Behavioural and physiological responses of shelter dogs to long-term confinement

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Summary
In Italy, National Law (281/1991) prohibits euthanasia of shelter dogs if they are not dangerous or suffering seriously. Adoption rates in rescue shelters are often lower than entrance rates, leading inevitably to overcrowded facilities where animals are likely to spend the rest of their lives in kennels. In this situation, housing conditions (i.e. space provided, environmental, and social stimulation) may have an impact on canine welfare. In this research project, the effects of two different forms of housing (group- and pair housing) on long-term shelter dogs were compared using behavioural and physiological parameters. Observational data and saliva samples were collected from dogs exposed to both experimental settings; behaviour and cortisol concentration levels were used as welfare indicators. Pair housing offered fewer social and environmental stimuli and behavioural analysis showed a significant decrease in locomotor, exploratory, and social behaviour. Cortisol levels show that this parameter varied independently of housing conditions. Although this study found no evidence suggesting that one form of confinement reduced animal welfare more than the other (e.g. in terms of abnormal behaviour, or higher cortisol concentrations), the type of confinement did affect the expression of a variety of behaviours and these variations should not be ignored with respect to housing decisions for long-term shelter dogs.

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Introduction

Canine populations are increasing worldwide and in many countries free-roaming dogs represent a significant public health problem due to the risks of dog attacks on people and livestock, zoonoses and car accidents. Different strategies can be applied to the management of free-roaming populations, shelters being one of the most important (8). The confinement of an animal generally implies physical restriction, impoverished environments, social isolation, and little control over, or ability to predict, events. Italian National Law (19) on 'companion animals and stray dog population control' prohibits euthanasia of shelter dogs if they are not dangerous or seriously suffering. This leads inevitably to overcrowded facilities where animal welfare is a major issue. It is therefore important to move toward a model of dog management and housing based on high standard levels of welfare.

Centuries of artificial selection have generated high levels of genetic and morphological diversification in the domestic dog. Breed, temperament, and previous experience of confinement have all been shown to play a significant role in dogs' ability to cope with subsequent confinement (6, 12, 13, 14), and these variables should normally be taken into account when studying dogs' adaptation to kenneling. However, when studying shelter populations, animals are mostly mixed breed adults of unknown background. Hence, in these circumstances, welfare measures should be able to detect the state of each animal in that specific environment regardless of its history.

Previous studies have succeeded in describing welfare indicators of dogs confined in different kennel environments, mainly based on behavioural and physiological parameters (10, 15, 27, 28). Behavioural parameters also give important information on animal needs and preferences, while being non-invasive and easily observable. Beerda and colleagues (3) have identified specific behavioural patterns shown in response to experimentally induced stress challenges, and similar results were found in later research (14, 23). Specifically, it has been demonstrated that

consequences of inappropriate confinement conditions and of social isolation are a decrease in activity, excessive autogrooming and vocalisations, and alterations in exploratory and locomotor behaviour and sleep patterns. Other studies, analysing the effects of confinement on dogs, suggest that kennel size *per se* does not affect significantly the quantity of behaviour but does influence the quality of activities (7, 11). It is now widely accepted that the quality of the housing environment plays a crucial role in animal welfare (16); the ideal environment should offer sufficient stimuli to motivate the expression of most normal canine behaviour. Dogs in shelters, however, typically spend most their time inactive (16, 21). Hughes and Campbell (18) reported an average time of 30-90 min a day dogs spent active regardless of cage size (1 m^2 vs 7 m^2) or access to a large outdoor pen. What differed between housing conditions was the distance travelled per day, suggesting that larger spaces may encourage the dogs to run or trot. Activity increases when dogs are socially or environmentally stimulated (17). Group-housing, for example, provides a relatively complex environment that encourages locomotor activity, olfactory exploration, and social interaction. Previous studies that focused on the effect of group-housing in comparison to isolation (4, 5, 15) demonstrated that isolation has negative effects on dogs' welfare. Other studies have highlighted improved welfare indicators with pair-housed dogs compared to those housed individually (6, 11). Some authors, however, report that group-housing is often avoided since it seems to increase the risk of disease transmission and aggressive behaviour between conspecifics (29).

Glucocorticoids, in the form of cortisol, are physiological markers commonly used for the assessment of welfare (2) since their concentrations reflect reliably the activity of the stress responsive hypothalamic-pituitary-adrenal axis (20). Higher cortisol concentrations have been found in confined dogs compared to pet dogs living at home, and in socially isolated dogs compared to dogs housed in groups (6, 13, 26). Urinary and salivary cortisol are reliable and less invasive alternatives to plasma cortisol (2).

There is evidence that the length of time in a rescue shelter influences the behaviour of kennelled dogs, however the effects of long-term confinement on canine welfare are still unclear and need further investigation (12, 30). The present study builds on earlier work in this area and seeks to provide further insight on how housing affects the welfare of shelter dogs in the long-term. More specifically, the effects of two different forms of confinement were analysed by means of established behavioural and physiological parameters.

Materials and methods

Animals and experimental conditions

Seventeen dogs (7 females, 10 males) were chosen among those housed at the animal shelter of Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' in Teramo, Italy. All were adult dogs (between 5 and 9 years old) that had lived in the shelter for four years or more at the time of observation. All dogs were medium-large size and not ascribable to any specific breed, although the majority could be described as crosses of herding or shepherd breeds. All animals were spayed or neutered, and declared healthy by shelter veterinarians.

Groups of dogs were formed during a pre-experimental phase that lasted 4 months. Daily sessions of group socialisation were carried out in order to identify compatible dogs. At the end of this process, the four experimental groups were identified, and they were housed in their experimental pens. To allow habituation to the new environment, data collection started one month after the introduction to the new housing. The first data collection (Time 1, T1) was carried out in the following experimental setting: dogs were all housed in the same confinement conditions, in groups of 4-5 animals of both sexes, in four outdoor enclosures of about 35 m^2 each. See-through wire mesh ran along all sides of the enclosures, cemented into a 50 cm high concrete wall. The pens were adjacent to one another. An 11 m^2 roof, with beds underneath, covered a portion of the pen to give protection from the sun and bad weather. The ground was unpaved soil. Dogs were fed once in the morning with dry pellets, and fresh water was available at all times. To avoid competitive behaviour, dogs were accustomed to being tethered to the fence with a 1 m leash during feeding. Dogs remained confined in their pen environment at all times.

Once the first data collection period was over, eight dogs (4 females and 4 males) housed in two of the four enclosures, were transferred in pairs to smaller enclosures (6 m^2). These dogs were defined as the experimental group, while the remaining nine dogs were left in the same two original outdoor enclosures as a control group. To avoid management problems and undesired aggressive behaviour that could compromise dogs' safety or the accomplishment of the study, pen-mates were selected based on prior histories of positive interaction within the group-housing condition. Pairs were all composed of one male and one female. The smaller enclosures were totally covered by a roof, and had visually transparent fencing at the front. Adjacent pens were separated by 1 m high solid partitions with wire mesh above.

Wooden sleeping platforms (100 x 120 cm) were placed on the floor of each pen as resting places. These enclosures had access to a common fenced area (120 m^2) where dogs were allowed to exercise in pairs for 2 h/day, usually in the morning during cleaning routines. The feeding routine was the same as previously described. Dogs were allowed to acclimatize to the new confinement condition for one month before commencement of the second data collection (Time 2, T2).

Data collection

Data collection was standardised for all groups of dogs during both T1 and T2. Behavioural data were collected by video recording dogs' activities during two daily sessions, for three consecutive days: 40 min in the morning before feeding time (06.15h-06.55h) and 40 min in the afternoon (17.15h-17.55h). All recordings were carried out in the absence of staff activities, cameras were fixed and an operator was

Table I. *Behavioural categories and variables recorded during the study and measured as frequencies (f) or durations (d) of occurrence.*

Category	Behaviour	Definition	*f/d*
Active behaviour	Walking	Ambulatory gait	*d*
	Trotting	Trotting gait	*d*
	Jumping	Jumping on the kennels' roof	*d*
	Hind legs	Standing on hind legs using forelegs against a wall/fence to support the body	*d*
Inactive behaviour	Lying	Sternal or lateral recumbence	*d*
	Sitting	Sit on hind legs	*d*
	Standing	Standing on four legs	*d*
	Resting	The subject is sleeping, or lying with head touching the ground	*d*
Active repetitive	Circling	Repetitive circling around pen	*d*
	Pacing	Repetitive pacing usually along a fence	*d*
	Social pacing	Repetitive pacing along fence in parallel with a dog on the other side	*d*
	Tail chasing	Repetitive chasing of tail	*d*
	Wall bouncing	Repetitive jumping at wall, rebounding off it	*d*
Social interaction[b]	Amicable	Lick, paw or allogrooming dog, often with tail wag	*d*
	Play	Bow, short charges with bouncing gait, play face, wrestle, play chase	*d*
	Threat	Raise hackles, aggressive vocalisations, lunge toward dog	*d*
	Rigid/high posture	Focus animal is standing with rigid posture, head and tail are elevated high, mouth is shut, no or very narrow tail wagging, T or parallel position with other dog	*d*
	Defensive	Avoid dog, increase distance, or cower, roll over	*d*
	Mount dog	Focal dog mounting/ mounted of/by another dog	*d*
	Sniffing dog[a]	Focal animal noses another dog	*d*
	Social look[a]	Focal animal orients toward another dog and keeps eye contact, usually this is associated with a change in tail movement and/or tail position	*d*
Other	Autogrooming	Behaviour directed towards the subject own body, like scratching, licking and self-biting	*d*
	Digging	Dig at ground with fore paws	*d*
	Out of sight	The subject is not visible, usually inside a kennel or behind a barrier	*d*
	Shade[a]	The subject is in a poor light so it is not possible to see facial expressions	*d*
	Barking	Staccato, short vocalisations	*f*
	Shaking off	Oscillate vigorously the head and body on its longitudinal axis	*f*
	Stretching	Stretching of the body and limbs	*f*
	Prolonged vocalisations[a]	Including howling and whining	*d*
Tail	Tail wagging[a]	Repetitive wagging movements of the tail	*d*
	Tail low/curled[a]	Tail is curled between hind legs, and posture is usually low	*d*
Environment exploration	Visual exploration[a]	The subject observing the environment or the pennmates inside the enclosure	*d*
	Olfactory exploration[a]	Nose moved along the ground or other objects with clear sniffing movements	*d*
Alimentary	Drinking	Drink water	*f*
	Chewing	Chew non nutritive material	*f*
	Urinating	Urinate with one leg cocked or in squatting position	*f*
	Defecating	Excreting the contents of the bowels	*f*
	Coprophagy	Eat own or other dog's foeces	*f*

(a) Non-exclusive behaviour: can occur together with other behaviours.
(b) When recording social behaviour, the recipient was also recorded, identifying it as: a same sex pen-mate; an opposite sex pen-mate; a dog in the adjacent pen.

in charge of turning them on and off, leaving the place during recordings. Since dogs can react more intensely to the arrival of a human attendant (18), the first and the last 5 min of each recording session were discarded from the analysis. Video analyses were carried out using a dedicated data recording system (The Observer XT 8.0, Noldus, The Netherlands) on the basis of an existing ethogram (15). A total of 38 behaviours were observed, and related patterns of behaviour were grouped together into distinct categories (Table I). Behavioural frequencies and duration of occurrences were recorded continuously during each 30 min observation bout, for a total of 180 min of recordings for each time period and for each of the 17 dogs included in the study.

Cortisol was assayed from saliva samples taken from all dogs in the study. To control for within-subject variability, cortisol was sampled from each dog for three consecutive days during both observation periods of the study (T1 and T2), immediately after the morning video recordings and when dogs were tethered before food administration. The three-day average value was considered as representative of the cortisol level of each dog during each observation phase. Saliva samples were collected from dogs' cheek pouches using Salivette® cortisol system (Starstedt, Verona, Italy). Saliva collection was carried out by the same shelter veterinarians familiar with the dogs, and it was done in a standardised way in less than 3 min to avoid measuring biased cortisol levels induced by manipulation. Samples were stored at –25°C until further analysis. Cortisol determination was carried out through immunoassay using the commercially available kit Salivary Cortisol (Salimetrics, State College, USA) and following the guidelines of the manufacturer.

Data processing and statistical analysis

Before statistical analysis could be carried out, data adjustments were applied for both behavioural and cortisol measures. Since dogs were at times not visible (e.g. inside a kennel or behind a visual barrier), and supposing that dogs' behaviour was the same when not within sight of the camera, raw behavioural data were calculated as a percentage of time during which the focal animals were visible. Each variable was corrected multiplying it for an adjustment coefficient (k) equal to the total observational time (T_t = 10,800 sec) divided by the visible time (T_t - x), x being the time the animal was not visible (i.e. out of sight, hidden in shade). Some of the behaviours listed in the ethogram were either never recorded (i.e. jump, mount dog, chew, social pace, tail chase, wall bounce) or recorded just once (tail low/curled between hind legs, coprophagy); these behaviours were therefore discarded from further analysis. Due to the low percentage of expression, some behaviours were also pooled and considered as single variables: for example, circle and pace were analysed as active repetitive category; amicable, play, threat, rigid/high posture, defensive were analysed as social interaction category; sniff dog and social look (non-exclusive behaviour) were analysed as communicative signals. For all behaviours included in the ethogram category social interaction, data were weighted according to the number of subjects present in each enclosure, and the recipient was recorded as either same sex pen-mate, opposite sex pen-mate, or dog in adjacent pen.

To test for each main effect (group and time) as well as their interaction, a two-way ANOVA with repeated measures on one factor was performed for all variables. Given that the same subject was observed twice (T1 and T2), time was treated as the within-subjects factor while group (experimental or control) was considered the between-subject factor. Although statistical analysis was considered sufficiently robust (since the assumption of homogeneous samples was respected), data distributions were visually inspected through box-plots to detect any deviations from normality.

Differences between and within groups were also calculated by 95% confidence intervals (CI) pair-wise comparisons, according to (1). As three comparisons were performed (two comparisons between times within the same group and one comparison between groups at T1), to control for the overall error rate, confidence intervals were calculated using Bonferroni correction (so the type I error rate was corrected to 0.05/3=0.0167). Whenever variable distributions appeared to be different from normal based on visual inspection, non-parametric

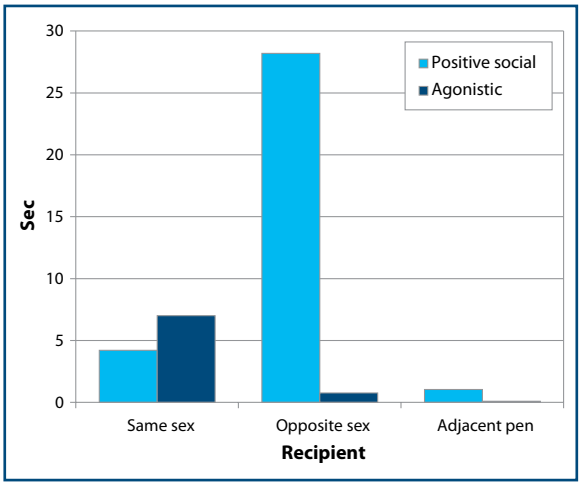

Figure 1. *Social behaviour at Time 1.* Recipients of positive (play, amicable) and agonistic (rigid/high posture, defensive, threat) social behaviour at T1 during group housing.

statistical tests (Wilcoxon Rank Sum Test for paired and/or independent samples applying Bonferroni correction) were also performed.

Due to small sample sizes, between-sex statistical comparisons of the effects of housing were not carried out. All data analyses were carried out using R-2.13.0 for Windows software.

Results

To obtain an overview of the general activity of dogs housed in groups, a preliminary descriptive analysis was carried out looking at the data collected for all 17 dogs at T1. Dogs housed in groups spent an average 90% of their time inactive, 6.5% of their time active and all other behaviours were shown for less than 3% of the time (e.g. social interactions, alimentary behaviour). Although the greater portion of the time was spent inactive, only for 42% of that time were dogs actually resting or asleep. The remaining time they were attentive, and scanning the environment visually. Social interactions were shown for only 0.3% of the total observation time. Dogs showed play behaviour for the greatest portion of the time spent in social interactions (77.33%), especially towards opposite sex pen-mates (Figure 1). Agonistic interactions (threat, defensive, and rigid/high posture) were shown less frequently (17.84%) and almost always towards same sex conspecifics (Figure 1).

Behavioural comparisons

All statistical values and levels of significance for each factor of the two-way ANOVA are presented in Table II. The between-subjects test revealed no significant overall differences between groups of dogs at T1, with the exception of resting behaviour. However, this difference was not confirmed by post-hoc analysis. This variable was also highly significant for ANOVA factor 'time', post-hoc showed a significant increase in duration at T2 compared to T1 for both groups (CI control: U=5,230.3; L=297.3; CI experimental: U=7,926.1; L=2,693.8).

'Time' and 'time by group interaction' factor significantly affected six variables (communicative signals, lying, standing, trotting, visual exploration, and walking). A significant drop in duration emerged for two active behaviours, trotting and walking (Figure 2), when experimental dogs were transferred from group confinement to pair housing. On average, dogs walked 86% less and trotted 95% less (Table III). The expression of three other behaviours

Table II. *Two-Way ANOVA with repeated measures on one factor (Time).*

Variable	Between-subjects effects		Within-subjects effects			
	Group		Time		Interaction	
	F-statistic	*p*-value	F-statistic	*p*-value	F-statistic	*p*-value
Active repetitive	1.82	0.197	1.66	0.217	2.42	0.141
Autogrooming	4.20	0.058	6.15	**0.025**	1.36	0.261
Barking	0.24	0.635	2.14	0.164	2.72	0.120
Communicative signals	1.77	0.203	4.82	**0.044**	11.35	**0.004**
Defecating	0.06	0.809	0.12	0.738	0.89	0.360
Digging	2.83	0.113	2.40	0.142	5.43	**0.034**
Drinking	0.38	0.545	0.08	0.778	12.55	**0.003**
Hind legs	1.84	0.195	3.17	0.095	0.91	0.355
Lying	1.31	0.270	24.13	**0.000**	5.60	**0.032**
Olfactory exploration	0.08	0.780	0.76	0.398	5.86	**0.029**
Prolonged vocalisations	0.08	0.779	7.31	**0.016**	2.20	0.159
Resting	6.16	**0.025**	35.36	**0.000**	3.64	0.076
Shaking off	1.65	0.218	0.13	0.720	2.44	0.139
Sitting	2.13	0.165	0.06	0.810	0.06	0.813
Social interaction	1.27	0.278	1.84	0.195	1.61	0.224
Standing	0.59	0.453	12.97	**0.003**	10.86	**0.005**
Stretching	0.33	0.573	1.86	0.193	0.00	0.992
Tail wagging	0.12	0.739	0.34	0.568	4.75	**0.046**
Trotting	0.06	0.811	7.59	**0.015**	8.90	**0.009**
Urinating	0.83	0.378	0.14	0.718	13.49	**0.002**
Visual exploration	0.38	0.549	16.07	**0.001**	12.17	**0.003**
Walking	0.06	0.811	8.57	**0.010**	14.65	**0.002**

Signifcant P values (< 0.05) are in bold.

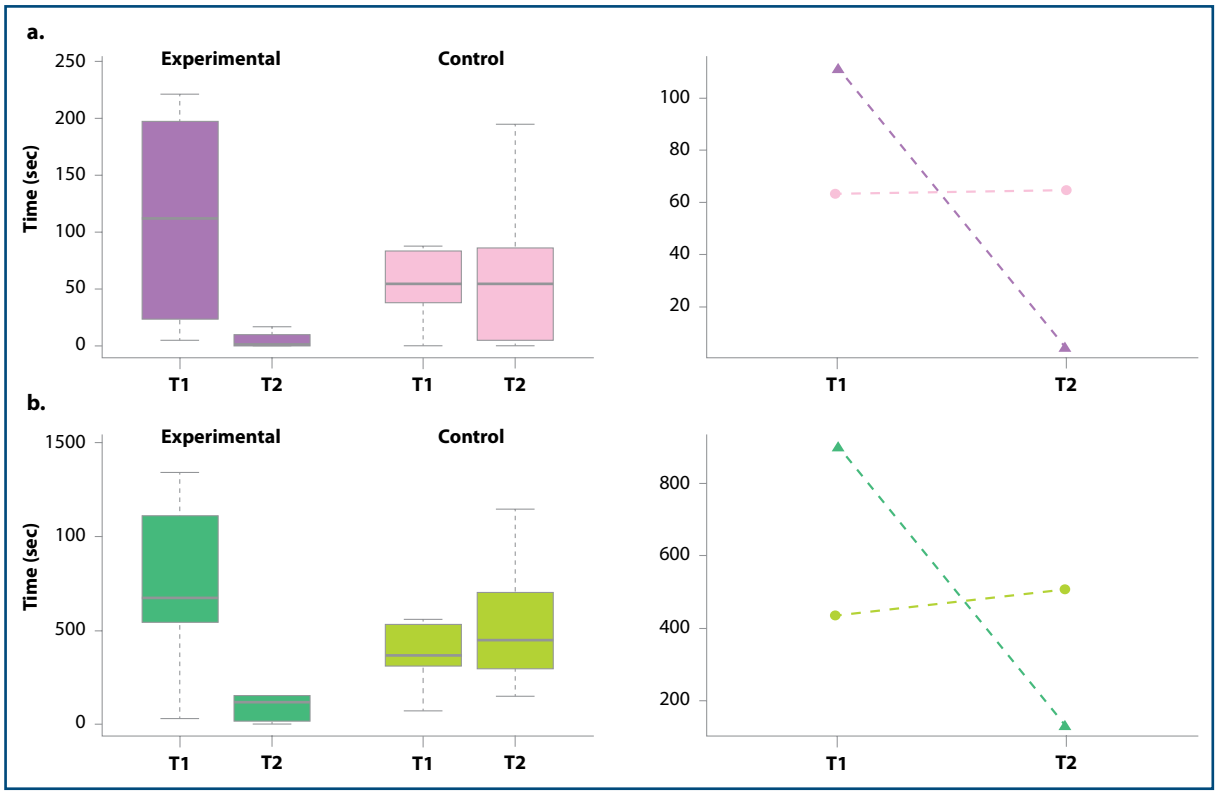

Figure 2. *Behavioural variations.* For the behavioural variables (a) trotting and (b) walking, box-plots represent changes in the duration of the behaviour between the two phases of the study (T1 and T2) for both experimental and control dogs (within-group comparisons); interaction plots represent the direction of the behaviour (increasing or decreasing dashed lines) from T1 to T2 for both experimental (solid triangles) and control (solid circles) group.

Table III. *Mean osservational time (±SEM) recorded for each behaviour.*

Variable	Control group		Experimental group	
	T1 (s)	T2 (s)	T1 (s)	T2 (s)
Active repetitive	7.2±1.1	13.7±2.06	111.5±26.6	4.9±1.7
Autogrooming	30.8±6.1	169.0±29.6	210.7±52.9	583.5±61.7
Barking	140.0±11.2	149.1±22.1	355.9±38.8	34.7±4.7
Communicative signals	70.9±5.8	114.4±13.7	295.2±18.8	39.3±4.7
Defecating	0.4±0.1	0.8±0.2	0.6±0.1	0.4±0.1
Digging	0	7.2±1.8	44.9±8.1	0
Drinking	0.4±0.1	3.4±0.3	3.9±0.5	1.0±0.2
Hind legs	238.8±49.2	156.8±26.0	35.5±5.3	12.0±3.6
Lying	6406.1±233.2	7470.4±185.0	6222.7±214.3	9168.9±145.2
Olfactory exploration	299.7±14.4	487.1±53.7	654.7±37.2	198.0±16.3
Prolonged vocalisations	9.6±1.0	21.9±4.3	0	40.3±6.4
Resting	2110.9±215.4	4874.7±165.9	2565.2±221.1	7875.1±249.2
Shaking off	1.7±0.1	4.2±0.3	7.8±0.4	3.2±0.4
Sitting	577.0±88.9	574.6±77.1	209.5±35.0	146.1±20.8
Social interaction	33.3±6.6	67.0±10.5	11.6±7.2	11.8±3.4
Standing	1723.8±70.9	1605.9±95.1	2248.2±136.1	556.8±75.4
Stretching	2.1±0.4	3.7±0.2	2.9±0.3	4.5±0.7
Tail wagging	436.6±45.6	734.6±73.7	803.0±106.0	216.0±49.3
Trotting	63.5±4.7	64.5±6.7	111.3±11.2	5.1±0.8
Urinating	0.4±0.1	4.1±0.3	6.1±0.9	1.0±0.1
Visual exploration	4170.0±280.8	3798.0±199.5	5521.4±159.0	1539.3±138.2
Walking	435.5±31.4	510.1±34.2	898.2±92.8	132.5±18.7

T1 represents the first observational period where all dogs were group-housed in outdoor pens.
T2 represents the second observational period where dogs of the experimental group were pair-housed while the control group was left in the same outdoor pens.

(lying, standing, and visual exploration) was also influenced by the change in the confinement conditions (Figures 3 and 4a). Experimental dogs in pair housing spent significantly more time lying (CI T2-T1: U=4,504.9; L=1,387.5) and significantly less time standing (CI T2-T1: U=-755.6; L=-2,627.3) and showing visual exploration (CI T2-T1: U=-1,953.8; L=-6,010.4) compared to group-housing. After being transferred to the smaller enclosure, the duration of the expression of communicative signals (i.e. sniff dog, social look) by experimental dogs dropped on average by 86.7% (Table III). Despite this, the difference was not significant according to the post-hoc test applying Bonferroni correction (p=0.039).

ANOVA results highlighted a significant p-level for the olfactory exploration variable (Table II). Wilcoxon pair test detected a significant decrease between T1 and T2 in the time spent by the experimental group showing this behaviour (V=1, p= 0.015). An increase in the average time spent autogrooming was observed in both groups of dogs (Table III). However, post-hoc tests revealed that this increase was only significant for control dogs (V=44, p=0.008).

Other overall significant values emerged from ANOVA analysis, concerning digging, tail wagging, prolonged vocalizations, and alimentary behaviour. However, post-hoc comparison did not confirm these differences.

Cortisol comparison

Five saliva samples, out of a total of 102 collected during the two phases of the study, were discarded due to insufficient physiological material. Analysis was carried out on the remaining 97 samples. ANOVA test revealed that the two groups of dogs did not differ in cortisol levels at T1 (F=0.15, n.s.), but time had a significant effect (F=18.47, p<0.001). Figure 4 shows that there was a decrease in cortisol levels from T1 to T2, and that this change was consistent for both groups of dogs (interaction: F=0.01, n.s.).

Discussion

Rescue shelters should be temporary refuges for stray and abandoned dogs waiting to be re-homed. Unfortunately, adoption systems are often insufficient to overcome the large numbers of dogs entering shelters, while no-kill policies ensure that, if a dog fails to find a new home, it is likely to spend the rest of its life in shelter housing. In the Italian context there are currently around 150,000 shelter dogs 41% of which are estimated to be adult dogs (over 4 years old) with almost no

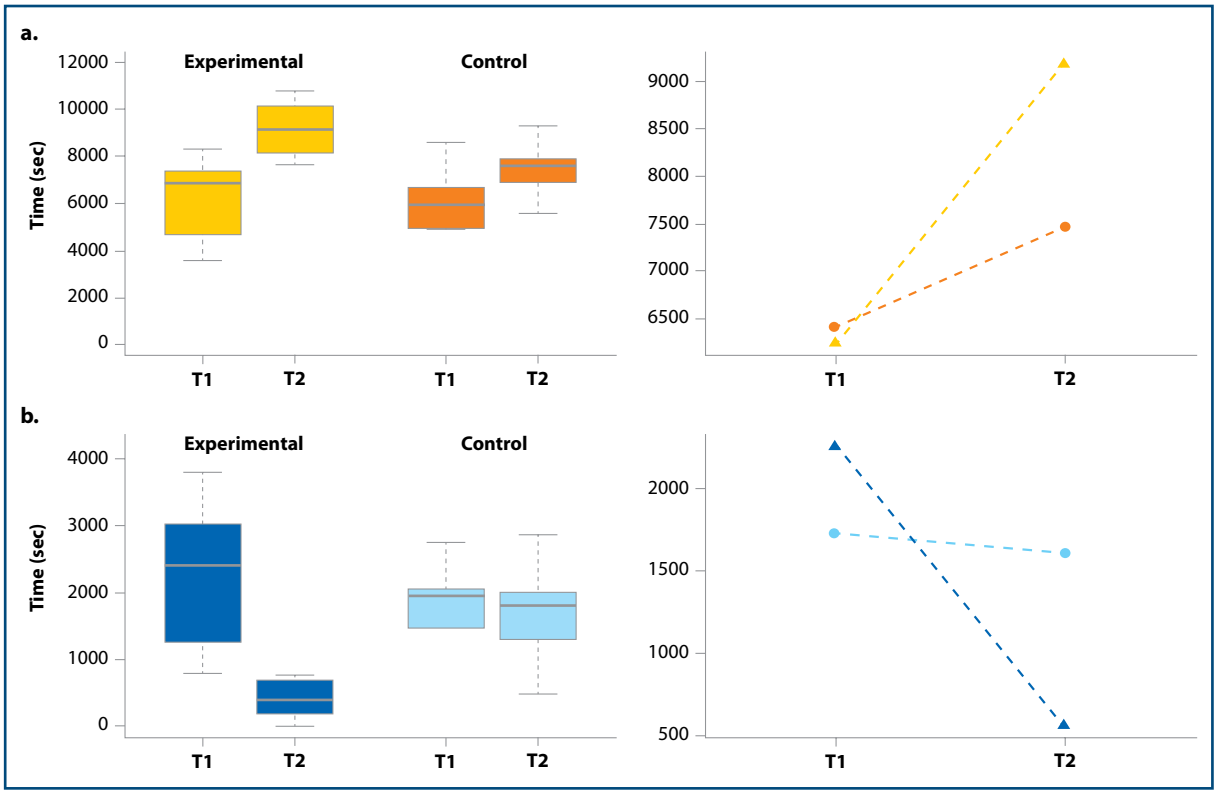

Figure 3. *Behavioural variations.* For the behavioural variables (a) lying and (b) standing, box-plots represent changes in the duration of the behaviour between the two phases of the study (T1 and T2) for both experimental and control dogs (within-group comparisons); interaction plots represent the direction of the behaviour (increasing or decreasing dashed lines) from T1 to T2 for both experimental (solid triangles) and control (solid circles) group.

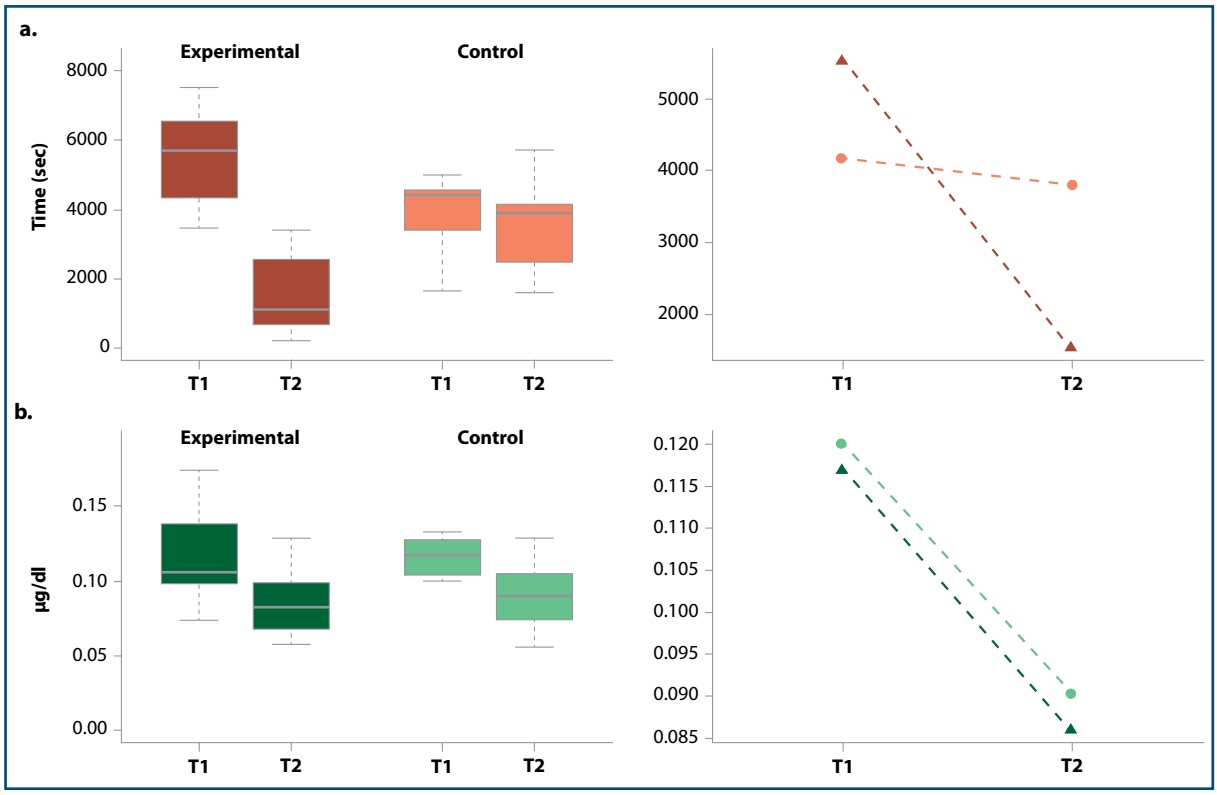

Figure 4. *Behavioural and cortisol variations.* For the behavioural variable (a) visual exploration and for the (b)cortisol concentration levels, box-plots represent changes between the two phases of the study (T1 and T2) for both experimental and control dogs (within-group comparisons); interaction plots represent the direction of the behaviour or cortisol concentration (increasing or decreasing dashed lines) from T1 to T2 for both experimental (solid triangles) and control (solid circles) group.

chance of being adopted (unpublished data). In this situation, housing conditions (i.e. space provided, environmental and social stimulation) may have a considerable impact on canine welfare. In this paper, the potential welfare effects of two different housing conditions on long-term shelter dogs were examined.

When dogs were housed in groups they spent most of the observed time inactive, and activity levels (6.5%) in general were lower than those recorded in previous studies. For example, Hubrecht and colleagues (15), found that shelter dogs housed in large outdoor enclosures (744 m^2) spent 23.5% of their time active, while laboratory group-housed dogs in smaller indoor pens (6.7 m^2) spent 19.1%. The age of the subjects may help to explain some of these differences: in the current study animals were older adults (from 5 to 9 years old), whereas in Hubrecht's (15) study subjects were younger (mean age 1.7 years old). Since age and time spent in shelters seems to affect activity levels of dogs (30), these elements should be taken into consideration when determining confinement strategies for dogs. Dogs in the current study spent little time in locomotor activities, and trotting was almost absent when housed in the smaller enclosures compared to the larger ones. These results concur with those of previous studies, showing decrease in activity and locomotion due to social and spatial restriction (11, 15). Other authors (6, 13) found an increase in locomotor activity in more austere housing conditions. This increase was associated with high stress levels, underlining the fact that activity *per se* is not necessarily a good indicator of welfare. The quality of activity may be important in this respect. Stereotypic locomotor activities are usually a sign of chronic stress and poor welfare conditions when associated with long-term housing (17). Stereotypies were described in the active repetitive category of the ethogram. Repetitive activities are not always a direct reflection of poor welfare, but they might be part of a strategy to cope psychologically with stressful conditions (12, 13). In the present study active repetitive behaviours (i.e. pacing and circling) were shown sporadically (0.3% of total observation time) by some individuals mainly in group-housing, probably in response to moments of high arousal caused by external stimuli.

Inactive behaviour differed between housing conditions: dogs housed in pairs, spent more time in a lying position while dogs housed in groups spent more time in a standing posture. The standing posture was more advantageous in the outdoor enclosures since the concrete wall around the

perimeter did not allow visibility of the external environment from a recumbent position. Moreover, dogs in the group-housing condition spent more time active, walking or trotting, increasing the time spent standing rather than lying down. Although it wasn't always possible to see if a dog's eyes were open or closed, when dogs were lying with their head down (recorded as resting behaviour), it was likely that subjects were either sleeping or resting. There is evidence that a return to normal sleep patterns in different species is an indicator of the animal's adaptation to a new environmental situation (24). Hetts and colleagues (11) found that subjects confined in more austere conditions (i.e. socially isolated) slept less. We did not record dogs' sleeping patterns, but it was observed that subjects in the pair housing condition spent longer time resting (on average 38.1% more) compared to when they were group-housed. Poor environment could inhibit most of the animal's activity leading to an impairment of its welfare. However, the same trend emerged also for the dogs in the control group, and no effect of housing condition was detected in ANOVA. Therefore this variation cannot be considered on its own an indicator of impaired welfare. In future research, accurate physiological measures of dogs' sleep patterns may provide a reliable tool for assessing their rate of adaptation to new or different housing conditions.

An increase in autogrooming is usually observed as a consequence of social and spatial restriction (4). Although the current findings detected some variation in this behaviour, this was not related to the change in housing. Previous studies have also observed dogs engaging in more self-grooming activity in association with decreased stress-related behaviour, allertness and attention-seeking, perhaps reflecting improved welfare (13, 23). In the current study, only a significant effect of time was observed in the control group. The increase observed in resting time could suggest that these variations were related to habituation and lower arousal of dogs.

When assessing animal welfare, attention is usually focused on stress indicators and negative emotional states (32). However, good welfare is also reflected by positive emotional states: play and affiliative behaviour, for example, are often considered indicators of good welfare (9). Although social behaviour was shown for only 0.3% of the total observation time, the description of the type of social interaction and to whom it was directed was informative of the social dynamics of group-housed dogs in confinement. When interacting with conspecifics, subjects housed in groups spent most of their social interaction time playing. Social play was shown mainly toward opposite sex pen-mates. Agonistic behaviours (threat, rigid/high posture) were rare, and when they occurred they were mainly directed toward same sex pen-mates. Although pairs to be transferred into smaller enclosures were not chosen randomly, these results tend to support the choice of matching opposite sex and compatible animals to avoid management and aggression problems. In the pair housing condition, social interactions were always positive (play and amicable) while agonistic behaviour was never recorded. An appropriate pen-mate in a confinement situation may help both animals to cope with the new environment, but further research is needed to determine the value of this role.

As highlighted in previous works, the presence of other conspecifics, and of an enriched environment, can elicit the expression of natural behaviour (22). Group confinement offered more social and environmental stimuli (e.g. soil, furniture, trees) compared to pair housing, and as expected, in this situation dogs performed more exploratory behaviours (visual and olfactory). Overall statistical comparisons and mean values showed that dogs were more motivated to express behaviours such as digging the ground, giving communicative signals, and tail wagging when housed in the outdoor pens compared to pair housing. However, these differencese were not confirmed by post-hoc analysis; a larger data collection could help to clarify these results.

Saliva cortisol is considered a valid measure for the assessment of acute, but also chronic, stress in dogs (2, 5), although high concentrations are also produced in response to moments of sustained arousal (13). The current findings detected a significant decrease in cortisol levels, between the first and the second data collection periods for both groups of dogs, that was independent of the type of confinement. Although cortisol levels could have been affected by food anticipation, arrival of kennel staff, or physical handling, we are confident that saliva sampling was carried out by shelter veterinarians in a highly standardised fashion at both time points and, therefore, sampling is unlikely to have been the source of this difference. Looking more in detail at basal salivary cortisol levels reported in other studies, we found that data are rather variable. Beerda *et al.* (5) recorded an average basal level during outdoor group housing of 0.08 ± 0.01 µg/dl with significant variations between morning hours (before 10.00 h) and the rest of the day. In another study (3) basal level was on average 0.22 µg/dl and sampling was carried out after 10.00 h. Horvath *et al.* (14) recorded an average baseline of 0.12 ± 0.11 µg/dl during morning sampling and 0.07 ± 0.07 µg/dl during afternoon sampling. In the present study, cortisol level was on average 0.12 ± 0.002 during T1 and 0.09 ± 0.001 during T2. We could conclude that external

factors (e.g. seasonal variations, environmental conditions not recorded as part of the study) may have affected this physiological change, but that overall values remain within basal levels of saliva cortisol concentration recorded for this species. More frequent sampling of cortisol levels (see for example procedure in (5) might have provided a more informative adaptation curve to the housing conditions.

In terms of practicality, pair housing enclosures are more functional: animals can be managed more easily, there is a greater degree of control over sanitation and health, and the risks of agonistic interactions between pen-mates are reduced (25, 31). However, careful management and monitoring of group housing facilities can reduce most of the risks associated with this housing system. In the present study, no severe attacks occurred between group-housed dogs, and clinical data revealed no increased prevalence of health problems when compared with pair housing.

A general decrease in most activities (e.g. locomotor, social, and exploratory) was recorded when dogs were transferred from the group to pair housing condition, confirming that spatial restriction and partial social deprivation can increase inactivity of adult long-term shelter dogs. Nevertheless, it should be noted that, in the present sudy, pair-housed dogs had daily access to outdoor runs, and the behaviour expressed during that time was not recorded. It is possible that running and playing during exercise periods may have reduced the desire for exploratory, social or locomotor activities when in kennel. Although significant variations in behaviour were associated with the different confinement conditions, there were no other evident signs that one form of confinement reduced the welfare of these animals more than the other.

Identifying a life-long confinement condition for shelter dogs that is both economically sustainable and ethically acceptable is a considerable challenge. Many factors concur to help a dog in the coping process when a new environment or challenging situation is presented. The results of this study provide further insights into the effects of confinement on long-term shelter dogs, focusing on the reactions of adult animals that had experienced kennelling for 4 years or more. They also confirm that behavioural parameters are sensitive indicators of dog responses to new housing environments. Current management procedures and further investigation in this area should focus on individual variability and on the identification of standardised animal-based measures (e.g. health, physical condition, behaviour, etc.) that can provide a clear welfare assessment system for shelter dogs.

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