Behavioural and faecal glucocorticoid metabolite responses of single caging in six cats over 30 days

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ABSTRACT

Objectives: The aim was to characterize the behaviour and faecal glucocorticoid metabolite (FGM) responses of six cats (Felis catus) to single caging for 30 days.
Design: In this observational study, changes in outcome measures were monitored with habituation to caging. Continuous focal observations of the activity, location in the cage, and posture were conducted from video recordings for one 24-hour period/week/cat. Cat-Stress-Scores were recorded daily. All faecal samples were collected for analysis of FGM.
Results: The percentage time spent eating increased, while percentage time spent grooming decreased, from week 1 to week 2. Cat-Stress-Score declined significantly from week 1 to week 2. FGM concentrations were significantly greater in week 1 than in week 5. A posthabituation time budget of the behaviour of the cats in the single cages showed that inactivity dominated the time budget and the cats were located on the shelf almost half of the time.
Conclusions: Results suggests that a shelf was a resource of value to the cats, and that its inclusion in enclosure design is important. Quantitative and qualitative behavioural data indicated that there was an initial stress response to caging that stabilised after the first week, while FGM concentration took longer to stabilise.

INTRODUCTION

Cats can be caged in several situations, including animal shelters, boarding establishments, animal hospitals and research laboratories. Although there is increased use of multi-cat accommodation, there are still situations where cats are kept in single cages. For example, when there is either a requirement for single accommodation or it is beneficial for a particular cat. When a cat is confined in a novel environment, such as an animal shelter, it can be stressful (McCobb and others 2005) and some of this stress is likely to be due to confinement in a single cage (Cauvin and others 2003). In animal shelters, in particular, the stress of confinement might be associated with an increased risk of disease conditions, such as those affecting the respiratory and urinary tracts (Westropp and others 2006, Dinnage and others 2009, Tanaka and others 2012).

There have been several studies of behavioural and physiological responses of cats to caging; however, these studies did not assess both the quantitative and qualitative behaviour of the cats simultaneously with a reliable measure of the physiological stress response. Many studies (Kessler and Turner 1997, Gourkow and Fraser 2006, Dybdall and others 2007) have used a qualitative behavioural scoring system, the Cat-Stress-Score (CSS), to determine the stress responses of cats, but few studies have shown how this score relates to the physiological stress response. One study of 120 cats in a shelter (McCobb and others 2005) did not find a statistically significant correlation between the urine cortisol-to-creatine ratio and the daily morning CSS. A reduction in the CSS over the first three to eight days after single caging suggests that cats habituate to single caging (Kessler and Turner 1997, Hawkins and others 2004, Dybdall and others 2007, Moore and Bain 2013).

A valuable development in assessing the responses of animals to housing has been the use of faecal glucocorticoid metabolite (FGM) measurements. A validated and reliable assay method to determine FGMs in cats is now available (Palme and others 2001). An obvious advantage of this method over blood sampling to measure plasma cortisol concentration is that the method is non-invasive and the cats do not require handling for sample collection. In addition, the faecal concentrations are not affected by the circadian rhythm of plasma cortisol and they represent the accumulated cortisol metabolites excreted over a period of about one day (Buchanan and Goldsmith 2004). Although urine cortisol concentrations have been measured in several studies in cats (Carlstead and others 1993,

Cauvin and others 2003), the major route of excretion of cortisol metabolites in cats is in the faeces (86 per cent) rather than in urine (14 per cent) (Graham and Brown 1996). In studies on cats, 86 per cent of radioactive cortisol was found to have been excreted in the faeces within 22.3 hours (Graham and Brown 1996) and after an adrenocorticotropic hormone (ACTH) injection, peak FGM concentrations occurred after 25 hours (24–37 hours) (Schatz and Palme 2001).

Information on complete 24-hour time budgets for quantitative behaviour assessments of single caged cats is surprisingly limited. Carlstead and others (1993) reported information on durations spent alert/awake and in exploratory/play behaviour. However, other reports (De Monte and Le Pape 1997, Kry and Casey 2007, Ellis and Wells 2010) are based upon limited observations during only part of a 24-hour day. There have been a number of sleep studies of cats in single cages that have shown that cats can sleep for 48–55 per cent, are drowsy for 14–28 per cent, and alert and active for 18–33 per cent of a 24-hour day (Chamblin and Drew 1971, Lucas and Sterman 1974, Kuwabara and others 1986, Lancel and others 1991).

The aim of the current study was to use simultaneous measurements of quantitative and qualitative behaviour and FGMs to (a) examine whether cats show stress responses to single caging that subsequently decline over a 30-day period, and (b) to provide a 24-hour time budget for cats habituated to single caging.

MATERIAL AND METHODS
Subjects
This study was conducted on six domestic cats (*Felis catus*), three entire males and three females. The cats had been at a local shelter for a mean of 2.6±1.92 days (±sd), had a mean estimated age of 2.5±1.05 years, and all had been singly housed. Two cats had been surrendered by their owner and four of them were strays. For inclusion in this study cats had to be deemed adult, healthy, and safe to handle by shelter staff and the university veterinarian. The funding for this study imposed limitations on the study size, source of the animals and on the methodology used.

Housing and management
On arrival from the shelter, each cat was placed into a stainless steel cage (58×79×79 cm). The cages were in two rows of three cages approximately 1 m apart and facing each other (Fig 1). Within each cage, there was a shelf (55×20 cm) elevated 32 cm off the floor, a towel on the shelf, a litter pan (33×28×13 cm), a small plastic ball with a bell inside, and food and water dishes secured to the cage door (Fig 2). Starting at 06.00, 12 hours of fluorescent light was provided followed by 12 hours of darkness, supplemented with infrared lighting (880 Infra Red Illuminator, Dennard, UK). The cats were offered 50 g of dry cat food (Adult Indoor, Nutrience by Hagan) at 09:30 and at 16:30. Litter pans were cleaned, and water bowls were filled by animal care technicians between 08:00 and 09:00, and between 15:00 and 16:00. The room was maintained at a temperature of 20°C (range±2°C). Cats were housed onsite for 30 days at a time, between March 24, 2010 and May 26, 2010. The start of observations on a particular cat was dependent on the availability of cats at the local shelter that met the inclusion criteria. The cats did not all begin and end the study on the same days. However, at all times there were at least four cats present in the six cages.

FIG 1: Two banks of cages, facing each other
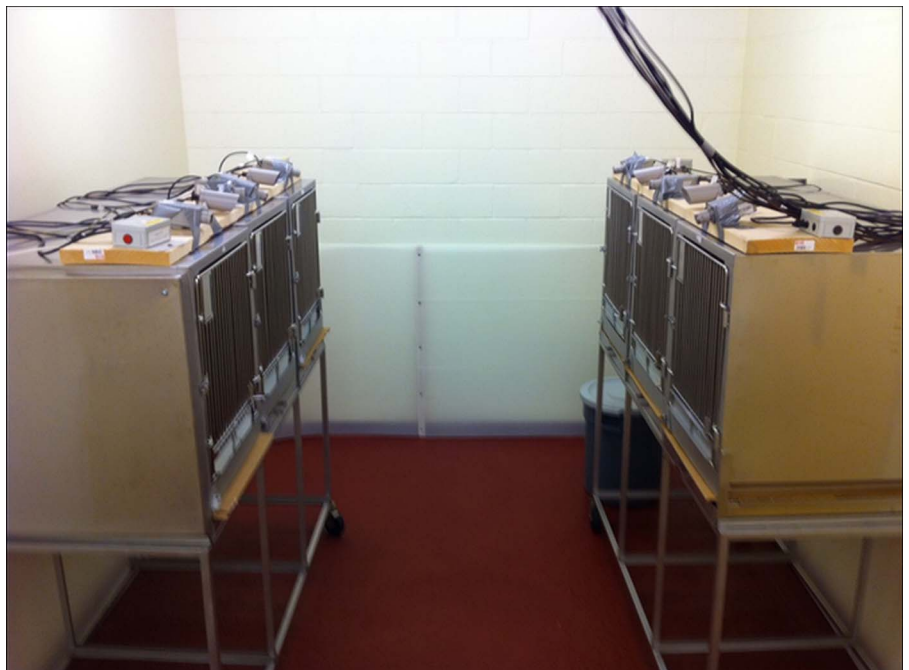
FIG 2: *Interior view of a cage*

Continuous quantitative behavioural observations

Cats were continuously video recorded at 50 frames/s using black-and-white closed-circuit television (CCTV) cameras (Panasonic, Germany) attached to a multiplexer (Sprite dx, Dedicated Micros, UK) and video recording equipment (CTR-3024, Computar, UK) for 30 days. One 24-hour recording every seven days, starting on the day of arrival, was analysed for each cat's general activity, location within the cage and posture using continuous observation. The ethogram for location, posture and activity is given in Table 1. As some behaviours have slight pauses in the middle of their exhibition that do not necessarily signal the end of their exhibition (eg grooming, eating and locomoting), a behavioural bout was considered to have concluded if the animal ceased to perform the behaviour for at least five seconds. This length of time was selected as it was deemed biologically relevant for all behaviours in this species (Lehner, 1996). The behavioural recordings were viewed at 10 times the actual speed by two observers, using Observer 5 software (Noldus Information Technology, Wageningen, The Netherlands). The recordings were split evenly between the observers in terms of week and cat.

CSS Recordings

CSSs were recorded once daily (at 16:00), using the method described by Kessler and Turner (1997). Briefly, two observers simultaneously stood in front of the cages for 10 minutes and then independently assigned the cats a score between 1 (fully relaxed) and 7 (terrorised) using the 11 behavioural/postural categories (such as tail position, pupil dilation and vocalisations) defined by Kessler and Turner (1997). These behavioural/postural categories and the provided definitions are available in Table 2. The assessment was then repeated within an interval of 15 minutes.

TABLE 1: Ethogram for quantitative behavioural observations (adapted from UK Cat Behaviour Working Group 1995)

Behavioural class	Behaviour	Definition
Activity	Resting	Cat remained generally inactive
	Grooming	Cat licked its body or licks its paw and passed the paw over its head
	Eating	Cat consumed (or appeared to consume) food
	Drinking	Cat lapped water
	Manipulation	Cat manoeuvred or attempted to manoeuvre an object with its paw
	Locomotion	Cat moved position within its enclosure
	Out of cage	Cat was not present in cage
	Other activity	Any activity not defined above
Location	On shelf	Cat was positioned on top of a towel covered shelf
	In litter pan	Cat was positioned in the provided litter pan
	Behind litter pan	$\geq$50% of the cat was positioned behind the provided litter pan
	Cage front	Cat was positioned at the cage bars at the front of the enclosure
	Floor other	Cat was positioned on the cage floor, other than at the cage front or behind the litter pan
	Out of cage	Cat was not present in cage
	Other location	Any location not defined above
Posture	Lying head down	One side of cat was in complete contact with the ground, head on side or extended
	Lying head up	One side of cat was in complete contact with the ground, head not in contact with ground
	Sitting	Pads of the front paws were on the ground with the front legs straight and the rump on the ground
	Standing	Cat was positioned with four paws on the ground, rump raised
	Locomotion	Cat moved position within its enclosure
	Out of cage	Cat was not present in cage
	Other posture	Any posture not defined above

Note. Behaviours within each behavioural class were discrete, and all behavioural classes were measured simultaneously

FGM measurement
Faecal collection
When litter pans were cleaned all faecal samples present were collected, homogenised, and placed into 30 ml Nalgene tubes. The samples were then temporarily stored at -4°C and then stored at -20°C until extraction. A mean of 30±8.45 samples were collected from each cat over their 30-day study period.

Hormone extraction and analysis
Samples were extracted and analysed according to Möstl and Palme's (2008) protocol using an enzyme immunoassay for 9.3 11-oxoaetiocholanolone (Lab-code: 72-alt, Palme and Möstl 1997) previously validated for use in cats (Palme and others 2001). In the current study, interassay and intra-assay coefficients of variation were 8.9 per cent (n=12) and 3.5 per cent (n=383), respectively.

Statistical analyses
Continuous qualitative behavioural observation
The interobserver agreement of the two observers was evaluated for agreement during six 24-hour periods of behavioural observation (one of each cat during the first week) using methods of percentage of agreement, Pearson's r, and Cohen's κ statistic (Jansen and others 2003; mean ±sd). Minimum acceptability of results was set at $\geq$80 per cent for percentage agreement (Cooper and others 2007), $\geq$0.8 (or a strong effect) for Pearson's r (Ferguson 2009), and >0.6 (or substantial to almost perfect) for Cohen's κ statistic (Landis and Koch 1977). For each behavioural class, percentage of time spent performing each of the specific behaviours was calculated for each 24-hour period analysed. Two-way analysis of variance models were used to investigate the effect of cat (represented by cat identity (ID)) and week on each outcome variable, and Bonferroni adjustments for assessing multiple behaviours were carried out within each behavioural class. Where necessary to satisfy model assumptions, data were transformed by the arcsine square root function. When model assumptions could not be met, two Friedman's tests were carried out for that variable, switching cat ID and week between treatment and block, in order to generate test statistics for each variable. To assess the impact of within-cat correlation structure, repeated measures analyses of variance were run and, generally, no changes in the result were found. Furthermore, for the two outcome variables with a significant effect of week, linear mixed models showed no evidence of autocorrelation within cats. For variables where week was found to be a statistically significant factor, Bonferroni pairwise comparisons were used to compare weeks.

CSS recordings
Daily CSSs were compared between researchers with a Wilcoxon signed rank test. Weekly median values of the

TABLE 2: Cat-Stress-Score, reproduced from Kessler and Turner (1997), with permission from Universities Federation for Animal Welfare

Score	Body	Belly	Legs	Tail	Head
1 Fully relaxed	i: laid out on side or on back a: NA	Exposed, slow ventilation	i: fully extended a: NA	i: extended or loosely wrapped a: NA	Laid on the surface with chin upwards or on the surface
2 Weakly relaxed	i: laid ventrally or half on side or sitting a: standing or moving, back horizontal	Exposed or not exposed, slow or normal ventilation	i: bent, hindlegs may be laid out a: when standing extended	i: extended or loosely wrapped a: tail up or loosely downward	Laid on the surface or over the body, some movement
3 Weakly tense	i: laid ventrally or sitting a: standing or moving, back horizontal	Not exposed, normal ventilation	i: bent a: when standing extended	i: on the body or curved backwards, may be twitching a: up or tense downwards, may be twitching	Over the body, some movement
4 Very tense	i: laid ventrally, rolled or sitting a: standing or moving, body behind lower than in front	Not exposed, normal ventilation	i: bent a: when standing hindlegs bent, in front extended	i: close to the body a: tense downwards or curled forward, may be twitching	Over the body or pressed to the body, little or no movement
5 Fearful, stiff	i: laid ventrally, or sitting a: standing or moving, body behind lower than in front	Not exposed, normal or fast ventilation	i: bent a: bent near to surface	i: close to the body a: curled forward close to the body	On the plane the body, less or no movement
6 Very fearful	i: laid ventrally, or crouched directly on top of all paws, may be shaking a: whole body near to ground, crawling, may be shaking	Not exposed, fast ventilation	i: bent a: bent near to surface	i: close to the body a: curled forward close to the body	Near to surface, motionless
7 Terrorised	i: crouched directly on top of all paws, shaking a: NA	Not exposed, fast ventilation	i: bent a: NA	i: close to the body a: NA	Lower than the body, motionless

Score	Eyes	Pupil	Ears	Whiskers	Vocalizations	Activity
1 Fully relaxed	Closed or half opened, may be blinking slowly	Normal	Half back (normal)	Lateral (normal)	None	Sleeping or resting
2 Weakly relaxed	Closed, half opened, or normal opened	Normal	Half back (normal) or erected to front	Lateral (normal) or forward (normal)	None	Sleeping, resting, alert or active, may be playing
3 Weakly tense	Normal opened	Normal	Half back (normal) or erected to front or back and forward on head	Lateral (normal) or forward	Meow or quiet	Resting, awake or actively exploring
4 Very tense	Widely opened or pressed together	Normal or partially dilated	Erected to front or back and forward on head	Lateral (normal) or forward	Meow, plaintive meow or quiet	Cramped sleeping, resting or alert, may be actively exploring, trying to escape
5 Fearful, stiff	Widely opened	Dilated	Partially flattened	Lateral (normal), forward or back	Plaintive meow, yowling, growling or quiet	Alert, may be actively trying to escape
6 Very fearful	Fully opened	Fully dilated	Fully flattened	Back	Plaintive meow, yowling, growling or quiet	Motionless alert or actively prowling
7 Terrorised	Fully opened	Fully dilated	Fully flattened back on head	Back	Plaintive meow, yowling, growling or quiet	Motionless alert

i, inactive; a=active, NA=Not applicable

two observer's scores were used for further analyses. Friedman's test was used to analyse median weekly CSS across weeks, with the week in study blocked by cat ID. Sign tests were used to analyse CSS differences between specific weeks.

FGM measurement

If more than one faecal sample was produced by a cat in one day, an average of the FGM concentration was used (Touma and Palme 2005). FGM concentration values were $\log_e$ transformed and linear models were used to investigate the effects of cat ID and week. Bonferroni pairwise comparisons were used to compare weeks.

Time budget

The percentages of time that each cat spent performing each of the specific behaviours in all three behavioural classes were calculated for the four 24-hour observations conducted during weeks 2 to 5.

RESULTS
Continuous behavioural observation

The interobserver agreement of the two observers for durations of behaviour within a 24-hour period satisfied all of the minimum acceptability standards (percentage agreement=87±7, Pearson's r=1.00 (range=0.99–1.00), Cohen's κ=0.85±0.08). Cat ID was found to be a significant factor for a number of behaviours: in the behavioural class activity, cat ID was significant for drinking, grooming and locomotion; in the behavioural class location, cat ID was significant for 'in litter pan' and 'on shelf'; in the behavioural class posture, cat ID was significant for lying head down, lying head up, sitting and standing. Week was also a significant factor for eating and grooming (Table 3). The percentage of time spent per day eating on the first day after arrival (week 1) was significantly less than during weeks 2 (P=0.035), 3 (P<0.001), 4 (P=0.002) and 5 (P<0.001) but, after week 1, there were no statistically significant differences between weeks (P>0.5). The percentage of time per day spent grooming on the first day after arrival (week 1) was significantly greater than during weeks 2 to 5 (P<0.001), but, after week 1, there were no further statistically significant differences between weeks (P≥0.9; Table 4).

CSS recordings

A one-sample Wilcoxon signed rank test showed no statistically significant difference (P=0.8, n=64), between daily CSS recorded by each observer, and a mean of both observers' scores was calculated to create one daily CSS value for each cat. Weekly median values of these daily scores were used for further analyses. Friedman's test (week blocked by cat) revealed a statistically significant difference in CSS across weeks (S=16.31, d.f.=4, P=0.003). Sign tests revealed that the CSS on the first day after arrival (week 1) was significantly greater than during weeks 2 to 5 (P=0.031), but after week 1 there were no statistically significant differences between weeks (P>0.1; Table 4).

FGM measurement

There was a statistically significant effect of week on the $\log_e$ FGM concentration (P=0.012). Week 1 was

TABLE 3: Effects of 'cat' and 'week in study' on the percentage of time spent per 24-hour period engaged in each behaviour

Behavioural class	Behaviour	Test	P value Cat ID	Week
Activity	Drinking	2-way ANOVA	0.004	0.084
	Eating	2-way ANOVA	0.490	0.004
	Grooming	2-way ANOVA	0.014	0.004
	Manipulation*	2-way ANOVA	0.511	1.000
	Locomotion*	2-way ANOVA	0.049	1.000
	Other	Friedman's test	1.000	1.000
	Resting	2-way ANOVA	0.630	1.000
Location	Behind litter pan	Friedman's test	0.720	1.000
	Cage front	Friedman's test	0.714	0.792
	Floor other	Friedman's test	0.444	1.000
	In litter pan	Friedman's test	0.024	0.582
	On shelf	2-way ANOVA	0.018	1.000
	Other	Friedman's test	1.000	0.552
Posture	Lying head down	2-way ANOVA	0.024	1.000
	Lying head up	2-way ANOVA	0.048	1.000
	Other	Friedman's test	1.000	0.552
	Sitting	2-way ANOVA	0.004	0.774
	Standing	2-way ANOVA	0.001	0.189

P values were Bonferroni corrected for multiple testing of variables within each behavioural class
*Transformed using arcsine square root
ANOVA, analysis of variance

TABLE 4: Weekly summary statistics for percentages of time spent per 24-hour period eating and grooming, CSS, and FGM (n=6)

	Eating Mean (±sd)	Grooming Mean (±sd)	CSS Median (IQR)	Log $_e$ FGM (ng steroid/g faeces) Mean (±sd)	Back transformed FGM Mean
Week 1	1.4[a] (0.94)	5.4[a] (2.26)	2.4[a] (0.88)	5.4[a] (1.07)	355.0
Week 2	2.8[b] (0.55)	2.3[b] (0.89)	2.1[b] (0.23)	4.5[ab] (1.01)	139.7
Week 3	3.6[b] (1.34)	2.2[b] (1.43)	2.0[b] (0.12)	4.4[ab] (0.73)	104.5
Week 4	3.5[b] (0.80)	2.6[b] (0.80)	2.0[b] (0.07)	4.5[ab] (0.65)	100.4
Week 5	3.4[b] (0.61)	2.1[b] (1.40)	2.1[b] (0.27)	4.1[b] (1.03)	95.7

[ab]Within a column, different superscripts indicate a statistically significant difference between weeks (P<0.05)
CSS, Cat-Stress-Score; FGM, faecal glucocorticoid metabolite; IQR, interquartile range

significantly greater than week 5 (P=0.011). There were no other statistically significant differences between weeks (P>0.1; Table 4).

Time budget

On the first day after arrival (week 1), the percentages of time spent eating and grooming were significantly different from those during weeks 2 to 5, but the percentages of time spent performing other behaviours were not significantly different between week 1 and weeks 2 to 5. Therefore, the percentages of time spent eating and grooming during week 1 are shown in Table 4, whereas the percentages of time spent on each behaviour in weeks 2 to 5, that is after habituation, are shown in Table 5. In the behavioural class 'activity', cats spent the largest percentage of their time 'resting'; in the behavioural class 'location', cats spent the largest percentage of their time 'on shelf'; and in the behavioural class 'posture', cats spent the largest percentage of their time 'lying head down'.

DISCUSSION

This study presents some novel and interesting findings on the habituation of cats to single caging and on their behaviour post habituation. The significant (24 per cent) decrease in the FGM concentration between arrival (week 1) and five weeks after single caging provides evidence of an initial stress response to a potential combination of movement, novelty and caging, followed by habituation. However, this result was obtained from only six cats that showed considerable between-cat variation and there could have been uncontrolled factors – other than length of caging – that could have affected the FGM concentration (i.e. factors such as temperament or previous experience could have contributed to the fact that many of the outcome variables were significantly different between cats). Although the faeces were collected under standard conditions, twice a day, so as to avoid undue disturbance, it was possible, that the FGM concentration might have changed after the faeces were voided (Palme, 2005). Lepschy and others (2010) provided evidence that the concentration of FGM is a useful

representation of adrenal corticoid activity, independent of the volume of faeces produced. However, it is always possible that at a time when eating was reduced, the adrenal corticoid activity may not have increased and the same amount of faecal glucocorticoid metabolites were present in the digestive tract, but the FGM concentration was greater because of the associated reduction in the weight of faeces produced.

The CSS provides an assessment of a cat at a particular time point. Therefore to provide weekly scores comparable with the other types of measurements, the daily scores were averaged. The significant reduction in the CSS from that after arrival in week 1 (2.4) to 2–2.1 in weeks 2 to 5, provides some supporting evidence for an initial stress response followed by habituation. It is

TABLE 5: Percentages of time spent per 24-hour period engaged in each behaviour, within each behavioural class, in weeks 2 to 5, n=6

Behavioural Class	Behaviour	Median % (IQR)
Activity	Drinking	1 (1.0)
	Eating	3 (1.2)
	Grooming	2 (2.0)
	Manipulation	1 (0.8)
	Locomotion	2 (0.8)
	Other	0 (0.1)
	Resting	91 (2.4)
Location	Behind litter pan	0 (11.8)
	Cage front	11 (6.1)
	Floor other	23 (39.5)
	In litter pan	1 (0.3)
	On shelf	53 (50.3)
	Other	0 (0.0)
Posture	Locomotion	2 (0.8)
	Lying head down	54 (14.3)
	Lying head up	29 (14.6)
	Other	0 (0.0)
	Sitting	11 (8.5)
	Standing	2 (2.3)

For each cat, the median of each weekly 24-hour value over week 2 to 5 was calculated and then the median and IQR for each behaviour, over weeks 2 to 5, for the six cats was calculated.
As not all behaviours had normal distributions, medians were presented for consistency IQR, interquartile range

acknowledged that many of the descriptors in the CSS refer to fear rather than stress (McMillan 2012). However, fear is likely to have been a component of the initial response to novelty and the emotional components of both stress and fear can stimulate stress responses (Levine, 2008). For example, the percentage of time spent by some cats either in or behind the litter pan might have been associated with an attempt to hide in response to a fearful or stressful situation (Carlstead and others 1993). The descriptors in the Kessler and Turner scoring system (Kessler and Turner 1997) categorises a score of 2.4 as indicative of a cat that was weakly relaxed to weakly tense. Therefore, the median values of the CSS in the current study were not indicative of a marked qualitative behavioural stress response. In other studies, the CSS was reported to have ranged from 3 to 5 after arrival in a shelter or boarding cattery (Kessler and Turner 1997, 1999, Dybdall and others 2007).

The quantitative behavioural changes for percentage time spent eating and grooming with duration of caging were interesting and consistent with many previous reports. It has been suggested that cats respond to novel and stressful situations, such as caging, by inhibiting normal activity patterns and/or reducing overall activity, including a reduction in eating (Gooding and others 2012). Tanaka and others (2012) reported reduced food intake and weight loss in cats during the first week in a shelter. They reported that for at least the first three days in the shelter, there was a significant negative correlation between the CSS and food intake. The explanation of the reasons for the increased grooming observed on the first day after arrival compared with that during later weeks are not straightforward and there are some contradictions in the literature on potential reasons for this increased grooming. It has also been suggested that increased grooming occurs when cats are stressed (Willemse and Spruijt 1995, van den Bos 1998, Iki and others 2011), but others have suggested that there can be a decrease in grooming (Overall and Dyer 2005). Grooming is a normal and frequent behaviour in cats that is undertaken in response to peripheral stimulation and acts to remove unwanted material and to maintain the insulation properties of the coat (Eckstein and Hart 2000, Virga 2005). However, in cats, excessive grooming might be indicative of a psychological reaction to an aversive situation and it has been suggested that it could be considered as a form of displacement activity that occurs in a conflict situation (Virga 2005). Virga (2005) suggests that "Displacement grooming can distract an animal from stressors, lower its level of arousal, or deflect social conflict or agonistic interactions. Displacement grooming may also occur as a stress response in the absence of sufficient social or environmental stimuli." In rats, increased grooming is observed in a novel environment. Grooming can occur following secretion of ACTH in the brain and be reduced by the anxiolytic action of benzodiazepines (Dunn 1998). However, in cats, excessive grooming appears to be

associated with decreased cortisol activity in the brain, rather than increased activity (Randall 1988).

There was more space available for resting at floor level than that available on the shelf, but the cats spent a numerically greater percentage of time on the shelf (53 per cent) than they did on available parts of the floor (44 per cent). However, there was large between-cat variation. Podberscek and others (1991) reported that communally housed cats used raised structures significantly more than the floor of the pen. The results suggest that a raised platform is a resource of value to a cat, and provides confirmatory evidence for the recommended practice (McCune 1995) to include shelving in enclosure design for cats. However, the shelf in this particular study was covered by a towel, so it is possible that the characteristics of this material enhanced the cat's preference for the shelf, and there is evidence (Smith and others 1994) that fabric substrates are preferred as resting surfaces by cats compared with metal substrates.

The finding that the cats spent 91 per cent of the time 'resting' is consistent with, but slightly higher than, that found in previous studies on cats in single cages where they found that the cats were inactive for 67–82 per cent of a 24-hour period (Chamblin and Drew 1971, Lucas and Sterman 1974, Kuwabara and others 1986, Lancel and others 1991). In the current study, if the cats are assumed to have been sleeping when they were lying down with their head down, the percentage time spent in this posture (54 per cent) was similar to that reported in the above studies for the percentage of time that the cats spent sleeping (48–55 per cent). In either a domestic situation (Piccione and others 2013) or if in a large, multi-cat cage (Podberscek and others 1991), cats show far greater activity than they do in a single cage. Rochlitz and others (1998) reported that cats kept in a single, but larger, cage with additional features also spent 90 per cent of their time inactive. Uetake and others (2013) found no effect of cage size on the activity of single caged cats, but a significantly greater activity was reported in multi-cat cages compared with single cat cages. Although inactivity might indicate relaxation and rest, it occupied most of the cats' time and it could have been associated with negative emotional states, such as fear (resulting in hiding behaviour), boredom or depression (Meagher and others 2013). The inactivity seen in cats kept in single cages might be indicative of an inadequate environment that does not provide sufficient social stimulation and/or sufficient physical features to motivate activity.

Analysis of the continuous behavioural observations revealed that cat ID was a significant factor affecting behaviour, which reveal a significant degree of difference between individuals. This is not surprising, due to the well-developed concept of individuality in the domestic cat. There is the potential for many factors to contribute to a cat's individuality, from more obvious distinctions such as age and sex, to concepts that are more difficult to define and/or study such as genetic

differences, behavioural styles and experience. The limited sample size may have reduced the power to identify subtle differences between certain weeks, but some larger differences between weeks were observed. As there was variation within the subjects in terms of cat-level characteristics it is possible that these differences may have influenced their response to the initial shelter conditions and then cage confinement during the study. Notably, Dybdall and others (2007) found that surrendered cats experienced significantly greater behavioural stress after entering shelter conditions than stray cats. As both types of cats were included in the current study, these differences could have potentially influenced the results.

Although the sample size was small, generalisability may be limited, and findings must be interpreted with caution, the results indicate that the percentages of times spent per day grooming and eating might have been related to the initial stress (positive and negative relationships, respectively) of caging. The quantitative behavioural results and the FGM results lend support to the CSS as a method of assessing stress in the domestic cat. Furthermore, the results of the quantitative behavioural observations, the CSS and the FGM indicated that the cats experienced an initial stress response to caging followed by habituation. The cats made use of a shelf within the cage for resting, but this inactivity dominated the time budget of the cats in a single cage.

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