviour. This is the second study to suggest that deprivation of enrichment may lead to an increase in frustration, exhibited as an increase in stereotypical behaviours (Latham and Mason, 2006). It could therefore be valuable to use deprivation in other enrichment studies to investigate the value of such resources.

During the deprived evening observation, compared with baseline, all dogs spent significantly more time gazing at the exit and standing, but there was no difference between the two groups in either behaviour. I would suggest that there are two reasons for both groups showing an increase in these behaviours. Firstly, observations taken at midday show that enriched dogs are displaying anticipatory behaviours ahead of their enrichment provision. These behaviours are likely to influence the control dogs because the two groups were not segregated, thus the increase in standing and gazing at the exit by the control dogs may be explained by contagion. Secondly the increase in time spent gazing by the control dogs may be an experimental effect. Unfortunately, due to the small number of video cameras available, the duration of gazing could be measured only from the enriched dogs on the actual day of enrichment deprivation. For control dogs the corresponding duration was measured the day after, and on this day the enriched group had been re-enriched and given their feeding devices. It is therefore possible that control dogs spent time observing enriched dogs with their devices which would have been measured as time spent gazing at the exit.

During the midday observation, comparing grooming behaviour during deprivation to that observed during enrichment, half of the control dogs spent longer grooming whilst only an eighth of the enriched dogs were observed to spend longer. Upon deprivation, none of the control dogs decreased the time spent grooming, however, a quarter of the enriched dogs did. Grooming is a behaviour generally not observed when an animal is subjected to high levels of stress (Friend, 1990) suggesting that the enriched dogs may have been experiencing some level of stress in the absence of enrichment.

In response to the observer, the treatment groups differed in the change in barking behaviour upon deprivation; control dogs either barked in the enrichment phase only or did not change their behaviour, whereas significantly more enriched dogs did not change their behaviour. An increase in barking behaviour in response to the observer upon deprivation may have been predicted, as barking can indicate frustration (Bradshaw and Nott, 1995) and changes in other behaviours i.e. stereotyping and grooming, suggests that dogs were frustrated in the absence of their enrichment. However, it is likely that the dogs did not associate the enrichment with myself, as it was the kennel assistant who provided dogs with their device over the four months. Thus, I would not predict that my presence would elicit behaviours indicative of frustration. It is instead possible that this was an effect of habituation, with both groups becoming familiar with me and thus non-responsive to my presence. This is further supported by observations of control dogs, fewer of which made eye contact with me during the deprivation phase compared to the enrichment phase. The same change was evident when comparing enrichment with baseline, suggesting that dogs do gradually habituate to novel people over time.

6.1.4.3 The effect of enrichment and its subsequent deprivation upon levels of C/C

Mean C/C levels were significantly higher during both the enriched phase and the deprivation phase, compared with levels measured during the baseline phase. Based upon findings from Chapter 2, one could conclude from the significantly elevated levels of cortisol that enrichment has resulted in compromised welfare. However there was no significant increase upon deprivation as compared to enrichment as shown in Hiby's study (2005). Furthermore, there was no difference between the mean C/C levels of enriched or control groups in any of the three phases, which would be expected if enrichment was compromising welfare. In the light of the above, I suggest that the changes may have been caused by a temporal or seasonal effect upon C/C levels of the population as a whole. Within this current study the majority of baseline data was collected during the summer months, whilst the enriched and deprived data was collected during late winter and early spring. It is acknowledged in the literature that seasonality has many diverse effects upon cortisol (Lane, 2006). However, increases in levels of cortisol have been associated with low ambient temperatures (e.g. mice; Strack unpublished data as cited in Dallman, 2001). Harsh environmental factors will increase metabolic stressors and require catabolic production of energy resources; a process requiring increased production of glucocorticoids (Lane, 2006). None of the dogs used in this study were housed in heated kennels; thus it is probable that during the late winter and spring months low ambient temperatures may have affected C/C levels. This is further supported by Hiby (2005) who found that in two separate studies of kennelled working dogs, C/C increased significantly as temperature decreased. Thus, any effect of enrichment upon physiology may have been masked by a much greater seasonal effect, unfortunately beyond the control of this study.

6.1.4.4 The effect of enrichment upon health

The relationship between health and the provision of environmental enrichment appears complex. Previous studies of two working dog populations (Chapter 4) provided contrasting results. In one population, more dogs provided with toys in their kennel tended to have been referred to the veterinarian for stress related symptoms since arriving at their current site (4.4.2.2). In the other population no relationship between health and toys was seen. In this current study, the provision of enrichment had neither a detrimental or beneficial effect as the health status, measured by the change in veterinarian visits before and after enrichment, did not differ between enriched and control dogs. This suggests that providing enrichment is not detrimental to health, but that when provision of toys is optional, kennel assistants may be providing enrichment to those dogs which they think are stressed, and that due to the same stress, these are the dogs most likely to be referred to the veterinary surgeon.

6.1.5 Conclusion

Contrary to Hiby's suggestion, that an extended period of enrichment would result in greater effects upon welfare (6.1.1), overall, enrichment had little influence upon the behaviour of military working dogs, with no change in any behaviour indicative of improved or good welfare. Several reasons are proposed for this;

1. Toys have little influence upon the welfare of dogs housed in rescue shelters, and Wells (2004b), suggests toys, are unlikely to substantially enrich a kennelled environment but rather result in one which is less impoverished. Thus, any improvement in welfare may be undetectable in an otherwise unstimulating environment. I suggest that this is also applicable to feeding devices.
2. The influence of other factors in the kennelled environment critical to welfare are likely to limit the effects of enrichment. Only when these factors are improved might feeding enrichment lead to a discernable increase in welfare.
3. The influence of operational factors may have caused additional stress which could not be considered within this study.
4. Large individual variations in behaviour will be likely due to differences in socialisation, rearing environment, susceptibility to stress etc. which will contribute both to errors in between-groups comparisons in behavioural and physiological responses to stress, and also may lead to variations between individual dogs in the value of feeding enrichment.
5. The indicators of welfare used within this study may have failed to identify subtle changes in improvement.

The removal of enrichment did however affect behaviour; the increase in stereotypic behaviours immediately following deprivation and their sustained performance over the day suggests removal of the device can be detrimental to welfare, since some individuals become frustrated as a result. Similarly, the decrease in time spent grooming upon deprivation by some of the enriched dogs also suggests that those individuals did experience some degree of stress in the absence of their enrichment device. This is further evidence that the provision of the feeding device was valued by the dogs. Removal of resources such as feeding devices and the effect upon subsequent behaviour may be a further useful tool to assess their relative value.

The introduction of enrichment similarly had no measurable effect upon physiology, and neither did deprivation, as there was no difference between the C/C levels of enriched and control dogs in any of the three phases. There was however a large significant increase in C/C between the baseline phase and both the enrichment and deprivation phase implying temporal or seasonal effects upon C/C, which may have masked any effect of the enrichment. Likewise health was not affected by the provision of enrichment and importantly, contradicting some trainers' and handlers' beliefs, no aggressive incidents occurred during the trial, although this was helped by constant monitoring for "possessiveness" by husbandry staff.

In summary, enriched dogs interacted with the feeding device daily, demonstrating that the devices were a resource which retained its value even after four months of use. However, the provision of enrichment did not influence indicators of welfare in a quantifiable way, although its subsequent discontinuation did. Thus, feeding enrichment may be valuable to military working dogs, but handlers and kennel managers should not solely rely upon such devices to improve welfare, since other changes to housing and husbandry may be more critical. Most importantly, should feeding enrichment devices be given, provision should be both predictable and consistent, as discontinuation causes stress.

Although both myself and Hiby (2005) have demonstrated that feeding devices are valued resources, handlers and trainers of military working dogs are often concerned that the provision of this type of enrichment may detrimentally affect working ability (Rooney and Bradshaw, 2004), health (e.g. due to choking) and increase dog-human aggression within the kennel. Therefore in the following chapter (6.2) I describe how I investigated the effects upon perceived working ability, health and aggression, using a questionnaire completed by handlers.

Chapter 6.2

The effect of feeding enrichment upon reported working ability, behaviour and health

The contents of this chapter have been accepted for publication in the Journal of Forensic Sciences.

6.2.1 Introduction

In 6.1.1, I described previous research which demonstrated the beneficial effects of feeding enrichment upon the welfare of individual military working dogs (Hiby, 2005) and also presented results from my own study using a similar population of dogs (Chapter 6.1). In my study, although feeding enrichment devices did not influence the overall welfare of dogs in a quantifiable way (6.1.3.2), all dogs clearly valued the feeding enrichment, interacting with the devices daily even after four months of provision (6.1.3.1). Additionally, discontinuation of the enrichment device appeared detrimental to the welfare of some dogs, tending to increase the time spent stereotyping and decrease the time spent grooming (6.1.3.2). Thus, based on these results, feeding enrichment may be a valuable addition to the kennelled environment for working dogs.

However, even though dogs have been shown to value enrichment and the welfare of some individuals may be enhanced, there is often reluctance to provide enrichment of any kind within the kennels of military working dogs. Unreported data, collected for the purposes of Chapter 4, revealed that out of 92 military working dogs housed at 16 different stations, only 33% were given continual access to some form of enrichment (toys and/or bones) whilst 61% were never given enrichment devices; the remaining 6% were provided with enrichment some of the time. In the UK military, most working dogs are trained, both initially and throughout their working life, using a reward of play with their handler. This play usually centres around a toy (usually a ball); hence a high level of playfulness is desirable in potential military working dogs (Rooney et al, 2004). Consequently, many trainers and handlers believe that access to any form of enrichment inside the kennel could decrease a dog's motivation to play with a toy during training, and would ultimately decrease their working ability. Furthermore, it is widely believed that the uncontrolled provision of enrichment may lead to increased guarding behaviour, manifest as aggression towards kennel assistants and handlers within the kennelled environment, or may lead to health problems, since dogs may chew and choke on pieces of the enrichment device. In this chapter, I describe a study which aimed to investigate whether these concerns are substantiated.

Empirical examination of working ability was not possible within this study due to time and operational constraints. However, a previous study conducted by myself and others (Rooney et al, 2007a) has shown that subjective ratings made by regular handlers or trainers are highly correlated with objective measures of military working dog ability. Since this is a time efficient yet viable alternative, in this study handlers were asked to rate their working dogs on criteria relating to working ability, behaviour and health using a questionnaire. Subsequently, as described in Chapter 6.1, some of these dogs were provided with a feeding enrichment device, a Kong™, filled with gravy soaked biscuits, every week-day evening over a period of four months. The remaining dogs were provided with a biscuit only, to provide equivalent contact with husbandry staff. At the end of this period, handlers were asked to rate their dogs again using the same criteria. These ratings were then compared with those made prior to enrichment provision, to gauge the effect of prolonged enrichment on reported working ability.

6.2.2 Methods

6.2.2.1 Experimental design

At the beginning of the study (pre-enrichment), questionnaires (6.2.2.5) were distributed to the handlers of the 43 dogs, housed at sites 1, 2 and 3 as described in Table 6.1.1 (Chapter 6.1). Each handler was asked to rate their working dog on 11 criteria (Table 6.2.1) relating to working ability, health and behaviour. The questionnaire was returned to the experimenter within 39 days and dogs were then split into two groups: enriched (n=22) and control (n=21), balanced for sex, age and working role. Enrichment devices (6.2.2.6) were provided daily to each of the enriched dogs for a period ranging from 115 to 125 days (mean = 120.6 ± 4.5). At the end of this period (post-enrichment), handlers rated the same working ability and behavioural characteristics as they had done previously. Unexpected subject loss, and changes in handlers throughout the study, resulted in a reduced sample of eight enriched and 14 control dogs.

6.2.2.2 Subjects

Dogs used in this study comprised 20 German Shepherd Dogs (GSDs), one Belgian Malinois and one GSD Belgian Malinois cross breed. Fifteen were male and seven female. Of the 22 dogs, nine were neutered, six were entire and the neuter status of seven was unknown. The dogs were aged between 26 and 104 months (mean =56.7 ±26.6). Two of the dogs were used as station mascots and for public relations activities, the remainder were PATrol Arm True (PATAT) (1.1).

6.2.2.3 Housing and husbandry

The dogs were housed at three different sites (1 to 3, Table 6.1.2), one located in England (n=5), one in Northern Ireland (n=8) and one in Scotland (n=9). The total living area available to the dogs ranged from 18.3 to 19.7 m^2 (mean=18.9 ± 0.6). Each dog had a wooden kennel (1.1m^2) in the centre of the living area. None of the kennels was heated so dogs were provided with a piece of veterinary bedding (Profleece®, Derbyshire, UK).

During the study, PATAT dogs received between 0 and 30 minutes of exercise per day from their handlers, whilst dogs used as station mascots or for public relation activities were exercised by kennel assistants for between 10 and 25 minutes per day. Kennels were cleaned daily in the morning and all dogs were fed once daily in the morning.

6.2.2.4 Questionnaire respondents

The data was provided by 22 working police dogs handlers and trainers, 19 males and three females aged between 20 and 42 years (mean=25.5 ± 5.4). Dog handling experience ranged from 0.5 to 24 years (mean=4.9 ± 5.7).

6.2.2.5 Questionnaire

Questionnaires (presented as Appendix 6) were distributed to handlers via senior personnel within the dog section. The questionnaires consisted of three pages. The first page described the overall purpose of the study. The second page contained ten questions that collected information about the handler and their dog, including breed, sex, age and years in service. On the third page, 11 attributes pertaining to the dog's working ability and behaviour were listed. Nine were adapted from characteristics which Rooney and Bradshaw (2004) and Rooney et al (2004) derived from interviews with 37 dog experts and used to compare breed and sexes. Two extra attributes were added to describe the dog's contentment in the kennel environment (Table 6.2.1). For each attribute the handler rated the dog as either; 1=very low; 2=low; 3=intermediate; 4=high; 5=very high. When handlers first completed the questionnaire at the beginning of the study (pre-enrichment) they were told that its purpose was to determine ratings of dogs' behaviour and working ability, but not that the questionnaire formed part of a subsequent study to look at the effects of enrichment. The identical questionnaire completed at the end of the study (post-enrichment) stated that the purpose was to see if the ratings had changed over time. At this point handlers could have been aware that some dogs within their section, and possibly their own, (although they had not been directly informed of this), had been provided with enrichment.

6.2.2.6 Feeding enrichment

Each enriched dog was provided with a feeding device as described in section 6.1.2.3.2. Control dogs received no enrichment but in order to balance the amount of contact with husbandry staff and number of feeding occasions, they were given a biscuit at the same time of day as the enriched group received their enrichment device.

6.2.2.7 Data analysis

For each of the 11 working ability attributes (Table 6.2.1), the change in working ability over the study period was calculated (score for attribute rated post-enrichment minus rating made pre-enrichment), for each individual dog. One-sample t-tests comparing the mean change in each attribute (treatment groups combined) against zero, were used to investigate whether any attributes had changed significantly over the study period. A one-way between groups multivariate analysis of variance (MANOVA) was then performed to compare overall differences in working ability attributes between the two treatment groups. Finally independent t-tests were used to examine the extent to which the mean change in each individual attribute differed between enriched and control treatment groups.

6.2.3. Results

On average scores for the eleven attributes changed very little over the study period (Table 6.2.1). Only one of the attributes changed significantly; *Ability to learn from being rewarded* rose slightly (mean score; pre-enrichment=4.0, post-enrichment=4.32, $t=2.3$, $p=0.03$) (Table 6.2.1). Changes for the enriched and control groups were virtually identical ($F_{(11, 10)}=0.22$, $p=0.99$), and the mean change in rating did not differ significantly between treatment groups for any of the 11 attributes individually ($t<0.66$, $p>0.33$, Table 6.2.1). In particular, the global measure *Overall working ability* was essentially unchanged across the study (mean score pre-enrichment=4.0, post-enrichment=4.14, p=0.42) and virtually identical between treatment groups (mean change in attribute; enriched=0.13, control=0.14, p=0.96) (Table 6.2.1).

Table 6.2.1 Mean change in attributes rated by dog handlers before and after enrichment period (score for attribute post-enrichment period minus pre-enrichment): (i) Testing changes within the entire population; one sample t-test value (t) and levels of significance presented; (ii) Comparing the change in attribute between the two groups, enriched and control; independent t-test value (t) and levels of significance presented.

Attribute	**(i) Mean change in attribute for entire population**			**(ii) Mean change in attribute comparing enriched and control groups**			
	Absolute change	**Test statistic value – (t)**	**P value**	**Enriched**	**Control**	**Test statistic value – (t)**	**P value**
Overall work ability	0.14	-0.83	0.42	0.13	0.14	0.05	0.96
Health	0.18	1.3	0.21	0.13	0.21	0.30	0.77
Ability to learn from being rewarded	0.32	2.3	0.03	0.50	0.21	-1.0	0.33
Consistency of behaviour from day to day	0	0	1.0	-0.13	0.07	0.45	0.66
Motivation to chase an object	-0.09	-0.44	0.67	-0.13	-0.07	0.12	0.91
Playfulness	0.14	0.9	0.4	0.13	0.14	0.06	0.96
Interest in toys or objects	0.18	0.9	0.38	0	0.29	0.66	0.51
Motivation to retain possession of a toy	-0.14	-0.62	0.54	0	-0.21	-0.46	0.65
Level of aggression towards humans	0	0	1.0	0.25	-0.14	-0.67	0.51
Willingness to enter kennel at end of shift	-0.14	-0.8	0.45	0	-0.14	-0.57	0.58
Contentment in the kennel environment	0.09	0.49	0.63	0.13	0.07	-0.14	0.89

6.2.4. Discussion

This study indicates that the provision of feeding enrichment had no discernible effect upon the reported working ability or general behaviour of a population of trained military working dogs. The popular claim that the provision of enrichment with manipulable objects results in a decrease in overall working ability has not been substantiated, as the mean change in rating of *Overall working ability* following feeding enrichment was essentially identical between enriched and control dogs.

The change in *Consistency of behaviour from day to day* following enrichment similarly did not differ between enriched and control dogs. Likewise the changes in ratings for all of the attributes describing the dog's toy-oriented behaviour: *Motivation to chase an object, Playfulness, Interest in toys and objects* and *Motivation to retain possession of a toy* were virtually identical between enriched and control dogs. This indicates that the provision of enrichment within a kennelled environment does not affect a working dog's motivation to play with, or retain, a toy used subsequently as a reward during training, as is often supposed. I suggest that it is unlikely that military working dogs would associate enrichment in their kennel with a toy provided in a training scenario. In the kennel, a feeding device serves as a way of occupying the dog's time, a source of stimulation and an outlet for natural rewarding behaviours; feeding and chewing. In contrast, during training, a toy serves as a reward, a focus for play, and a means of obtaining attention and interaction with the handler. Hence, these differing motivations mean that in kennel enrichment, provision has little or no effect upon a dog's motivation to play or work for a reward. In addition, I propose that, when provided in the way described in this study, dogs do not utilise Kongs as toys but instead as feeding devices. As described in 6.1.3.1, once emptied, dogs did not return to, or play with, the devices within the observed time. This proposition is supported by findings from a similar study which showed that the percentage of time spent interacting with food filled Kongs was considerably higher immediately following their provision compared to three hours later (Hiby, 2005). Furthermore, previous research suggests that habituation to toys may occur quickly with interest waning considerably over a few days (Wells, 2004b). In contrast, in the current study, dogs continued to utilise the food-filled devices even after four months (6.1.3.1), further suggesting that they are not used primarily as play items, but as a source of food-enrichment.

Handler's reports of aggression levels towards humans post-enrichment did not differ from pre-enrichment nor between enriched and control dogs, and thus the assertion that the provision of enrichment within a kennel can lead to increased aggression towards handlers and kennel assistants appears unsubstantiated. I suggest two reasons for this. Firstly it is important to note that the guidelines provided to the kennel assistants for the use of the devices (Appendix 5), stated how to avoid situations where aggression could potentially occur. Therefore kennel assistants never tried to remove the Kong when the dog was present, but only in its absence. Secondly, the Kongs were only removed from the kennel once they saw all of the food had been extracted, and so at this point the dog's value of the device was likely to be very low. Hence the potential for the dog to monopolise or display possessive aggression towards the device was minimal. I suggest that if feeding enrichment is provided, compliance to similar guidelines is critical to ensure its safe use.

Similarly, concerns regarding dangers to health were seen to be unfounded, as the mean change in *Health* rating did not differ between the two treatment groups. This is consistent with findings of Chapter 6.1; the provision of enrichment had neither a detrimental nor a beneficial effect as the health status, measured by the change in veterinarian visits before and after enrichment did not differ between enriched and control dogs (6.1.3.5). Furthermore, no detrimental incidents were reported (by handlers or kennel assistants) to occur during the trial e.g. choking on small pieces of the Kong. I suggest that this is a result of the close monitoring conducted by kennel assistants who checked each device daily to ensure it was not fragmenting.

The provision of enrichment did not increase scores of the dog's level of *Contentment within the kennel* nor its *Willingness to enter the kennel at the end of the shift*. Similarly, as demonstrated in Chapter 6.1, overall, enrichment had little influence upon the behaviour of military working dogs, with no change in behaviour indicative of improved or good welfare (6.1.3.2). Whilst this may be taken as evidence that the feeding device had no marked effect on welfare, in Chapter 6.1, the deprivation of enrichment did affect indicators of welfare in some dogs with an increase in stereotypic behaviour immediately following deprivation, sustained for at least a day, as well as a decrease in grooming. Both changes may be indicative of deteriorated welfare (stereotypies; 1.4.2.5, grooming; Friend, 1990, Hiby, 2005 (1.4.2.8a)) and hence I would argue that some individuals may benefit from feeding enrichment. However, effects may not be detectable at a population level unless large samples are used. Furthermore, there are many factors within a kennelled environment which can greatly decrease welfare (1.5.2) e.g. social isolation, high noise levels, low temperatures, unpredictability of events, lack of control of the environment, and the impact of these may well mask any effects of enrichment (6.1.5).

It is possible that had the handlers rated their dogs using an additional questionnaire during the deprivation period, a significant change in scores might have been observed when compared to scores post-enrichment. Unfortunately, this was not possible as it would have required completion of questionnaires on the day following deprivation and the 22 handlers were not available at this time and not all would have worked their dogs on this day. As enrichment was subsequently provided, completion of questionnaires on any other day would not have accurately reflected the dog's behaviour during deprivation.

Ability to learn from being rewarded was the only attribute which changed significantly over time. Whilst not significant, the increase was slightly higher in enriched dogs than in control dogs, which is the opposite of what would be expected if enrichment had interfered with training, or if the handlers of the enriched dogs had discovered that their dogs had been enriched, and down-rated their dogs according to the perception that enrichment is detrimental to training. This suggests that learning ability increased over the duration of the study, potentially due to training, and additionally, that possible beneficial effects of feeding enrichment on learning ability may warrant further investigation on a larger sample of dogs. The enriched dogs tended to score higher, and since ability to learn is a desirable trait in working dogs, this may suggest a beneficial effect of enrichment on working ability. Links between enrichment and enhanced learning ability have previously been found in both rodents (Barbelivien et al, 2006) and dogs (Milgram et al, 2005). Discrimination and reversal learning has been shown to significantly improve in environmentally enriched laboratory beagles (Milgram et al, 2005) and I would therefore suggest that this is an area worthy of further research in military working dogs.

6.2.5 Conclusion

In summary, in light of these findings, it seems that if correctly managed, enrichment, and specifically feeding enrichment, can be introduced to kennelled working dogs without any reported detrimental effects upon working ability, health or general behaviour, and with consideration of the findings reported in Chapter 6.1, there will be potential benefits to the welfare of at least some of the animals concerned.

Chapter 7

General discussion

7.1 Chapter aim and outline

In this thesis, I aimed to understand how factors of housing and husbandry relate to the welfare of kennelled military working dogs, identifying those which were the most influential and subsequently measuring effects of altering some of these upon welfare and working ability. In this final chapter, I first discuss the indicators of welfare utilised within this study (7.2). I then address my two hypotheses; '*factors of housing and husbandry influence welfare, some factors are more influential than others*' (7.3.1) and *changes to the most influential factors improve the welfare and working ability of military working dogs* (7.3.2). In section 7.3.3, I discuss the practical application of key findings. In section 7.4, I present a summary of the key findings and finally discuss areas which, in light of my findings, appear worthy of further research (7.5).

7.2 Measurement of welfare in kennelled dogs

In this section, I discuss some of the indicators of welfare utilised throughout this thesis; urinary C/C (7.2.1), kennelled behaviour (7.2.2), the relationship between behaviour and urinary C/C (7.2.3), and health (7.2.4).

7.2.1 Is urinary C/C a useful indicator of chronic stress and welfare?

It was proposed that resting or basal levels of C/C may be unreliable as indicators of chronic stress and welfare and that instead, C/C in response to a challenge should be measured (2.1). Therefore, I undertook a study whereby a challenge test for dogs was evaluated, a standardised veterinary examination (2.2.3.2). The C/C response to that challenge was then measured in three different populations of dogs (2.2.1), all differing in regular housing conditions (2.2.2). Whilst some of the individuals responded physiologically to the challenge (2.3.1), overall, the challenge did not activate the HPA system of all dogs (2.3.2). As a result, the challenge could not be used in the subsequent studies. Furthermore, the C/C response to a challenge could not be determined in chronically stressed dogs, in which the HPA axis might, based on studies of other species (e.g. pigs, Jarvis et al. 2006), respond abnormally.

Basal levels of C/C appeared to differ between the three populations (2.3.2.1) and findings were in agreement with Beerda et al (2000) that dogs housed in conditions of the highest austerity had the highest levels of C/C, thereby suggesting that elevated levels of C/C are indicative of chronic stress. In this thesis, dogs housed in kennels, compared to dogs in domestic environments had the highest levels of C/C and so this provides evidence to suggest that the welfare of kennelled dogs is poor.

Using this interpretation of basal C/C, it was found that kennelled dogs locked in the sleeping compartment during the cleaning routine were more likely to be stressed; levels of C/C were higher on average (4.4.2.1). Being locked in a confined space is potentially an acute stressor and if such practice occurs with regularity and over a prolonged period of time, it could be expected to lead to chronic stress.

Levels of C/C tended to be higher in dogs housed at sites which experienced lower temperatures at the time of study, indicating that they were physiologically stressed (4.4.2.1). This result is in agreement with previous studies of mice (1.5.2.5) and similar populations of military working dogs (Hiby, 2005) which suggest the interpretation of C/C is robust. However, it is not clear whether or not such stress has a psychogenic component.

However, one apparently counter-intuitive relationship between welfare and C/C was found. In Chapter 4, when relating housing and husbandry to welfare, dogs housed at those sites with the smallest kennels were likely to have the lowest C/C, suggesting that being housed in a small kennel was less stressful than being housed in a larger one (4.4.2.2). This apparently contradicts the elevated C/C associated with being confined in a small space described above, although one is a repeated acute stressor whereas the other is chronic. Responses to space may therefore differ between acute and chronic restrictions suggesting further research investigating the effect of space allowance and C/C is required.

In Chapter 5, I investigated the effect of sixty minutes of exercise upon levels of C/C provided in one of two ways; ten minute walks, six times a day or a single sixty minute walk. The subjects had been in kennels for only a short period of time, on average 23 days. Transfer into a kennelled environment is a known acute stressor (Rooney et al, 2007b) but most dogs tend to adapt to this stressor within 10 days (Hennessy et al, 1997, Rooney et al, 2007b). Those dogs which are unable to adapt to this stressor due to persistent challenges; separation from social attachment figures (1.5.2.8), unpredictability (1.5.2.16), lack of control over the environment (1.5.2.16), low temperatures (1.5.2.5) and limited opportunities for inter and intra-specific contact (1.5.2.8 and 1.5.2.9 respectively) are likely to be chronically stressed. It was therefore feasible that, if adaptation had not occurred, some of the dogs in Chapter 5 were chronically stressed. Results of Chapter 2 suggested that elevated levels of C/C were indicative of chronic stress and so one would assume that a reduction in stress and an improvement to welfare would result in a decrease in C/C. When additional exercise was provided, C/C was observed to decline, suggesting that in early chronic stress states, C/C responds as predicted. Whether this is the case for long-term stress states is not yet understood.

In Chapter 6.1, whilst it appeared initially that the provision of enrichment was associated with a significant increase in C/C, this increase was evident in both enriched and control dogs, suggesting that another factor, not measured in the study, was contributing. This may have been temperature; the majority of baseline data was collected during the summer months whilst the enriched data was collected during late winter and early spring, thus I suggest that low ambient temperatures may have caused the increase in C/C. Hence, I conclude that longitudinal studies of kennelled dogs should carefully consider the impact of ambient temperature upon C/C.

Overall, based upon the findings in this thesis, elevated levels of C/C appear to be indicative of chronic stress in kennelled dogs. However, caution needs to be exercised with their interpretation as even with apparently normal levels of C/C, one cannot rule out chronic stress (Beerda et al, 2000). For example, levels of cortisol in pigs, separated from their social group, returned to pre-stress levels even though other indicators of stress suggested that the subjects had not adapted to the stressor (Schrader and Ladewig, 1999). For that reason, in dogs, I suggest that the use of C/C as an indicator of chronic stress can be useful, but much more research is required to fully understand its utility. This is developed further in section 7.5.1.1.

7.2.2 Kennelled behaviour

7.2.2.1 How should we sample kennelled behaviour?

Prior to this study, the effects of human observers on behaviour, and diurnal variations in behaviour, had not been explored in kennelled dogs. Findings from Chapter 3 indicated that for comprehensive welfare assessment, observations should be made in both the presence and absence of an observer, and at various times of day. Furthermore, one-zero sampling was more accurate than instantaneous sampling, when recording instances of stereotyping (Appendix 4). These findings have been implemented throughout this thesis. Behavioural indicators collected in Chapters 4, and 6.1 were conducted in the presence and absence of an observer and at various times of day whilst studies described in Chapter 5, investigating effects of exercise, employed one-zero sampling to ensure that an accurate record of stereotypic behaviour was obtained. I would suggest that these methods are incorporated into future studies for accurate assessment of kennelled dog welfare.

7.2.2.2 Is activity state a useful indicator of welfare?

Activity state has been measured in a large number of studies of kennelled dogs (1.4.2.3) and is often used to infer stress and welfare state. For example, when exposed to classical music, dogs spent more time resting (defined as laid down with eyes open) and less time standing than when exposed to any other type of music, leading Wells et al (2002b) to conclude that the welfare of dogs may be enhanced through appropriate forms of auditory stimulation, with classical music being particularly beneficial. Beerda et al (2000) observed that dogs kept in the most austere environments, and likely to be experiencing chronic stress, were the most active. In these two examples, high levels of activity appear to be related to poor welfare, yet in contrast, Meers et al (2004) showed that dogs which were exercised the most, were more active, moving around the kennel more, concluding that welfare was improved. Interpretation of activity state may therefore be problematic and Hiby (2005) showed that in some cases it could be complex; when introduced into a kennelled environment, dogs which showed an increase in C/C over a period of days spent more time on average walking or trotting, but on days when individual dogs produced the most C/C they spent a relatively small percentage of time walking or trotting. On days where they walked or trotted the most, their C/C levels were relatively low.

In this thesis, activity state was found to relate to a number of factors e.g. dogs at sites where higher levels of noise were recorded during the day were more likely to lie down during the quieter times of day (4.4.2.1) and dogs with larger kennels were more likely to lie down, again during the quieter times of day (4.4.2.1). The latter result suggested that inactivity: lying down and sitting still, was indicative of good welfare. Additional exercise (5.3.1.1) resulted in more time spent lying down and less time standing during the day providing additional evidence.

However, the use of activity in the assessment of dog welfare has lead to concern. In a recent review of the kennel environment and its influence upon dog welfare, Taylor and Mills (2007) criticised conclusions based on activity state being used to inform decisions about welfare. They propose that recording the activity state of a dog provides very little information about its welfare status and additionally, because behaviour is often an adaptive response, it is difficult to suggest at which point a percentage of activity reflects poor welfare. Within this thesis, I have not attempted to suggest at what duration or proportion of time is indicative of good or poor welfare. Rather, as in Chapter 5, I implemented a within dog experimental design which allowed the assessment of basal activity and changes in activity in response to treatment to be explored. I did predict however, that additional exercise would improve welfare, and an increase in time spent lying down as a result led me to conclude that lying down was indicative of good welfare. Yet, I support Taylor and Mills (2007) in their criticisms about activity state because, ultimately, making a decision about the welfare state, based upon a change in activity, still depends upon what it is believed that lying down or sitting represents for the dog. Whilst it is often assumed that lying down is a restful behaviour, inferring a positive subjective state, there are cases where lying down can indicate negative subjective states e.g. apathy or learned helplessness, and thus compromised welfare. This has been observed in dogs which have spent over five years in kennels (Wells et al, 2002a).

Nonetheless, based upon the findings in this thesis, I believe that measures of activity do have a role within welfare assessment, if interpreted carefully, and that inactivity within the subjects studied in this thesis was indicative of good welfare.

7.2.2.3 Are stereotypies useful indicators of welfare?

Studies in Chapter 3 demonstrated that the performance of stereotypies differed dependent upon eliciting stimuli (time of day and observer presence) concurring with recent findings by Denham (2007). Furthermore, also in agreement with Denham (2007), the HPA system's responsiveness appeared to be related to whether or not a dog stereotyped in response to stimuli (2.3.6). Unfortunately, further investigation of these relationships were beyond the scope of this thesis but the causation of stereotypies and their link to physiology is worthy of further scientific enquiry and I would recommend such a study.

In Chapter 6.1, in response to enrichment deprivation, previously enriched dogs tended to spend more time stereotyping than control dogs, and this effect tended to be sustained across to the next day. This increase in stereotyping appears to demonstrate the value of enrichment so was a useful indicator and thus may be a useful measure in future studies to investigate the value of resources. I suggest that studies which investigate the impact of any resource e.g. intra and inter-specific contact, bedding, etc, should incorporate a period of deprivation where an increase in such behaviour may be evident.

Nonetheless, the complexity of these indicators should not be overlooked. Section 1.4.2.5 has already introduced some of these complexities and, in particular, the problem that the welfare of some dogs which stereotype may be better than that of their counterparts which do not. Within this thesis, I have referred to spinning, bouncing, circling and pacing behaviours as stereotypical i.e. invariant and repetitive, with no obvious function or goal (Mason, 1991). However, there is the possibility that some apparently stereotypical behaviour is, in fact, functional. For example, it is feasible that spinning or bouncing behaviours, commonly observed in the presence of people (Denham, 2007), are in fact learned strategies to gain human attention and are sustained due to their performance being intermittently reinforced; some incidences of spinning etc will correspond with rewarding events, such as feeding or being removed from the kennel for exercise. Such behaviour therefore has a function and by definition cannot be a stereotypy but is instead simply a reinforced repetitive behaviour. Determining which behaviours are truly stereotypical may be difficult but testing susceptibility to extinction may determine whether some are learned strategies or true stereotypies. At present, there is little research in this area and it is obviously warranted.

I do however, agree with Mason et al (2007) that extensive performance of repetitive behaviour is a cause for concern and so should continue to be used as an indicator of welfare. Large numbers of kennelled dogs are observed performing repetitive behaviour, between 46% (Hiby, 2005) and 93% (Denham, 2007), and they can result in tail injuries and lameness

(Jennings, 1991, Rooney et al, submitted) impinging upon biological functioning and thus welfare.

7.2.3 The relationship between behaviour and C/C

The relationship between behaviour and levels of C/C was tested in two studies within this thesis; in response to a veterinary examination (Chapter 2) and in response to additional exercise (Chapter 5). In neither study was a relationship found between the behaviour and the physiology. These findings are concurrent with those of other studies (Beerda et al, 1997; 1998, van Vonderen et al, 1998, Rooney et al, 2007b). There are numerous reasons as to why the behaviour observed was not associated with the physiology measured; behaviour may be dissociated from physiology as a result of selective breeding for other behavioural traits (2.4.4, Bradshaw et al, 2005) whilst dog's individual coping strategies and the behavioural expression of stress may differ (5.4.2, Rooney et al, 2007b) and so a multi-measure approach should always be taken.

7.2.4 Is health a useful indicator of welfare?

As introduced in section 1.4.1.6, immunosuppression, as a result of chronic stress, can lead to an increased susceptibility to both disease and infection. Within Chapters 4 and 6.1, data was collected pertaining to those symptoms which could be related to, or be a result of, stress, e.g. diarrhoea, self biting, tail damage, foot problems and skin complaints. The presence and absence of these symptoms was found to be linked to various factors within housing and husbandry (4.4.2). Dogs housed at sites where higher levels of exercise were provided were less likely to have been taken to the veterinarian with one of these symptoms in the past year, and dogs with higher levels of intra-specific contact were more likely to have been ill with one of these symptoms in the month preceding the study. Neither of these provides direct evidence for immuno-suppression due to psychogenic factors: exercise has a direct effect on health, and the probability of transmission of disease increases with increased contact with conspecifics. When feeding enrichment (6.1.2.3.2) was provided to dogs over a four month period, health was neither beneficially nor detrimentally affected (6.1.3.5). This is most likely because, overall, welfare was unaffected by enrichment provisioning, thus it is difficult to comment upon the utility of this measure based upon experimental evidence from this thesis alone.

7.3 General hypotheses

7.3.1 General hypothesis 1; *'Factors of housing and husbandry influence welfare, some are more influential than others'*

Two population surveys were conducted, gathering potential indicators of welfare and factors which may influence it. As hypothesised, factors of housing and husbandry did influence the welfare of kennelled dogs with many relationships between housing, husbandry and welfare indicators evident (4.4.2). Associations differed between the two populations of kennelled working dogs studied (4.5) probably because some elements of housing and husbandry were found to vary more between-population than within-population. As a result, critical factors for dog welfare have been identified for each population separately. Within each population, I suggest that changing specific critical factors is likely to improve welfare but changing the same factor in the other population might not necessarily have the same effect. For example, if we wanted to increase the time dogs spent lying down in population one, we would increase the space allowance (4.4.2.1). However, in population two, increasing space allowance is likely to have little influence upon the time spent lying down, as space allowance appeared to be adequate in this population and not a critical factor for the welfare of those dogs. However, in population two, we could influence the time spent lying down by increasing the level of intra and inter-specific contact, since dogs with higher levels of intra and inter specific contact were more likely to lie down (4.4.2.2).

Even with a limited number of populations, several factors have been identified which appear more influential than others. Exercise, noise and predictability of routine related to similar behaviours in both populations. For example, dogs provided with higher levels of exercise were less likely to bark (4.5.1.4) whilst dogs provided with higher levels of predictability within routines were more likely to exhibit behaviours of stress and frustration, coprophagy, restlessness and vocalisation in population one (4.4.2.1) and panting in population two (4.4.2.2). Changes to the levels of exercise, noise and predictability are therefore likely to improve the welfare of both populations and I predict, other populations of kennelled dogs.

7.3.2 General hypothesis 2; '*Changes to the most influential factors improve the welfare and working ability of military working dogs*'

In section 1.5, I hypothesised that changes in the most influential factors of housing and husbandry would result in improvements to welfare. Furthermore, because compromised welfare can result in reduced working ability, I hypothesised that changes to the most influential factors may also improve working ability. Evidence from Chapter 4 led me to hypothesise that three factors would have influence upon the welfare of kennelled working dogs; exercise (4.5.1.4), noise (4.5.2.1) and predictability of routine (4.5.1.5). Due to time and logistical constraints (4.6), of the three, only exercise was investigated. In addition, previous research on the welfare of military working dogs suggested that feeding enrichment might enhance welfare, particularly if provided long term (Hiby, 2005). In light of findings from Chapter 4 revealing complex relationships between enrichment and welfare (4.5.1.3), this factor was considered worthy of investigation. As a result, two studies of within-dog manipulations were conducted and their effects upon welfare (Chapter 5 and 6.1) and working ability (Chapter 6.2) measured. It has previously been noted that that these are severely lacking (Taylor and Mills, 2007), hence these studies fill an important void in the literature.

7.3.2.1 Exercise

When comparing between kennel sites, the study in Chapter 4 revealed that in general, dogs provided with the longest and most frequent walks were less likely to bark (4.5.1.4) suggesting that higher levels of exercise may be beneficial to welfare and a decrease in exercise may lead to frustration. Hence I investigated the effect upon welfare of forty minutes of additional exercise provided in two different ways; ten minute walks six times a day or a single sixty minute walk (Chapter 5). At the end of each regime dogs were walked for twenty minutes only. Overall, additional exercise appeared beneficial. Over the study period C/C levels consistently decreased (5.3.1.3) and restful behaviours increased (5.3.1.1). An interruption to additional exercise did not affect behaviour as predicted (5.3.3) although this maybe because the effects were only sampled on one day, or because twenty minutes of exercise were sufficient to mask any effect. I propose that an effect might have been observed had exercise been eliminated completely.

Originally I had intended to conduct this study using the shepherd dogs described in Chapter 4, as relationships between exercise and welfare were evident in these dogs. But due to operational commitments (5.4.1.1), this was not permitted. Gundogs procured as arms and explosive search dogs (1.1) were used. These dogs differed in breed, age and time in kennels compared with subjects in Chapter 4. Breed differences can have a significant effect upon behaviour (Hart 1995) so it is possible that the behavioural responses to additional exercise may have differed if the same study was conducted using the same populations as Chapter 4. Nonetheless, the differences are likely to be subtle. Five different activity states were used to assess welfare; lie down, sit, stand, stereotype and other active behaviour (5.2.5.1). Questionnaire studies have suggested that 'reactivity' (derived from factor analysis of which activity and playfulness were original variables) does not differ between Labrador Retrievers, Springer Spaniels and German Shepherds, since within the UK, each breed was rated as average for reactivity (Bradshaw et al, 1996). Therefore differences in activity between Labrador Retrievers, English Springer Spaniels and German Shepherds are unlikely. Furthermore, exercise is likely to be important to all dogs, independent of breed, because the home range of their ancestral species is large and much time within it is spent active (5.1). Thus the beneficial effect observed is likely to be evident in most other breeds.

7.3.2.2 Feeding enrichment

Intersite comparisons suggested that the relationship between the presence of environmental stimulation within kennels and welfare was complex (4.5.1.3). What is more, many of the relationships suggested that the welfare of dogs with toys and bones was poorer than those without e.g. dogs with a higher level of environmental stimulation within the kennel were more likely to have been referred to the vet since arriving at the site (4.4.2.2) and were more likely to lick their lips (4.4.2.1), a behaviour indicative of acute stress (1.4.2.8a). Previous research has pointed to the beneficial effects of enrichment (6.1.1) so in this study, the decision to provide enrichment by kennel assistants and handlers could have been based upon the stress indicators which the dogs displayed. Hence a study was conducted to investigate the effects of environmental stimulation upon welfare. Feeding devices were chosen, as previous research in other species e.g. maned wolves (6.1.1, Cummings et al. 2007) and domestic dogs (6.1.1) suggested they would be more beneficial than toys, and Hiby (2005) saw trends, when used in similar domestic dog populations, compatible with enhanced welfare.

Overall, dogs did not habituate to feeding devices, interacting with them on a daily basis even after four months (6.1.3.1 & 6.1.3.4), and there was objective evidence which suggested that they were anticipating the arrival of the devices (6.1.3.2). Anecdotally, one dog's aggressive behaviour ceased following enrichment provision (6.1.3.4). Yet, feeding devices did not significantly change the indicators of welfare measured; neither behaviour (6.1.3.2) nor C/C levels (6.1.3.3) significantly changed in enriched dogs compared to controls. Several reasons for the lack of change were proposed; 1) feeding devices are unlikely to substantially enrich a kennelled environment but rather result in one less impoverished, 2) other critical factors may have limited the effect of feeding enrichment, 3) operational factors, not measured in the study, may have caused additional stress and 4) changes resulting from enrichment were too subtle to be measured.

The best evidence for a beneficial effect of feeding enrichment came from the subsequent deprivation of enrichment. Stereotypic/repetitive behaviour tended to increase immediately and was sustained over to the next day, whilst the duration of grooming tended to decrease in previously enriched dogs (6.1.3.2). So whilst welfare, in general, was not measurably improved, the feeding devices were clearly valued resources, as in some dogs, deprivation resulted in the immediate expression of behaviours indicative of compromised welfare.

In Chapter 6.2, no effect upon working ability was found following the provision of enrichment. This result contradicts the belief held by many trainers and handlers that access to any form of enrichment inside the kennel can decrease a dog's motivation to play with a toy during training, ultimately decreasing working ability (6.2.1). However, it could be argued that the measures used within this study were unreliable due to their subjective nature; handlers were asked to rate their dogs' working ability and general behaviour using 11 criteria (Table 6.2.1). Subjective measures of working ability can be highly correlated with those obtained objectively (Rooney et al, 2007a) which tends to refute this argument, but subjective measures do not reveal the same level of information as those measured objectively (Rooney et al 2007a). Within the enrichment study, due to time constraints, it was not possible to assess the dog's working ability objectively and this is an area which in police and patrol dogs has yet to receive interest and so is worthy of further enquiry (7.5.2).

7.3.3 Practical applications

7.3.3.1 Exercise

The findings from Chapter 5 suggest that additional exercise is beneficial to kennelled dogs and that the effect is cumulative, at least initially. However, it is unlikely that kennel assistants will be able to provide the additional duration of exercise tested here. Additionally, the provision of a single daily walk may lead to distress in those dogs which prefer not to eliminate in their kennels. I would therefore recommend that at least two short walks are provided as conducted in this study (5.2.4), so that dogs have regular and frequent opportunities to eliminate.

7.3.3.2 Feeding devices

As discussed in section 7.3.2.2, evidence from deprivation studies showed that feeding devices were valuable resources to some of the dogs studied, so even though objective and measurable improvements to welfare indicators were not observed during enrichment, their daily provision appears justified. However, the effects of deprivation indicate that their provision should be both predictable and consistent, otherwise the welfare of the dogs could become compromised. I recommend that establishments should only provide feeding enrichment if they can guarantee daily provision. Starting and then failing to sustain provision could lead to a decrease in the welfare state of individuals which greatly value feeding enrichment.

7.4 Summary

This thesis has provided additional evidence that kennelling can compromise the welfare of dogs. The basal C/C level of long-term kennelled dogs was significantly higher than that of dogs housed in a domestic environment. I suggest that the differences indicate that kennelled dogs are more stressed and by definition, their welfare is poorer.

This thesis has revealed information which can be used to improve the recording of kennelled dog behaviour. Evidence has shown for the first time that kennelled dog behaviour differs according to time of day and is affected if the observer is visible. The use of a single technique or recording at one time of day could lead to incomplete or inaccurate welfare assessment.

Stereotypical/repetitive behaviours described in other populations of kennelled dogs were also present within the subjects studied in this thesis and were present in a substantial proportion of each population studied. This allowed the use of stereotyping for the measurement of welfare state in all studies. Activity was also a useful measure of welfare and it was apparent that related behaviour, such as lying down, was consistent, at least within this thesis, with improved welfare.

The hypothesis that factors of housing and husbandry influence welfare and that some factors are more influential than others was supported. Levels of exercise, noise and predictability of routine appeared the most influential in both populations. Environmental stimulation within the kennel had a complex relationship with welfare indicators, and requires further investigation.

The hypothesis that changes in the most influential factors of housing and husbandry would result in improvements to welfare and working ability was supported for some factors but not for others. Increasing the amount of daily exercise improved welfare. Providing enrichment did not affect perceived working ability but was a valued resource for the majority of subjects as indicated by their response to its removal. The lack of effect of enrichment upon working ability is significant as the belief that enrichment detrimentally affects working ability was not substantiated and hence there is no reason for establishments not to provide this resource.

7.5 Further research

7.5.1 Indicators of welfare

7.5.1.1 Urinary C/C

In the studies reported in this thesis, urinary C/C appeared to reveal useful information about the welfare state of subjects. For example, in Chapter 2, the C/C levels of kennelled dogs were higher than those of pet dogs, in agreement with Beerda et al (2000), who concluded that elevated levels of C/C are indicative of chronic stress. However, for how long these elevated levels are maintained is unknown. It would appear unlikely that elevated levels are sustained, as long term elevation of GCs can have major deleterious effects on the immune and central nervous systems (Lane, 2006). There are no studies, to my knowledge, which have measured GCs in dogs over a period of more than one year and so there is a current void in this area.

Longitudinal studies measuring GCs may greatly increase our understanding of this measure, helping us to understand their temporal nature and if elevated levels are sustained, their effects upon specific physiological systems. In addition, such studies may identify differences due to individual and genetic variation, breed differences and early experience as seen in other species (Mormède et al, 2007). Such studies may be suited to highly controlled dog populations such as laboratory or in working dog breeding programmes.

This study failed to reveal differences in responsiveness of the HPA axis between groups of dogs apparently differing in chronic stress state (Chapter 2). One explanation may be that the imposed stressor was not uniformly perceived as such by all the subjects. I would suggest that the development of a more universal stressor is explored, perhaps a physical stressor such as exercise (2.4.7). A successful challenge may reveal changes in the HPA system and responsiveness at various stages of stress e.g. what is the response during a state of acute stress? Does it differ to that during chronic stress? Does responsiveness during early chronic stress differ to that during late chronic stress?

7.5.1.2 Additional indicators of welfare

In 6.1.5 and 7.3.2.2, I suggested that the welfare indicators I used may have been inadequate or failed to identify subtle changes indicative of improved welfare, and it has recently been recommended that more sophisticated welfare indicators be developed for dogs so that more subtle positive changes in well-being can be assessed (Taylor and Mills, 2007).

Neurotransmitters e.g. phenylethylamine, may be useful indicators as their release causes feelings of elation, euphoria and exhilaration; positive interaction between humans and dogs e.g. talking softly to, touching and stroking, lead to increased levels of phenylethylamine in the dog (Odendall and Lehmann, 2000). Metabolites of other hormones indicative of emotive state e.g. serotonin, although at present in their infancy, may also prove to be effective markers.

I would also suggest that preference testing (1.4.3) be considered for dogs. Conducting such tests would reveal how important both environments and resources are to dogs and what they actually want. There are recognised problems associated with these tests (1.4.3) but currently there is a void in the use of preference testing of dogs.

7.5.2 Measures of working ability

Within this thesis, it was difficult to conduct within dog single manipulations on operational subjects and so I was only able to investigate the effects of feeding enrichment upon working ability (Chapter 6.2). In Chapter 6.2, time allowed only for subjective ratings of working ability. Police dogs are often tasked to search buildings and areas, much like arms and explosive search dogs, so a test using similar concepts to that developed by Hiby (2005) where spatial memory was tested, could be utilised. Alternatively, components of search ability e.g. search behaviour, thoroughness and location ability could be empirically tested using a standard search task such as that developed by myself and others (Rooney et al, 2007a).

7.5.3 The influence of noise and predictability of routine upon welfare

Manipulation of noise and predictability of routine was unfortunately beyond the scope of this thesis. As both factors were significantly linked to similar indicators of welfare in the two populations of dogs (4.4.2) their influence is likely to be important and therefore worthy of further investigation. I would suggest that manipulation of noise levels and their effect upon welfare is perhaps best conducted in a non-operational environment where a greater level of control over extraneous noise can be exercised. On the other hand, the predictability of routine can be controlled easily within an operational environment requiring the manipulation of positive events e.g. changing the timing of feeding and exercise so would be suited to enquiry within a similar population to that studied in Chapter 5.

7.5.4 The influence of pair and group housing upon welfare

None of the subjects studied in Chapter 4 were pair-housed and so it was not possible to explore differences in welfare between those dogs singly housed and those pair housed. This is a factor which is likely to be of great significance (1.5.2.9) and as stated by Taylor and Mills (2007), many researchers have proposed that the welfare of dogs could be improved via pair or group housing. There is resistance within the military to pair-house dogs due to concerns of a loss in operational capability, should a fight occur which leads to injury.

Measures could be put in place to minimise this aggression, for example by testing for amicable pairs, increasing the kennel space thus allowing dogs to be at a distance from one another, and by giving additional resources (toys, feeding bowls and bedding) to prevent monopolisation. Even though it was not possible to explore differences in welfare between those dogs singly housed and those pair housed, intra-specific contact appeared to be an important influence on many of the relationships that were evident. I therefore suggest that pair and group housing be investigated.

7.5.5 Can we identify factors which affect the welfare of dogs in general?

Within this thesis, I have studied military working dogs; shepherds, retrievers and spaniels in a working dog kennelled environment. To identify factors which affect the welfare of dogs in general, it should be considered that firstly, there are a variety of reasons as to why dogs are kennelled; rescue, veterinary hospitals, laboratory research, teaching, breeding, service work, assistance training (Taylor and Mills, 2007), and secondly, that the breeds which are kennelled are likely to vary considerably. The populations studied within this thesis appeared superficially similar but in fact their housing and husbandry varied substantially. Thus, considering the variety of reasons as to why dogs are kennelled, the way in which dogs are housed and cared for, in other kennelled establishments, is likely to vary considerably. As introduced in section 1.1, different breed types have been selected for varying purposes and so their behavioural traits are likely to differ. Some dogs may be more susceptible to the cold e.g. short haired breeds, whilst some breeds may find it more difficult to cope with a kennelled environment than others, and this is often said to be true of the Belgian Malinois (Jennings, 1991). For these reasons, it is possible that some breeds may differ in which factors have the most influence upon their welfare. To identify factors which affect the welfare of dogs in general would require a large study where factors within a range of kennelled establishments and the welfare of different breeds are studied. Whilst this may be initially expensive and labour intensive, it would have advantages. It has recently been highlighted in the literature, that there are a large number of factors within the kennelled environment which could impact upon kennelled dog welfare and have yet to be studied in detail (Taylor and Mills, 2007). Due to limited budgets and the time taken to conduct studies, it will be impossible to study every possible factor. Instead a large scale study, using the approach described would identify the most important factors.

Similar survey studies have been undertaken within other species, albeit on smaller scales. For example, European brown bear (*Ursus arctos arctos*) behaviour at six different zoological parks has been studied in detail, revealing factors which influence stereotypies (Montaudouin and Le Pape, 2004). The effects of different approaches to housing and management upon the welfare of laying hens (Whay et al, 2007) and risk factors related to feather pecking in free range hens (Nicol et al, 2003) have been explored. In commercially farmed chinchillas (*Chinchilla lanigera*) a questionnaire survey revealed six risk factors which influenced the incidence of fur chewing; breeder experience, facility volume, space index, number of breeding rooms, allocation of rooms for reproduction and fur production and wood shaving changes per week (Ponzio et al, 2007).

Conducting a similar study in kennelled dogs would allow detailed hierarchical analysis and the identification of generic factors. Single variable manipulation studies of these pertinent factors, which have been severely lacking, may then help us to improve the welfare of kennelled dogs in general; rescue shelters, laboratories, boarding and quarantine kennels. Such a study would have considerable value and so I suggest that such a study is conducted.

I conclude as Taylor and Mills (2007);

'It is unfortunate that the environment of an animal which was the first to be domesticated and with which we have arguably the strongest relationship has been so poorly studied, especially when we appreciate what they have given to humans.'

Table A.1 Minimum space allowance per dog (m^2) recommended in existing guidelines (adapted from Prescott et al, 2006 to include revised legislation).

Guidelines	Notes	Weight <10	<15	>20	>30
Home Office 1989[a]	Housed singly	4.50	4.50		6.50
	Housed in groups	1.90	2.25		3.25
Home Office 1995[b]	Post weaned stock	1.50	2.00	2.25	
Council of Europe 1986[c]		0.70 (1.4)	1.2 (1.6)	1.7 (1.9)	2.0 (2.0)
Council of Europe 2006[d]	For 1-2 dogs		4.0	8.0	
	For each additional animal		2.0	4.0	
	Post weaned stock	1.50	2.0	4.0	
CCAC 1993[e]	Housed singly	0.75		1.20	2.23
	Housed in groups	1.50		2.0	3.0
NRC[f]			0.72	1.08	>2.16
DEFRA 2002[g]		1.10 (3.7)		1.40 (5.5)	1.40 (7.4)
CIEH[h]					1.90 (3.34)
UK government 1983[i]	Housed singly	1.0			
	Each additional animal	0.25			

[a]Home Office (1989) Code of Practice for the Housing and Care of Animals Used in Scientific Procedures

[b]Home Office (1995) Code of Practice for the Housing and Care of Animals in Designated Breeding and Supplying Establishments

[c]Council of Europe (1986) European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, ETS No. 123

[d]Council of Europe (2006) Revised Appendix A to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, ETS No. 123

[e]Canadian Council on Animal Care (1993) CCAC Guide to the Care and Use of Experimental Animals, Vol. 1, 2nd edn

[f]National Research Council (1996) Guide for the Care and Use of Laboratory Animals

[g]Department for Environment, Food and Rural Affairs (2002) DEFRA Voluntary Code of Practice for the Welfare of Dogs and Cats in Quarantine Premises

[h]Chartered Institute of Environmental Health (1995) Model Licence Conditions and Guidance for Dog Boarding Establishments: Animal Boarding Establishments Act 1963

[i]UK Government (1983) Pet Animals Act 1951 (Amendment) Act 1983

Individual sample dog questionnaire

Name:	**Age:**	**Sex (N/E):**
Site:		
Number (in study):		
Date arrived at site:		
Date of qualification as police dog:		

1. When did the dog last work (day and time)?

Enrichment

2. Does the dog have access to toys?

Continuously ☐

Sometimes ☐

Occasionally ☐

Never ☐

Details of toys:

3. Does the dog have access to bones?

Continuously ☐

Sometimes ☐

Occasionally ☐

Never ☐

Behaviour

4. Does the dog spin in the kennel?

Spinning – Dog turns repetitively in a tight circle pivoted around the back legs

Several times per day ☐

Every day ☐

Most days ☐

Only in the past ☐

Never ☐

5. When do you see him spin?

6. Does the dog circle in the kennel?

Circling – Dog walks repetitively around the inside perimeter of the kennel

Several times per day ☐

Every day ☐

Most days ☐

Only in the past ☐

Never ☐

7. When do you see him circle?

8. Does the dog pace in the kennel?

Pacing – Dog walks repetitively back and forth immediately adjacent or parallel to the kennel on either side of the subject dog OR across the front of the kennel

Several times per day ☐

Every day ☐

Most days ☐

Only in the past ☐

Never ☐

9. When do you see him pace?

10. Does the dog bounce off walls in the kennel?

Bouncing – Dog jumps at wall in kennel and re-bounds off the wall

Several times per day ☐

Every day ☐

Most days ☐

Only in the past ☐

Never ☐

11. When do see him bounce?

12. Does the dog bite itself?

Current (minor sore) ☐

Current (major sore) ☐

Has in the past ☐

Never ☐

13. Has the dog ever had tail damage?

Current ☐

In the past ☐

Never ☐

14. Has the dog ever been coprophagic i.e. eaten his own faeces?

Current ☐

In the past ☐

Never ☐

15. Has the dog ever bitten a member of staff?

Y ☐

N ☐

Details:

16. What is the dog's behaviour towards you?

Always friendly ☐

Usually friendly ☐

Consistently neutral ☐

Unpredictable ☐

Aggressive ☐

17. What is the dog's behaviour to less familiar carers?

Always friendly ☐

Usually friendly ☐

Consistently neutral ☐

Unpredictable ☐

Aggressive ☐

18. Does the dog ever bark for prolonged periods?

Every day ☐

Most days ☐

Has in the past ☐

Never ☐

19. Does the dog ever whine/howl for prolonged periods?

Every day ☐

Most days ☐

Has in the past ☐

Never ☐

20. Does the dog chew his kennel?

Every day ☐

Most days ☐

Has in the past ☐

Never ☐

Health:

21. Is the dog currently on any medication?

22. What diet is the dog given?

23. Has the dog had diarrhoea in the past month?

Y ☐

N ☐

24. Has the dog had foot problems in the past month?

Y ☐

N ☐

25. Does the dog have evidence of baldness on the chest?

Y ☐

N ☐

26. Does the dog have tail damage?

Y ☐

N ☐

Health Records (Details of incidences) :

Diarrhoea:

Foot problems:

Self-mutilations:

Tail damage:

Skin complaints:

General husbandry questionnaire

This questionnaire was used to obtain information about the husbandry routine at each site.

Site name

Date

Kennel assistant

Kennel routine

1. How many people are responsible for the care of the dogs?

2. What hours do you work on an average day?

3. What is the daily routine for the kennel staff at this site?

4. Does this differ on any particular weekday?

5. Do you work at weekends?

6. If no, who cares for the dogs on a weekend?

7. Does the weekend routine differ from that on a weekday?

8. How often do you feed the dogs?

Once daily ☐

Twice daily ☐

Time of day:

9. How often do you groom the dogs?

Weekly

Daily

Other

10. Are the dogs muzzled whilst groomed?

Y ☐

N ☐

11. How often do you clean the kennel?

Once daily ☐

Twice daily ☐

12. Do all dogs defecate and urinate in their kennel?

13. Are dogs transferred into other kennels during cleaning?

Y ☐

N ☐

14. If no are the dogs left in the run or locked in sleeping area?

15. Is the kennel hosed daily?

Y ☐

N ☐

16. How many people do the dogs see on a (normal) daily basis:

Kennel enrichment

17. Do the dogs have access to toys in their kennel?

Continuously ☐

Sometimes ☐

Occasionally ☐

Never ☐

Details of toys:

18. Do the dogs have access to bones in their kennel?

Continuously ☐

Sometimes ☐

Occasionally ☐

Never ☐

Heating

19. Are the kennels heated?

Y ☐

N ☐

20. What time of day is the heating is turned on?

21. What time of day is the heating is turned off?

Exercise

22. How many times are the dogs walked per day? 1☐ 2☐ 3+

23. Does this differ on a weekend?

24. Is this the same when the dog is on duty or is the handler then responsible for his/her dog`s exercise?

25. How much time of the walk is spent on lead?

26. How much time of the walk is spent off lead?

27. Do the dogs have access to a loose exercise area?

Daily ☐

Occasionally ☐

Weekly ☐

28. How much time do the dogs spend, on average, in the loose exercise area?

29. Do the dogs have access to toys in the loose area?

Y ☐

N ☐

30. Are the dogs exercised, off lead, in pairs in the loose area?

Y ☐

N ☐

31. Does this happen:

Daily ☐

Occasionally ☐

Weekly ☐

32. Are the dogs ever walked together on lead in pairs (must be able to have contact with one another)?

Y ☐

N ☐

33. Does this happen:

Daily ☐

Occasionally ☐

Weekly ☐

34. What would be the maximum length of time a dog would be left between walks?

35. Would the dog's routine change should the handler be on leave?

Bedding

36. What type of bedding are the dogs provided with?

37. Are they provided with bedding?

Always ☐

Usually ☐

Only if cold (i.e. Winter) ☐

Occasionally ☐

Never ☐

Music

38. Do you ever have music playing in the kennel area?

39. Is that music played?

Every day ☐

Sometimes ☐

Never ☐

Operational Duties (over a period of a month)

40. What is the typical shift pattern worked by the handler and dog?

41. How many hours does the handler and dog work per day?

42. What is the general routine for the dog whilst on duty (if known)

Day

Night

43. What would be the maximum length of time a dog would be left between duties?

Without leave

With leave

Diet

44. Are all dogs fed the same diet?

Y ☐

N ☐

Details

Kennels

45. Do dogs ever change kennel position within block?

Y ☐

N ☐

Comparing methods for sampling the stereotypic behaviour of kennelled dogs

A4.1 Introduction

As introduced in section 1.4.2, behaviour has been shown to be an easily observable manifestation of stress and welfare in several species (Mench & Mason, 1999). One type of behaviour commonly suggested as indicative of stress and compromised welfare is stereotypies (1.4.2.5). A stereotypy is defined as a behaviour pattern that is invariant and repetitive, with no obvious function or goal (Mason, 1991). They generally develop in situations where an animal is frustrated, stressed, fearful, restrained or receiving a lack of stimulation (Mason, 1991) and so are common in kennelled dogs. Between 46 (Hiby, 2005) and 93% (Denham, 2007) of long term kennelled military working dogs have been observed to stereotype i.e. pace, circle, spin, tail chase and wall bounce. Some stereotypies exhibited by kennelled dogs can be short in duration, elicited by short lived stimuli e.g. the passing of the kennel by a person or conspecific and depending upon the stimuli which elicit them, some are rare. It is therefore paramount that behaviour is sampled in a way which ensures that a representative record of stereotyping is obtained.

The most accurate method for behavioural sampling is continuous recording. This method provides an exact record as it measures every occurrence of each behavioural pattern (Martin and Bateson, 1993). However, this type of sampling is very time consuming and so alternative sampling rules are often sought. As described by Martin and Bateson (1993), time sampling allows behaviour to be sampled periodically and can be sub-divided into two principal types: instantaneous sampling and one-zero sampling. Both sampling types require the observation session to be divided into short sample intervals. During instantaneous sampling, on the instant of each sample point, the observer records whether or not the behaviour is occurring. During one-zero sampling, instead of on the instant of each sample point, the observer records whether or not the behaviour pattern has occurred in the preceding sample interval.

For the analysis of stereotyping, one-zero sampling is likely to be more accurate than instantaneous sampling, since all occurrences of stereotypical behaviour during the sample interval are recorded. However, if the sample interval is short in relation to the typical bout length of stereotyping, then it is likely that the majority of occurrences of stereotypic behaviour will overlap the sample point and thus the approximation using instantaneous sampling will relate to that obtained using one-zero sampling.

To examine this, recordings of kennelled dog behaviour were divided into one minute sample intervals allowing both instantaneous and one-zero sampling of stereotypic behaviour. The aim was to determine whether the records of stereotyping using instantaneous and one-zero sampling were significantly related. If records were significantly related, observations could be sampled using an instantaneous method which would be less time-consuming than one-zero sampling.

A4.2 Methods

A4.2.1 Subjects

Two groups of dogs, A (N=4) and B (N=7) procured by the Defence Animal Centre (DAC, 1.2.2) as potential arms and explosive search dogs (1.1) were used in this study. The same population were also used to investigate the effects of exercise upon welfare described in Chapter 5.

Group A comprised one Labrador Retriever, one Labrador Retriever cross and two English Springer Spaniel (ESS) aged between 13 and 22 months (mean=15.5 ±4.4) procured between 17 and 25 days (mean=19.5 ±3.7) before the study commenced. Three were from rescue shelters and one donated by a member of the general public. Group B comprised four Labrador Retrievers and four ESS aged between 10 and 21 months (mean=14.4 ±3.1), procured between 19 and 36 days (mean=26.4 ±6.3) before the study started, four donated by members of the general public, two from rescue shelters and two from dealers. Group A was studied in September 2006 and B in October 2006.

See Chapter 5.2.2 for details of housing and husbandry.

A4.2.2 Recording and analysis of stereotypical behaviour

The kennelled behaviour of each dog was recorded using two waterproof cameras with light emitting diodes (LEDs) for night recording (RF Concepts, model 2020, lens 3.6mm); one placed in the sleeping compartment and one in the outdoor run of the kennel (Figure 5.2).

Each camera was linked to a 16 channel multiplexer and a time lapse videotape recorder. The video recorder was started at 08:00am and continued recording for 24 hours.

Nine days of kennelled behaviour were recorded in total; from each 24 hour recording, two ten hour periods were analysed; daytime; 09:30 to 19:30 and night time; 20:30 to 06:30, using "The Observer" version five, software package (Noldus Information Technology). The period between 06:30 and 09:30, when behaviour was disrupted by routine husbandry, was not recorded.

Daytime observations were divided into one minute sample intervals for both instantaneous and one-zero sampling. Using instantaneous sampling, on the instant of each sample point, the presence of stereotyping was recorded whilst, simultaneously, using one-zero sampling, on the instant of each sample point, the presence of stereotyping during the preceding sample interval was recorded. The number of samples where the dog was absent or not visible was recorded so that proportions of visible time stereotyping could be calculated (see below)

As the occurrence of stereotypical behaviour was predicted to be lower during the night time, the period between 20.30 and 06.30 was sampled less frequently, divided instead into ten minute sample intervals. Both instantaneous and one-zero sampling was conducted in the same way as during daytime. Only one dog stereotyped during the night-time observation so variables describing stereotypical behaviour during this observation were not analysed further.

Using behaviour observed during the daytime, two variables were calculated:

Proportion of sample points visible stereotyping = number of sample points on which stereotyping occurred/ (maximum number of samples - number of samples where dog was either absent or not visible).

Proportion of sample intervals visible stereotyping = number of sample intervals during which stereotyping occurred/ (maximum number of samples - number of samples where dog was either absent or not visible).

In addition, for each observation, a further variable was calculated: the ratio between the two sampling methods (instantaneous to one-zero): *Proportion of sample intervals visible stereotyping /proportion of sample points visible stereotyping.*

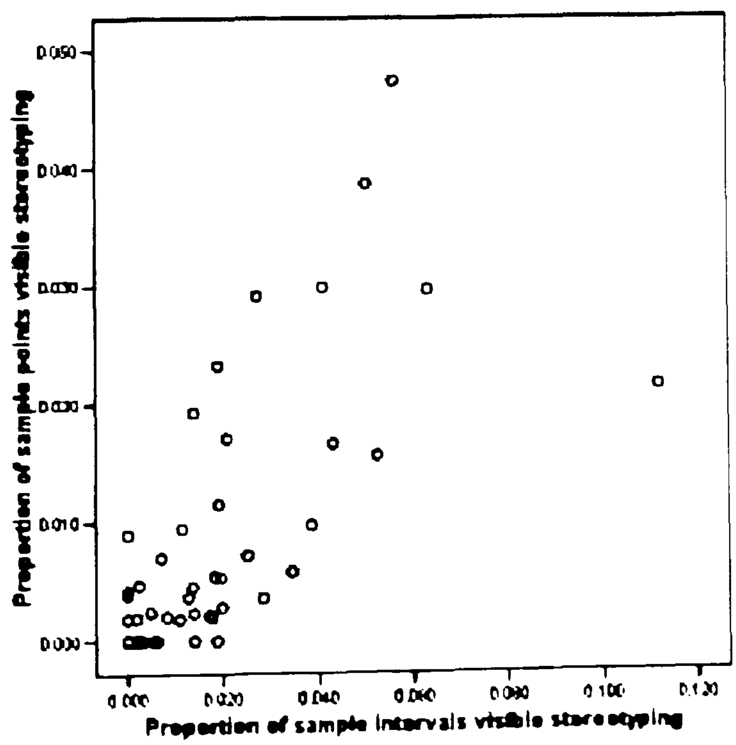
A4.2.3 Statistical analysis

To examine whether instantaneous sampling produced a record which approximated that of one-zero sampling, Spearman rank correlations were used to test the relationship between the two stereotyping variables. To provide a measure of the relative efficiency of the two methods, the range and average of the ratio between the two sampling methods was calculated.

A4.3 Results

Both proportion of sample points visible stereotyping and proportion of sample intervals visible stereotyping were highly correlated (Spearman rank correlation; Rho=0.77, $p<0.001$, Figure A3.1). Fifty nine percent of the variation in one measure was accounted for statistically by the variation in the other, thus the two measures were highly consistent in the sampling of stereotypies.

Figure A4.1 The relationship between the proportion of sample points visible stereotyping and proportion of sample intervals visible stereotyping.



The ratio *proportion of sample intervals visible stereotyping to proportion of sample points visible stereotyping* (one-zero to instantaneous sampling) ranged from 0 to 9 (mean = 3.0 ±2.5).

A4.4 Discussion

In this study, the recording of stereotypic behaviour was sampled both instantaneously and using a one-zero rule to determine whether the occurrence of stereotyping using instantaneous sampling approximated that of one-zero sampling. Both sampling types were highly correlated suggesting that either would be useful for measuring the occurrence of stereotypies. This is not surprising because the sample interval used to divide the observation session was very short (one minute), and thus it is highly likely that the occurrence of stereotyping throughout the observation overlapped the sample point and so was recorded by both sampling methods. Had the sample interval been of a longer duration then it is likely that instantaneous sampling would have been less accurate relative to one-zero sampling. However, one-zero sampling was more effective at recording the occurrence of stereotypies as on average, three times as many occurrences were observed compared to when only instantaneous sampling was used. For example, during one observation of the same dog, 20 occurrences of stereotyping were recorded using one-zero sampling whilst only five were recorded using instantaneous sampling.

The high level of agreement between the two methods suggests that instantaneous sampling could be used to record the occurrence of stereotypies in Chapter 5. However, one-zero sampling appears to be more effective in recording the occurrence of stereotyping and as I wanted the most accurate record, one-zero sampling was used instead.

Although this study has shown that the sampling of stereotypic behaviour using instantaneous sampling can be highly correlated to records obtained by one-zero sampling, it is important to recognise that this type of sampling will always lead to an under-estimate of the occurrence and this was demonstrated by the average ratio of 3:1 between the two methods. So, the method may not be suitable for studies where accurate records of rare behaviours such as stereotypies are required, for example, during welfare assessments or site comparisons.

Instead, instantaneous sampling may be more useful where comparisons, before and after an imposed treatment, in the overall levels of behaviour, are of interest.

It should also be considered that one-zero sampling is itself problematic as it can introduce systematic bias (Martin and Bateson, 1993). Durations of stereotyping may be over-estimated because the behaviour is recorded as though it was performed throughout the sample interval when in fact it may have occurred for only a few seconds. In contrast, an under-estimate of the behaviour can also occur as more than one occurrence of stereotyping could be performed during each sampling interval, so these issues should always be considered when the data is being interpreted.

A4.5 Conclusion

In light of these findings, I would suggest that where time is constrained, the sample interval is short, and accurate reports of behavioural patterns are not required, instantaneous sampling can be used, instead of one-zero sampling, as the record approached that obtained using one-zero sampling. However, both techniques can lead to bias and so careful interpretation of results is required.

Guidelines for food enrichment

I have recently visited your dog section and studied a number of dogs in detail measuring their behaviour and physiology. I would now like to use these dogs for another study designed to look at the effects of long term food enrichment (Kongs filled with part of the dog's normal diet) upon the behaviour of working police dogs. Kongs will be provided to dogs for at least four months at which point I will return to the section and measure each dog's behaviour and physiology again.

I have split the dogs housed at your section into two groups, one of which will be provided with food enrichment (dogs to receive enrichment are listed in Table 1).

As well as studying dogs at your station, I am also conducting the study at another three stations. It is therefore important that the guidelines provided are closely followed so that I can ensure that each dog at your station is treated the same as each study dog at the other three stations.

- It is very important that only those dogs selected for the study (Table 1) are provided with food filled Kongs. However, all other dogs housed at the station, can be given a small treat e.g. biscuits at the same time as the study dogs receive their Kongs.

- Kongs are only to be filled with the dog's normal diet e.g. Eukanuba premium/intestinal/dermatosis (details of how to prepare the filling are provided at the end of this document). This is to ensure that all study dogs, both at this station and those study dogs at other stations, are provided with the same type of filling.

- Kennel staff should provide each study dog with a food filled Kong at the end of their working day just before they go home; Monday to Friday.

- Kongs should be collected from kennels first thing the following morning, on a daily basis so that they can be cleaned and checked for damage. We suggest collecting the feeding devices at a time when the dog is not present in the kennel e.g. during exercise or kennel cleaning

- Dogs are likely to value the feeding devices at times when they are full so staff should never attempt to remove the Kong from the dog at this time. If when empty, you have a dog which won't readily give up his Kong, encourage the dog to drop the Kong by offering another favourable resource such as a tasty treat. Once the dog has dropped the Kong, reward the behaviour and remove the dog from the kennel before returning to collect the Kong.

- If it is apparent that a Kong needs replacing due to excessive chewing then please use one of the spares and contact myself so that I can send a replacement.

- If at any time, there is concern regarding the provision of Kongs to any of the study dogs then please contact me straight away.

- At the end of each week please complete the table provided (Table 2) to show that each study dog has received the Kong.

    - If the dogs has received a Kong every day Monday to Friday then please tick the third column
    - If for some reason, a dog does not receive a Kong on one of the week days then please state which day(s) and why.

- If a dog is not given a Kong at all that week, for any reason, then please write this down.

- Similarly if a dog experiences anything which is not part of his/her normal routine e.g. visits the Vet, competes in trials, then please fill in the space provided on the chart.

Table 1 Study dogs receiving food enrichment; these dogs must receive a food filled Kong Monday to Friday from the x to the y.

Preparing the filling

- Each day, place enough of each type of food for all the study dogs with a small amount of gravy granules into a bowl. If any food is left over then this can be refrigerated for the next day.
- Pour hot water over the food at least one hour before filling the Kong
- Leave food to soak until all the biscuits are soft
- Once soft, mash up the food with a fork
- Using a spoon, stuff the Kong until full
- Place one Kong into each study dog's kennel.

Table 2 Provision of enrichment

Station:

Week commencing:

Dog's Name	Dog received a Kong every day this week (please tick)	Dog not received a Kong every day this week. Please state which day(s) and reason(s) why	Please provide details of any events experienced by the dog which are not part of the normal routine

Military Working Dog Questionnaire

I am a scientist at the Defence Science and Technology Laboratory (dstl), where I study the behaviour of military working dogs.

I am currently researching how housing and husbandry affect the behaviour and working ability of dogs. As part of this project, I have visited a large number of kennel establishments, including your own. During these visits I have collected behavioural and physiological data from a number of dogs and have also spent time with the personnel who care for these dogs.

As a final part of this study, I would now like to ask you, as a handler, a number of questions about your own dog. I would be very grateful if you could take about five minutes to fill in this questionnaire, placing it in the attached envelope (to retain confidentiality) and return to the head of your section as soon as possible but definitely within the next seven days.

Many thanks for your help

Sam Gaines

This questionnaire has been designed to find out about attributes of military working dogs and to look at how individual dogs change over time.

We will ask you to complete another similar questionnaire in several months time so please be as accurate as you can now.

There are no right or wrong answers and no one else will see your responses so please give frank and open responses.
Please answer the following questions

Name:________________________________

Station: ______________________________

Age (years):________

Nationality: ___________________________

Sex: Male Female

Number of years as a dog handler ____________

Dog's name: _________________________________

Breed:______________________________________

Age (years): ____________

Years of service: ____________________________

Please indicate the level your dog possesses of each of these attributes, using the scale below. For example if you consider that your dog possesses an attribute at an extremely high level then please circle 5.

extremely high	high	intermediate	low	extremely low
5	4	3	2	1

	High — Low
1. Overall work ability	**5----4----3----2----1**
2. Health	5----4----3----2----1
3. Ability to learn from being rewarded	5----4----3----2----1
4. Consistency of behaviour from day to day	5----4----3----2----1
5. Motivation to chase an object	5----4----3----2----1
6. Playfulness	5----4----3----2----1
7. Interest in toys or objects	5----4----3----2----1
8. Motivation to retain possession of a toy	5----4----3----2----1
9. Contentment in the kennel environment	5----4----3----2----1
10. Level of aggression towards humans	5----4----3----2----1
11. Willingness to enter kennel at end of shift	5----4----3----2----1

Thank you very much for your time

I would be very grateful if you could check that you have not omitted to rate any of the eleven attributes.

Thank you.

dstl

AZI

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