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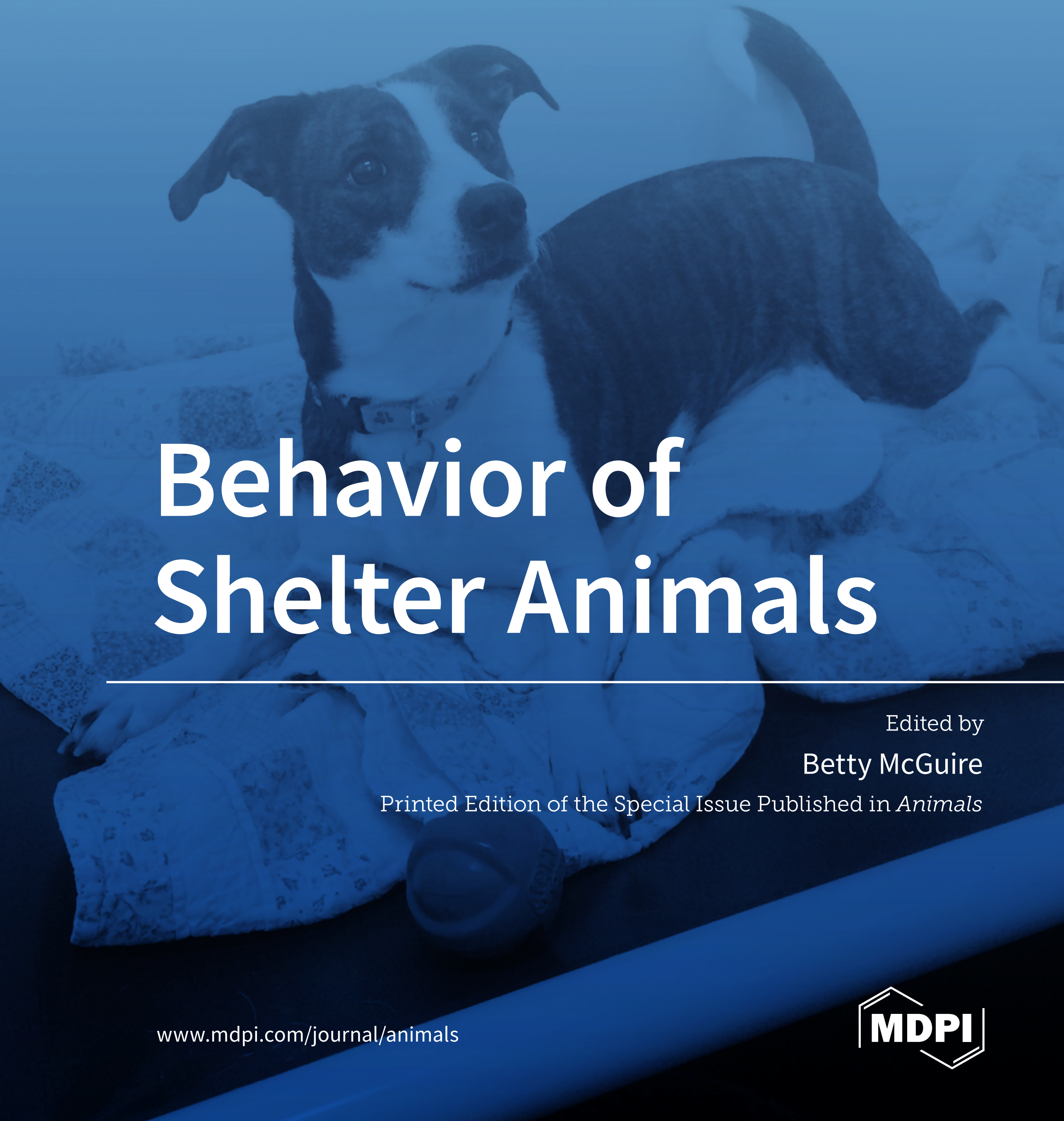

Behavior of Shelter Animals

Edited by

Betty McGuire

Printed Edition of the Special Issue Published in *Animals*

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Editor

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This is a reprint of articles from the Special Issue published online in the open access journal *Animals* (ISSN 2076-2615) (available at: https://www.mdpi.com/journal/animals/special_issues/Behavior_of_Shelter_Animals).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-0872-6 (Hbk)
ISBN 978-3-0365-0873-3 (PDF)

Cover image courtesy of Betty McGuire.

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About the Editor

Betty McGuire is a Senior Lecturer in the Department of Ecology and Evolutionary Biology at Cornell University, Ithaca, NY, USA. She received her undergraduate training at Pennsylvania State University, and completed her doctoral degree at the University of Massachusetts Amherst. For many years, she studied social behavior, reproduction, and ecology of small mammals, including voles, rats, and shrews, using both laboratory and field approaches. More recently, her research has focused on the behavior of shelter dogs, particularly scent-marking and food-guarding behaviors, and human–dog interactions. She has been recognized for training research undergraduates, with whom she frequently publishes. Having co-authored textbooks in human biology and animal behavior, she is currently collaborating on a vertebrate biology text. She is an Editorial Board Member for *Animals*.

Preface to "Behavior of Shelter Animals"

Each year animal shelters receive, care for, and rehome dogs, cats, and other companion animals. Even under the best of circumstances, however, shelters can be challenging environments for the animals admitted. Often, animals are housed in close proximity in unfamiliar locations, sometimes with limited space and, typically, high noise levels. Additionally, shelter animals interact with many unfamiliar people and experience a lack of predictability and control in their daily lives. Some animals enter shelters with behavioral problems, while others may develop problematic behaviors as a result of shelter experiences. Collaborative research programs between academic institutions and animal shelters have provided new data on the welfare of shelter animals. Data are now available on how shelter environments impact the welfare of resident animals, and the effectiveness of shelter programs in reducing stress and providing enrichment. Studies also address whether shelter behavioral evaluations are useful tools for predicting behavior and assessing adoptability. This Special Issue focuses on the behavior of dogs and cats while they are either in shelter environments or adoptive homes. The eleven papers focus on stress and behaviors associated with stress; the effectiveness of shelter enrichment programs in reducing stress; the usefulness of shelter behavioral evaluations in predicting behavior in other contexts; and human–dog interactions. The goal is to provide information that will inform shelter programs and policies, and thereby improve the welfare of shelter animals.

Betty McGuire
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Review

Psychological Stress, Its Reduction, and Long-Term Consequences: What Studies with Laboratory Animals Might Teach Us about Life in the Dog Shelter

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Received: 12 October 2020; Accepted: 4 November 2020; Published: 7 November 2020

Simple Summary: Experiments in laboratory animals have provided the basis for studies of stress, its reduction, and its long-term consequences in shelter dogs. Stressors often used in laboratory experiments, such as uncontrollable noise and novelty, are also inherent in shelters where they produce similar physiological reactions, including elevations of circulating levels of glucocorticoid stress hormones. We review how experiments demonstrating a social partner can reduce glucocorticoid responses in the laboratory guided studies showing that human interaction can have similar positive effects on shelter dogs. We also describe recent work in which human interaction in a calming environment reduced aggressive responses of fearful shelter dogs in a temperament test used to determine suitability for adoption. Finally, we present evidence from the laboratory that stress can produce long-term effects on behavior (e.g., reduced socio-positive behavior) that may be due to glucocorticoids or other factors, and which may not occur until long after initial stress exposure. We suggest that the possibility of similar effects occurring in shelter dogs is a question deserving further study.

Abstract: There is a long history of laboratory studies of the physiological and behavioral effects of stress, its reduction, and the later psychological and behavioral consequences of unmitigated stress responses. Many of the stressors employed in these studies approximate the experience of dogs confined in an animal shelter. We review how the laboratory literature has guided our own work in describing the reactions of dogs to shelter housing and in helping formulate means of reducing their stress responses. Consistent with the social buffering literature in other species, human interaction has emerged as a key ingredient in moderating glucocorticoid stress responses of shelter dogs. We discuss variables that appear critical for effective use of human interaction procedures in the shelter as well as potential neural mechanisms underlying the glucocorticoid-reducing effect. We also describe recent studies in which enrichment centered on human interaction has been found to reduce aggressive responses in a temperament test used to determine suitability for adoption. Finally, we suggest that a critical aspect of the laboratory stress literature that has been underappreciated in studying shelter dogs is evidence for long-term behavioral consequences—often mediated by glucocorticoids—that may not become apparent until well after initial stress exposure.

Keywords: shelter dog; stress; hypothalamic–pituitary–adrenal; cortisol; glucocorticoid; social buffering; enrichment; early-life stress; individual differences; animal welfare

1. Introduction

There is a vast literature documenting the consequences of psychological stress in laboratory animals. Many of these studies are translational in that they use rats, mice, or other species as models to provide insight into how stress exposure in humans can impair emotional wellbeing and promote the development of mental as well as physical disorders. A common procedure is to expose animals to one or more stressors that are uncontrollable and often unpredictable. These may include isolation, noise, separation from companions, and confinement or restraint. These manipulations are often found to increase behaviors thought to share processes underlying human psychopathology such as anxiety, depression, or post-traumatic stress disorder, as well as to reduce some cognitive abilities [1–7]. Even limiting the amount of nesting material provided to lactating female rats, which then disrupts the treatment the females provide their litters, has multiple negative outcomes on the offspring at later ages [8].

Now consider the experience of a dog (or lactating bitch and pups) that suddenly find themselves confined in an animal shelter. While housing conditions vary substantially across shelters, stressors like those that contribute to serious deleterious consequences for laboratory animals are still unavoidably inherent to some degree across shelter environments. From the perspective of a laboratory that has split its efforts between basic and translational studies of psychological stress with laboratory animals and studies to measure and reduce stress and its effects in shelter dogs, the similarities between shelter conditions and laboratory paradigms designed to induce adverse emotional and behavioral outcomes are impossible to dismiss. Both shelter housing and laboratory stress paradigms induce physiological stress responses, perhaps most importantly, though not exclusively, activation of the hypothalamic–pituitary–adrenal (HPA) axis, e.g., [6,9]. Not only can HPA activation be taken as a sign of stress in shelter dogs, but the repeated or prolonged activation of the HPA system, and particularly of the glucocorticoid hormones that are the endpoint of the HPA response, may serve as a mechanism underlying many of the long-term effects of prior psychological stress [10,11].

Fortunately, basic research has also suggested means by which the impact of stressors can be minimized. Prominent among these is the process referred to as "social buffering", or the ability of a companion to moderate physiological stress responses. Social buffering is both a basic phenomenon we have studied over the years as well as a strategy we have used to attempt to improve welfare and preclude later adverse consequences of shelter confinement. In the remaining portions of this paper, we will view our work with dogs and related studies by other investigators within the broader context of basic and translational research on stress and social buffering.

2. HPA Responses and Social Buffering in the Shelter

2.1. How Stressors Like Those in the Shelter Affect HPA Activity

It has long been known that the HPA system is especially sensitive to psychogenic stressors, that is stressors that pose no actual physical harm [12]. Events that are novel, unpredictable, or out of an individual's control indicate that the current situation is not fully understood and can suggest that harm is likely forthcoming. How this perception activates the HPA axis is complex and still not fully understood, see [13]. However, in brief, cortical regions detecting psychogenic stressors (e.g., medial prefrontal cortex—mPFC) together with associated limbic and brain stem regions including amygdala nuclei, portions of the bed nucleus of the stria terminalis and hippocampus, as well as the nucleus of the solitary tract, relay neural signals to the region of the paraventricular nucleus of the hypothalamus (PVN). Here, they excite (or block inhibition of) neurons containing arginine vasopressin and especially corticotropin-releasing hormone (CRH), which are then released into the hypothalamic–hypophyseal portal system. Upon reaching the anterior pituitary, these peptides bind receptors to spur release of adrenocorticotropin hormone (ACTH) into the general circulation. In several minutes, ACTH reaches the adrenal cortex to trigger synthesis and release of glucocorticoid hormones, notably cortisol and corticosterone. Glucocorticoids bind with Type 1 (MR) and (particularly during stress) Type 2 (GR) receptors throughout the body, including the brain, to induce multitudinous actions on target tissues. Activation of GR receptors in the hippocampus and elsewhere also mediates negative feedback to suppress HPA activation as the stressor passes. In addition, just as some cortico-limbic inputs excite the PVN, others (from, e.g., prefrontal cortex (PFC), hippocampus) actively suppress activity of CRH and vasopressin neurons. In principle then, there are many potential routes by which social buffering can inhibit HPA activation, either by inhibiting excitatory inputs or by exciting inhibitory inputs to the PVN. Investigation of stimuli that induce HPA activation began with Selye [14]. Although social buffering is now a widely accepted concept across a number of species [15–17], investigation of the ability of social partners to reduce stress developed decades after Selye's original work.

2.2. Brief History of Social Buffering in Nonhuman Primates

The first publication addressing social buffering of HPA activity appears to have been Hill et al.'s [18] study of surrogate-reared Old-World rhesus macaque monkeys during the first year of life. When these infants were removed from the home cage and placed into a novel cage for an hour, they had higher plasma cortisol concentrations when alone than when accompanied by their rearing surrogate. That is, the presence of the artificial mother appeared to buffer the response of the HPA axis to disturbance and exposure to novelty. If a surrogate mother could buffer cortisol responses of infants, then one would certainly expect an actual mother to be effective as well. Indeed, later studies confirmed that cortisol elevations of rhesus infants that had been handled or handled and exposed to novel surroundings were significantly reduced when in the presence of their biological mother as compared to when they were alone [19,20]. This effect of the mother's presence was not restricted to just rhesus or other Old World monkeys. In New World squirrel monkeys, both infants [21] as well as their mothers [22] had higher plasma cortisol concentrations following disturbance if tested without the other member of the dyad than if mother and infant remained together. On the other hand, not all affiliative social companions appeared capable of buffering HPA responses. In squirrel monkey troops, some of the most amicable interactions occur among juvenile peers which avidly engage in play, and in adult females which spend much time in close proximity with one another. Yet, juveniles did not reduce the cortisol response of familiar juveniles, and adult females did not reduce the cortisol response of familiar, even preferred, adult females [23,24].

Together these results suggested there was something special about the mother–infant relationship that enabled the partners to buffer each other's stress responses. The most obvious possibility was simply that the degree of social connection between partners was critical; specifically, that the intensity of the relationship between mother and infant was greater than the affiliation among other friendly partners. Notably, the data did not allow one to exclude other unspecified attributes that might be characteristic of only mothers and infants. However, a second New World primate, the titi monkey, provided some insight. Unlike squirrel monkeys, titi monkeys are monogamous, with adults typically spending long periods of time in quiet contact with their pair-mate. Additionally, whereas young squirrel monkeys ride on the back of their mothers, and never their fathers, titi infants ride on the backs of both parents, especially the father [25]. Moreover, in preference tests, the mother as well as the father more often chose to be near each other than to be near their infant, and infants, in turn, preferred being near their father rather than their mother [25]. This very unusual pattern of familial preferences (Table 1) permitted experimental dissociation of social attraction or relationship intensity from other characteristics specific to the mother–infant relationship. For these experiments, entire family groups were captured and then placed back into the home cage either alone or with a specific partner(s). While all family members showed a pronounced elevation of cortisol levels following capture when returned to the home cage alone, adult males and females showed significant reductions in cortisol only when returned with each other, i.e., their adult pair-mate, and not when returned with their infant [25]. Infants displayed a reduction in cortisol concentrations when returned only with their mothers, and a further significant reduction to the level seen in undisturbed infants when returned only with their fathers [26]. Thus, the likelihood of social buffering occurring corresponded perfectly to the strength of the social attraction between the partners (Table 1). Since the time these early experiments were conducted there have been numerous demonstrations of buffering in other species, some of which involve partners with no prior social relationship whatsoever, e.g., pairs of unfamiliar adult male rats; [27,28]. Yet, the intensity of the positive relationship between partners remains the best predictor of whether an individual will buffer the glucocorticoid response of a companion [17,28,29].

Table 1. Relative preference for, and buffering by, specific titi monkey family members.

Subject	Preference for	Buffering by
Mother	Father > Infant	Father yes; Infant no
Father	Mother > Infant	Mother yes: Infant no
Infant	Father > Mother	Both yes, Father > Mother

Data from Mendoza and Mason [25] and Hoffman et al. [26].

2.3. Social Buffering of HPA Responses in Dogs

With this general principle of the importance of the strength of the relationship in mind, our first study of social buffering in dogs compared the ability of a long-term conspecific kennelmate and the human caretaker in reducing glucocorticoid elevations in adult dogs. The dogs (7–9 years old), which had been maintained in littermate pairs continuously since ~8 weeks of age, were examined in a novel environment either alone, with their kennelmate, or with their life-long human caretaker. Although we had anticipated that the caretaker might have some effect, we were nevertheless struck by the differential influence of the two companions. Whereas, the passive presence of a dog's human caretaker reduced the plasma glucocorticoid elevation to the novel environment, the dog's sibling and long-term kennelmate was without effect [30]. This finding certainly seemed to speak to the affinity that dogs have evolved for humans over thousands of generations. In addition, the results documented a tangible effect of human presence on the stress physiology of dogs that might then be leveraged to reduce stress and, therefore, improve welfare of dogs confined in shelters.

The first step, however, was to determine how a stay in an animal shelter affected glucocorticoid levels. The initial experiments confirmed what was expected: The psychological stressors encountered upon entering an animal shelter powerfully activated the HPA axis. Circulating cortisol levels were nearly three times as high as those of pet dogs sampled in their home and remained so for 3 days before gradually waning [31]. Subsequent work indicated that cortisol concentrations did not decline to levels like those of pet dogs sampled under resting conditions until sometime after 10 days [9]. Work from other laboratories has generally found cortisol levels to be elevated at least about this length of time or longer [32–37], though variability among individual dogs is common, e.g., [35,37].

In our first attempt to buffer this response [31], dogs were taken from their kennels and had a blood sample collected. They were either petted or returned to their kennel for 20 min, and then a second blood sample was collected to estimate the effect of the petting. Initial results were disappointing, showing that petting had no overall effect. However, when in a follow-up analysis dogs in the petting group were partitioned based on the sex of the individual doing the petting, those dogs petted by a man showed an increase from the first to the second blood sample, while those petted by a woman showed no change across samples [31]. It appeared, therefore, that interaction with a woman prevented the sampling procedure required to collect the first blood sample from elevating cortisol levels obtained in the second sample, but interaction with a man had no buffering effect. This differential influence was determined to depend on the nature of the petting administered by men versus women. When men were trained to pet in the more soothing and quiet manner characteristic of the women, the men were as effective as the women in preventing the initial blood sampling procedure from elevating cortisol levels in the second sample [38]. This was the second lesson we learned about social buffering in dogs: Not only are humans particularly effective in buffering the glucocorticoid response of dogs, but seemingly subtle differences in how the human interacts with the dog can determine whether or not the HPA response is reduced. Still, however, we were only effective in reducing the response of the dogs to an additional minor stressor—the initial blood sampling—rather than mitigating the response to the shelter itself.

After a series of further unsuccessful attempts to reduce the glucocorticoid response to shelter housing, we came upon what appeared to be a third lesson about social buffering in shelter dogs; that is, in addition to how you interact, the location where you interact also is critical. In this study, we were able to secure a quiet, secluded room in the rear of the shelter that was farther away from the commotion of the housing and public areas than in any of our previous studies. Here, we found that it did not matter if a person petted, played with, or passively sat near the dogs. In all cases, plasma cortisol levels were reliably reduced when a person (woman) was present [39]. If the dog was simply isolated in the secluded room, there was no reduction in cortisol levels. In other words, the secluded room alone had no effect, but a person in the room, even sitting quietly with the dog, suppressed the cortisol response to shelter housing. Ours was not the first laboratory to find human interaction to reduce the cortisol response to shelter conditions. During the time of our unsuccessful attempts, two other laboratories had found human interaction to such buffering effects [33,36]. The forms of human interaction in these studies were more complex, involving a variety of activities in different locations, but both included some time outdoors, removed from the commotion of the shelter. In all, these studies document how social buffering in the form of human interaction can readily mitigate the physiological stress response imposed by inherent features of shelter housing. Yet, this strategy has clear limits in that the effect is temporary. When dogs are returned to their kennel, cortisol levels elevate to their pre-interaction levels within an hour [40]. A recent approach that greatly prolongs the beneficial effect of interaction is to foster shelter dogs to a private home for a night or two. In shelters in which this procedure is implemented, urinary cortisol levels are reduced throughout the fostering period, though here too cortisol concentrations elevate to pre-interaction levels when returned to the shelter environment [41].

2.4. Mechanism of Social Buffering of HPA Responses

Oxytocin appears to be the most likely mediator of social buffering of dogs' HPA response by human interaction. Release of oxytocin both stimulates, and is stimulated by, engaging social behaviors such as gentle touch and prolonged gazing [42,43]. Furthermore, while oxytocin's influence is much more complex than simply enhancing sociability, there is a wealth of data on how oxytocin can promote socio-positive or bonding-related behaviors [43–45], including those of dogs with humans or other dogs [46,47]. These effects often may be due to oxytocin reducing anxiety or wariness to engage in social activity [48]. Oxytocin can also reduce HPA activity more directly by, for instance, inhibiting excitatory input to the PVN or via inhibitory GABA interneurons connecting oxytocin neurons to corticotropin releasing hormone cells in the PVN [43,49]. Indeed, in the monogamous prairie vole, the ability of an adult male to buffer the HPA response of his female partner is inhibited by pharmacologically blocking oxytocin receptors in the PVN [50] (Figure 1, top).

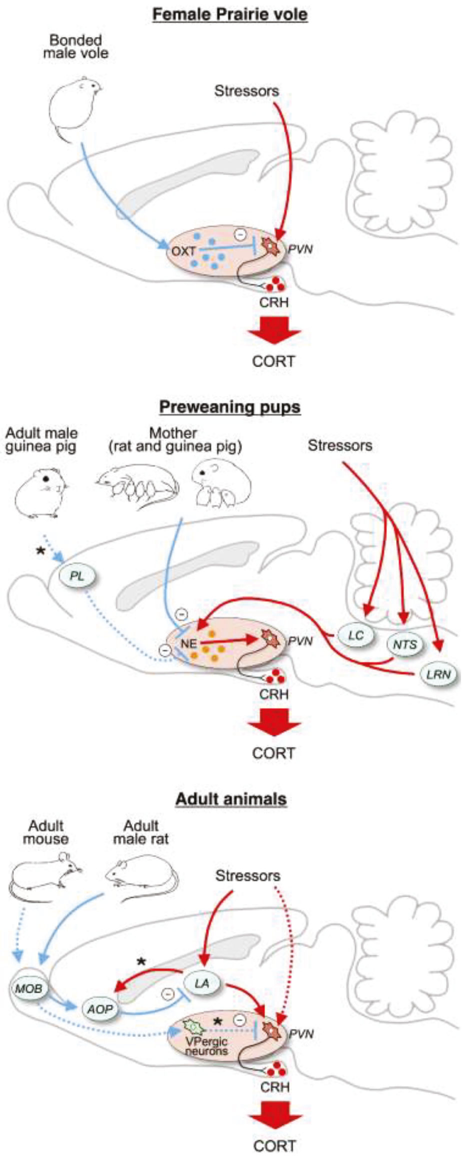

Figure 1. Summary of findings regarding neural circuits underlying social buffering. (**Top**) Presumed neural mechanisms underlying social buffering by mates in female prairie voles. (**Middle**) Possible neural mechanisms underlying social buffering in rodent pups. (**Bottom**) Possible neural mechanisms underlying social buffering by adult conspecifics other than mother and mates. Solid and dashed lines represent pathways proposed in each experimental model. However, the pathways do not necessarily imply direct anatomical connections. Hypothetical buffering pathways are marked by asterisks. AOP, posterior complex of the anterior olfactory nucleus; CORT, cortisol or corticosterone; CRH, corticotropic releasing hormone; LA, lateral amygdala; LRN, lateral reticular nucleus; MOB, main olfactory bulb; NE, norepinephrine; NTS, nucleus of the solitary tract; OXT, oxytocin; PI, prelimbic cortex; PVN, paraventricular nucleus of the hypothalamus; VP, vasopressin. Figure redrawn from [28].

However, studies in laboratory rodents have identified a number of other potential mediators that could act independent of, or in conjunction with, oxytocin. For lactating rats, evidence indicates that the mother's buffering of HPA activity of her pups is due to inhibition of excitatory noradrenergic input to the PVN from brainstem [51]. In guinea pig pups, both the mother and an unfamiliar male can buffer HPA responses and do so through different mechanisms. The presence of the mother, even when anesthetized, reduces pups' cortisol response during exposure to novelty [52] quite possibly again by inhibiting noradrenergic input [53]. In contrast, adult males reduce pups' cortisol response in a novel environment when the male is awake and actively engaging the pup, but not, unlike the case for the mother, when then male is anesthetized [54]. The active male increases excitation in the pup's PFC, which may activate known inhibitory connections to the PVN [55] (Figure 1, middle). Finally, in adult rats and mice, the ability of companions to reduce HPA responses appears due to the companion activating olfactory connections to the amygdala or directly to the PVN [56–58] (Figure 1, bottom). Thus, at this point it would be premature to conclude that oxytocin mediates the reduction in HPA activity in dogs interacting with humans. Furthermore, as the guinea pig data above suggest, it is even possible that different forms of human interaction (e.g., soothing touch, play) suppress HPA activity through different pathways.

3. Stress Effects on Behavior

3.1. The Challenge of Detecting Behavioral Consequences of Stress in Shelter Dogs

Stress in shelters is of concern in large part because of the possibility it will increase readily apparent behaviors such as stereotypy, hyperactivity, fearful behaviors, and continual barking that will either discourage adoption or prompt recent adopters to return their dog to the shelter, e.g., [59,60]. However, the stress endured by shelter dogs may have less conspicuous effects on behavior that are more difficult to verify experimentally. Major obstacles to the necessary experiments include the impossibility of achieving random assignment, undesirability of invasive procedures, need to accommodate shelter procedures in experimental designs, the hugely divergent past experiences of dogs who end up in shelters, and the difficulty of distinguishing effects of stress as opposed to other aspects of the shelter environment. However, while effects that are unequivocally due to stress are difficult to document, the existing literature in laboratory animals clearly points to a variety of ways that psychological stressors like those experienced in the shelter may both reduce desirable behavior and lead to later emerging behavioral and emotional repercussions, at least for some dogs. To take some examples from the broader literature, juvenile rats exposed to social instability (15 days of repeated periods of isolation followed by housing with unfamiliar conspecifics) showed lower levels of social behavior, both immediately after treatment and in adulthood [61,62]. In another study, periods of maternal separation prior to weaning led to inhibited social behavior and abnormal PFC development in juvenile female rats, whereas for males these effects did not appear until adolescence [63]. Adult mice housed individually for 8 weeks performed more poorly on several measures of cognition than did mice housed in groups [64], and rhesus macaques, whose mothers had been exposed to unpredictable noise bursts during pregnancy, scored worse than controls on measures of attention and neuromotor maturation during the first 3 weeks of life [65] and played less in adulthood [66]. Findings such as these raise concern that desirable traits, such as sociality and cognition, may be compromised as a result of the shelter experience.

Other potential long-term consequences may be more insidious. Much of the current surge in laboratory studies of lasting biobehavioral effects of stress has been driven by the increasing realization that stress at a particular life stage (primarily but not exclusively during prenatal, early postnatal, or adolescent phases) can alter the course of later development, which in humans leads to increased vulnerability to a variety of mental and physical disorders [67]. These include increased susceptibility to major depression, anxiety disorders, post-traumatic stress disorder, and schizophrenia [11,68–70]. Importantly, these outcomes may not emerge in humans until years later, often after a mental or physical challenge at the later age, a pattern commonly referred to as the "2-hit" model [68,70,71] because a second major stressor or "hit" is required to unmask the long-term effect. The first hit is thought to sensitize some aspect of underlying stress physiology so that the second stressor produces a larger, more prolonged, and/or unregulated stress response that then gives rise to the mental disturbance. These effects can be modeled in laboratory animals. For example, exposing adolescent mice to 12 days of unpredictable stress increased measures of anxiety-like and depressive-like behavior when the mice were placed in stressful situations 30 days later [72]. Similarly, two 3-h periods of isolation near the time of weaning increased depressive-like behavior of guinea pigs when isolated again in early adolescence [5]. If such a model applies to some extent to dogs confined in animal shelters, it implies that behavioral and welfare consequences of the stress of shelter housing may not occur until exposed to a subsequent stressor that then engages the now sensitized stress physiology, perhaps well after the dog has been adopted. One piece of evidence supporting such concern derives from an early study in our laboratory in which dogs were exposed to a highly novel stressful situation before and after 8 weeks of shelter housing. Whereas dogs that received regular sessions of human interaction throughout the 8-week period showed comparable plasma cortisol responses to the two stress sessions, those deprived of the supplemental interaction showed a significantly greater cortisol response to the second stressor [34] (Figure 2).

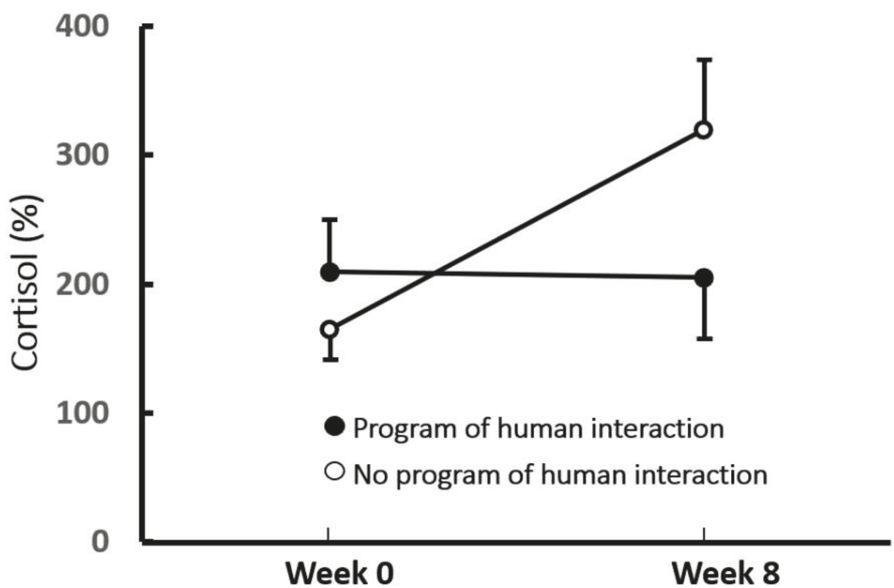

Figure 2. Mean per cent increase in plasma cortisol levels in response to a highly novel situation prior to and following an 8-week period in which shelter dogs either received or did not receive supplementary human interaction (5 weekly, 20-min sessions). Vertical lines represent standard errors of the means. Dogs receiving standard care, but not dogs receiving supplemental human interaction, exhibited enhanced cortisol responsiveness over the 8-week period ($p < 0.05$). Figure redrawn from Hennessy et al. [34].

Glucocorticoids are, in fact, one mediator of lasting behavioral effects of stress exposure, e.g., [73,74], including on social behavior [75]. In utero effects of stress exposure appear mediated by maternal glucocorticoids acting on the fetus [76,77]. Even glucocorticoids received through the mother's milk may influence social and cognitive development [78]. Another mediator of long-term effects is stress-induced neuroinflammatory signaling. Early-life stress upregulates central inflammatory activity in later life [79,80] and, in humans, increased inflammatory activity promotes development of stress-related disorders [81,82]. Seemingly analogous processes have been demonstrated in laboratory rodents [83,84]. Although stress in early life can affect a variety of brain regions, threat-related circuits [85] including connections between the mPFC, amygdala, and hypothalamus appear to be critical. Among the most robust effects of early-life stress is sensitization of cortico-amygdalar circuitry [86,87]. One way in which inflammatory signaling appears to promote human psychopathology is by enhancing amygdala activity, which then appears to further increase inflammation, creating a positive feedback loop promoting greater susceptibility to stress-related disorders [86,88]. Moreover, increased amygdala activity that escapes regulatory control by the PFC can then affect hypothalamic control of the HPA axis and sympathetic nervous system [89] to further promote development of stress-related pathologies [90–93].

One would not expect all dogs to be equally susceptible to the stress of shelter housing. To the extent that outcomes such as those outlined above pertain to shelter dogs, young dogs or the unborn fetuses of pregnant bitches would be most vulnerable. Further, as others have emphasized, e.g., [94], even in dogs of the same age, we should expect substantial individual differences in stress responsiveness and vulnerability. Due to a combination of experience and temperament, some dogs react much more strongly than others to admittance to a shelter. One form of enhanced reaction is aggression.

3.2. Reduction in Fear-Induced Aggression in the Shelter

Dogs exhibit a variety of initial reactions upon entering a shelter. While most show signs of fear, for some, the fear is extreme. These dogs may tremble, cower in the back of the kennel, and keep their tail tucked firmly between their legs. They may also show signs of fear-induced aggression [95,96], a situationally dependent form of aggression that occurs in some individuals when fear is high, and escape is thwarted. As this aggression only occurs when dogs are severely frightened, such dogs may be excellent candidates for adoption as a pet in a typical home, but in the shelter they are often in great peril. With shelters understandably concerned about the injuries and damage to the shelter's reputation as a source of quality pets that an aggressive dog might cause, preventing dangerous dogs from being adopted becomes a priority. Some form of a "temperament test" is often used for this purpose. The SAFER® (hereafter SAFER) is one such instrument. Though designed to be but one of several sources of information used to determine suitability for adoption [97], busy shelters may rely solely on the outcome of the test, or a modified version of it, to determine the fate of their confined dogs. If the test is administered a few days after entry to the shelter—before initial fear may have a chance to abate—dogs exhibiting fear-induced aggression are likely to fail and be euthanized rather than adopted.

Both our own work and that of others, e.g., [98,99], suggested that some form of human contact might help reduce the fear and aggression, and ultimately the euthanasia of such dogs. Accordingly, the second author initiated an enrichment program centered around responsive human interaction for fearfully aggressive dogs at a local shelter. The program appeared successful anecdotally and so prompted an experimental evaluation of its effectiveness [100]. This enrichment was provided in a secluded room as in our earlier work [40]. Dogs also had access to toys and were given small treats. In addition, oil of lavender was misted into the room and classical music was played softly in the background in light of the reported calming effect of these forms of stimulation [101,102]. Only dogs that exhibited signs of both a high level of fear and aggression were enrolled. Enrichment was conducted by the second author, a board-certified Associate Applied Animal Behavior Specialist with extensive experience working with shelter dogs. Dogs in a treatment group received the enrichment for 15 min, twice a day, for 5–7 days. Control dogs received normal shelter care. The day following the final treatment, or on the same average day in the shelter for controls, dogs were administered the modified version of the SAFER used by this shelter, administered by shelter staff as per shelter operating procedure. This version of the test assessed the dog's reaction to eye contact, sensitivity to touch, movement and sound during play, response to having its paw squeezed and having its food bowl manipulated during eating, and the presence of another dog. The staff were unaware that performance on the SAFER was an outcome that we measured in the study.

In an initial experiment, we found that just 10 of 30 fearful dogs in the control group passed the SAFER test, whereas 23 of 30 fearful dogs in the treatment condition passed, a difference that was highly significant [100]. These results were replicated in a second experiment in which only 2 of 16 fearful control dogs and 15 of 16 fearful dogs receiving our enrichment passed the SAFER. For comparison, we also included groups of non-fearful dogs, nearly all of which passed the SAFER regardless of whether they received enrichment or not [100] (Table 2). While we certainly do not encourage those without appropriate qualifications to work with shelter dogs exhibiting fear-induced aggression, we do believe these results are an example of enrichment centered around human interaction buffering meaningful behavioral effects of stress in the shelter environment. They also highlight the value of attending to individual differences in designing treatment strategies. In addition, they align with conclusions of others [103] advocating for the discontinuation of temperament tests for testing adoption suitability (as has been done in the shelter in which we conducted our work).

Table 2. Number of fearful enrichment and control dogs that passed and failed the SAFER test in the first experiment of Willen et al. [100].

	Pass	Fail	% Pass
Experiment 1			
Fearful			
Enriched***	23	7	77
Control	10	20	33
Experiment 2			
Fearful			
Enriched***	15	1	94
Control	2	14	12
Non-Fearful			
Enriched***	14	2	87
Control	15	1	94

*** differs from total fearful control dogs, $p < 0.001$.

4. Conclusions

Lasting behavioral consequences of stress exposure in translational laboratory experiments or in the dog shelter are rightfully considered to be negative outcomes if they serve as models of human suffering or pathology, or in the case of the shelter, reduce welfare or the likelihood of successful adoption. Yet many such outcomes appear to have derived from behaviors that were adaptive in natural environments. If stressful conditions experienced by a young animal, or by its mother prenatally, are an indication that conditions are likely to be stressful in that environment as the individual matures, it can be beneficial for that animal to alter its development to best address the expected future environment. Thus, behavioral plasticity, which can allow for changes in the developmental trajectory to better suit the predicted environment—whether plasticity is induced by glucocorticoids or by other means—will be subject to natural selection [104,105]. This notion of "predictive adaptive responses" is thought to describe a common evolutionary process [76,106–108]. In a stressful, competitive environment, behavioral traits such as reduced sociality, increased reactivity and aggression, and a re-focusing of cognition on skills relevant to basic survival at the expense of "higher level" skills might all be adaptive and, indeed, all have been documented to develop disproportionally following early stressful conditions [108–110]. While specific behavioral outcomes vary by species and situation, there is no reason to expect dogs to be exempt from these influences. We cannot be sure that the stress of shelter exposure or the glucocorticoid elevations or other stress-related physiological changes induced by stress encountered in the shelter are sufficient to produce such changes in behavior development. However, this remains a possibility, particularly for dogs that are especially sensitive to stressors.

The concept of predictive adaptive responses might afford a useful perspective from which to consider stress in the shelter and its outcomes in future studies. From a practical point of view, the chance that stressors encountered in the shelter may be shaping later behavior in unwanted ways reinforces continued efforts to reduce shelter stress, such as reviewed here, even if a later negative behavioral or welfare outcome cannot be documented at the present time.

Author Contributions: Conceptualization, M.B.H.; writing—original draft preparation, M.B.H.; writing—review and editing, R.M.W., P.A.S. All authors have read and agreed to the published version of the manuscript.

Funding: The writing of this review was supported by grant HD100825 from the National Institute of Child Health and Human Development.

Acknowledgments: The authors acknowledge the many colleagues and students participating in the experiments from our laboratory reviewed here. The continued support of the Montgomery County Animal Resource Center in Dayton Ohio is gratefully acknowledged.

Conflicts of Interest: The authors have no conflict of interest to declare.

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animals

Article

Investigating the Impact of Brief Outings on the Welfare of Dogs Living in US Shelters

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Academic Editor: Betty McGuire
Received: 8 February 2021; Accepted: 15 February 2021; Published: 19 February 2021

Simple Summary: Animal shelters can be stressful places for dogs to live. Social isolation is likely one component of the environment that contributes to poor welfare but spending time out of the kennel with a person has been shown to temporarily ameliorate that stress. In this study, 164 shelter-living dogs at four animal shelters across the United States were taken on two-and-half-hour outings with a person and physiological measures of stress and physical activity captured by accelerometer devices were compared before, during, and after this short-term outing. We found that dogs' stress was higher when they were away on these field trips and their activity changed, including less time spent in low activity and more time in higher activity. While measures of physiology and activity were found to return to pre-field trip levels the following day, these results suggest that outings of this duration do not provide the same reduction in stress as previously shown with temporary fostering. Nevertheless, short-term outings may provide shelter dogs with greater adoption visibility and assist in foster recruitment and, thus, should be further explored.

Abstract: Social isolation likely contributes to reduced welfare for shelter-living dogs. Several studies have established that time out of the kennel with a person can improve dogs' behavior and reduce physiological measures of stress. This study assessed the effects of two-and-a-half-hour outings on the urinary cortisol levels and activity of dogs as they awaited adoption at four animal shelters. Dogs' urine was collected before and after outings for cortisol:creatinine analysis, and accelerometer devices were used to measure dogs' physical activity. In total, 164 dogs participated in this study, with 793 cortisol values and 3750 activity measures used in the statistical analyses. We found that dogs' cortisol:creatinine ratios were significantly higher during the afternoon of the intervention but returned to pre-field trip levels the following day. Dogs' minutes of low activity were significantly reduced, and high activity significantly increased during the outing. Although dogs' cortisol and activity returned to baseline after the intervention, our findings suggest that short-term outings do not confer the same stress reduction benefits as previously shown with temporary fostering. Nevertheless, it is possible that these types of outing programs are beneficial to adoptions by increasing the visibility of dogs and should be further investigated to elucidate these effects.

Keywords: dogs; animal shelter; cortisol; stress; welfare; human-animal interaction; activity

1. Introduction

Between 4.0 and 5.5 million dogs enter animal shelters each year in the US [1,2]. Considerable efforts have been made over the past two decades to improve outcomes for dogs facing this experience (for a review, see [3]), leading to more dogs being adopted and returned to their owners and reduced levels of euthanasia [2,4].

More recently, animal welfare organizations have begun focusing on the standard of care that dogs receive while in the shelter [5]. In part, this is in recognition of the potential stressors within the environment, including excessive noise [6–9], spatial restriction [10–12], social isolation [13], loss of attachment figures [14], loss of control [15] and lack of a daily routine [16]. One way to mitigate the impact of these stressors is through the use of enrichment interventions intended to improve welfare [17–19].

The most commonly studied enrichment intervention in sheltering is interactions with people [15,16, 20–32]. The majority of these interventions occur at the shelter but out of the kennel and are 15–45 min in duration. Often, their impacts are measured by changes in physiology and behavior.

Cortisol is one of the most widely utilized physiological markers of stress in dogs [33]. Previous studies have found elevated cortisol levels for dogs living in shelters as compared to those in homes [29,34] as well as for dogs from homes entering kennels for the first time [35]. Resting activity or sleep can also be a useful component in welfare assessment. Dogs in shelters have been observed to sleep less during the day than dogs in homes [36,37] and had more activity during their most and least active hours than owned dogs [38], suggesting a lack of restful activity for shelter dogs.

A handful of human-interaction interventions, such as those by Hennessy et al. [22], Gunter et al. [32], and Fehringer [28], describe dogs leaving the animal shelter which may provide greater relief to dogs than in-shelter interactions. Gunter et al. [32] reported the impacts of one- and two-night stays in volunteers' homes and measured dogs' cortisol levels before, during, and after those stays. Dogs were found to have lower cortisol levels in homes; and while dogs' cortisol increased upon return to the shelter, it was no higher than baseline levels. Additionally, dogs' bouts of uninterrupted rest were longest when in the foster home, but still remained longer upon return to the shelter than prior to fostering. Fehringer [28] also found that placement in a home resulted in lower cortisol compared to in-shelter levels, and dogs' cortisol steadily declined over the first three days in foster care.

Little is known about how short-term outings of a few hours in duration without an overnight stay could impact the welfare of dogs awaiting adoption. Considering that previously tested in-shelter interventions of less than one hour have been shown to reduce cortisol and improve behavior [15,16,21,26, 27,29–31], it is possible that out-of-shelter outings of a slightly longer duration could confer even greater benefits. However, it is worth noting that many of these aforementioned interventions took place in living room-like settings at the shelter and involved calm interactions with the dog lying down and being petted by a person.

Conversely, short-term outings into the community such as field trips allow for increased physical activity for the dog. Yet a prior study failed to find clear evidence of behavioral improvement following physical activity when provided to dogs at the shelter. Protopopova, Hauser, Goldman, and Wynne [39] compared interventions of exercise and reading offered for 15 min daily for two weeks. Both interventions were followed by increases in dogs' back-and-forth motion in the kennel, a locomotor behavior associated with reduced welfare and longer lengths of stay [40]. While a greater reduction in door jumping was observed after exercise as compared to the reading intervention, dogs were more often at the front of their kennels and barking less often after volunteers read to them for the same duration. Thus, it seems likely that the activity a person engages in with a shelter dog could differentially affect its welfare.

In the present study, we explored whether short-term outings with a person away from the animal shelter would influence dogs' urinary cortisol:creatinine (C/C) ratios in the afternoon of the intervention as compared to ratios collected in the shelter before and after these outings. Additionally, dogs' physical activity was monitored throughout the study to detect potential differences in activity intensity.

2. Materials and Methods

2.1. Shelters

Data were collected at four animal shelters in the United States: Spokane County Regional Animal Protection Service (SCRAPS, June 2019); Fulton County Animal Services in Atlanta, GA (FCAS, July 2019); the Regional Center for Animal Care and Protection in Roanoke, VA (RCACP, August and October 2019); and Detroit Animal Care and Control (DACC; September 2019). The shelters varied in their geographical location and annual dog intake but all were open-admission facilities (Table 1).

Table 1. Shelter admission type and state where located, canine intake for the prior year, and number of dogs, complete sequences, and sample details.

Shelter	State	Prior Year Canine Intake	Subject Dogs	Complete Sequences *	Samples Collected	Samples Removed **
SCRAPS	WA	3074	41	36	204	8
FCAS	GA	6321	40	34	200	7
DACC	MI	4063	41	36	205	6
RCACP	VA	2027	42	37	210	6

Note. * Complete sequences are dogs in which all five collection timepoints were obtained and used in the analysis. ** Samples were removed from data analysis when urinary cortisol:creatinine ratio values were three standard deviations above the shelter's mean.

These shelters had existing short-term outing programs prior to data collection, although their weekly usage varied, ranging from one or less at RCACP to five or less per week at SCRAPS, FCAS, and DACC. Additionally, DACC held monthly events in which typically sixty-five or more dogs would leave on short-term outings with shelter volunteers. Data collection at DACC began 19 days after their last event.

In addition to providing short-term outings, all shelters in this study had walking programs for their dogs in which dogs would spend time out of their kennels interacting with volunteers in and around the facility. Both FCAS and DACC had temporary fostering programs in which dogs would leave the shelter for stays in a caregiver's home. Dogs at all four shelters had access to elevated, cot-style beds in their kennels and also received sporadic food enrichment. At SCRAPS and FCAS, dogs interacted with each other outdoors in supervised groups.

Staff at each of the facilities determined which dogs would participate in this study. To minimize the likelihood of injury or harm to individuals carrying out these field trips, shelters selected dogs without histories of aggressive behavior. Dogs enrolled in the study at FCAS and RCACP had not previously experienced an outing, while it is possible that dogs at SCRAPS and DACC that entered the shelter prior to data collection may have done so. Dogs that were fearful during urine collection were not included due to the researchers' inability to obtain samples for cortisol analysis.

2.2. Short-Term Outings

Dogs experienced approximately two-and-a-half-hour-long outings with a person between 11:00 a.m. and 3:00 p.m., off the property of the animal shelter. (Less than two percent of outings occurred outside of these times.) Volunteers, staff, and members of the public were eligible to take dogs on outings but were required to meet organizational requirements prior to participation, including being over 18 years of age and providing a driver's license for identification. Shelter staff provided participating individuals with handling instructions and supplies, and the authors discussed the purpose of this study. When dogs returned to the shelter, the research team asked these individuals to complete a questionnaire about their outing, and dogs were placed back into their kennels.

2.3. Collection Timeline

Study enrollment lasted three days for each dog with five collections per dog. Beerda et al. [12] found that both urinary and salivary cortisol levels of kenneled dogs were higher in the mornings than in the evenings. However, in studies in which working dogs were active in both the morning and evening as compared to dogs that only had daytime activities, this across-the-day reduction in cortisol was not present [41]. Given this uncertainty regarding the presence of a circadian influence with the cortisol levels of shelter dogs, collections occurred in both the morning and afternoon to capture potential changes over time as well as those caused by the outing.

Morning collection on Days 2 and 3 was conducted between 7:00 a.m. and 9:30 a.m. These times are consistent with those used by Gunter et al. [32]. Afternoon collections on Days 1–3 occurred between 3:30 p.m. and 6:00 p.m. Table 2 provides the experimental timeline for collection. Less than one percent of samples fell outside these collection windows due to dogs not urinating when walked or providing an inadequate volume of urine (minimum 1.5 mL). In these cases, dogs were provided a mixture of wet food and water and walked until the dog urinated.

Table 2. Experimental timeline for urine collection.

Window	Morning Collection (7:00–9:30 a.m.)	Short-Term Outing (11:00 a.m.–3:00 p.m.)	Afternoon Collection (3:30–6:00 p.m.)
Day 1			Sample 1
Day 2	Sample 2	Outing: ~2.5 h	Sample 3
Day 3	Sample 4		Sample 5

Note. Due to the time window in which outings could occur, collection for Sample 3 commenced two hours after the dog had returned from its outing. Additionally, collection for Sample 5 was attempted at a time that was between dogs' collection times for Samples 1 and 3.

2.4. Urine Collection

The research team collected dogs' urine before and after their outings for C/C analysis. Dogs were removed from their kennels on leash and walked to locations outside of the shelter for urine collection and returned to their kennels after samples were obtained. Olympic Clean-Catch™ plastic trays taped to 36 inch (91 cm) "Pickup and Reach" tools (Harbor Freight, Calabasas, CA, USA) were used for urine collection. Samples were poured from the collection trays into 10 mL plastic tubes with snap caps for storage. Trays were rinsed with water and air-dried or wiped with sterile KimWipes™ (Kimberly-Clark, Irving, TX, USA) between collections. Samples were immediately placed in a cooler with ice after collection, and in a freezer within two hours at a temperature of −18 °C.

Frozen urine samples were shipped overnight on dry ice to ZNLabs Veterinary Diagnostics (Louisville, KY, USA) for C/C analysis. Analysis was conducted using an automated wet biochemistry analyzer (AU680, Beckman Coulter, Brea, CA, USA) for measurement of creatinine. Bio-Rad Liquid Human Urine Precision Chemistry Controls 1 and 2 (Bio-Rad Laboratories, Inc., Hercules, CA, USA, Control Level 1 #397, Control Level 2 #398) were run on each day of urine sample testing and stored according to manufacturer instructions. Cortisol was measured using a commercially available product designed for an enzyme-amplified chemiluminescence assay system (Immulite 2000 XPi, Siemens Healthcare Diagnostics, Inc., Newark, DE, USA). Cortisol:creatinine ratios (measured in μmol/L: μmol/L) $\times 10^{-6}$ were then calculated.

2.5. Activity Monitoring

Whistle FIT activity monitors (Whistle Lab Inc., San Francisco, CA, USA) were attached to collars and placed on the dogs, allowing for collection of their movement via the triaxial accelerometer. Placement of collar-mounted monitors occurred during the morning of the dogs' first day in the study and worn for the study's duration, unless battery loss or malfunction resulted in removal and replacement with a new device. Data from the Whistle FITs were transmitted to Whistle servers via each shelter's wireless network and Bluetooth.

Yashari, Duncan, and Duerr [42] assessed the validity of Whistle devices as a measure of canine activity by comparing data generated from these devices with that of Actical, a previously validated activity monitor. They found that measurements of dogs' activity intensity and total activity were highly correlated between the two devices, 0.81 and 0.93, respectively.

For the purposes of this study, dogs' activity was calculated using the raw data recorded by the Whistle devices. These triaxial accelerometers collected the x, y, and z components of a vector representing dog movement, $M = (M_x, M_y, M_z)$, at a rate of 50 times per minute. The magnitude of this vector, $M = \sqrt{}\ (M_x{}^2, M_y{}^2, M_z{}^2)$, was used to indicate dogs' composite movement at each time period. Magnitude calculations were then summed over one-minute epochs as an estimate of the dogs' activity during that minute. Magnitude-per-minute values ranged from 0.89 to 8102.

To characterize this activity, all magnitude-per-minute (m/m) values were categorized into one of five, evenly apportioned activity levels. Quintile thresholds were derived from the complete set of magnitude values obtained throughout the study. Each quintile thus contains approximately 37,576 records. The magnitude-per-minute thresholds and associated activity categories, Q1 (lowest) through Q5 (highest), are shown in Table 3.

Table 3. Activity level categorization as based on each quintile's magnitude-per-minute thresholds.

Activity Level	Low Threshold	High Threshold
Q1 (Low Activity)	0.0001	548.14
Q2	548.14	1217.75
Q3	1217.93	2475.77
Q4	2475.82	3058.04
Q5 (High Activity)	3058.04	8102.00

M/m values were calculated for the four hours prior to the five urine sample collections, a time window based on the previously demonstrated reflection period of canine urinary cortisol [43]. Dogs' total minutes in each activity level as well as the proportion of time spent in each of those levels were calculated. Data from dogs with at least 200 m/m values for the four hours prior to the urine collection were used in our analysis.

2.6. Statistical Analysis

To investigate whether dogs' cortisol differed across time, by shelter or in a shelter-by-timepoint interaction, we analyzed C/C ratios obtained for the dogs at our four study sites with a linear mixed model.

Dog and intercept were entered as random effects with timepoint, shelter, and a timepoint-by-shelter interaction along with the covariates of age, weight, length of stay (LOS), and the activity categories included as fixed effects. These covariates were included in the model as they have been previously found to influence cortisol [32]. A variance covariance matrix was employed, and a diagonal covariance matrix for the repeated measure of timepoint. The method of Restricted Maximum Likelihood (REML) was used for estimating parameter values.

To explore whether the number of minutes dogs spent in the five activity categories changed throughout this study, by shelter or in a timepoint-by-shelter interaction, we performed a doubly multivariate analysis with a general linear model. Dogs' weight, age, and mean LOS (across the three days of the study) were entered as covariates. For the within-subjects variable of time, the contrast was polynomial and for shelter, simple.

With both models, dogs' age and LOS were log transformed to ensure the normal distribution of variables. When post-hoc comparisons were conducted in our analyses, a Sidak correction was utilized to reduce the likelihood of false positives when multiple comparisons were made. A statistical significance level of $p < 0.05$ was used throughout.

2.7. Ethical Statement

Procedures carried out at SCRAPS, FCAS, DACC, and RCACP were approved by the Arizona State University Institutional Animal Care and Use Committee (IACUC: 17-1552R).

3. Results

3.1. Descriptive Statistics

When characterizing the dogs at SCRAPS, FCAS, DACC, and RCACP, they were more often male (56.1%), with most dogs arriving to the shelter as a stray (76.2%). Nearly one-fifth of dogs (19.5%) were surrendered by their owners or returned after a failed adoption. On average, dogs were slightly over three years of age (M = 39.00 months, SD = 30.96) and weighed 23.96 kg (SD = 7.55). The number of days dogs were living in the shelter at the time of the study ranged from 1.50 to 252.50 days (M = 38.95, SD = 42.45).

In total, 164 short-term outings were conducted as part of this study, with 40 dogs participating at FCAS, 41 each at both SCRAPS and DACC, and 42 at RCACP. In an effort to better understand what transpired during these outings, we collected information about the individuals that provided these outings as well as the activities they engaged in with the dogs and where those activities occurred.

Nearly two-fifths of individuals that took dogs on short-term outings were public participants (37.80%), with the remaining individuals being shelter staff and volunteers or members of the research team, and most often those that were providing outings were female (86.59%). Over half of all outings (51.83%) included just one person and the shelter dog. Over one-third of short-term outings (35.37%) did not involve any additional people interacting with the dog, such as petting, playing, or offering the dog a treat, while more than half (53.05%) involved additional interactions with 1–5 people. Shelters varied by the individuals taking dogs on these outings and their interactions (Table 4).

Table 4. By shelter, individuals taking dogs on short-term outings that were members of the public, that were female; outings that included more than one person participating; outings where no additional people interacted with the dog; and outings where 1–5 additional people interacted with the dog.

Shelter	% Public of Outing Participants *	% Female of Outing Participants	% Outings with >1 People on Outing	% Outings where No Additional People Interacted with Dog	% Outings Where 1–5 Additional People Interacted with Dog **
SCRAPS	31.70	78.05	53.66	21.95	63.41
FCAS	80.00	92.50	52.50	40.00	55.00
DACC	4.88	82.93	43.90	48.78	50.00
RCACP	35.71	92.86	42.86	30.95	43.90

Note. * Individuals responsible for taking dogs on short-term outings that were not members of the public were either shelter staff and volunteers or part of the research team. ** Outings in which more than five people interacted with the dog account for the remaining percentage of outings not reported here.

Over three-quarters of dogs (75.60%) spent time outdoors on their field trip, such as visiting a park, where they walked, hiked, or jogged with the person, and almost 30% of dogs (29.90%) visited a pet-friendly store or restaurant in the community. Fewer than half of the dogs (43.90%) visited the person's home while on the field trip, with only 22.00% of dogs exclusively spending time in a home during the outing.

3.2. Cortisol Analysis

Dogs at SCRAPS, FCAS, DACC, and RCACP yielded 793 cortisol values that were statistically analyzed across the five urine collections in this study to detect an effect of time of collection, shelter, or shelter-by-timepoint interactions with dogs' weight, (log) age, (log) LOS and minutes spent in each activity category added into the model as covariates.

With this model, the variables of shelter, timepoint, shelter-by-timepoint interaction, weight, and log (LOS) were significant (at $p < 0.05$), with log(age) marginally significant at $p = 0.061$. None of the activity categories were statistically significant but were retained in the model to account for the effect of activity on C/C ratios.

The main effect of timepoint tested was significant, F (4, 560.42) = 6.29, $p < 0.001$, demonstrating that the dogs' cortisol changed across the study. In post-hoc comparisons, dogs were found to have significantly higher cortisol values on the afternoon of the field trip as compared to the afternoon of the day before ($p <$ 0.001) and the afternoon of the day after ($p = 0.001$). Figure 1 presents the estimated marginal means and standard errors of the cortisol values for the five timepoints across the three days of the study.

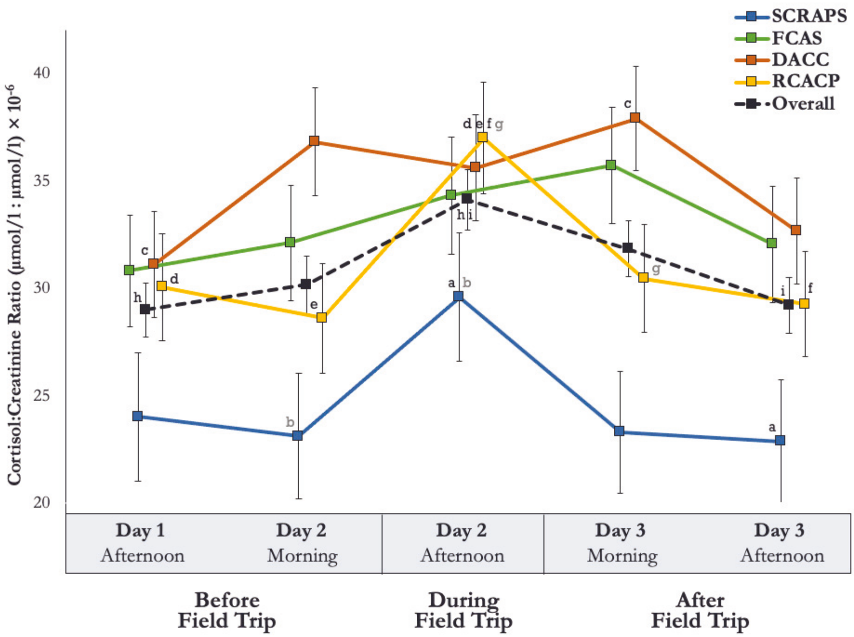

Figure 1. Estimated marginal means of dogs' cortisol:creatinine ratio values and standard errors for the five study timepoints by shelter. "Overall" represents the estimated marginal means and standard errors at each timepoint, regardless of shelter. All comparisons (shared letters: a–i) are significant at $p < 0.05$, except for comparisons b ($p = 0.051$) and g ($p = 0.068$).

A main effect of shelter was also detected, F (3, 149.15) = 3.19, $p = 0.026$, revealing that the estimated marginal means for cortisol varied amongst the shelters. In post-hoc comparisons, dogs at SCRAPS had the lowest C/C ratios, which were significantly different from dogs at DACC ($p = 0.017$). Table 5 includes the average estimated marginal means of C/C ratios and standard errors for the dogs at SCRAPS, FCAS, DACC, and RCACP. When this analysis was repeated, excluding cortisol values from the afternoon of the field trip to examine only pre- and post-intervention timepoints in the shelter, this effect was slightly more pronounced, F (3, 149.79) = 4.00, $p = 0.009$. Post-hoc comparisons and subsequent differences between dogs at SCRAPS and those at DACC ($p = 0.007$) were marginally greater. %endparacol

Table 5. Mean cortisol:creatinine ratio values, standard errors, *F* test statistics, and *p* values for five timepoints before and after a short-term outing at four US animal shelters.

			Pre-Field Trip						Post-Field Trip					
			Afternoon before (Day 1)		Morning before (Day 2)		Afternoon of Field Trip (Day 2)		Morning after (Day 3)		Afternoon after (Day 3)			
	Average		1		2		3		4		5		Test Statistics	
Shelter	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	*F*	*p*
SCRAPS	24.58	2.55	24.02	3.00	23.12^{b}	2.93	29.59 ^{a,b}	3.00	23.30	2.85	22.87^{a}	2.86	2.79	0.026
FCAS	32.00	2.25	30.81	2.60	32.11	2.68	34.32	2.72	35.71	2.71	32.05	2.70	1.58	0.177
DACC	34.81	2.03	31.09 ^{c}	2.46	36.81	2.52	35.59	2.47	37.89 ^{c}	2.43	32.66	2.46	3.03	0.017
RCACP	31.07	2.10	30.06 ^{d}	2.48	28.60 ^{e}	2.55	36.99 ^{d,e,f,g}	2.60	30.46 ^{g}	2.52	29.26 ^{f}	2.46	4.00	0.003
Overall			28.99 ^{h}	1.26	30.16	1.33	34.12 ^{h,i}	1.39	31.84	1.31	29.21 ^{i}	1.29	6.29	<0.001

Note. All comparisons (shared letters: a–i) are significant at p = 0.05 or less except for comparisons b (p = 0.051) and g (p = 0.068).

The interaction of shelter-by-timepoint was significant, F (12, 537.12) = 2.01, p = 0.022, indicating that dogs' cortisol values differed at each of the shelters at the various study timepoints. When examining these shelter-specific timepoint differences, dogs at SCRAPS had significantly higher cortisol values on the afternoon of the field trip as compared to the following afternoon (p = 0.043) and marginally so the morning before the field trip (p = 0.051). Dogs at DACC had significantly higher cortisol on the morning of the day following the field trip as compared to the afternoon of the day before the field trip (p = 0.020). At RCACP, dogs had significantly higher cortisol during the afternoon of the field trip as compared to the afternoon of the day before the field trip (p = 0.020), the morning prior to the field trip (p = 0.007), the afternoon of the day after the field trip (p = 0.005), and marginally so the morning after the field trip. Figure 1 presents the estimated marginal means and standard errors of the cortisol values at each shelter across the study timepoints.

When investigating differences between shelters at the same collection timepoint, dogs at DACC had significantly higher morning cortisol values on the days pre- and post-field trip than dogs at SCRAPS (p = 0.004 and p = 0.001, respectively). The morning values post-field trip on Day 3 were also significantly higher at FCAS (p = 0.017) and marginally so at DACC (p = 0.064) than values obtained at SCRAPS. Table 5 includes the estimated marginal means of C/C ratios and standard errors at each collection timepoint at SCRAPS, FCAS, DACC, and RCACP.

3.3. Activity Analysis

At SCRAPS, FCAS, DACC, and RCACP, 121 dogs provided 710 readings of minutes spent in each of the five activity categories. Minutes were analyzed across the five study timepoints to detect an effect of time, shelter, or a shelter-by-timepoint interaction with dogs' weight, (log) age, (log) meanLOS entered as covariates in the model.

With this model, Box's Test of Equality of Covariance Matrices was shown to be violated (p < 0.001), indicating that the covariance matrices of the dependent variables were not equal. As such, test statistics are reported here are using Pillai's Trace as it is considered to be the most robust test to violations of model assumptions [44]. Mauchly's Test of Sphericity was also violated for four of the five activity categories (p < 0.001), with the exception of activity category Q3 (p = 0.369), thus sphericity was not assumed, and Greenhouse–Geisser tests were used to determine statistical significance.

We found that timepoint significantly influenced dogs' minutes in the five activity categories, F (20, 95) = 41.78, p < 0.001, demonstrating that their activity varied across the three days of this study. In post-hoc comparisons, dogs spent less time in the lower activity categories of Q1 and Q2 during the afternoon of the field trip than any other time in the study (p < 0.001). Conversely, dogs spent significantly more time in the higher activity categories, Q4 and Q5, during the afternoon of the field trip than all other timepoints ($p < 0.001$). Figure 2 presents the estimated marginal means and standard errors of minutes spent in the five activity categories for the five timepoints across the three days of the study.

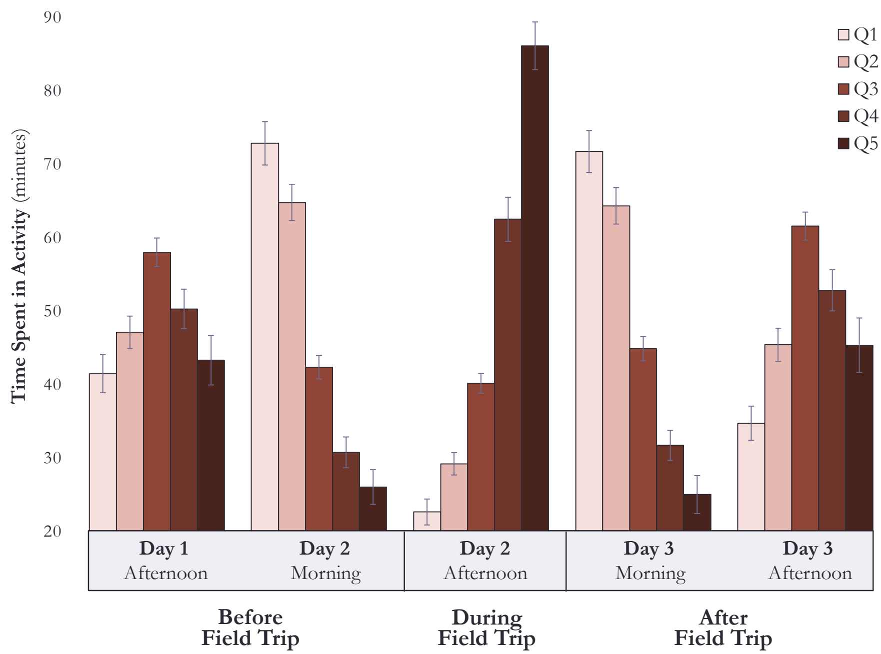

Figure 2. Estimated marginal means and standard errors of minutes spent in the five activity categories during the four hours prior to each of the five urine collection timepoints.

A significant effect of shelter was also found, F (15, 336) = 2.20, p = 0.006, indicating that the minutes dogs spent in the various activity categories differed amongst the shelters; however, in post-hoc comparisons, only one difference was found between shelters. Dogs spent more time in Q3 activity at DACC than dogs at RCACP (p = 0.007).

A significant shelter-by-timepoint interaction was detected, indicating that the time dogs spent in each of the activity categories differed between shelters at the various study timepoints. (SCRAPS: F (20, 95) = 8.91, $p < 0.001$; FCAS: F (20, 95) = 5.97, $p < 0.001$; DACC: F (20, 95) = 12.53, $p < 0.001$; RCACP: F (20, 95) = 15.88, $p < 0.001$.) When examining shelter-specific activity levels, two patterns were apparent. Firstly, activity during the afternoon of the field trip was generally different from other timepoints; and secondly, the activity recorded in the mornings and afternoons differed from each other, mirroring the timepoint post-hoc comparisons previously reported (see Figure 2). One additional difference was seen at SCRAPS, where dogs spent less time in the lowest activity category (Q1) during the afternoon of the day after the field trip than the afternoon of the day before the field trip (p = 0.018).

When exploring whether dogs' activity varied across study timepoints at the shelters, the timepoint-by-shelter interaction was significant, with the exception of the morning of the day after the field trip, indicating that minutes of time spent in the various activity categories at each of the study timepoints differed by shelter. (Timepoints 1: F (15, 336) = 2.39, p = 0.003; 2: F (15, 336) = 1.54, p = 0.088; 3: F (15, 336) = 2.18, p = 0.007; 4: F (15, 336) = 1.41, p = 0.138; 5: F (15, 336) = 2.10, p = 0.003.) We found that dogs at SCRAPS had more low activity (Q1) than dogs at DACC during the afternoon of Day 1 (p = 0.034), fewer Q1 minutes in the morning of Day 2 as compared to the dogs at DACC (p = 0.036) and RCACP (p = 0.013). Dogs at RCACP also had significantly more minutes of Q1 low activity during the afternoon of Day 3 than dogs at DACC (p = 0.004).

When exploring the second-lowest activity category (Q2), the only differences detected were during the afternoon of the field trip: dogs at FCAS had more Q2 activity than dogs at SCRAPS (p = 0.039) and RCACP (p = 0.048). With regards to moderate Q3 activity, DACC dogs spent more time in this category during the first afternoon of this study than dogs at FCAS (p = 0.001) and RCACP (p = 0.007) and marginally more time than RCACP dogs during the morning of Day 3 (p = 0.054). Only two differences were found in dogs' higher activity (Q4), and those were between the same shelters in the mornings before and after the field trip: dogs at SCRAPS had more higher activity than dogs at RCACP (p = 0.008 and p = 0.002, respectively). Lastly, dogs at RCACP spent more time in the highest activity (Q5) during the field trip than dogs at FCAS (p = 0.001).

4. Discussion

In our investigation of short-term outings, we found that this intervention increased the stress of shelter dogs and decreased their resting activity. Even after accounting for activity in our cortisol analysis, we found that dogs' C/C values were significantly higher during the afternoon of intervention as compared to the prior and following afternoons. However, cortisol did return to pre-outing levels by the next day. Additionally, we found that dogs of greater weight had lower cortisol values, and older dogs had higher cortisol values (as previously shown by Zeugswetter et al. [45] and Rothuizen et al. [46], respectively). These new findings support the previously reported relationships of dogs' weight and age to cortisol [32]. Dogs with shorter lengths of stay also had higher cortisol values than those with longer stays in the shelter.

In our analysis of time dogs spent in the five activity levels across this study, we found similar apportioning of activity in the mornings and afternoons prior to and after field trips. During the mornings, dogs were spending the most time in low activity (see Table 3). Not surprisingly, these are early morning hours prior to staff arrival when dogs are often sleeping, supporting prior findings about the activity of dogs in shelters [36,38]. Conversely in the afternoons pre- and post-outing, dogs were more active than the morning, spending more minutes in the higher and mid-activity levels of Q5, Q4, and Q3.

4.1. Duration, Activities, and Location of Human Interaction with Shelter Dogs

To our knowledge, the present study is the first investigation of an intervention in which dogs leave the shelter for a few hours and then are returned to their kennels. More commonly in the scientific literature, human interaction is provided to dogs while remaining at the shelter. In these studies [15,16,21,26,27,29–31], interactions with the person ranged from 15 to 45 min in duration and dogs' cortisol was found to temporarily decrease, a finding that was not replicated in the present study despite the increased duration (2.5 h) of human interaction.

It may not be the duration of the interaction, though, that is as important as the location and activities undertaken between the person and the dog. In the aforementioned studies where the dogs' cortisol levels were lower following the intervention, particularly those by Hennessy et al. [15], Hennessy et al. [16], Shiverdecker et al. [27], Dudley et al. [29], Willen et al. [30], and McGowan et al. [31], dogs were removed from their kennels and the interaction occurred at the shelter but in a quiet, secluded room. In the present study, less than one-quarter of the dogs returned to the person's home for their outing, while the majority of dogs spent time walking, hiking, or jogging in public. When Gunter et al. [32] and Fehringer [28] reported reductions in cortisol in their fostering interventions, dogs were taken to homes for one and two or three nights, respectively.

When examining dogs' activity across the three days of this study, dogs were more active in the greater intensity categories during the short-term outings. Specifically, they spent nearly twice as long in Q5 activity as any other afternoon in the study. Previous research by Radosevich et al. [47] demonstrated that as the intensity and duration of exercise increased so did dogs' cortisol. This arousing effect of activity on cortisol has also been found with sled dogs [48,49] and dogs off leash at a dog park [50].

Contrariwise, cortisol reductions observed by Hennessy et al. [15], Hennessy et al. [16]; Shiverdecker et al. [27], Dudley et al. [29], Willen et al. [30], and McGowan et al. [31] were induced after dogs received calm petting, with the person even massaging and talking soothingly to the dog during the intervention (with the exception of the stranger and play conditions in Shiverdecker et al. [27]). Similarly, Gunter et al. [32] reported a relaxing experience in the home, with dogs having their longest bouts of uninterrupted rest during temporary fostering.

4.2. Psychological Stress and Increases in Cortisol

Based on the statistical analysis, however, activity alone does not explain this rise in cortisol during the afternoon of the outing, allowing for the possibility that the difference in cortisol was related to the psychological stress of the field trip. Instead of a quiet room where dogs could escape the stressors of the shelter or spend time resting in a home, dogs were active on these field trips and exposed to a variety of novel sights and sounds. If visual, auditory, and olfactory stimuli in the shelter have been found to negatively impact dogs' welfare [17], field trips that include activities such as outdoor dining, hiking, or visiting a store could be stressful, too. Future studies, where volunteers exclusively take dogs to homes or other quiet locations during the outings and calmly interact with them instead of partaking in more energetic activities may find the type of reduction in cortisol that was reported in previous studies.

Increases in cortisol, as reported here with short-term outings, do not necessarily indicate poorer welfare for dogs. Owned dogs engage in a variety of preferred activities that positively impact their welfare and are accompanied by higher cortisol levels, such as attending the dog park [50], competing in agility [51] or hunting [52]. Certainly, these activities are arousing; but we propose that it is the environments in which these dogs are living that should be considered. After such activities, owned dogs return to their homes while dogs awaiting adoption return to the stressful environment of the shelter. Zeugswetter and colleagues [45] identified that the median morning C/C ratio for healthy owned dogs is just 16 (μmol/L: μmol/L $\times 10^{-6}$), while in the present study, the median cortisol value in the morning was 27.1 (μmol/L: μmol/L $\times 10^{-6}$).

4.3. Proximate Welfare Interventions

Our research and that of others [29,34] indicate that shelter dogs have highly elevated cortisol levels, and these levels likely persist for a prolonged period of time [18]. In addition to physiological measures of stress, shelter dogs spend significantly less time resting than dogs living in homes [38]. By all accounts, it would seem that instead of further arousing dogs that are already coping with an unpredictable environment, enrichment interventions should be aimed at reducing dogs' cortisol levels and other physiological and behavioral measures indicative of compromised welfare, even if these reductions are transitory. A recent review of canine enrichment in the shelter by Gunter and Feuerbacher (under review) [53] characterized a variety of interventions that can positively impact the lives of shelter-living dogs.

In addition to the previously mentioned in-shelter human interactions and fostering programs where the dog leaves the kennel and spends time with a person in an effort to improve their welfare, Gunter and

Feuerbacher [53] suggested that dogs would benefit from more enriched living conditions while in the shelter, such as beds to lie upon and objects that they prefer to chew, such as soft, plush toys. With regards to social interaction with other dogs, Gunter et al. (in prep) [54] recently found that when dogs were provided three, 15 min conspecific sessions a day, their levels of Secretory Immunoglobin-A, an immune function antibody, were lower when compared to days when no dog contact was provided, suggesting that time with other dogs could also be beneficial to their welfare.

4.4. Individual Shelter Differences

While we were able to detect an overall effect of the intervention on cortisol values across our study, we were also able to detect overall shelter differences as well as variability in how the intervention affected dogs at the individual shelters. For example, cortisol values of dogs at SCRAPS were, on average, lower than those at other shelters. Differences in the magnitude of the intervention's impact were also seen. Specifically, the arousing effect of the field trips, as indicated by increased C/C ratios, was detected most strongly at SCRAPS and RCACP, likely because of their lower in-shelter cortisol values. Yet, dogs at DACC and FCAS that also had higher afternoon cortisol values during field trips did not have statistically significant differences in pre- and post-outing comparisons.

We also found that cortisol values obtained at SCRAPS and RCACP failed to demonstrate evidence of a circadian rhythm effect: both morning and afternoon cortisol values were similar. One shelter, DACC, showed the strongest potential evidence of reduction across the day; however, a possible explanation for this reduction may be related to their operating hours. While SCRAPS and RCACP remained open until the early evening, DACC closed to potential adopters, volunteers, and most staff by 3:30 p.m. Hewison, Wright, Zulch, and Ellis [55] found that closing a shelter to the public during afternoons led to reductions in noise, increases in dogs' sedentary behavior, along with decreases in locomotor and stereotypic behaviors. Thus, the reduction in cortisol across the day at DACC may be related to an absence of late-day stressors. If so, it may be possible that shelter dogs' cortisol levels could decrease across the day, but the stimulating nature of human traffic at adoption facilities in the afternoons and early evenings may be preventing this from occurring.

4.5. Distal Effects on Welfare

While we have not found evidence here for the proximate welfare advantages of brief outings, it is possible that short-term outings may benefit shelter dogs in ways we did not measure. Outings with a volunteer, staff person, or member of the public may support adoptions and foster recruitment efforts by increasing the visibility of adoptable dogs in the community, which could benefit dogs' ultimate welfare by enabling them to permanently leave the shelter and live in a home. In an ongoing investigation evaluating the deployment and implementation of short-term outing programs in animal shelters, preliminary analyses indicate that over 5% of dogs that experience a field trip are adopted by the person taking them on that trip. Future studies that explore these distal effects would aid shelters in the evidence-based care they provide to homeless dogs by identifying which enrichment interventions improve dogs' daily experiences and which programs are associated with decreased lengths of stay and increased likelihood of adoption.

4.6. Limitations

When considering the limitations of our study, not all dogs at these shelters were eligible to participate, particularly dogs that were unable to walk on leash, fearful of the urine collection tools, or deemed unsafe to handle by shelter staff. While efforts were made to conduct this study at shelters that were representative of animal sheltering in the US, the four participating shelters were open-admission facilities. It is possible that facilities with more managed intake policies and lower intake numbers may have dogs in different living conditions that would respond differently than the findings reported here.

Additionally, the individuals taking dogs on short-term outings, though they were most often shelter staff and volunteers and members of the research team, did vary in their dog knowledge and handling skills, which may have affected the stress that dogs experienced during their field trips. Additionally, it is possible that a dog's familiarity with the individual providing the short-term outing may further impact its experience [56]. However, it is worth noting that Gunter et al. [32] showed a consistent reduction in stress when dogs left the shelter during one and two nights of temporary fostering, which were provided by members of the public and shelter volunteers.

5. Conclusions

This study demonstrates that shelter dogs' urinary cortisol concentrations increased during the afternoon of a short-term outing, even when accounting for their activity throughout the study. During the afternoon of the intervention, dogs' high-intensity activity increased, and low-intensity activity decreased. These changes in cortisol and activity, however, were temporary, and both returned to pre-outing levels by the following day.

The magnitude of the intervention's effect on cortisol and activity varied between the participating shelters with values differing amongst shelters as well as at this study's various timepoints, suggesting that the living conditions at these facilities also influence dog welfare. In all, our findings indicate that short-term outings as tested here do not provide the reductions in stress achieved with temporary fostering in a home. Nevertheless, it is possible that outings of this type benefit shelter dogs' distal welfare by increasing adoption prospects within the community and should be investigated further to understand this effect.

Author Contributions: Conceptualization, L.M.G., E.N.F., and C.D.L.W.; methodology, L.M.G., E.N.F., R.J.G., and E.M.B.; validation, R.J.G., E.M.B., R.T.B., and J.M.P.; formal analysis, L.M.G. and R.T.B.; investigation, L.M.G., R.J.G., E.M.B., R.T.B., E.N.F., and J.M.P.; original draft preparation, LMG; writing—review and editing, L.M.G., R.J.G., E.M.B., R.T.B., E.N.F., J.M.P., and C.D.L.W.; visualization, L.M.G., R.T.B., and C.D.L.W.; supervision, L.M.G., E.N.F., and C.D.L.W.; funding acquisition, C.D.L.W., E.N.F., and L.M.G. All authors have read and agreed to the published version of this manuscript.

Funding: This research was funded by Maddie's Fund.**Institutional Animal Care & Use Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Animal Care and Use Committee of Arizona State University (Protocol 17-1552R, approved on 10 February 2017).

Acknowledgments: Thank you to the staff and volunteers of Spokane County Regional Animal Protection, Fulton County Animal Services, the Regional Center for Animal Care and Protection, and Detroit Animal Care and Control for their generosity and support of this research. Special gratitude to Havalah Moran, Lindsey Soffes, Meera Solomon, Michelle Harmon, Belinda Bell, Lara Hudson, Margo Butler, Dana Eldred, Renena McCaskill, Meghann Cords, Melinda Rector, and Mike Warner for their logistical and data collection support. Additional thanks to laboratory staff, graduate students, and research assistants, including Lindsay Isernia, Vlada Markov, Madeline Hadank, Bre Johnson, Becca Rafalowski, Anjali Patel, Meagan Samele, Tayo Oduyelu, Michelle Dwyer, Allie Kaufman, Chelsea Hughes, and Alysha Verba. Without the support of these individuals and organizations, this research would not be possible.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of this manuscript, or in the decision to publish the results.

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Effects of Olfactory and Auditory Enrichment on Heart Rate Variability in Shelter Dogs

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Received: 20 July 2020; Accepted: 6 August 2020; Published: 10 August 2020

Simple Summary: Many pet dogs end up in shelters, and the unpredictable and overstimulating environment can lead to high arousal and stress levels. This may manifest in behavioural problems, and decreased welfare and adoption chances. Heart rate variability is a non-invasive method to measure autonomic nervous system activity, which plays an important role in the stress response. The sympathetic nervous system is responsible for increasing the dog's arousal in response to stress and the parasympathetic nervous system is responsible for counteracting the arousal and calming the dog. Environmental enrichment can help dogs to be more relaxed, which is likely to be reflected by increased parasympathetic activity. Dogs' heart rate variability responses to three enrichment methods capable of reducing stress—music, lavender and a calming pheromone produced by dogs, dog appeasing pheromone and a control condition (no stimuli applied) were compared. Exposure to music appeared to activate both branches of the autonomic nervous system, as dogs in that group had higher heart rate variability parameters reflecting both parasympathetic and sympathetic activity compared to the lavender and control groups. We conclude that music may be a useful type of enrichment to relieve both the stress and boredom in shelter environments.

Abstract: Animal shelters can be stressful environments and time in care may affect individual dogs in negative ways, so it is important to try to reduce stress and arousal levels to improve welfare and chance of adoption. A key element of the stress response is the activation of the autonomic nervous system (ANS), and a non-invasive tool to measure this activity is heart rate variability (HRV). Physiologically, stress and arousal result in the production of corticosteroids, increased heart rate and decreased HRV. Environmental enrichment can help to reduce arousal related behaviours in dogs and this study focused on sensory environmental enrichment using olfactory and auditory stimuli with shelter dogs. The aim was to determine if these stimuli have a physiological effect on dogs and if this could be detected through HRV. Sixty dogs were allocated to one of three stimuli groups: lavender, dog appeasing pheromone and music or a control group, and usable heart rate variability data were obtained from 34 dogs. Stimuli were applied for 3 h a day on five consecutive days, with HRV recorded for 4 h (treatment period + 1 h post-treatment) on the 5th and last day of exposure to the stimuli by a Polar® heart rate monitor attached to the dog's chest. HRV results suggest that music activates both branches of the ANS, which may be useful to relieve both the stress and boredom in shelter environments.

Keywords: dog; heart rate variability; shelter; stress; arousal; lavender; dog appeasing pheromone (DAP); music

1. Introduction

Animal shelters are stressful environments due to novelty, loud noises, unpredictability and lack of control [1,2]. This overstimulating environment can lead to high arousal levels and stress in shelter animals. The stress response is multifactorial and includes activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic branch of the autonomic nervous system (ANS), with behavioural [3] and physiological changes [4] produced. Behavioural responses to stress consist of increased arousal [5,6], which in turn results in heightened sensory sensitivity and alertness, the production of corticosteroids and increased heart rate (HR) [7]. Stress in animals can be monitored in various ways, such as behavioural observation, which provides external indicators of an animal's internal state [8] and the response to its surroundings, physiological measures such as the amount of circulating glucocorticoids [9] and heart rate variability (HRV) [10]. HRV is a useful indicator of ANS activity [11] and has the advantage of being measured non-invasively [10,12] (externally and without puncturing the skin).

The ANS is divided into two branches: the sympathetic nervous system (SNS), which is excitatory, and the parasympathetic nervous system (PNS or vagal), which is inhibitory [13]. When there are no apparent threats, the PNS is dominant, which helps to maintain low levels of arousal and a stable heart rate [13]. PNS activity is mediated by acetylcholine neurotransmission released by the vagus nerve [14] and it produces a rapid response in cardiovascular function [15]. The SNS becomes dominant in situations of psychological or physical stress, leading to arousal that helps the individual to respond to environmental challenges [13]. SNS activity is mediated by epinephrine and norepinephrine [14], producing changes in cardiovascular function in a slower time course than PNS [15].

HRV is the fluctuation of time intervals between successive heart beats [16] and reflects the interaction between both branches of the ANS on the sinoatrial node of the heart [10]. A healthy heart has irregular time intervals between beats [17,18], therefore a high variability in sinus rhythm suggests better health and cardiovascular adaptability [19]. Low variability can indicate abnormal cardiac activity or an ANS imbalance leading to poor adaptability to psychological and physiological challenges [19]. HRV is assessed through several time domains, frequency domains and non-linear parameters (Table 1).

Table 1. Heart rate variability (HRV) parameters and their physiological origin.

Analysis Methods	Parameter	Units *	Description	Assumed Physiological Interpretation of Parameter
Time domain	Mean RR	ms	Mean time duration between successive RR intervals (two consecutive R waves of the electrocardiogram (ECG)) [9]	Increases with vagal activity and decreases with sympathetic activity [20]
	Mean HR	bpm	Mean heart rate	Increases with sympathetic activity and decreases with vagal activity [20]
	SDNN	ms	Standard deviation of RR intervals [9]	Mix of vagal and sympathetic activity [21]
	RMSSD	ms	Root mean square of differences between successive RR intervals [12]	Increases with vagal activity [11]
	pNN50	%	Percentage of successive RR interval pairs which differ by more than 50 ms [9]	Increases with vagal activity [11]
Frequency domain	LF	ms^2 and n.u.	Low frequency band of the power spectral density analysis of the HR fluctuation (0.067–0.235 Hz) [22]	Mix of vagal and sympathetic activity [23]
	HF	ms^2 and n.u.	High frequency band of the power spectral density analysis of the HR fluctuation (0.235–0.877 Hz) [22]	Increases with vagal activity [21]
	LF/HF		Low frequency/high frequency ratio	Mix of vagal and sympathetic activity [16]
Non-linear	SD1	ms	Standard deviation 1 of the Poincare Plot—short-term HRV [11]	Increases with vagal activity [24]
	SD2	ms	Standard deviation 2 of the Poincare Plot—long-term HRV [11]	Mix of vagal and sympathetic activity [24]

* Unit abbreviations: ms: milliseconds, bpm: beats per minute, %: percentage, ms^2: milliseconds squared, n.u.: normalised units.

HRV can be recorded using standard electrocardiogram (ECG) equipment, such as Holter systems, and wearable devices such as Polar® heart rate monitors [11]. The recorded RR intervals (duration between two consecutive R waves of the ECG) are then analysed using HRV software such as Kubios. The measurement of HRV can be challenging in terms of accuracy and interpretation. One key challenge is determining whether all traces are valid or if some are artefacts. Artefacts are recordings that appear like heartbeats but are not produced by sinoatrial node depolarisations and therefore are abnormal [16]. They can occur due to technical factors, such as poorly placed or fastened electrodes, movement of the subject and/or long recordings [25–27]. Artefacts can also occur due to physiological factors, such as ectopic beats, ventricular tachycardia and atrial fibrillation [25,26]. Data should be corrected for artefacts [11], as otherwise they can affect the reliability of the results [25,27]. HRV is influenced by many factors, such as respiration, posture and physical activity, and therefore the conditions under which data are collected should be standardised (i.e., stationary subject) [11] to allow treatment effects to be identified.

Studies in cattle (*Bos taurus*) [28], horses (*Equus caballus*) [20] and dogs (*Canis familiaris*) [29] have investigated the association between stress and HRV. In calves, root mean square of differences between the successive RR interval (RMSSD) was significantly reduced in those with external stress load (ambient temperature >20 °C and insect disturbance) and internal stress load (diarrhoea) compared to animals without obvious stress load. Standard deviation of RR intervals (SDNN) was significantly reduced in calves with internal stress load compared to those experiencing external stress load or no evident stress load [28]. In horses, there was a significant increase in HR, low frequency (LF) and the LF/HF ratio and a significant decrease in HF when they were forced to move backwards for 3 min compared when at rest or when forward walking [20]. In dogs approached by a stranger in the absence of their owner, HR increased and SDNN decreased [29]. Maros et al. [30] found that when dogs looked at their favourite toy, SDNN significantly increased, possibly indicating elevated attention. Kuhne et al. [31] found that when dogs had increased HR and decreased RMSSD compared to baseline values, they performed more appeasing and redirected behaviours. Moreover, low percentage of successive RR interval pairs that differ by more than 50 ms (pNN50) has been associated with aggression; this parameter was significantly lower in dogs with bite histories compared to dogs without them [32]. These results indicate that HRV is a useful tool to assess physiological and emotional stress.

As mentioned above, shelters can be challenging places for dogs and it is important to try to mitigate stress and arousal levels to avoid chronic stress that may impact welfare [33]. Moreover, stress and high arousal levels can increase the development of undesirable behaviours [3,34–36]. These reduce the likelihood of adoption [37], increase the risk of being returned to the shelter after adoption [38,39] and elevate the risk of euthanasia [2]. Sensory environmental enrichment, which consists of stimulating one or more of an animal's senses [40] is a useful tool to help reduce stress and arousal levels.

In humans, music has been effective in reducing anxiety in patients during hospitalisation [41], and can enhance relaxation by masking unpleasant noises [42]. Music can also reduce anxiety in patients with coronary heart disease, cancer patients and those awaiting surgery [43]. Music has been effectively used in animal studies as a form of environmental enrichment, for example, Western lowland gorillas (*Gorilla gorilla gorilla*) tended to perform more behaviours suggestive of relaxation when exposed to classical music compared to a no auditory stimulation control [44]. Additionally, Asian elephants *(Elephas maximus)* showed less stereotyped behaviours when exposed to classical music compared to a no auditory stimulation control [45]. Kennelled dogs have been experimentally exposed to different types of auditory enrichment. Kogan et al. [46] examined the effects of different types of music and found that with classical music dogs spent more time sleeping and less time barking than with heavy metal, bespoke music specifically designed for dog relaxation, or no music. Bowman et al. [47] used a variety of music genres (Soft Rock, Motown, Pop, Reggae and Classical) and found that when any type of music was played, dogs spent less time standing and more time lying (with the exception of Reggae). Wells et al. [48] played different types of music (Classical, Heavy Metal and Pop), as well as human conversation, and found that dogs exposed to classical music spent more time resting and less time standing than dogs exposed to the other treatments. In Bowman et al. [9], the initial effects of classical music compared to a silent control, were a reduction in vocalisation and increase in time lying down, but dogs habituated to the stimuli by the second day of exposure.

Lavender exposure has been associated with increased relaxation [49] and reduced anxiety in humans [50,51], and has also been shown to have beneficial effects in different animal species. In pigs (*Sus scrofa domesticus*), lavender straw appeared to reduce the severity of travel sickness [52]. Horses exposed to humidified air mixed with lavender essential oil had lower heart rates, after an acute stress response, than horses exposed to humidified air alone [53]. In mice (*Mus musculus*), lavender was shown to have a sedative effect after inhalation, reflected by decreased motility of the animals [54,55]. Similarly, lavender has been used in dogs in different environments. Graham et al. [56] used diffused essential oils in a rescue shelter, and found that dogs spent more time lying down and less time moving when exposed to lavender and chamomile oils compared to rosemary and peppermint oil and a control (no odour). Wells [57] studied the effects of lavender for travel-induced excitement in dogs. Dogs were exposed to a lavender impregnated cloth and a control cloth (no odour) while going on car journeys. Dogs exposed to lavender spent more time resting and less time vocalising and moving. A study in sheep *(Ovis aries)* showed that lavender effects depended on the sheep temperament. Calm sheep exposed to lavender oil had a lower agitation score and vocalised less than calm control sheep, while nervous sheep exposed to lavender vocalised and attempted to escape more than nervous control sheep [58].

Dog appeasing pheromone (DAP) is a synthetic compound based on fatty acids secreted by the mammary gland of bitches after parturition [59]. The effect of the DAP diffuser use has been studied in puppies with disturbance (i.e., vocalisation and continuous door scratching at night) and house-soiling issues. Puppies exposed to DAP cried significantly less than those exposed to a placebo, but there were no effects on the number of nights that puppies soiled inside [60]. Dogs using impregnated DAP collars showed some improvement in behaviour while in car journeys. The greatest improvement was in dogs that had shown motion sickness signs (vomiting and salivating), while the least was in excitable dogs (those who had shown behaviours as barking, jumping and whining) [61]. In a veterinary clinic setting, DAP diffusers appeared to reduce anxiety signs, but there was no evidence of aggression reduction during a clinical exam with a single exposure to the pheromone [62]. In shelter dogs, DAP diffused for 7 days reduced barking amplitude and frequency when people walked by the kennels [63]. DAP collars have been used in puppies during training sessions where they appear to result in less fearful and more sociable behaviour, and improved learning [64]. This literature shows that sensory environmental enrichment can help to reduce stress and arousal signs in different settings and different animal species.

This study is part of a larger project investigating enrichment effects in shelter dogs (methodology and behaviour results are reported in Amaya et al. [65]). The first part of this project analysed the behaviour of sixty dogs when exposed to music, DAP, lavender or a control [65]. Dogs performed fewer vocalisations and increased calmer body postures when exposed to any of the treatments compared to the control, although the effect was weaker for the lavender treatment [65].

The current paper reports on the physiological data collected from the shelter dogs during the study described in Amaya et al. [65] and the aim was to determine if the stimuli have physiological effects that are detectable through HRV recordings. We hypothesized that HRV parameters influenced by vagal activity will be higher in shelter dogs exposed to music, lavender and DAP than those in the control group.

2. Materials and Methods

2.1. Subjects

The subjects enrolled in the study consisted of 60 shelter dogs; 35 males and 25 females, all desexed. Mean (± SD) dog age was 3.2 ± 2.4 years, ranging from 6 months to 11 years. They came from different sources and most were mixed breed. Their mean length of stay in the shelter was 45.9 ± 29.8 days, range 8–150 days (Appendix A). On admission to the shelter, all dogs were given a veterinary clinical examination and a standardised behaviour assessment as described in Clay et al. [66]. Each week, the RSPCA Qld Behaviour Team identified dogs for the study, with inclusion criteria being those showing high arousal-related behaviours, such as air snapping, mouthing, attempts to bite their lead or handler, excessive activity, constant vocalisation and over-reaction to other dogs. The selection was made based on information of their kennel behaviour, as provided by shelter staff working with them regularly. Shelter staff were responsible for placing the selected dogs into the study kennels; they were blind to the treatments and assigned dogs at random to each kennel as they became available.

2.2. Study Environment

This study was conducted at the Royal Society for the Prevention of Cruelty to Animals Queensland's (RSPCA Qld) Animal Care Campus at Wacol, Brisbane, Australia, between August and November 2017. Shelter activities took place as usual (cleaning, feeding and walking) and therefore shelter staff and volunteers were regularly present around the kennel blocks. Two kennel blocks were used for this study, each consisting of 16 kennels divided into two rooms of 8 kennels (two rows of four) and separated by a door. Each kennel had dimensions of 1.6 m × 4 m, and included a crate measuring 0.72 m × 1.55 m and a bed. Both sides had plastic walls that prevented dogs from seeing each other. The back of the kennel had thin metallic bars from roof to floor and the front door had a solid section at the bottom and the same metallic bars from the top of the solid section to the top of the door. For housing details refer to Amaya et al. [65]. The dogs were fed dry food twice a day and had water ad libitum. They were taken for walks twice a day by volunteers for 10 min each time (during the morning cleaning and the afternoon spot cleaning) and had occasional contact with volunteers at other times, except for the 3 h treatment period and 1 h post-treatment.

2.3. Study Design

Dogs were exposed to one of three forms of enrichment: music (n = 14), lavender (n = 15) and DAP (n = 16) or a control condition (no stimuli applied; n = 15). Dogs were exposed to the stimuli in their kennel for 3 h/day on 5 consecutive days, but the HRV measurement only took place on the final day of exposure to the stimuli. Treatments were conducted between approximately 10.30 am and 13.30 pm, depending on when all morning activities were complete. Dogs were also monitored for one-hour post-treatment.

For the music treatment, a databank of 301 songs was downloaded from Spotify (www.spotify.com/au/), and filters were applied to these songs to select music believed to be most suitable for the dogs. Songs were included if they matched the following criteria: tempo of 70 or fewer beats per minute, valence from 0 to 0.5 and energy less than 0.2 (these two last on scales of 0–1.0) [67]. The piano was the sole instrument, except in 6 tracks in which there was accompaniment by violins for part of the tracks [65]. Previous research suggests that single instruments require less neurological processing than multiple instruments [68]. The resulting selection of 51 tracks with a total 183-min duration was played with random track selection order on a mobile phone (Motorola® mobility (Google), Moto G (1st generation), Mountain View, CA, USA) connected to a mobile wireless stereo speaker (Logitech®, X300, Lausanne, Switzerland), with a set volume throughout the experiment. The speaker was placed in a plastic holder hung on the crate's door (in the middle of the kennel). The music was played at 70 dBA, measured from the kennel's door (700 cm of distance from speaker) using a sound level meter (Digitech®, QM-1589, Stanford, CT, USA) at the beginning of each treatment period.

For the lavender treatment, one ultrasonic diffuser (Select Botanicals, Gladesville, New South Wales, Australia) was placed in the crate and another at the back of the kennel. The dilution was 4 drops of 100% organic Bulgarian lavender (*Lavandula angustifolia*; Select Botanicals, Gladesville, New South Wales, Australia) in 60 mL of water. For the DAP treatment, 3 pumps of a synthetic analogue of the canine appeasing pheromone (15.72 mg/mL; Adaptil®, Ceva, Glenorie, New South

Wales, Australia) were sprayed on a bandana worn by the dog and 2 pumps on the dog's bedding as recommended by the manufacturer. Three additional pumps were sprayed at different points of the kennel's floor (1 at each of the back corners and 1 the front door). The control dogs did not receive any extra sensory stimulus.

2.4. Data Collection and Analysis

On the 5th day of every research week, the dogs were fitted with a heart rate monitor. Four human heart rate monitors were used throughout the study, randomly allocated to treatments. Two different models were used: 3 Polar® RS800CX (Polar Elctro, Kempele, Finland) and 1 Polar® V800 (Polar Elctro, Kempele, Finland). They consisted of a wearlink strap, a watch-computer and a wireless integrated network device. The Polar® RS800CX has been validated for measuring heart rate variability of dogs [69–72] and employed in studies using music as environmental enrichment [9,47,73]. The Polar® V800 has been validated for measuring heart rate variability in humans [74].

The heart rate monitor was positioned on the left side of the thorax at the third intercostal space and secured with adhesive bandages (ZebraVet®, Rocklea, Queensland, Australia). The area of attachment was shaved and cleaned with methylated spirits to allow good contact between the device and the skin. Ultrasound liquid was generously applied to the device to help with the transmission. The watch-computer was secured to the dog's collar. The heart rate monitor recorded for 20 min before commencing the treatment to capture a baseline of the heart rate. It then recorded for three hours while the dog was being exposed to the treatment. An extra hour was recorded after the treatments had finished to measure after-effects. Every 45 min the watches were checked to make sure they were still recording. If they had stopped, more ultrasound liquid was added and the recording restarted. The procedure for the heart rate monitor positioning and securing with adhesive bandages was the same for dogs in the four treatments.

Once the recording finished, data were transmitted via a bidirectional infrared interface to the Polar® Protrainer 5 software (Polar Electro, Kempele, Finland) for the Polar® RS800CX and via USB connection to the Polar® FlowSync software (Polar Electro, Kempele, Finland) for the Polar® V800. These data were then exported as text files to Kubios software (Standard Version 3.1.0. Kubios Oy (limited company) Departments of applied Physics, University of Eastern Finland, Kuopio, Finland).

Dogs were video recorded in their kennels using two mini cameras with charge-coupled devices and infrared capability (Signet®, Electus Distribution Pty. Ltd., Rydalmere, New South Wales, Australia), one at the front and one at the back of the kennel. Behaviours were recorded continuously (24 h/d during the 5 d of stimuli exposure). Observations were divided in three periods: the treatment period (3 h) 5 min observed every 15 min, i.e., 12 separate observations lasting 3600 s in total; the post-treatment period (4 h), 5 min observations every 30 min, i.e., 8 separate observations lasting 2400 s in total and the night period, 5 min of each hour were observed, i.e., 16 separate observations lasting 4800 s in total. Boris® behaviour coding software (version 6.0.4. for Windows, Torino, Italy) was used to record behaviour in an ethogram [65]. There was a single coder for all the videos and they were not blind to the stimuli as specific objects of each treatment were visible in the videos (i.e., bandana, speaker and diffusers). Time values were then transformed into % values (duration of behaviour/total observation time $\times$ 100, in s). Videos were observed for a second time during the baseline, treatment and post-treatment periods of the 5th and last day of exposure to the stimuli, when HRV was recorded, to find segments where the dogs were lying down for 5 consecutive minutes. This position was chosen as movement can interfere with the recordings and create artefacts. It has been recommended to obtain HRV during conditions when the subject is stationary, with unchanging motor activity [11,14]. As the dogs were freely moving in the kennel, the only possible segments of 5 consecutive minutes in the same position were obtained when the dogs were lying down. Five 5-min segments that fit the position criteria and had the smallest percentage artefact correction were chosen for each dog during the treatment period; this segment length has been recommended to standardise HRV studies [11,14]. Data were analysed either uncorrected or corrected using the 'very low threshold' option of this software

(0.45 s) and only segments with less than 5% corrected beats were included in the analysis following Kubios [75] recommendations. Of the 60 dogs originally enrolled in the study, 5 were excluded from the HRV analysis for the uncorrected data analysis, and 26 from the corrected data analysis. One dog was adopted the day before the HRV analysis took place. For the corrected data, the excluded dogs either did not fit the requirement of having segments with less than 5% artefact correction while lying down (n = 17) or did not meet the criteria mentioned above and also had missing data due to technical issues with the Polar® straps and/or watches (n = 8). No attempt was made to interpolate data for missing dogs. Therefore, data from 55 and 34 dogs were included in the uncorrected and corrected HRV analysis of treatment effects, respectively: music (n = 12 and 6), lavender (n = 13 and 10) and DAP (n = 16 and 9) or the control condition (n = 14 and 9). Baseline and post-treatment data were not used as only 14 and 7 dogs, respectively, had segments fitting the standard requirements. Baseline values would have been a useful measure as the dog's own control, but it was only recorded for 20 min and therefore it was hard to find 5 min segments when dogs were lying down and furthermore, with less than 5% artefact correction. Due to the large imbalance in dog numbers between treatments, the baseline data could not be statistically analysed.

2.5. Statistical Analysis

The HRV data were statistically analysed using Minitab 18 software (Minitab. LLC, State College, PA, USA). Mixed effects models were constructed using dog and heart rate monitor (HRM) as random factors, with dog nested within HRM, and treatment as a fixed factor. Dependent variables were Mean RR (ms), Mean HR (bpm), SDNN (ms), RMSSD (ms), pNN50 (%), standard deviation 1 of the Poincare Plot—short-term HRV (SD1), standard deviation 2 of the Poincare Plot—long-term HRV (SD2), LF/HF ratio, LF band (0.067–0.235 Hz) and HF band (0.235–0.877 Hz). These bands were estimated specifically for dogs by Behar et al. [22]. Both bands are expressed in absolute values of power (ms^2) and normalised units (n.u.). Artefacts were also fit as a dependent variable.

Assumptions were checked via plotting, and square root transformations were used for absolute LF power (ms^2), absolute HF power (ms^2) and LF/HF ratio, to achieve normal distribution of residuals. Assumptions were met after transformation. R-squared values for all models were high (>68% for HRV parameters and 65% for artefacts). When omnibus tests were significant ($p < 0.05$), differences between individual treatments were examined using Tukey's tests, which adjust for multiple comparisons. Trends were considered if $p \leq 0.10$ but >0.05.

In the first study from this project [65], treatment effects on the behaviour of dogs (n = 60) were analysed using mixed effects models constructed using dog as a random factor and dog number (entry time to the study), treatment and day as fixed factors. Only a subset (n = 34) of the dogs from that study were able to be included in the current dataset, therefore to assist in the interpretion of HRV treatment effects, the behaviour was reanalysed using the same statistical model but only using results from dogs with both behaviour and HRV data.

3. Results

3.1. Artefact Correction and Model Selection

There was no significant treatment effect on artefact correction ($F_{3,26} = 0.75$, $p = 0.53$) with mean correction levels of 1.88%, 1.42%, 1.42% and 2.23% (SED = 0.49) for music, lavender, DAP and control treatments, respectively. We selected the model that used artefact correction because that method is generally recommended by those working in this field [11,25,27]. R-squared values were high (>70%) for both uncorrected and corrected models.

3.2. Treatment Effects on HRV during the 3 h Treatment Period

Absolute LF power (ms^2) was higher in dogs exposed to music compared to those in the lavender and control groups (Table 2). SD2 (ms) was higher in dogs exposed to music compared to those in the lavender group. There were trends for treatment effects on mean HR, mean RR (ms) and SDNN (ms) (p = 0.10, 0.08 and 0.07, respectively). Inspection of the means suggest that these trends are largely influenced by the music group, which had the lowest mean HR, and highest mean RR/SDNN of the treatment/control groups. There were no significant treatment effects for any of the other HRV parameters.

Table 2. HRV parameters of dogs (n = 34) exposed to lavender, music, dog appeasing pheromone (DAP) or a control treatment in a shelter environment, during the treatment period. For square root (√) transformed parameters, back transformed values are also reported in parentheses. Means that do not share a superscript letter are significantly different (p < 0.05) by Tukey's test.

Parameters	Control	Music	Lavender	DAP	SED	F-Value (d.f. 3,29-32)	*p*-Value
Time Domain							
Mean RR (ms)	605	763	641	644	27.5	2.55	0.08
Mean HR (bpm)	103.7	80.3	95.4	96.8	3.96	2.26	0.10
SDNN (ms)	96.4	135.8	81.2	100.7	11.58	2.58	0.07
RMSSD (ms)	138	195	115	137	19.9	1.88	0.16
pNN50 (%)	52.6	69.5	50.2	52.2	6.85	1.49	0.24
Frequency Domain							
LF Power, √ (ms^2)	46.1 ^{b} (2125)	74.8 ^{a} (5595)	39.2 ^{b} (1537)	52.7 ^{ab} (2777)	6.49	5.52	0.003
LF Power (n.u.)	35.7	36.4	35.6	39.2	5.85	0.09	0.96
HF Power, √ (ms^2)	72.6 (5271)	103.3 (10671)	58.4 (3411)	72.6 (5271)	12.07	2.10	0.12
HF Power (n.u.)	64.1	63.3	64.2	60.5	5.85	0.09	0.96
LF/HF ratio, √	0.77 (0.59)	0.79 (0.62)	0.82 (0.67)	0.86 (0.74)	0.12	0.08	0.97
Non-linear							
SD1 (ms)	97.4	137.9	81.2	96.9	14.09	1.88	0.16
SD2 (ms)	96.1 ^{ab}	130.4 ^{a}	80.2 ^{b}	102.3 ^{ab}	10.47	3.88	0.02

Mean RR (ms; mean time duration between successive RR intervals (two consecutive R waves of the electrocardiogram (ECG)), Mean HR (bpm; mean heart rate), SDNN (ms; standard deviation of RR intervals), RMSSD (ms; root mean square of differences between successive RR interval), pNN50 (%; percentage of successive RR interval pairs that differ by more than 50 ms). LF/HF (low frequency/high frequency ratio), LF (low frequency) band (0.067–0.235 Hz) and HF (high frequency) band (0.235–0.877 Hz), both expressed in absolute values of power (ms^2) and normalised units (n.u.). SD1 (ms; standard deviation 1 of the Poincare Plot—short-term HRV) and SD2 (ms; standard deviation 2 of the Poincare Plot—long-term HRV).

3.3. Treatment Effects on Behaviour of Subset of Dogs (n = 34) during the 3 h Treatment Period

Reanalysis of the behaviour data from the previous study with the current animal cohort resulted in some differences to the statistical outcomes (Table 3 and Appendix B). For 11 of the 20 behaviours analysed, the deduced statistical significance (significant: $p < 0.05$, trend: $0.05 < p \leq 0.10$, or non-significant: $p > 0.10$) was the same. Two behaviours were no longer significant in the subset cohort (lie down total and sniff ground) and two lost significance and became trends (lie down-head down and body shake; Table 3) Two behaviours were no longer trends (stand and walk) and two became trends (groom and tail still). One behaviour reached criterion for significance in the subset cohort but did not in the full behavioural study (lie down-head up). It is important to note that while the HRV data was only recorded on the 5th and last day of exposure to the stimuli and only segments when dogs were lying down were analysed, the behaviour data belongs to the 5 days of treatment exposure (3 h/d) and therefore includes all the observed behaviours (i.e., standing).

Table 3. The behaviour of a subset of dogs (n = 34) exposed to lavender, music, DAP or a control treatment, during the 3 h treatment period on 5 consecutive days. For square root (√) transformed parameters, back transformed values are also reported in parentheses. Means that do not share a superscript letter are significantly different ($p < 0.05$) by Tukey's test.

Behaviour	Control	Music	Lavender	DAP	SED	F-Value (d.f. 3,17)	p-Value
Activity							
Lie down total, % of time	47.8	62.2	58.0	61.2	5.769	0.93	0.45
Lie down-head down, % of time	28.9	51.8	43.1	34.5	6.017	2.91	0.07
Lie down-head up, √ % of time	4.12 ^{ab}	3.04 ^{b}	3.61 ^{b}	5.09 ^{a}	0.437	4.79	0.01
	(17.0)	(9.24)	(13.0)	(25.9)			
Stand, % of time	34.3	30.1	31.1	26.2	4.302	0.48	0.70
Walk, √ % of time	2.69	2.12	2.27	2.26	0.248	0.67	0.58
	(7.23)	(4.49)	(5.15)	(5.10)			
Standing exit door, √ % of time	2.10 ^{a}	0.82 ^{b}	0.65 ^{b}	0.87 ^{b}	0.240	6.03	0.005
	(4.41)	(0.67)	(0.42)	(0.76)			
Sit, √ % of time	1.43	0.52	1.21	1.53	0.377	1.23	0.33
	(2.04)	(0.27)	(1.46)	(2.34)			
Vocalisation							
Vocalisation, % of time	7.90 ^{a}	0.27 ^{b}	1.40 ^{b}	3.71 ^{b}	1.427	12.2	< 0.001
Other behaviours							
Pant, √ % of time	1.07 ^{a}	0.02 ^{b}	0.30 ^{ab}	0.33 ^{ab}	0.426	4.01	0.03
	(1.14)	(0.0004)	(0.09)	(0.11)			
Body shake, √ events per hour	2.82	3.56	4.42	6.99	2.481	2.64	0.08
	(7.95)	(12.7)	(19.5)	(48.9)			
Sniff ground, √ % of time	0.29	0.19	0.22	0.44	0.144	1.25	0.32
	(0.08)	(0.04)	(0.05)	(0.19)			
Groom, √ % of time	0.41	0.34	0.54	0.82	0.271	2.80	0.07
	(0.17)	(0.12)	(0.29)	(0.67)			
Tail position and movement							
Tail low, % of time	60.2	71.6	60.2	53.8	5.353	1.26	0.32
Tail medium/high, % of time	16.3	16.1	21.1	14.4	3.828	0.26	0.86
Tail movement, % of time	11.0 ^{a}	1.38 ^{b}	6.82 ^{ab}	6.63 ^{ab}	2.164	3.37	0.04
Tail still, % of time	78.3	90.6	86.5	81.2	3.104	3.17	0.05
Location in kennel							
Front, % of time	31.0	27.2	22.6	16.6	6.623	0.66	0.59
Back, % of time	31.9	31.0	31.4	34.9	7.497	0.07	0.98
Crate, % of time	19.5	26.9	34.5	37.7	8.445	1.31	0.31
Middle, √ % of time	2.15	2.41	1.46	1.41	0.504	1.13	0.37
	(4.62)	(5.80)	(2.13)	(1.99)			

In the subset of dogs, those in the DAP group laydown with their head up more than dogs in the music or lavender groups. Dogs in the control group stood more on their hind legs with their front legs on the exit door and vocalised more than dogs in the three stimuli groups. Dogs in the music group panted and wagged their tail less than those in the control group. There was a trend for treatment effects on lie down-head down. Inspection of the means suggest that these trends are influenced by the music group, which spent the most time lying down with the head down out of the treatment/control groups. In both datasets, dogs exposed to the stimuli showed more behaviours related to relaxation compared to the control group, but in the full dataset the lavender group did to a lesser extent compared to the other two stimuli.

4. Discussion

4.1. Stimuli Effects on HRV Parameters

Dogs from the music group had a higher absolute LF power (ms^2) than dogs in the lavender and control groups. The interpretation of the LF band has been debated in the literature. Some studies [76–79] consider it an index of sympathetic activity only, while others suggest that this band reflects a mix of parasympathetic and sympathetic activity [23,80–90]. This second argument is based on research that shows conditions associated with sympathetic activity and therefore an increase in LF power would be expected, but instead a decrease in LF power has been observed [14], for example, during myocardial ischemia [80] and exercise [80,91,92]. Moreover, pharmacological interventions to enhance or reduce sympathetic activity in the heart do not produce consistent changes in the LF power [90,91,93]. Based on this, we have interpreted the LF band as a parameter that is influenced by both parasympathetic and sympathetic activity.

A relationship between the LF band and music has been previously established in humans. It was found that the LF component increased with the number of music sessions people were exposed to, for both calming and excitative music, and decreased when no music was played [94]. It was concluded that the LF component increases with music listening regardless of music type, and musical stimuli might activate both parasympathetic and sympathetic nervous systems as even brief exposure to music can produce perceptible cardiovascular effects [95] and the beat of music alone can cause a response in the ANS [43]. Yet, both calming music and silence produced subjectively relaxing moods [94]. These results concur with our findings, as the absolute LF power (ms^2) of the music group was higher than for lavender and control, two non-auditory conditions. This suggests that this parameter reflects the presence of musical stimuli. This possibly activated both branches of the ANS due to its varied effects temporally, with different rhythms and cadences in the tracks used.

The first study of this project compared the effects of the three stimuli and a control condition on behaviour [65]. Although the HRV results suggest that music activates both branches of the ANS, dogs from that group spent significantly more time lying down with their head down and less time standing on their hind legs with their front legs resting against the exit, vocalising and panting compared to the control group (Appendix B). These results were not identical when behaviour analysis was run in the subset of dogs drawn from the first study for the present analysis. However, in both analyses dogs in the music group stood on their hind legs with their front legs resting against the exit, vocalised and panted less than the control group. In this subset of dogs there was also a trend for a treatment effect on lying down with the head down, which appeared to be highest in the music group (Table 3). All of these behaviours are associated with increased relaxation, which corresponds with the data in the previous study using the full cohort of dogs.

SD2 is a non-linear parameter that describes long-term variability and is correlated with SDNN and RMSSD [96]. It is influenced by both sympathetic and parasympathetic input [24,96–99]. Tulppo et al. [99] found that SD2 decreased during exercise after parasympathetic blockade and therefore attributed it to sympathetic activation. Consequently, an increase in this parameter is thought to indicate a decrease in sympathetic activity. Previous studies have found higher SD2 in dogs exposed to classical music compared to a silence control [9,73]. Bowman et al. [9] interpreted it as a decrease in sympathetic activity, associated to decreased anxiety in the dogs. In our study, dogs in the music group had a higher SD2 compared to lavender. As mentioned above, dogs in the music group in this subset of dogs had a trend to lie down with their head down more than the other three groups. Moreover, the lavender group showed behaviours associated with increased relaxation and reduced arousal compared to the control group to a lesser extent than the music and DAP groups in the full dataset analysis. This suggests that the difference in SD2 could be due to lower sympathetic activity in the music group or increased sympathetic activity in the lavender group. However, the lack of difference in other parameters specific to vagal activity makes any firm conclusions difficult.

In humans, several studies have tested the effects of lavender on HRV and other cardiac parameters (i.e., heart rate, systolic and diastolic blood pressure), with no significant effects [100]. However, a dog study had some significant findings. Dogs received either a dermal application of lavender or a placebo during four 3.5 h periods while monitoring HRV. In dogs exposed to lavender, there was a significant increase in HF power and a significant decrease in heart rate, but only during the 3rd and 4th periods, respectively [101]. These results suggest that topical exposure to lavender oil had some effect on vagal activity. The difference in results with our study might be due to the fact that lavender was administered through diffusers rather than on the skin, which may decrease any anxiolytic effect. Further research would be recommended on the effect of application method.

There was a trend for dogs in the music group to have lower mean HR and higher mean RR, which reflects increased vagal activity [20], and higher SDNN, which is influenced by both parasympathetic and sympathetic activity [21] and estimates overall HRV [14]. Bowman et al. [9] found a reduced mean HR, and increased mean RR and SDNN in dogs when initially exposed to classical music. They attributed these changes to a possible increase in vagal activity but also to the fact that the dogs spent a lot of time lying down while music was played. However, RMSSD and pNN50, both of which reflect vagal activity [11,16], were also increased, suggesting that the HRV changes resulted from increased vagal activity due to music exposure. In our study, HRV was only analysed when the dogs were lying down, but this was standardised across the four treatments, indicating that the trend in the music group were possibly driven by increased vagal activity compared to the other groups. This is supported by the trend of dogs in this group to show more behaviours indicative of relaxation. As shelters are very busy environments during the day, being able to rest more may indicate improved welfare [102]. Moreover, when physical activity is controlled, SDNN could be a good sign of increased attention [29,30]. This suggests that while dogs in the music group possibly had increased vagal activity, they could have more intently perceived the stimulus than dogs in the other groups, as music is constantly changing, opposed to DAP and lavender, which are constant. This increased attention could be reflected in some sympathetic activity, also inferred by the higher absolute LF (ms^2) power, indicating activation of both branches of the ANS.

No significant differences between enrichment groups were found in RMSSD, pNN50 or LF/HF ratio. Köster et al. [73] did not find significant effects in RMSSD and pNN50 in dogs exposed to classical music compared to those in a silent control during a canine clinical examination practice. In that study, dogs exposed to music had higher SDNN, but lower mean RR than dogs in the control, possibly indicating that exposure to music was a novel experience rather than calming. Neither Köster et al. [73] or Bowman et al. [9] measured LF and HF bands individually, therefore it is not possible to compare directly with our results. However, they did measure the LF/HF ratio and one found it was not significant [73] while the other found it was not consistently affected by music [9].

In our previous behaviour study, dogs exposed to DAP spent more time lying down and stood on their hind legs with their front legs resting against the exit less than those in the control group. Thus dogs exposed to DAP showed more behaviours associated with increased relaxation and reduced arousal compared to the control group. The absence of any significant effects of DAP on cardiac activity is therefore surprising, but they cannot be compared with other studies, as to the authors' knowledge, no other studies have looked into DAP effects on HRV measurements.

4.2. Study Limitations

One limitation for this study was the small number of dogs with baseline data and the large imbalance in dog numbers between treatments. This baseline would have been useful as an index of each subject's autonomic state, with stressed dogs potentially having lower vagal activity before enrichment exposure [11]. The small number was in part due to the use of artefact correction, with many dogs having more than 5% artefact correction, which made them ineligible for inclusion. Having a smaller number of dogs reduced power and created imbalance across treatments, although significant differences between treatments were still apparent. Further research on the optimum level of artefact correction for studies with dogs is warranted.

Another possible limitation was the need to use an adhesive bandage to keep the heart rate monitor strap in place during the recording. Studies have shown that pressure wraps such as the ThunderShirt® (Durham, NC, USA) [103] and telemetry vests [104] help to reduce heart rate and anxiety related behaviours of dogs that wear them. The bandages could have produced an anxiolytic effect and therefore reduced treatment effects. However, all the dogs (control and treatment) had the bandages applied so if there was an effect, all groups would have experienced it. Moreover, the time that the dogs had the Polar® heart rate monitors attached to them might have been too long, allowing more technical issues and mechanical artefacts to occur. In the future, it would be recommended to have shorter HRV recording periods [105] to have more control over these issues. However, very short recordings would not be recommended either, as placing and adjusting the monitors might be stressful for the dogs and could influence recordings.

Motor activity can influence HRV and it can also mask emotional and health processes and produce more artefacts [16], therefore, HRV measures should be taken when stationary [11,14]. The correlation between Polar® heart rate monitors and echocardiogram decreases as motor activity increases in humans [106], horses [107], pigs [108] and dogs [70]. Moreover, when the aim is to compare non-motor (psychological) components of cardiac activity, only recordings obtained during similar behavioural patterns should be used [11]. As the recordings were taken from dogs freely moving around the kennel, the only possible segments of 5 consecutive minutes in the same position were obtained when the dogs were lying down, and therefore the results reflect ANS activity only for this body posture. Despite a highly standardised protocol and obtaining the HRV measure only of stationary dogs, Essner et al. [69] found that the Polar heart rate monitor missed intervals that the echocardiogram detected and therefore some HRV results can be inaccurate. Parker et al. [107] and Marchant et al. [108] also pointed out some problems with the validity and reliability of Polar® heart rate monitors and particularly when recording data in ambulatory conditions.

It is important to take into account the equipment used and its possible limitations. Following Kubios [75] advice, we used the lowest possible artefact correction level (very low threshold). However, these automatic correction levels were originally developed for human heart rate data, so there is no certainty that they can appropriately correct dog heart rate data [109].

It is possible that because HRV was only measured on the fifth day, dogs had habituated to the stimuli by then. However, based on previous behaviour observations over time [65], there was no evidence of habituation to any of the stimuli over the 5 days of exposure.

5. Conclusions

From the three stimuli dogs were exposed to, music produced the most changes in HRV, seemingly by activating both branches of the ANS and therefore producing significant changes in HRV parameters that reflect both parasympathetic and sympathetic activity. There were also trends for dogs in this group to have lower heart rate and consequently increased RR intervals. These results combined with the behaviour results from the first study and the behaviour results of this subset of dogs, indicate that dogs in the music group were more relaxed. There is evidence from the HRV that this was related to an increased vagal activity compared to dogs in the other groups. Shelters could consider using similar methods of music enrichment, as is it the easiest and cheapest stimulus to apply and produces both behavioural and physiological positive effects in dogs. It may help to relieve both the stress and boredom in shelter environments. As for the other stimuli, their effect might have not been strong enough to produce measurable changes in cardiac activity.

Wearable devices such as the Polar® RS800CX and the Polar® V800 can be useful tools to measure autonomic responses in dogs. However, many variables should be taken into account when using HRV as a physiological parameter to measure stress. These include the recording quality, the dog's motor activity while collecting the data and artefacts [106].

Author Contributions: Conceptualization, V.A., C.J.C.P. and M.B.A.P.; methodology, V.A., C.J.C.P. and M.B.A.P.; software, V.A.; formal analysis, V.A. and K.D.; investigation, V.A.; resources, C.J.C.P. and M.B.A.P.; data curation, V.A.; writing—original draft preparation, V.A.; writing—review and editing, C.J.C.P., M.B.A.P. and K.D.; visualization, V.A.; supervision, C.J.C.P., M.B.A.P. and K.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We acknowledge the financial support and provision of resources by the School of Veterinary Science and the Centre for Animal Welfare and Ethics, University of Queensland, and RSPCA Queensland for lending their facilities and all the staff and volunteers who helped during the study, especially Joshua Bryson and Annie Cross. We are grateful too for the technical support of John Mallyon.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Dogs (n = 34) included in the HRV analysis.

Dog	Age (in Months)	Source	Days in Shelter at Beginning of Trial	Treatment	Heart Rate Monitor (HRM)	Number of 5-min HRV Segments
Diesel 1	15	Owner surrendered	33	Control	2	2
Sasha	37	Owner surrendered	46	Control	3	1
Diesel 2	73	Transferred from other shelter	45	Control	3	5
Koda	12	Stray	58	Control	3	4
Tyra	68	Stray	20	Control	4	5
Buffy	18	Impounded by council	30	Control	4	1
Walter	10	Impounded by council	43	Control	4	3
Chumps	28	Impounded by council	39	Control	4	2
Alf	65	Impounded by council	93	Control	2	1
Gem	50	Impounded by council	59	Lavender	4	5
Spencer	13	Owner surrendered	21	Lavender	1	1
Bob	20	Impounded by council	64	Lavender	4	1
Bronson	26	Transferred from other shelter	67	Lavender	2	2
Eugene	18	Impounded by council	22	Lavender	4	5
Chloe	18	Returned after previous adoption	22	Lavender	3	2
Diesel 3	12	Brought in by shelter ambulance	150	Lavender	3	5
Tyson	48	Impounded by council	17	Lavender	2	2
Karter	60	Stray	58	Lavender	3	1
Pumpkin	12	Impounded by council	32	Lavender	3	5
George	13	Impounded by council	37	Music	2	5
Diesel 4	25	Owner surrendered	60	Music	4	3
Rusty	44	Owner surrendered	18	Music	1	2
Oscar	60	Impounded by council	22	Music	2	5
Belle	36	Owner surrendered	60	Music	1	3
Cadbury	18	Impounded by council	24	Music	4	1
Basil	9	Impounded by council	43	DAP	4	5
Pepper	15	Owner surrendered	20	DAP	4	3
Ellie	24	Impounded by council	36	DAP	2	5
Jenny	105	Brought in by shelter ambulance	60	DAP	1	5
Mia	27	Owner surrendered	25	DAP	1	4
Missy	46	Owner surrendered	41	DAP	2	3
Lisa	6	Brought in by shelter ambulance	11	DAP	3	5
Sheba	120	Impounded by council	34	DAP	4	5
Tackle	11	Returned after previous adoption	17	DAP	4	5

Appendix B

Table A2. The behaviour of shelter dogs (n = 60) exposed to olfactory and auditory stimuli or a control treatment for 3 h/d on 5 consecutive days [65]. For square root (√) transformed parameters, back transformed values are also reported in parentheses. Means that do not share a superscript letter are significantly different ($p < 0.05$) by Tukey's test.

Behaviour	Control	Music	Lavender	DAP	SED	F-Value (d.f. 3,41)	p-Value
Activity							
Lie down total, % of time	44.4 ^{b}	61.3 ^{ab}	52.6 ^{ab}	61.7 ^{a}	4.64	3.29	0.03
Lie down-head down % of time	29.4 ^{b}	49.9 ^{a}	38.7 ^{ab}	43.6 ^{ab}	4.72	4.46	0.008
Lie down-head up, √ % of time	3.58	3.13	3.52	4.01	0.337	1.24	0.31
	(12.8)	(9.79)	(12.4)	(16.1)			
Stand, % of time	39.0	29.5	33.4	26.6	3.44	2.44	0.08
Walk, √ % of time	2.67	2.00	2.31	2.04	0.189	2.37	0.09
	(7.14)	(4.02)	(5.33)	(4.17)			
Standing exit door, √ % of time	1.67 ^{a}	0.55 ^{b}	0.86 ^{ab}	0.51 ^{b}	0.164	4.35	0.009
	(2.79)	(0.30)	(0.74)	(0.26)			
Sit, √ % of time	1.39	1.16	1.90	1.65	0.316	0.81	0.49
	(1.93)	(1.35)	(3.60)	(2.74)			
Vocalisation							
Vocalisation, √ % of time	2.42 ^{a}	1.30 ^{b}	1.12 ^{b}	1.27 ^{b}	0.291	6.90	0.001
	(5.87)	(1.70)	(1.26)	(1.61)			
Other behaviours							
Pant, √ % of time	1.30 ^{a}	0.12 ^{b}	0.48 ^{b}	0.36 ^{b}	0.267	7.26	0.001
	(1.69)	(0.01)	(0.23)	(0.13)			
Body shake, √ events per hour	0.33 ^{b}	0.30 ^{b}	0.42 ^{b}	0.72 ^{a}	0.197	6.38	0.001
	(0.11)	(0.09)	(0.17)	(0.51)			
Sniff ground, √ % of time	0.27 ^{ab}	0.09 ^{b}	0.25 ^{ab}	0.37 ^{a}	0.115	3.47	0.03
	(0.071)	(0.007)	(0.061)	(0.13)			
Groom, √ % of time	0.42	0.37	0.52	0.67	0.199	1.65	0.19
	(0.18)	(0.14)	(0.27)	(0.45)			
Tail position and movement							
Tail low, % of time	61.3	70.3	58.2	60.0	4.15	1.62	0.20
Tail medium/high, √ % of time	3.89	3.15	3.74	3.35	0.397	0.37	0.78
	(15.1)	(9.93)	(14.0)	(11.2)			
Tail movement, % of time	10.10 ^{a}	5.30 ^{b}	8.11 ^{ab}	5.45 ^{b}	1.659	3.59	0.02
Tail still, % of time	81.4	87.6	85.9	87.3	2.42	2.08	0.12
Location in kennel							
Front, √ % of time	4.90	4.70	4.23	3.51	0.501	2.10	0.12
	(24.0)	(22.1)	(17.9)	(12.3)			
Back, % of time	35.2	38.1	39.1	36.0	5.78	0.15	0.93
Crate, √ % of time	3.80	3.86	4.35	5.45	0.640	1.49	0.23
	(14.5)	(14.9)	(18.9)	(29.7)			
Middle, √ % of time	1.97	2.41	1.51	1.52	0.438	1.94	0.14
	(3.87)	(5.81)	(2.27)	(2.32)			

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Abilities of Canine Shelter Behavioral Evaluations and Owner Surrender Profiles to Predict Resource Guarding in Adoptive Homes

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Received: 18 August 2020; Accepted: 17 September 2020; Published: 20 September 2020

Simple Summary: Some domestic dogs guard resources and display behaviors such as growling, snarling, or biting when approached. Most animal shelters test for food-related aggression and some consider dogs assessed as food aggressive to be unadoptable and candidates for euthanasia. We surveyed adopters of 139 dogs assessed as either resource guarding (n = 20) or non-resource guarding (n = 119) at a New York (NY) shelter to determine whether shelter identification as food aggressive was associated with guarding in adoptive homes. We also examined whether description of resource guarding in owner reports completed when surrendering a dog to the shelter predicted guarding in adoptive homes. Statistically, shelter assessment as resource guarding and owner-supplied information indicating resource guarding were each associated with guarding in adoptive homes. However, more than half of dogs either assessed by shelter staff or described by surrendering owners as resource guarding did not guard in adoptive homes. Our data indicate that information from surrendering owners, while potentially helpful, is not always predictive of a dog's behavior in an adoptive home, and most importantly, that shelters should not consider all dogs assessed as resource guarding to be unadoptable because many of these dogs do not display guarding behavior post adoption.

Abstract: Some shelters in the United States consider dogs identified as food aggressive during behavioral evaluations to be unadoptable. We surveyed adopters of dogs from a New York shelter to examine predictive abilities of shelter behavioral evaluations and owner surrender profiles. Twenty of 139 dogs (14.4%) were assessed as resource guarding in the shelter. We found statistically significant associations between shelter assessment as resource guarding and guarding reported in the adoptive home for three situations: taking away toys, bones or other valued objects; taking away food; and retrieving items or food taken by the dog. Similarly, owner descriptions of resource guarding on surrender profiles significantly predicted guarding in adoptive homes. However, positive predictive values for all analyses were low, and more than half of dogs assessed as resource guarding either in the shelter or by surrendering owners did not show guarding post adoption. All three sources of information regarding resource guarding status (surrender profile, shelter behavioral evaluation, and adopter report) were available for 44 dogs; measures of agreement were in the fair range. Thus, reports of resource guarding by surrendering owners and detection of guarding during shelter behavioral evaluations should be interpreted with caution because neither source of information consistently signaled guarding would occur in adoptive homes.

Keywords: dog; food aggression; food guarding; resource guarding; animal shelter; behavioral evaluation; adoption; owner surrender

1. Introduction

Resource guarding aggression represents a suite of behaviors, such as growling, freezing, snapping, and biting, shown by some domestic dogs that are possessive of food, toys, or sleeping sites [1]. According to one survey of 77 animal shelters in the United States, most shelters test for food guarding during behavioral evaluations and about half consider dogs identified as food aggressive to be unadoptable and therefore candidates for euthanasia [2]. In contrast, successful re-homing of most shelter dogs assessed as food aggressive has been reported, although more than one effort at adoption was sometimes needed because dogs that displayed severe guarding during assessments were returned more frequently by adopters [3]. Even so, shelters that make food aggressive dogs available for adoption often restrict who can adopt them, which can result in longer shelter stays [4,5]. Because resource guarding can affect both public safety and dog welfare, it is important to determine whether dogs assessed as food aggressive during shelter behavioral evaluations display food aggression in adoptive homes. This topic is especially relevant given recent critiques regarding the usefulness and predictive abilities of shelter behavioral evaluations with regard to tests for resource guarding and other behaviors [4,6–8].

Four studies, two examining several types of behavior and two focused exclusively on resource guarding, have investigated whether tests conducted under shelter conditions successfully predict behavior in adoptive homes. Van der Borg et al. [9] developed and administered 21 tests to 81 dogs in five different shelters and surveyed adopters after dogs had spent 1–2 months in the home. The authors compared shelter test results with 72 reports from adopters. For aggression displayed over food or a bone, shelter tests were consistent with adopter reports about 43% of the time. Clay et al. [10] examined whether results from 11 tests run at one shelter predicted post-adoption behavior of 120 dogs. Although results from some shelter tests, such as those assessing friendliness, fearfulness, and anxiousness, reliably predicted behavior in the home one month after adoption, results from tests for resource guarding did not (percentage of dogs assessed as food aggressive in the shelter and reported to be food aggressive in the adoptive home was not provided). Mohan-Gibbons et al. [2] identified 96 dogs assessed as food aggressive at one shelter, placed them on a food program (free-feeding and foraging enrichment), and contacted their adopters three times in the months following adoption. Food guarding was rarely reported in the first three weeks in the home: for example, of the 60 adopters who responded at least once, six reported at least one guarding incident. No guarding was reported by these six adopters at 3 months post adoption, although one new incident of guarding was reported by another adopter at this time. Marder et al. [5] followed 97 shelter dogs and compared results from behavioral evaluations at the shelter to adopter reports at least 3 months after adoption. Unlike the dogs followed by Mohan-Gibbons et al. [2], this sample included dogs assessed as food aggressive and dogs assessed as not food aggressive in the shelter, and dogs were not placed on a specific food program. Of the 20 dogs assessed as food aggressive in the shelter, 11 (55%) showed food aggression in the adoptive home. Of the 77 dogs assessed as not food aggressive in the shelter, 17 (22%) showed food aggression in the adoptive home. Thus, in the three studies reporting percentages for resource guarding, the percent of dogs assessed as food aggressive in the shelter that showed food aggression in the adoptive home ranged from about 10% to 55%.

Given the wide range in percentages reported from the three previous studies comparing shelter evaluation results with adopter reports in regard to resource guarding [2,5,9], we revisited this question at one animal shelter in New York. Our study design was most similar to that employed by Marder et al. [5] in that we contacted adopters of dogs assessed as food aggressive in the shelter as well as adopters of dogs assessed as non-food aggressive to determine whether guarding behavior was exhibited in the adoptive home, and dogs in our study were not maintained on a food program. Our study differs from that by Marder et al. [5] in that we contacted adopters 1–3 months post adoption whereas they contacted adopters at least 3 months post adoption and many were contacted one or more years after adoption. Additionally, whereas the adopter survey conducted by Marder et al. [5] focused on food and food-related items (rawhides and bones), we also asked adopters about the guarding of sleeping sites to determine whether dogs that displayed food-related aggression at the shelter would guard non-food items in the home. Finally, unlike the studies by Mohan-Gibbons et al. [2], Marder et al. [5], Van der Borg et al. [9], and Clay et al. [10], we also investigated whether information supplied by surrendering owners predicted behavior in the adoptive home. Specifically, we examined whether owner answers to questions concerning reasons for surrender, resource guarding, and aggression predicted guarding behavior in the adoptive home. Thus, for a subset of dogs, we were able to compare owner surrender profiles, shelter behavioral evaluations, and adopter reports for consistency in assessment of resource guarding.

Based on the evidence presented by Marder et al. [5], we predicted that about half of dogs that showed food-related guarding during shelter behavioral evaluations would show food-related guarding in their adoptive homes, and that about a quarter of dogs assessed as non-food guarding in the shelter would show food-related guarding in their adoptive homes. Given that resource guarding appears sensitive to context, setting, and type of resource [1,2,5], we did not expect an assessment of food-related aggression in the shelter to be associated with the guarding of sleeping sites in the adoptive home. Owners may under-report problematic behaviors at the time of surrendering their dogs to shelters [11], so the predictive ability of owner reports might be lower than that of shelter behavioral evaluations. Alternatively, because shelter evaluations are conducted under conditions that are unfamiliar and often challenging for dogs [6,7], owner reports might better predict behavior in adoptive homes, even though no two homes are identical. We viewed these outcomes as equally likely. Throughout this paper, we use the term "owner" to refer to a person surrendering a dog to the shelter and "adopter" to refer to a person taking a dog home from the shelter.

2. Materials and Methods

2.1. Study Shelter

We analyzed canine surrender profiles and shelter behavioral evaluations, and contacted adopters of dogs from the Tompkins County SPCA in Ithaca, NY, USA. The shelter is no-kill, with open-admission and scheduled intake. Shelter programs to increase dog adoptions include: a small number of foster homes; a large number of volunteers who participate in dog walking, in-kennel training and companionship, overnight fostering of dogs, and day-trips with dogs; playgroups for suitable pairs of dogs; and adoption promotion via off-site events, social media, local print, and by a volunteer group independently advertising dogs that have been on the adoption floor for a long time. This research was conducted from August 2018 through January 2020 under protocol 2012-0150, which was approved by Cornell University's Institutional Animal Care and Use Committee.

2.2. Dog Care and Housing

Upon intake, dogs were housed in the rescue building in chain link cages (indoor area, 2.2 m^2 and outdoor run, 3.5 m^2). All dogs were examined by veterinary staff at intake and received vaccinations, flea control, fecal exam, deworming, and a heartworm test. After the veterinary exam, each dog was scheduled for behavioral evaluation (Section 2.3). A day or two after behavioral evaluation, staff moved dogs to the adjacent pet adoption center where they were individually housed in one of 13 cubicles (from 5.2 m^2 to 7.3 m^2), which contained a raised bed, blanket, toys, and water bowl. Dogs were fed each day by staff between 08:00 and 09:00 h and again between 15:00 and 16:00 h. Staff and volunteers exercised dogs several times a day through leash walks and time in a large outdoor enclosure. Intact dogs were spayed or neutered before adoption.

2.3. Behavioral Evaluations

About 3 days after intake, each dog's behavior was evaluated by two shelter staff (one serving as evaluator and one as scribe) using nine tests based on Sternberg's Assess-a-Pet [12], with modifications described by Bollen and Horowitz [13]. Tests occurred in the following sequence: cage presentation;

sociability; teeth exam; handling; arousal; food bowl; possession; stranger; and dog-to-dog. The Assess-a-Hand was used only during the food bowl test and possession test, and most dogs were tethered to the wall for these two tests. Dogs that displayed significant fear in response to tethering and extremely small dogs whose movements would be impeded by the heavy clip of the tether were held on a leash by the scribe. Responses scored for the food bowl and possession tests are shown in Table 1. As described in McGuire [3], we classified dogs as showing resource guarding if they exhibited at least one of the following behaviors during either the food bowl test, possession test, or both tests: stiffened, exhibited whale eye, snarled, froze, growled, lunged, snapped, or bit the Assess-a-Hand (food bowl test: responses 6 and 7; possession test: responses 4 and 5; Table 1). Source shelters transferring dogs to the Tompkins County SPCA only occasionally sent results from behavior assessments at their shelter; any results received were considered for that particular dog and the dog was tested by Tompkins' staff as well.

Table 1. Possible responses during food bowl and possession tests on the canine behavioral evaluation at the shelter [1].

Response	Food Bowl Test	Possession Test
1	Stopped eating and backed away from dish	Readily dropped item
2	Continued eating without signs of uneasiness	Allowed Assess-a-Hand to take item
3	Moved muzzle deeper into dish and ate faster	Resisted letting go of item but did not show outward aggression
4	Stiffened slightly	Stiffened, exhibited whale eye, snarled
5	Moved muzzle toward Assess-a-Hand	Froze, growled, lunged, snapped, bit Assess-a-Hand
6	Stiffened, exhibited whale eye, snarled	—
7	Froze, growled, lunged, snapped, bit Assess-a-Hand	—

[1] Kibble and canned food were provided during the food bowl test and a food-related item, such as a raw hide chew or pig's ear, was provided during the possession test.

2.4. Adopter Survey

Standard practice at the shelter (and continued during our study) is for the behavior or adoptions staff to counsel adopters of dogs either described by previous owners as resource guarding or assessed as resource guarding during a shelter behavioral evaluation. Staff follow a conversation-based adoption process during which adopters are fully informed about the guarding behavior reported by previous owners or observed by shelter staff and how it could impact the household. Staff also offer printed handouts and links to online videos with training tips (e.g., using positive reinforcement to teach "leave it" and "drop it"). It is also standard practice for a long-term volunteer to contact (via phone, email, or text) adopters of all dogs approximately 2 weeks after the dog entered its new home. The volunteer asks questions about how the dog is adjusting, whether there are any concerns, and if the dog has been examined by a veterinarian. Beginning in summer 2018, upon completion of these standard questions, the volunteer asked adopters if they would be willing to participate in our study on post-adoption behavior. We received the contact information for adopters who agreed to participate and then contacted them at least 4 weeks after the adoption (range, 4–12 weeks; pilot data indicated that response rates decreased after 12 weeks). We tried to reach each adopter via two of three different methods (phone, email, or text) before recording "no response". We asked adopters to rank their dog's behavioral responses from 0 to 2 (where 0 = no visible signs of aggression; 1 = growling or snarling; 2 = snapping or biting) in five different situations in which a family member: (1) took away toys, bones, or other objects valued by the dog (e.g., rawhides); (2) took away the dog's food; (3) retrieved items or food taken by the dog; (4) approached the dog while it was eating; and (5) approached the dog at its favorite sleeping site (hereafter Q1–Q5; modified from the Canine Behavioral Assessment and Research Questionnaire, C-BARQ; [14] www.cbarq.org). We also asked three scripted questions (Q6–Q8; modified from Marder et al. [5]), beginning with whether the adopter considered their dog to be possessive in guarding food, toys, or space. If the adopter answered yes, then we asked whether they regarded their dog's guarding behavior as a major concern and whether they felt it was difficult to prevent or manage possessive behaviors in their dog.

Of the 205 adopters who agreed to participate in our study when called by the shelter volunteer 2 weeks post adoption, 139 responded to our survey (58 by email; 53 by text and 28 by phone) for an overall response rate of 67.8%. Demographic data (age class and source) for the 139 dogs whose adopters responded are shown by sex in Table 2. Based on results of shelter behavioral evaluations, 20 of the 139 dogs (14.4%) were assessed as resource guarding and 119 (85.6%) as non-resource guarding. On average (mean ± *SD*), adopters responded 5.6 ± 1.4 weeks after adopting their dog. Time to respond did not differ between adopters of dogs assessed as resource guarding at the shelter (5.5 ± 1.6 weeks; range, 4–12 weeks) and adopters of dogs assessed as non-resource guarding (5.6 ± 1.1 weeks; range 4–11 weeks; $t = 0.16$, *d.f.* = 22.47, $p = 0.87$).

Table 2. Demographic data for dogs ($n = 139$) whose adopters responded to our survey.

Demographic Information	Males	Females
Age class [1]		
Juvenile	13	11
Adult	54	39
Senior	12	10
Source		
Surrendered by owner	32	25
Transferred from another shelter	24	22
Picked up as a stray	11	5
Returned by adopter	7	8
Seized by animal control officer	5	0

[1] Juveniles, from 4 months to <1 year; adults, from 1 year to <8 years; and seniors, ≥8 years. We did not track behavior in the adoptive home for puppies because shelter behavioral evaluations for this age group differ from those for dogs at least 4 months of age.

2.5. Canine Owner Surrender Profiles

We reviewed surrender profiles for 51 of the 57 dogs that were owner surrendered and whose adopters responded to our survey described in Section 2.4; owners of the remaining six dogs did not complete a surrender profile. We focused on three sections of the 4-page profile form that we considered potentially relevant to resource guarding behavior and scored each section as yes/no (Table S1). The first section asked, "Why are you surrendering your dog to the shelter?", provided the options of behavioral problems, time commitment, family issues, health issues (owner), health issues (dog), and other, and instructed the owner to circle all that apply. We scored this as yes if the option, "behavioral problems", was circled. The second section asked, "What does your dog do when you or someone else:" and listed nine scenarios from which we chose two: (1) "go near the food bowl?"; (2) "try to take away toys, rawhides, or anything else of value?". We scored this as yes when at least one incident of growling, snarling, snapping, nipping, or biting was reported by surrendering owners. The scenarios not chosen in this section concerned responses to strangers in different settings and being hugged, reprimanded, and told to get off the sofa or bed. The third section began with, "Has your dog ever snarled at you or anyone else?" and provided lines where owners could check yes or no. In the four subsequent questions in this section, "snarled" was replaced with each of the following terms, respectively: "growled", "snapped", "nipped", and "bitten (broken the skin)". Owners were asked to explain the situation if they checked yes for any of the five behaviors. We scored this as yes when the owner reported the dog had displayed at least one of the five behaviors.

Of the 51 profiles available, 41 were mostly or fully completed (one question left unanswered or all questions answered). A few owner responses were either illegible or unclear in meaning and not included in the data set (e.g., an answer of "yes" or "no" to questions in the second section, such as "What does your dog do when you or someone else go near the food bowl?"). We made combined profiles for each of the six dogs that had more than one profile on file at the shelter during the time period of our study; these dogs had been returned to the shelter at least once. For combined profiles, we included all options circled on the different profiles as reasons for surrendering, and if a dog was reported as showing visible signs of aggression by one owner but not by another owner for questions in the second section ("What does your dog do when?") and third section ("Has your dog ever?"), then we scored the dog as having shown visible signs of aggression.

2.6. Statistical Analyses

We used JMP Pro 13 for all statistical analyses.

2.6.1. Relationships between Shelter Behavioral Evaluations and Adopter Reports

Relatively few adopters reported visible signs of aggression in their dogs at home, especially snapping or biting. Accordingly, we combined growling, snarling, snapping, and biting into a single category and scored adopter reports of their dog's behavioral responses to the five situations described in our survey as visible signs of aggression reported or not reported. We present descriptive information on the specific behaviors shown by dogs. We excluded from analyses of adopter responses to survey questions, cases in which adopters did not place their dog in the particular situation described (e.g., adopters who did not take toys, bones, or other valued objects away from their dogs were excluded from the analysis of responses to Q1 and adopters who did not take food away from their dogs were excluded from the analysis of responses to Q2, etc.; exclusions are reflected in the denominators shown in Table 3, Section 3.1, and described in the footnote). We used Fisher's exact test to examine whether resource guarding status of dogs based on shelter behavioral evaluations was associated with adopter reports of behavior in the home for the five situations surveyed (Q1–Q5). We also report positive predictive values and negative predictive values, as defined by Marder et al. [5], for each of the five questions. Positive predictive value is the likelihood that a dog that displayed food guarding during the shelter behavioral evaluation displayed food guarding in the adoptive home. Negative predictive value is the likelihood that a dog that did not display food guarding during the shelter behavioral evaluation did not display food guarding in the adoptive home. We include 95% confidence intervals for positive and negative predictive values.

2.6.2. Relationships between Owner Surrender Profiles and Adopter Reports

We used logistic regression to determine whether different types of information from owner surrender profiles predicted adopter reports of visible signs of aggression in the home when toys, bones, or other valued objects were taken away by a family member (Q1 on the adopter survey). The model included the following predictor variables from surrender profiles: (1) behavioral problems indicated as a reason for surrender (yes/no); (2) resource guarding described (yes/no) in the section "What does your dog do when you or someone else:"; and (3) visible signs of aggression reported (yes/no) in the section, "Has your dog ever?". For the second factor, we used information from the two scenarios ("What does your dog do when you or someone else goes near the food bowl" and "What does your dog do when you or someone else tries to take away toys, rawhides, or anything else of value") to be consistent with our method of scoring dogs as resource guarding or non-resource guarding based on shelter behavioral evaluations (i.e., we classified dogs as resource guarding if they exhibited particular behaviors during either the food bowl test, possession test, or both tests). Sample sizes were too small for us to analyze whether information from the 51 surrender profiles predicted adopters responses to Q2 through Q5 of the survey because within the 51 surrendered dogs, the number reported by adopters to have shown visible signs of aggression was sometimes one or zero for these questions. As a result, models for Q2 through Q5 were unstable, so we only report results for Q1.

2.6.3. Relationships between Shelter Behavioral Evaluations, Owner Surrender Profiles, and Adopter Reports

We compared shelter behavioral evaluations and owner surrender profiles in terms of prevalence of resource guarding and several measures of predictive ability with respect to behavior in the adoptive home (using responses to Q1 from the adopter survey). The following measures were defined in Patronek and Bradley [6] and we modified them for specific use with our data: sensitivity (proportion of adopted dogs that were correctly identified as resource guarding via the shelter evaluation or surrender profile); specificity (proportion of adopted dogs that were correctly identified as non-resource guarding via the shelter evaluation or surrender profile); false positive rate (proportion of adopted dogs identified by the shelter evaluation or surrender profile as resource guarding when they are not) and false negative rate (proportion of adopted dogs identified by the shelter evaluation or surrender profile as non-resource guarding when they are not). For shelter behavioral evaluations, we provide these five measures for all 139 dogs in the adopter survey. We also provide the five measures for only those dogs in the adopter survey that were owner surrendered to allow for a direct comparison between shelter behavioral evaluations and owner surrender profiles for the same group of dogs. For this comparison, we used two-sample proportion tests with a Yates' continuity corrected to test whether measures of prevalence, sensitivity, specificity, false positive rate, and false negative rate were significantly different between the shelter behavioral evaluations and the owner surrender profiles. Finally, we had a complete owner surrender profile, shelter behavioral evaluation, and adopter report for a total of 44 dogs. We present in tabular form resource guarding status for these 44 dogs based on each of our three sources of information and provide Kappa statistics regarding levels of agreement between sources.

3. Results

3.1. Relationship between Shelter Behavioral Evaluations and Adopter Reports

Assessment of resource guarding at the shelter was significantly associated with adopter reports of dogs showing visible signs of aggression when toys, bones, or other valued objects were taken away in the home ($p < 0.001$; Q1, Table 3). The positive predictive value was 47.4% (PPV = 9/(9 + 10); 95% CI 27.3–68.3%) and the negative predictive value was 88.9% (NPV = 104/(104 + 13); 95% CI 81.9–93.4%). For dogs that showed resource guarding during the shelter assessment, eight adopters reported growling or snarling when valued objects were taken away; one adopter reported snapping or biting. For dogs that did not show guarding during the shelter assessment, ten adopters reported growling or snarling when valued objects were taken away; three reported snapping or biting.

Assessment of resource guarding at the shelter was significantly associated with adopter reports of dogs showing visible signs of aggression when food was taken away in the home ($p < 0.001$; Q2, Table 3). The positive predictive value was 33.3% (PPV = 6/(6 + 12); 95% CI 16.3–56.3%) and the negative predictive value was 97.4% (111/(3 + 111); 95% CI 92.5–99.1%). For dogs that showed resource guarding during the shelter assessment, five adopters reported growling or snarling when they took food away from their dog; one reported snapping or biting. For dogs that did not show guarding during the shelter assessment, three adopters reported growling or snarling when they took food away; none reported snapping or biting.

Assessment of resource guarding at the shelter was significantly associated with adopter reports of dogs showing visible signs of aggression when family members retrieved items or food taken by dogs ($p < 0.02$; Q3, Table 3). The positive predictive value was 23.5% (PPV = 4/(4 + 13); 95% CI 9.6–47.3%) and the negative predictive value was 95.5% (107/(5 + 107); 95% CI 90.0–98.1%). For dogs that showed resource guarding during the shelter assessment, four adopters reported growling or snarling when they retrieved items from their dog as did five adopters of dogs assessed as non-guarding. No adopters reported snapping or biting in this situation.

There was a tendency for assessment of resource guarding at the shelter to be associated with adopter reports of dogs showing visible signs of aggression when approached while eating in the home ($p = 0.10$; Q4, Table 3). The positive predictive value was 10.0% (2/(2 + 18); 95% CI 2.8–30.1%) and the negative predictive value was 98.3% (116/(2 + 116); 95% CI 94.0–99.5%). For dogs that showed resource guarding during the shelter assessment, two adopters reported growling or snarling when they approached their dog at the food bowl as did two adopters of dogs assessed as non-guarding. No adopters reported snapping or biting.

Assessment of resource guarding at the shelter was not associated with adopter reports of dogs showing visible signs of aggression when approached at a favorite sleeping site ($p = 0.47$; Q5, Table 3). The positive predictive value was 5.0% (PPV = 1/(1 + 19); 95% CI 0.9–23.6%) and the negative predictive value was 97.5% (NPV = 115/(3 + 115); 95% CI 92.8–99.1%). One adopter of a dog that displayed resource guarding during the shelter assessment reported growling or snarling when they approached the dog at a favorite sleeping site. For dogs assessed as non-guarding at the shelter, two adopters reported growling or snarling when they approached their dog at a favorite sleeping site and one reported snapping or biting.

Table 3. Responses of adopters to survey questions about how their dog responds to five different situations in the home (Q1–Q5). Dogs are classified as resource guarding ($n = 20$) or non-resource guarding ($n = 119$) based on behavioral evaluations at the shelter before adoption.

Adopter Survey Questions and Resource Guarding Status of Adopted Dogs	No Visible Signs of Aggression Reported [1]	Visible Signs of Aggression Reported [1]
Q1: Toys, bones or other valued objects taken away?		
Resource guarding	52.6% (10/19)	47.4% (9/19)
Non-resource guarding	88.9% (104/117)	11.1% (13/117)
Q2: Food taken away?		
Resource guarding	66.7% (12/18)	33.3% (6/18)
Non-resource guarding	97.4% (111/114)	2.6% (3/114)
Q3: Taken items or food retrieved?		
Resource guarding	76.5% (13/17)	23.5% (4/17)
Non-resource guarding	95.5% (107/112)	4.5% (5/112)
Q4: Approached while eating?		
Resource guarding	90.0% (18/20)	10.0% (2/20)
Non-resource guarding	98.3% (116/118)	1.7% (2/118)
Q5: Approached at a favorite sleeping site?		
Resource guarding	95.0% (19/20)	5.0% (1/20)
Non-resource guarding	97.5% (115/118)	2.5% (3/118)

[1] Percentages represent number of dogs reported by adopters to either not show or show visible signs of aggression divided by number of dogs put in each situation (e.g., For Q1, one adopter of a dog classified as resource guarding at the shelter and two adopters of dogs classified as non-resource guarding at the shelter did not take toys, bones, or other valued objects away from their dogs and for Q2, two adopters of dogs classified as resource guarding at the shelter and five adopters of dogs classified as non-resource guarding at the shelter did not take food away from their dogs). Visible signs of aggression included growling, snarling, snapping, or biting.

Most adopters did not consider their dog to be possessive of food, toys, or space and classification as resource guarding at the shelter was not associated with adopters describing their dogs as possessive ($p = 1.00$; Q6, Table 4). Of those adopters who considered their dog possessive of food, toys, or space, most did not regard their dog's guarding behavior as a major concern (Q7; Table 4) and most did not find it difficult to prevent or manage possessive behaviors in their dog (Q8, Table 4). Sample sizes were too small for formal statistical analysis of responses to Q7 and Q8.

Table 4. Responses (no/yes) of adopters to the three scripted survey questions (Q6–Q8). Dogs are classified as resource guarding (*n* = 20) or non-resource guarding (*n* = 119) based on behavioral evaluations at the shelter before adoption.

Adopter Survey Questions and Resource Guarding Status of Adopted Dogs	No	Yes
Q6: Consider your dog to be possessive in guarding food, toys, or space?		
Resource guarding	85.0% (17/20)	15.0% (3/20)
Non-resource guarding [1]	82.2% (97/118)	17.8% (21/118)
Q7: If yes to Q6, regard your dog's guarding behavior as a major concern?		
Resource guarding	100.0% (3/3)	0.0% (0/3)
Non-resource guarding	71.4% (15/21)	28.6% (6/21)
Q8: If yes to Q6, difficult to prevent or manage possessive behaviors in your dog?		
Resource guarding	100.0% (3/3)	0.0% (0/3)
Non-resource guarding	76.2% (16/21)	23.8% (5/21)

[1] One adopter of a dog classified as non-resource guarding at the shelter left this question blank; thus, sample size is 118 for non-resource guarding dogs rather than 119.

3.2. Relationship between Owner Surrender Profiles and Adopter Reports

Of the 51 surrender profiles available, one was missing the page with the question, "Why are you surrendering your dog to the shelter?"; thus, the sample size was 50 profiles for this question. Table 5 shows the reasons owners provided for surrendering their dogs.

Table 5. Reasons provided by owners for surrendering their dog to the shelter, ranked from highest to lowest. Owners were asked to circle all that apply, so percentages do not add to 100%.

Reason for Surrender	Percentage of Surrender Profiles (*n* = 50)
Family issues	38% (19/50)
Other [1]	28% (14/50)
Behavioral problems	26% (13/50)
Owner's health	22% (11/50)
Time commitment	18% (9/50)
Dog's health	8% (4/50)

[1] For profiles in which the option "other" was circled, the reasons provided were owner housing issues (*n* = 7; moving, military deployment, eviction, and homelessness), dogs either not getting along with resident pets (*n* = 2) or needing more space (*n* = 2), inability to pay veterinary expenses (*n* = 1), and owner deceased (*n* = 1). One owner provided no explanation.

Forty-four of the 51 surrender profiles available had complete information for the section on resource guarding, with 70.5% (31/44) reporting no visible signs of aggression in their dogs and 29.5% (13/44) reporting at least one incident of growling, snarling, snapping, nipping, or biting when either the owner or someone else went near the food bowl or tried to take away toys, rawhides, or anything else of value (growling or snarling was reported in 11 dogs and snapping or nipping in two dogs).

Of the 51 surrender profiles available, 49 had complete information for the section on aggression, with 46.9% (23/49) reporting their dogs had never growled, snarled, snapped, nipped, or bitten the owner or anyone else and 53.1% (26/49) reporting their dogs had displayed at least one of the five behaviors listed (growling was the behavior most frequently reported by owners, 20 profiles, and biting was the behavior least frequently reported, one profile). Explanations supplied by owners for situations in which growling, snarling, snapping, nipping, or biting occurred typically fell into the following categories: resource guarding, aggression directed at either strangers or children, and handling sensitivities (e.g., growling during claw trimming).

Forty-four of the 51 profiles available had complete information for all three sections of interest (behavioral problems indicated as a reason for surrender, resource guarding described, and aggression described). Our logistic regression analysis revealed that description of resource guarding behavior in owner surrender profiles predicted visible signs of aggression when toys, bones, or other valued objects were taken away in the adoptive home ($X^2 = 5.57$, *d.f.* = 1, $p < 0.02$; Table 6). The positive predictive value was 38.5% (5/(5 + 8); 95% CI 17.7–64.5%) and the negative predictive value was 93.6% (29/(2 + 29); 95% CI 79.3–98.2%). Citing behavioral problems as a reason for relinquishing a dog was not a significant predictor of visible signs of aggression when toys, bones, or other valued objects were taken away in the adoptive home ($X^2 = 0.44$, *d.f.* = 1, $p = 0.51$; Table 6). The positive predictive value was 15.4% (2/(2 + 11); 95% CI 4.3–42.2%) and the negative predictive value was 83.9% (26/(5 + 26); 95% CI 67.4–92.9%). Similarly, description of visible signs of aggression in surrender profiles was not a significant predictor of visible signs of aggression when toys, bones, or other valued objects were taken away in the adoptive home ($X^2 = 0.06$, *d.f.* = 1, $p = 0.81$; Table 6). The positive predictive value was 21.7% (5/(5 + 18); 95% CI 9.7–41.9%) and the negative predictive value was 90.5% (19/(2 + 19); 95% CI 71.1–97.3%).

Table 6. Relationship between owner-supplied information at the time of canine surrender to the shelter and adopter reports of resource guarding behavior in the new home when toys, bones, or other valued objects were taken away (Q1 on the adopter survey).

Owner-Supplied Information on Canine Surrender Profile [1]	Adopter Report of No Visible Signs of Aggression When Valued Objects Taken Away	Adopter Report of Visible Signs of Aggression When Valued Objects Taken Away
Behavioral problems circled		
Yes	84.6% (11/13)	15.4% (2/13)
No	83.9% (26/31)	16.1% (5/31)
Resource guarding described		
Yes	61.5% (8/13)	38.5% (5/13)
No	93.6% (29/31)	6.4% (2/31)
Signs of Aggression described		
Yes	78.3% (18/23)	21.7% (5/23)
No	90.5% (19/21)	9.5% (2/21)

[1] A response of yes to owner-supplied information indicates the following: (1) In response to the question, "Why are you surrendering your dog to the shelter?", the owner circled the option behavioral problems (other options also could be circled); (2) In response to the question, "What does your dog do when you or someone else go near the food bowl or try to take away toys, rawhides, or anything else of value?", the owner described at least one incident in which the dog growled, snarled, snapped, nipped, or bit; and (3) In response to the questions, "Has your dog ever snarled (or growled, snapped, nipped, bitten) at you or anyone else?", the owner responded yes to at least one of the five behaviors.

3.3. Relationships between Shelter Behavioral Evaluations, Owner Surrender Profiles, and Adopter Reports

Table 7 shows prevalence and measures of predictive ability based on either shelter behavioral evaluations or owner surrender profiles in reference to behavior reported by adopters in survey Q1. For shelter behavioral evaluations, we provide values for both the total number of dogs in our study ($n = 139$) and for the subset of owner surrendered dogs for which we had complete data ($n = 44$). This allows a more direct comparison of the two sources of information—shelter behavioral evaluations and owner surrender profiles—for the same set of 44 dogs, although small sample size is problematic. Prevalence of resource guarding tended to be lower when based on shelter behavioral evaluations than when based on owner surrender profiles and measures of predictive ability were generally similar between these two sources of information (Table 7).

Table 7. Measures of prevalence and predictive abilities for shelter behavioral evaluations and owner surrender profiles with respect to behavior shown by dogs in adoptive homes when toys, bones, or other valued objects were taken away (Q1 on adopter survey). Measures for shelter behavioral evaluations are shown for all dogs included in the adopter survey (n = 139; column 2) and for only those dogs that were owner surrendered and for which we had complete data (n = 44; column 3). p value is from comparison of values in columns 3 and 4.

Measures [1]	Shelter Behavioral Evaluation (All Dogs)	Shelter Behavioral Evaluation (Only Owner Surrendered Dogs)	Owner Surrender Profile	p
Prevalence	14.4% (20/139)	18.2% (8/44)	29.5%(13/44)	0.32
Sensitivity	40.9% (9/22)	50.0% (4/8)	71.4% (5/7)	0.75
Specificity	91.2% (104/114)	88.9% (32/36)	78.4% (29/37)	0.37
False positive rate	52.6% (10/19)	50.0% (4/8)	61.5% (8/13)	0.95
False negative rate	11.1% (13/117)	11.1% (4/36)	6.5% (2/31)	0.81

[1] Measures based on Patronek and Bradley [6] and defined as follows: prevalence (number of dogs that either displayed resource guarding during the shelter behavioral evaluation or were described by owners as resource guarding at time of surrender to the shelter/total number of dogs either evaluated or surrendered); sensitivity (proportion of adopted dogs that were correctly identified as resource guarding via the shelter evaluation or surrender profile); specificity (proportion of adopted dogs that were correctly identified as non-resource guarding via the shelter evaluation or surrender profile); false positive rate (proportion of adopted dogs identified by the shelter evaluation or surrender profile as resource guarding when they are not) and false negative rate (proportion of adopted dogs identified by the shelter evaluation or surrender profile as non-resource guarding when they are not).

We had a complete owner surrender profile, shelter behavioral evaluation, and adopter report for 44 dogs (Appendix A). All three sources of information agreed with respect to resource guarding status (either yes or no) for 65.9% of dogs (29/44; dogs 1 through 29; Appendix A). Five of the 44 dogs, or 11.4%, were assessed as resource guarding by two of three sources (two dogs by surrendering owners and shelter; one dog by shelter and adopter; two dogs by surrendering owners and adopters; dogs 30 through 34; Appendix A). Ten of the 44 dogs, or 22.7%, were assessed as resource guarding by only one of the three sources (six dogs by surrendering owners; two dogs by shelter and two dogs by adopters; dogs 35 through 44; Appendix A). Finally, the level of agreement was slightly higher between shelter behavioral evaluations and adopter reports (k = 0.39; p = 0.01) than between either owner surrender profiles and adopter reports (k = 0.26; p = 0.06) or owner surrender profiles and shelter behavioral evaluations (k = 0.26; p = 0.06), although all three levels of agreement fell only in the fair range and Kappa can be less reliable when prevalence is low (Kappa of 1 = perfect agreement; 0 = agreement equivalent to chance; 0.21–0.40 = fair agreement; [15]).

4. Discussion

We found statistically significant associations between assessment as resource guarding during shelter behavioral evaluations and resource guarding reported in the adoptive home for three particular situations: taking away toys, bones or other valued objects; taking away food; and retrieving items or food taken by the dog. However, the positive predictive values for these three associations were low (47.4%, 33.3%, and 23.5%, respectively), meaning that from about one half to three quarters of dogs assessed as resource guarding in the shelter did not show guarding in these three situations in their adoptive homes. Thus, guarding behavior during the shelter assessment did not consistently indicate that guarding behavior would occur post adoption. Negative predictive values were high for these associations (88.9%, 97.4%, and 95.5%, respectively), indicating that almost all dogs assessed as non-resource guarding during shelter behavioral evaluations did not show guarding in these situations in their adoptive homes. Depending on the particular situation, from about 5–11% of dogs that did not show guarding during the shelter assessment were reported to show guarding post adoption. Results from shelter behavioral evaluations yielded a prevalence for resource guarding of 14.4% in our study population, which is similar to values reported for other shelter dog populations [2,4,5]. Conditions with low prevalence are associated with high negative predictive values and low positive predictive values [6], and our findings for resource guarding behavior fit this pattern.

Directly comparing our values with those from previous studies is somewhat challenging because adopters were asked slightly different questions and at different times post adoption. For example, we asked adopters separate questions about specific scenarios (e.g., taking away toys, bones or other valued objects; taking away food; and retrieving items or food taken by the dog) 4–12 weeks post adoption. Marder et al. [5] considered these scenarios (except the inclusion of toys) as one and categorized dogs as showing food aggression in the home when adopters reported visible signs of aggression over any of the following: a meal, delicious food items (such as bones and rawhides), or stolen food or table scraps; they surveyed adopters at least 3 months post adoption, with many adopters contacted more than 1 year after adoption. Nevertheless, our finding that 47.4% of dogs assessed as resource guarding in the shelter showed guarding in their adoptive homes when toys, bones, or other valued objects were taken away, is more similar to the 55% overall value reported by Marder et al. [5] and the 43% value reported by Van der Borg et al. [9] than the 10% value found by Mohan-Gibbons et al. [2]. One possible explanation for the lower percentage obtained by Mohan-Gibbons et al. [2] is that food aggressive dogs in their study were placed in a behavior modification program while in the shelter and also in their adoptive home, although compliance by adopters was low for at least some aspects of the program. Consistent with our prediction, assessment as resource guarding during either the food bowl test, possession test, or both tests was not associated with adopter reports of dogs showing visible signs of aggression when approached at a favorite sleeping site. This lack of an association likely reflects the sensitivity of resource guarding to context, setting, and type of resource [1,2,5]. This sensitivity was further demonstrated in our data by the differing proportions of dogs classified as resource guarding at the shelter that showed visible signs of aggression in the adoptive home across the five scenarios in our survey: 47.4% when toys, bones or other valued objects taken away; 33.3% when food taken away; 23.5% when taken items or food retrieved; 10.0% when approached while eating; and 5.0% when approached at a favorite sleeping site.

Regarding adopters' perceptions of resource guarding behavior, we found that irrespective of whether their dog was classified as resource guarding or non-resource guarding at the shelter, most adopters (82–85%) did not consider their dog to be possessive of food, toys, or space. Of those adopters who considered their dog possessive of these items, most did not regard their dog's guarding behavior as a major concern and did not find it difficult to prevent or manage possessive behaviors in their dog. Our findings on adopter perceptions of resource guarding agree with those described by Van der Borg et al. [9] and Marder et al. [5]. Mohan-Gibbons et al. [2] surveyed strength of bonds and found that most adopters of food guarding dogs described themselves as strongly bonded to their dog. These authors also reported that return rates at their study shelter for food guarding dogs were 5% as compared to 9% for the general dog population; it is worth noting, however, that food guarding dogs were screened to be highly adoptable based on scores from other tests in the shelter behavioral evaluation (i.e., having scores of one or two on other tests, indicating highly adoptable behavior, and scores of three, four, or five on the food bowl test, indicating stiff body language, growling, and attempting to bite). In a study of nearly 5 years of records from the Tompkins County SPCA, McGuire [3] differentiated resource guarding by level of severity, and found that guarding was a significant predictor of a dog being returned, with dogs that displayed severe guarding behaviors (lunging, snapping, biting) during the behavioral evaluation more likely to be returned (40.0%) than those showing mild to moderate guarding behaviors (e.g., growling, snarling, freezing; 18.2%) or no guarding behavior (17.5%). Given that dogs that show severe guarding behaviors typically make up 15–17% of resource guarding dogs at shelters [3,4], the majority of resource guarding dogs likely have return rates similar to those of non-resource guarding dogs. Additionally, many dogs that show severe guarding can be successfully placed, although more than one effort at re-homing may be needed [3]. Finally, we found that 11.1% of dogs assessed as non-resource guarding during the shelter behavioral evaluation showed guarding in their adoptive home; this value is somewhat lower than the 22% reported by Marder et al. [5]. Although larger sample sizes than we obtained are needed to fully address adopter attitudes regarding resource guarding (Table 4), it would be interesting to further examine whether attitudes differ between adopters who knowingly take home dogs assessed as resource guarding at shelters and find continued guarding in the home and adopters who take home dogs assessed as non-resource guarding at shelters and see guarding behavior in the home.

For the subset of dogs in our adopter survey that were relinquished by owners, we examined information provided by their owners on three parts of the shelter's surrender profile form: reasons for surrendering the dog; questions about resource guarding; and questions about visible signs of aggression toward either the owner or another person. Reasons for surrender to the Tompkins County SPCA included both human-related reasons (family issues; housing issues, which fell under "other"; time commitment; and health) and dog-related reasons (behavioral problems; health; space needs; and inability to get along with resident pets), as has been found in previous studies of dogs surrendered to shelters [16–19]. With respect to questions on resource guarding, 29.5% of owners reported at least one incident of growling, snarling, snapping, nipping, or biting when the owner or someone else either went near the food bowl or tried to take away toys, rawhides, or anything else of value. For questions about whether dogs had ever growled, snarled, snapped, nipped, or bitten the owner or anyone else, 53.1% of owners reported their dogs had displayed at least one of the five behaviors listed, with growling most frequently reported, and biting least frequently. Of the responses provided by owners to questions in these three sections of the surrender profile, only responses to questions about resource guarding behavior significantly predicted guarding in the adoptive home. However, the positive predictive value was 38.5% and the negative predictive value was 93.6%, suggesting that owner responses on the surrender profile indicating absence of guarding behavior in their dog may be more informative than owner responses indicating presence of guarding behavior. Stephen and Ledger [20] found information provided by surrendering owners to be of limited usefulness in predicting behavior in adoptive homes: eight of the 20 behaviors described by relinquishing owners were significantly correlated with incidence of these behaviors in the adoptive home at 2 weeks post adoption and six of the 20 were significantly correlated at 6 weeks post adoption (most correlation coefficients for these 14 behaviors were between 0.4 and 0.7, typically considered the moderate rather than high range). Unfortunately, resource guarding was not one of the 20 behaviors monitored. Possible explanations for dogs behaving differently in successive households include differences in characteristics of owners, adopters, and households and the intervening experience of living at an animal shelter [20].

Our measures of predictive abilities of the resource guarding component of shelter behavioral evaluations at the Tompkins County SPCA, in reference to subsequent behavior in adoptive homes (Table 7, column 2), are similar to those obtained by Marder et al. [5] and summarized in Patronek et al. [7] Figure 1. For example, the following values were obtained by us and by Marder et al. [5], respectively: sensitivity (40.9%, 39.3%); specificity (91.2%, 87.0%); false positive rate (52.6%, 45.0%); and false negative rate (11.1%, 22.1%). These similarities are interesting given differences in study shelters, behavioral evaluations, survey methods, and potential differences in dog populations, although additional values from other studies of resource guarding are needed to know if these measures are typical. We also examined owner surrender profiles, which allowed us to directly compare measures of prevalence and predictability between shelter behavior evaluations and surrender profiles for the same 44 dogs (a subset of those in the adopter survey; Table 7, columns 3 and 4). Unfortunately, these comparisons were compromised by small sample sizes and additional data are needed to draw firm conclusions. Nevertheless, the prevalence data for this subset of dogs (shelter behavioral evaluations, 18.2%, versus owner surrender profiles, 29.5%) suggested that owners were not under reporting resource guarding behavior by their dogs at time of surrender as has been suggested for owner-directed aggression and fear of strangers [11]. Finally, our data allowed us to compare levels of agreement between the three sources of information—owner surrender profiles, shelter behavioral evaluations, and adopter reports—for these 44 dogs. We found that level of agreement was slightly higher between shelter behavioral evaluations and adopter reports than between either owner surrender profiles and adopter reports or owner surrender profiles and shelter behavioral evaluations, although all three levels of agreement were in the fair range.

Limitations of our study include sample size issues linked, in part, to the fairly low prevalence of resource guarding in shelter dogs. Relatively few dogs in shelters display resource guarding during behavioral evaluations (average prevalence of 14% across 77 shelters in the United States, range 7–30%; [2]). Prevalence at our study shelter was 14.4% and this made it challenging for us to obtain sufficient numbers of resource guarding dogs. Over our 1.5 year study period, we were able to gather data from adopters of 20 dogs assessed as resource guarding in the shelter; adopters of another 11 dogs assessed as resource guarding agreed to participate in our survey but did not respond. Another limitation is that we considered as one group dogs that were tethered to the wall and dogs held on a leash by a staff member during the food bowl and possession tests at the shelter. Most dogs were tethered to the wall and the decision to hold a dog on a leash was made either for humane reasons (dogs showed significant fear of tethering) or test accuracy (the heavy clip of the tether inhibited movements of very small dogs). Nevertheless, dogs are sensitive to environmental context, and tethering/not tethering could have influenced their behavior during tests. We contacted adopters 1–3 months after adoption because pilot data indicated lower response rates after 3 months. While our time frame might seem too soon to assess a dog's behavior in a new home, our results were very similar to those of Marder et al. [5], who surveyed adopters at least 3 months postadoption and typically one or more years after adoption. Finally, over a nearly 5 year period (2014–2019) that partially overlapped with the current study, about 9% of dogs assessed as resource guarding at the Tompkins County SPCA were not made available for adoption; some were euthanized for behavioral reasons while others were transferred to rescue groups or returned to the owner [3]. Thus, it is possible that some of the dogs most likely to show resource guarding in an adoptive home were removed from the shelter population before reaching the adoption floor.

5. Conclusions

Although we found statistically significant associations between resource guarding during shelter behavioral evaluations and resource guarding reported in adoptive homes for three of five situations surveyed, positive predictive values were low, with at least half of dogs assessed as resource guarding in the shelter not showing guarding in adoptive homes. Our results, together with those from other research studies [2–5,9,10] and analyses put forth in critiques of shelter behavioral assessments [6,7] call into question the practice in some U.S. shelters of considering all dogs assessed as food aggressive on behavioral evaluations to be unadoptable and candidates for euthanasia. We also found that owner responses on surrender profiles to specific questions concerning resource guarding significantly predicted guarding in adoptive homes. Again, however, the positive predictive value was low, with more than half of dogs described as resource guarding by surrendering owners not showing guarding in adoptive homes. The negative predictive value was quite high (almost 94% of dogs described as non-guarding by surrendering owners did not show guarding post adoption), demonstrating the continued importance for shelters to collect information from surrendering owners, even if the information concerns behaviors not shown by the dog. For presence or absence of resource guarding behavior in the subset of owner-surrendered dogs, our measures of agreement from two-way comparisons of owner surrender profiles, shelter behavioral evaluations, and adopter reports fell in the fair range, perhaps reflecting the complexity of resource guarding behavior and importance of the setting in which it is evaluated. A source of information not studied here that might aid in predicting behavior in adoptive homes, is reporting from experienced staff or volunteers who temporarily foster a shelter dog in their home. We are unaware of any studies on the predictive abilities of reports from experienced fosterers in the context of behavior post adoption and encourage such research.

Supplementary Materials: The following is available online at http://www.mdpi.com/2076-2615/10/9/1702/s1, Table S1: Questions selected for analysis from three sections of the Tompkins County SPCA's Owner Surrender Profile Form. Our method of scoring owner responses is shown in parentheses.

Author Contributions: Author contributions were as follows: conceptualization, B.M.; supervision, B.M.; methodology, B.M. and S.P.; data collection and curation, B.M., D.O., and S.X.; statistical analyses, B.M. and S.P.; writing—original draft preparation, B.M. and S.P.; writing—review and editing, B.M., D.O., S.X., and S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We thank Jim Bouderau, Executive Director of the Tompkins County SPCA, for permission to analyze dog records. Emme Hones, Behavior Program Manager at the shelter, provided owner surrender profiles and PetPoint files from behavioral evaluations and shared her knowledge and expertise concerning behavioral evaluations and resource guarding. We thank volunteers Ann Staiger and Carol Turton for asking adopters during two-week call-backs if they would participate in our study and then passing along contact information for those who agreed. Katherine Bemis provided helpful comments on drafts of our manuscript. Finally, Samantha Rubio was instrumental in initiating this project.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. A comparison of resource guarding status based on owner surrender profiles, shelter behavioral evaluations, and adopter reports for 44 dogs. Shaded cells with a Y (for Yes) indicate resource guarding was reported by surrendering owner, shelter evaluator, or adopter. Unshaded cells with an N (for No) indicate no resource guarding was reported.

Dog	Owner Surrender Profile	Shelter Behavioral Evaluation	Adopter Report
1	Y	Y	Y
2	Y	Y	Y
3	Y	Y	Y
4	N	N	N
5	N	N	N
6	N	N	N
7	N	N	N
8	N	N	N
9	N	N	N
10	N	N	N
11	N	N	N
12	N	N	N
13	N	N	N
14	N	N	N
15	N	N	N
16	N	N	N
17	N	N	N
18	N	N	N
19	N	N	N
20	N	N	N
21	N	N	N
22	N	N	N
23	N	N	N
24	N	N	N
25	N	N	N
26	N	N	N
27	N	N	N
28	N	N	N
29	N	N	N
30	Y	Y	N

Table A1. *Cont.*

Dog	Owner Surrender Profile	Shelter Behavioral Evaluation	Adopter Report
31	Y	Y	N
32	N	Y	Y
33	Y	N	Y
34	Y	N	Y
35	Y	N	N
36	Y	N	N
37	Y	N	N
38	Y	N	N
39	Y	N	N
40	Y	N	N
41	N	Y	N
42	N	Y	N
43	N	N	Y
44	N	N	Y

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animals

Article

Comparison of Canine Behaviour Scored Using a Shelter Behaviour Assessment and an Owner Completed Questionnaire, C-BARQ

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Received: 23 August 2020; Accepted: 21 September 2020; Published: 3 October 2020

Simple Summary: In shelters, it is usual to conduct a standardised behaviour assessment to identify adoption suitability. The information gathered from the assessment is used to identify the behaviour of the dogs, suitability for adoption and to help to match the dog to an ideal home environment. We investigated if the dogs' behaviour in the home as reported by owners was reflected in the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Queensland behaviour assessment, conducted on the same dogs during a visit to the shelter. A total of 107 owners and their dogs aged 1–10 years were assessed in-home, by the owners, and in the shelter, by a researcher. The owners completed a questionnaire (Canine Behavioural Assessment and Research Questionnaire (C-BARQ)) prior to the standardised behavioural assessment conducted at the RSPCA Queensland. Regression analysis identified positive correlations between the two for fear, arousal, friendliness and anxiousness, identified in in-home behaviour and the behaviour assessment. This research therefore allowed a greater understanding of current canine behaviour assessment protocols used at the RSPCA Queensland in regard to the predictability of behaviour, behavioural problems and the efficiency and effectiveness of testing procedures.

Abstract: In shelters, it is usual to conduct a standardised behaviour assessment to identify adoption suitability. The information gathered from the assessment is used to identify the behaviour of the dogs, its suitability for adoption and to match the dog with an ideal home environment. However, numerous studies have demonstrated a lack of predictability in terms of the post-adoption behaviour in these assessments. We investigated if the owners' perception of dogs' behaviour in the home was reflected in the RSPCA Queensland behaviour assessment, conducted on the same dogs during a visit to the shelter. A total of 107 owners and their dogs aged 1–10 years were assessed in-home and in the shelter. The owners of the dogs completed a questionnaire (the Canine Behavioural Assessment and Research Questionnaire (C-BARQ) survey) 1–2 weeks before bringing their dog to the shelter for the standardised behavioural assessment conducted at the RSPCA Queensland. An ordinal logistic regression analysis identified positive correlations for fear, arousal, friendliness and anxiousness, identified in in-home behaviour and the behaviour assessment. Furthermore, the behaviours of friendliness, fearfulness, arousal, anxiousness, and aggression were positively predictive between home behaviour and tests in the behaviour assessment. This research therefore led to a greater understanding of current canine behaviour assessment protocols used at the RSPCA Queensland in regard to the predictability of behaviour, behavioural problems and the efficiency, effectiveness and predictability of current behaviour testing procedures.

Keywords: dog behaviour; behaviour problems; behaviour assessment; canines; shelters; predict; home behaviour

1. Introduction

The Royal Society for the Prevention of Cruelty to Animals (RSPCA) Australia accepted 33,863 dogs to its shelters during the period 2018–2019 [1]. Sources of admitted dogs in Queensland include councils, owner surrenders, humane officer admission (employees of the RSPCA with investigative powers under the Queensland Animal Care and Protection Act 2001) and euthanasia requests [2], with age at admission being variable, but with over 74% adult dogs. Dogs are surrendered for numerous reasons: human-related (unwanted, changed circumstances, financial, owner's health, and ex-commercial/racing), or dog-related (medical and behavioural problems) [3]. After surrender, dogs are housed in the shelter until their suitability for adoption is determined, and if suitable, adopted.

The procedures used to identify dogs suitable for adoption include a medical check, behavioural assessment, in-kennel monitoring, and monitoring by shelter staff when interacting with the dog. Behavioural assessments are the preferred method in many shelters to give an overview of the dog's behaviour for potential adopters [4,5]. They assess the dog's reactions to diverse novel stimuli typical of everyday life situations and their ability to cope in challenging situations [6], usually 3–5 days after entering the shelter [5].

The testing procedures have a risk of both false positives and negatives [7,8], that is, running the risk of falsely identifying a behavioural problem that does not exist or deeming a dog suitable for adoption when it is not. These problems may arise due to the stress experienced by the dog from living in the shelter [9], and because certain behaviours are multifactorial and a test carried out at a single point in time may not be able to accurately capture this behaviour. Few studies have evaluated the effect of the timing of behaviour assessments, for example immediately on shelter admission [10].

Measurements used in the assessments need to be appropriate and meaningful, providing both quantitative and qualitative data [11]. Qualitative measurements include history-taking measures, which provide a reflection of previous home environment and behaviour. Current procedures used by RSPCA Queensland are primarily quantitative measures, which are in line with the behaviour assessments reported in the literature that use a direct measure of behaviour by observing the dog's response to several testing procedures [4,12–17]. Other measures focus on the assessment of behaviours in everyday situations, using a questionnaire for the dog's owner to complete [18–21]. A widely used questionnaire is the Canine Behavioural Assessment and Research Questionnaire (C-BARQ), which includes items focusing on behaviour associated with aggression, fear and anxiety, trainability, excitability, separation, attachment, attention-seeking, and chasing [18]. It has been extensively evaluated and used to validate quantitative behaviour assessments focusing on areas of behaviour issues and service dogs [22–26].

In order to further investigate the accuracy with which behaviour assessments used in shelters identify behaviours exhibited elsewhere, this study adopted a novel approach to help to determine whether previous home behaviours are accurately reflected in these shelter assessments. The study asked owners to complete a validated questionnaire (C-BARQ) about their dog's behaviour and then to bring the dog into a shelter where the dog underwent the standardised behaviour assessment. The aim of this study was to determine if the dogs' behaviour in the home was reflected in the RSPCA Queensland behaviour assessment, conducted on the same dogs during a visit to the shelter.

2. Materials and Methods

2.1. Ethics

This study was conducted with the approval of the University of Queensland's Human and Animal Ethics Committees (approval numbers 2018001353 and SVS/290/18, respectively). The study complies with provisions contained in Australia's National Statement on Ethical Conduct in Human Research and with Queensland regulations governing experimentation on humans.

2.2. Subjects

Companion dog owners from the general public (n: 107) were invited via social media to participate in this study. The RSPCA and the University of Queensland media outlets were used to attract participants. Participants had to have owned their current dog for at least 6 months, be over the age of 18 years and willing to complete a questionnaire and bring their dog into the shelter to undergo a non-invasive behaviour assessment. Participants received an information sheet, and if willing to have their dog participate in the study, they signed a consent form outlining that the testing would be used for research purposes. Each participating dog was allocated a number which was used to tie the C-BARQ and assessments to the same dog. Apart from the consent form, all information was non-identifiable and most of the questions focused on information about the dog, not the owner. Owners of dogs had to complete and submit the C-BARQ questionnaire before an appointment was made for the shelter assessment. C-BARQ focuses on the dog's interactions in numerous situations. The shelter assessment used was the standardised assessment used on all in-coming dogs.

Dogs

Dogs were required to be older than 6 months and younger than 13 years of age. Any breed was allowed in the study. Dogs were also required to have no medical conditions nor be on any medication that had the potential to influence behaviour. Dogs previously adopted from shelters were allowed in the study and were initially categorised separately to identify any variability. However, there were no differences between groups, therefore, separate categories were dropped. All dogs were required to be with the owners for at least 6 months.

2.3. Behaviour Assessment

The dogs were brought into the shelter by their owner for the formal behaviour assessment. It was conducted in a room (4.5 m × 4.7 m) in a separate building, approximately 50 m from the shelter offices and kennels to minimise disturbance. The dogs were initially left in the room by themselves for 15 min to allow them to acclimatise to the room while the researcher watched their behaviour from the next room via a video link (4× Go pro Hero 4 Silver positioned an equal distance apart). The owner waited in an adjoining area for the period of acclimatisation and assessment.

The behavioural assessment used in this study was the standard assessment used by the RSPCA Queensland for shelter dogs. The assessments were conducted, recorded and scored by the lead researcher (LC), who was formally trained in the assessment regimen. Reviewed behaviours included room exploration, leash manners, sociability, tolerance, play behaviour with toys, the response to unusual/unpredictable stimuli, possessive behaviours, toddler and stranger interaction, time alone and social interactions with other dogs [27] (Appendix A). In each test, the dog's behaviours were scored for friendliness, socialisation, fearfulness, arousal and aggressiveness. The assessment comprised nine different tests performed over a 15 min period. The equipment used was in line with the RSPCA Queensland's protocol and included a 1.8 m leash, a tennis ball, a plush squeaky toy, rope, plastic hand on an extend pole, bowl, raw hide or bone, and the combination of wet and dry dog food. The details of the RSPCA Queensland assessment tests can be found in Clay et al. [27]. All the tests were recorded by video (Go Pro Hero 4, Model: HERO4 Black, Manufacture: Hong Kong, China) and reviewed later.

2.4. Owner Questionnaire, C-BARQ

Owners rated the behaviour of their dog at home based on behavioural interactions in relation to attachment or attention seeking, sociability, touch sensitivity, excitability, chasing, fear, aggression, and separation-related behaviours. The owners' information on their dog's behaviour was categorised into predetermined behavioural categories on a score of 0–4 (Appendix B). The C-BARQ questionnaire used had the 102 question format [24] and was scored on a scale between 0 and 4 (aggression: 0, none—4, serious, separately scored for stranger-, owner-, dog and familiar dog-directed aggression; fear: 0, no fear or anxiety—4, extreme fear, both stranger, non-social and dog fear; separation-related problems: from 0, never, to 4, always; attachment/attention-seeking: from 0, never, to 4, always; touch sensitivity: from 0, never, to 4, always; excitability: from 0, calm, to 4, extremely excitable; chasing, energy, and trainability: from 0, never, to 4, always).

2.5. Behaviour Scoring

The formal behaviour assessments were scored for dog behaviour during all tests, as described in Clay et al. [27]. The ethogram comprised 48 behaviours, determined following the preliminary observation of dogs during the formal behaviour assessment, classified as either long duration behaviours (for which the duration was recorded) or events (for which the number of occurrences was recorded). The behaviours focused on eight components: activities of the mouth, body, tail position, tail movement, ears, eyes, position in room, and movement (Table 1). The descriptions of each behaviour were presented in a previous study [27]. Behaviour recording was assisted by coding software BORIS [28], which recorded the frequency and duration of each behaviour using continuous input from the coder. Two behaviour variables with no or only one occurrence were discarded: squint and whale eyes. From the coded behaviours, using similar principles to our previous articles [27,29], the proportion of the time and frequency of the five behavioural categories (anxiety, fear, friendliness, arousal, aggression) were derived. The descriptions of each behaviour are presented in Table 1 and their connection to behavioural categories (anxiety, fear, friendliness, arousal, aggression) in Table 2 are based off the literature described in a previous article (27).

Table 1. Behaviours of dogs (n = 107) recorded for each body part, as well as the position in the room and movement types.

Mouth	Body	Tail	Tail Movement	Ears	Eyes	Position	Movement
Open/closed	Weight forward	Low	Wagging	Alert	Soft	Front	Pacing
Panting	Weight back	Med	Fast	Back	Hard	Bed	Sit/lay
Mouthing	Balanced	High	Stiff	Forward	Direct	Door	Stand
Lip lick	Relaxed	Tucked	Slow	Open	Squinting	Wall	Still
Snap	Tense		Loose		Whale eyes		
Bite	Lowered				Dilated		
Whining	Play bow				Targeted		
Barking	Jumping up				Diverted		
Growl	Lowered head						
Howling	Piloerect						
	Body curve						

Table 2. The behaviours contributing to the behavioural states fear, anxiety, aggression, arousal, and friendliness.

Fear															
Diverting	Ears back	Lip licking	Lowered body	Lowered head	Shiver	Stiff tail	Tail low	Tail tucked	Tense body posture	Weight back	Yawn				
Anxiety															
Fast tail	High tail	Jumping	Licking	Lip licking	Medium tail	Pacing	Panting	Stiff tail	Tense body	Weight back	Weight forward	Whining			
Aggression															
Biting	Ears forward	Growling	High tail	Lip licking	Lowered head	Medium tail	Snapping	Standing	Stiff tail	Still tail	Targeting	Vertical lip raise			
Arousal															
Barking	Diverting gaze	Fast tail	High tail	Jumping up	Jump off	Licking	Medium tail	Mouthing	Pacing	Panting	Weight forward	Whining			
Friendliness															
Balanced	Body curve	Direct eye	Ears forward	Ears open	Fast tail	Handler interaction	Jump	Medium tail	Play	Relaxed body	Slow tail	Sniff	Soft eye	Tail loose	Walking

2.6. Statistical Analysis

Statistical analysis was conducted using Minitab 18. Behaviours were analysed as the percentage of the total observation time (long duration behaviours) or the percentage of the frequency of occurrence (events) during the overall behaviour assessment and within the individual tests. The C-BARQ questionnaire has predetermined categories that were calculated after the 102 questions were complete. Descriptive analysis was used for behaviour in assessments.

Spearman's rank order correlations were computed between C-BARQ and the formal behaviour assessment variables. As comparisons with 79 other behaviours were made for each behaviour in each test of the behaviour assessment, results were corrected for false discovery using the Benjamini–Hochberg procedure [30]. The Bonferroni correction was rejected as it assumes the independence of the individual tests. The Benjamini–Hochberg procedure ranks the p values for each test and compares the p values to critical values [(rank/no. tests) × false discovery rate (selected as 0.20 as recommended by McDonald [30]). All p values up to the critical one were considered to indicate a significant difference [30].

Ordinal logistic regression was used to compare the temperament/behavioural information from owner-reported temperament/behaviour with derived behaviours from the shelter assessment, both overall and within the different tests. The Benjamini–Hochberg was used to correct for false discovery as with Spearman rank correlations.

3. Results

3.1. Descriptive Statistics

The sample included 107 companion dogs (males: 52, females 57, desexed: 103, intact: 6) who were over the age of 6 months and under 13 years (mean: 5 years 3 months). Sources of the dogs included: shelters (44.9%), breeders (23.8%), other (online, private sales, or did not disclose) (11.9%), neighbour, friend, or relative (10.1%), and under 5% were from pet stores or were stray dogs.

A variety of breeds were included in the study, determined by the C-BARQ questionnaire completed by the owners; mixed breeds (19.3%), Border collie (10.1%), Kelpie (8.3%), Staffordshire bull terrier (8.3%), German shepherd (5.5%), Australian cattle dog (3.7%), and Rottweiler (3.7%). All other breeds represented less than 3% of the population of dogs. Mean weight of the dogs was 21.8 ± 1.06 kg.

With respect to the household environment, 64.2% had other dogs in the household; 35.8% were single dog homes. Of the total population, 69.7% of the households had no children and 30.28% had children living in the home. With regard to the living arrangements for the dogs, 80.7% were classified as inside/outside, 12.8% were only inside, 4.6% were only outside and 1.8% had no classification.

3.2. Owner Questionnaire

All owners completed the C-BARQ questionnaire (107 participants). Many owners indicated that their dogs displayed no signs of fear (score 0) in situations with other unknown dogs (46%), strangers (68%) and non-social interactions (56%), with the second highest occurrence being the dog displaying minimal signs of fear (score 1) in the above situations (Appendix C). When owners did report that some fear was displayed, it was most likely to be dog directed, then non-social and least likely to be stranger directed.

It was mostly reported that little aggression was observed. In particular, owner-directed aggression was very rare, only 5% of owners reported this, and stranger-directed aggression was also quite rare, with only 28% of owners reporting this, and mostly at low levels. However, dog-related aggression (unfamiliar dogs) was relatively common, reported by 60% of owners, but less towards familiar dogs (34% of owners). Separation-related behaviours were even less common, reported by 23% of owners, but attention-seeking, chasing, excitable and energetic behaviours were relatively common, with most owners reporting some occurrence. Touch sensitivity was less common, with most owners reporting that it was never or seldom seen. Dogs were reported to be trainable most of the time, but never always.

3.3. Formal Behaviour Assessment

In the overall formal behaviour assessment, dogs spent 41.2% of their time in friendly behaviours, 28.4% displaying fear, 14.3% in a state of high arousal, 13.5% displaying anxiousness, and 2.5% in aggression. Considering the frequency of the behaviours, there was a mean of 37.6% incidents of friendly behaviours, 30.3% incidents of fear-related behaviours, 15.4% incidents of high arousal behaviours, 13.7% incidents of anxiety-related behaviours, and 3.5% incidents of aggressive behaviours.

In individual tests, the major behaviours that had the highest occurrences were friendly and fearful, whereas anxiousness, arousal and aggression had lower instances (Appendix D). However, there were higher instances of arousal in the toy interaction test which reflects the purpose of the test.

3.4. Relationships between Owner-Reported Dogs' Behaviour in the Home and Behaviours Derived from the Formal Behaviour Assessment in the Shelter

All correlations were corrected using Bonferroni correction and varied in strength. Considering the overall behaviour assessment, there were positive Spearman rank correlations between the fear displayed in the assessment and the fear in non-social situations and stranger situations reported by the owner (Table 3). A friendly classification in the shelter assessment correlated negatively with stranger-directed fear reports by the owner. Aggression in the shelter correlated positively with touch sensitivity reports by the owner, both in the overall assessment and in the touch sensitivity test. In the latter test, friendliness correlated with the non-social fear reports by the owner.

Table 3. Significant ($p < 0.01$) Spearman rank correlations between the owner-reported dogs' temperament/behaviour in the home and the behaviours derived from the formal behaviour assessment at the shelter.

Behaviour Assessment Test	Shelter Behaviours	Owner-Reported Temperament in the Home (C-BARQ)	Correlation Coefficient
Overall	Fear	Stranger-directed fear	0.34
		Non-social fear	0.36
	Friendliness	Stranger-directed fear	−0.32
	Aggression	Touch sensitivity	0.31
Touch sensitivity	Aggression	Touch sensitivity	0.27
	Friendliness	Non-social fear	−0.25
Play interactions	Fear	Stranger-directed fear	0.45
		Stranger-directed aggression	0.29
		Non-social fear	0.32
	Friendliness	Stranger-directed fear	−0.42
Response to Unusual/unpredictable stimulus	Fear	Stranger-directed fear	0.32
	Friendliness	Stranger-directed fear	−0.31
Food possession	Friendliness	Stranger-directed fear	−0.32
Toddler doll	Fear	Non-social fear	0.32
	Aggression	Touch sensitivity	0.32
			$p < 0.01$

In the Play interactions test in the shelter, fear correlated positively with stranger-directed and non-social fear and aggression in the home. Friendliness in this test correlated negatively with stranger-directed fear reports by the owner. In the Response to unusual/unpredictable stimuli test in the shelter, fear correlated positively with stranger-directed fear reports by the owner, which also correlated negatively with friendliness in the behaviour assessment. In the Food possession test in the shelter, friendliness correlated negatively with stranger-directed fear, and in the Toddler doll test, fear correlated positively with non-social fear reports by the owner, and aggression correlated with touch sensitivity reports by the owner.

3.5. Predictability of Behaviour Assessment

In the home environment, dogs whose owners reported low levels of stranger-directed fear had high levels of friendliness in the Overall shelter test and in the Response to Unusual/Unpredictable Stimulus, Food Possession, Stranger, and Toddler doll tests (Table 4). High levels of stranger-directed fear related positively to aggression in the Overall, Play interaction, Response to Unusual/Unpredictable Stimulus and Food Possession tests, to fearfulness in the Touch Sensitivity test and negatively to high arousal in the Toddler doll test. Owner-reported non-social fear and fear in the Exploration of room, Touch sensitivity and Response to unusual stimulus tests were related. Stranger-directed aggression reported by the owner was also related to fearfulness in the Touch sensitivity test. Owner-directed and reported aggression was negatively related to friendliness, fearfulness and high arousal in the Stranger test, and positively related to aggression in that test and the Toddler doll test. Familiar dog aggression reported by the owner was negatively related to friendliness, fearfulness and high arousal in the Toddler doll test and positively related to aggression in that test.

Touch sensitivity reported by the owner was negatively related with friendliness (Overall assessment, Response to unusual stimulus, Toddler doll, Time alone, Dog-to-dog interaction), high arousal (Overall assessment, Toddler doll, Touch sensitivity, Time alone), fearfulness (Touch sensitivity, Dog-to-dog interactions), and anxiety (Response to unusual stimulus, Toddler doll, Dog-to-dog interaction). There was a positive relationship between those related with aggression (Overall assessment, Touch sensitivity, Play interaction, Response to unusual stimulus, Toddler doll tests).

Attachment/attention seeking reported by the owner related negatively with friendliness (Response to unusual stimulus, Toddler doll), fearfulness (Overall assessment, Response to unusual stimulus, Toddler doll, Time alone), high arousal (Overall assessment, Play interaction, Response to unusual stimulus, Toddler doll), anxiety (Response to unusual stimulus, Toddler doll, Time alone). It related positively with aggression (Overall, Response to unusual stimulus, Toddler doll, Dog-to-dog interaction tests).

Excitability related negatively to fearfulness in Touch sensitivity, high arousal in Touch sensitivity, and it related positively to anxiousness in the Exploration of room, high arousal in the Exploration of room, and Time alone tests.

Energetic behaviour was related positively to high arousal in the Exploration of room, and aggression in Dog-to-dog interaction and negatively to friendliness in the Dog-to-dog interaction. Chasing was related negatively to anxiousness in the Toddler doll test.

Table 4. Significant ($p < 0.01$) relationships between the owner-reported temperament/behaviour and the behaviours derived from the overall behaviour assessment and individual tests, conducted in the shelter, determined by ordinal logistic regression.

Owner-Reported Temperament/Behaviour	Behaviour in Behaviour Assessment in Shelter	Coef.	Odds Ratio	Lower CI	Upper CI
	Overall				
Stranger-directed fear	Friendliness	0.20	1.22	1.07	1.41
	Aggression	−0.13	0.88	0.78	0.99
Touch sensitivity	Friendliness	0.16	1.17	1.03	1.33
	High arousal	0.12	1.13	0.99	1.30
	Aggression	−0.14	0.87	0.77	0.98
Attachment/attention-seeking	Fearfulness	0.13	1.14	1.01	1.30
	High arousal	0.17	1.19	1.03	1.36
	Aggression	−0.13	0.88	0.78	0.99
	Exploration of room				
Non-social fear	Fearfulness	−0.04	0.96	0.93	0.99
Excitability	Anxiousness	−0.06	0.94	0.89	1.00
	High arousal	−0.05	0.95	0.91	0.99
Energetic	High arousal	−0.04	0.96	0.92	1.00
	Touch sensitivity				
Stranger-directed fear	Fearfulness	−0.04	0.96	0.93	0.99
Non-social fear	Fearfulness	−0.03	0.97	0.94	0.99
Stranger-directed aggression	Fearfulness	−0.04	0.96	0.93	0.99
Touch sensitivity	Fearfulness	0.15	1.16	1.03	1.30
	Anxiousness	0.17	1.18	1.03	1.35
	High arousal	0.15	1.16	1.02	1.32
		−0.10	0.91	0.83	0.99
Excitability	Fearfulness	0.15	1.16	1.03	1.30
	High arousal	0.15	1.17	1.02	1.33
	Aggression	0.15	1.17	1.02	1.33
	Play interactions				
Stranger-directed fear	Friendliness	0.15	1.16	1.05	1.27
	Aggression	−0.12	0.88	0.81	0.97
Touch sensitivity	Aggression	−0.12	0.89	0.81	0.97
Attachment/attention-seeking	High arousal	0.12	1.13	1.02	1.25
	Response to unusual/unpredictable stimulus				
Stranger-directed fear	Friendliness	0.13	1.13	1.04	1.24
	Fearfulness	−0.04	0.96	0.94	0.99

Table 4. *Cont.*

Owner-Reported Temperament/Behaviour	Behaviour in Behaviour Assessment in Shelter	Coef.	Odds Ratio	Lower CI	Upper CI
	Aggression	−0.09	0.91	0.84	0.99
Non-social fear	Fearfulness	−0.03	0.97	0.95	1.00
Separation related behaviours	Aggression	−0.08	0.92	0.85	1.00
	Friendliness	0.09	1.09	1.01	1.19
Attachment/attention-seeking	Friendliness	0.15	1.16	1.05	1.29
	Fearfulness	0.10	1.10	1.02	1.20
	Anxiousness	0.12	1.13	1.03	1.23
	High arousal	0.11	1.12	1.02	1.23
	Aggression	−0.09	0.91	0.84	0.99
Touch sensitivity	Friendliness	0.10	1.11	1.02	1.20
	Anxiousness	0.13	1.14	1.02	1.27
	Aggression	−0.09	0.91	0.84	0.99
	Food possession				
Stranger-directed fear	Friendliness	0.13	1.14	1.02	1.28
	Aggression	−0.11	0.89	0.80	0.99
	Stranger				
Stranger-directed fear	Friendliness	0.10	1.10	1.01	1.21
Owner-directed aggression	Friendliness	0.12	1.13	1.02	1.25
	Fearfulness	0.12	1.12	1.02	1.24
	High arousal	0.13	1.13	1.01	1.27
	Aggression	−0.13	0.88	0.80	0.97
	Toddler doll				
Stranger-directed fear	High arousal	0.12	1.13	1.01	1.26
	Friendliness	0.09	1.10	1.00	1.20
Familiar dog aggression	Friendliness	0.12	1.13	1.03	1.24
	Fearfulness	0.11	1.11	1.01	1.22
	High arousal	0.13	1.14	1.03	1.28
	Aggression	−0.12	0.89	0.81	0.98
Owner-directed aggression	Aggression	−0.13	0.88	0.79	0.97
Attachment/attention-seeking	Friendliness	0.11	1.11	1.02	1.21
	Fearfulness	0.12	1.13	1.04	1.24
	Anxiousness	0.17	1.19	1.08	1.32
	High arousal	0.16	1.18	1.07	1.29

Table 4. *Cont.*

Owner-Reported Temperament/Behaviour	Behaviour in Behaviour Assessment in Shelter	Coef.	Odds Ratio	Lower CI	Upper CI
	Aggression	−0.12	0.89	0.82	0.97
Touch sensitivity	Friendliness	0.11	1.12	1.03	1.22
	Anxiousness	0.11	1.12	1.01	1.24
	High arousal	0.10	1.10	1.01	1.21
	Aggression	−0.11	0.90	0.83	0.97
Chasing	Anxiousness	0.11	1.11	1.01	1.23
	Time alone				
Attachment/attention-seeking	Fearfulness	0.11	1.12	1.01	1.24
	Anxiousness	0.15	1.17	1.04	1.31
Touch sensitivity	Friendliness	0.11	1.12	1.01	1.24
	High arousal	0.14	1.15	1.02	1.29
Excitability	High arousal	−0.04	0.96	0.92	1.00
	Dog-to-dog interaction				
Attachment/attention-seeking	Aggression	−0.08	0.93	0.86	1.00
Touch sensitivity	Friendliness	0.09	1.10	1.01	1.19
	Anxiousness	0.13	1.14	1.01	1.29
Energetic	Friendliness	0.09	1.10	1.01	1.20
	Aggression	−0.09	0.92	0.85	0.98

4. Discussion

Behavioural assessments are used in the RSPCA Australian shelters to identify behavioural problems, determine suitability for adoption and to monitor the behaviour of each dog over time while in the shelter. The use of the behavioural assessment as a tool in combination with surrender information (home environment, in-home behaviour, and behaviour towards other dogs), veterinary history, in kennel observations, and staff feedback is thought to provide some representation of the dog's behaviour. The behavioural assessment is not being used as a pass–fail tool, rather, it is used as one component of a toolbox to collect information over time. It is important to know how valid it is. The aim of this study was to determine if dogs' home behaviour, measured using information provided by owners using the C-BARQ, was accurately reflected in the standardised RSPCA Queensland behaviour assessment. The study was conducted with dogs owned by members of the general public and therefore not dogs potentially negatively affected by stress due to time in the shelter.

Major themes identified in this study are consistent with the previous findings and results reported in previous studies, particularly in relation to fear, arousal, friendliness, and anxiousness [27,29]. The major tests that were most predictive of behaviour in a home environment were the exploration of room, touch sensitivity, and Response to unusual stimulus in regard to non-social fear. Stranger-directed fear was predictive in tests of touch sensitivity, and response to unusual stimulus response. Touch sensitivity was reflected in the corresponding test in the assessment. Owner-directed aggression was predicted in the stranger and toddler doll tests. Stranger-directed aggression was only identified in touch sensitivity in relation to fear. Excitability and energy were predicted in the exploration of room, touch sensitivity, and time alone tests. Finally, attachment was predicted in the tests related to the response to unusual stimulus, and toddler doll.

Overall friendliness identified during the play interactions, response to unusual stimulus, food possession, stranger, toddler doll and dog-to-dog interactions tests were reflected in the low scoring of the categories of energetic, fear and aggressive-related issues in C-BARQ. Categories of the C-BARQ that were not predicted in the tests were dog rivalry, dog-directed aggression, separation-related behaviours, trainability, and chasing.

There are few studies on the ability of an assessment to reflect previous home behaviour; rather, most literature looks at predicting future behaviour [8,13,14,25,31–35]. In this study, behaviour reported in the home showed a relationship with certain aspects of the behavioural assessment including fear, friendliness, anxiety, arousal and aggression.

The relationship between fear displayed in the assessment and owners' indication of stranger-directed and non-social fear, aligns with previous findings of the predictability of fear [14,36]. In looking at C-BARQ categories, stranger-directed fear and aggression, and non-social fear in the home were related to fear observed in the exploration of room, touch sensitivity, and response to unusual stimulus. Non-social fear, stranger-directed fear, and aggression in the home were associated with increased odds of fearfulness in dogs in the assessment. This consistency of fear responses is to be expected, since the fear response is a manifestation of a survival response in the brain located in the amygdala, with the behavioural response created being very recognisable and easy to identify in all species [37]. Furthermore, the consistency of fear responses indicates a similarity of stimulus features and the demonstration of fearful behaviour requires appropriate environmental stimuli. One might expect to observe some consistency of fear responses in the home environment and shelter, even if people cannot categorise the motives/diagnosis of fear.

Mornement and co-authors [14] argued that general measures of anxiousness and fear measured in the Behaviour assessment for rehoming K9's (B.A.R.K) protocol significantly predicted "Fearful/inappropriate toileting" behaviours post adoption. These results outline the stable predictiveness of fear consistent over a shelter to a post-adoption environment and therefore suggests the stability of fear over longitudinal periods. Foyer and co-authors [38] further reflected this in a study looking at behaviour in the first year of life and in a later temperament test in dogs. Results from the study outlined that dogs scoring high in categories of stranger-directed fear, non-social fear, and dog-directed fear showed a significantly lower rate of success 3 months later in the temperament test due to fear [38]. Therefore, it is of no surprise to observe consistency in the fear response seen in this study.

In relation to the friendliness displayed in the home environment and behaviour assessment, it is no surprise that it reflects previous findings [14]. Mornement and co-authors [14] found that post adoption, dogs greeting visitors in a friendly manner could be predicted by friendliness scores in B.A.R.K. However, it did not appear to be a reliable predictor of problem behaviours, such as overall aggression or destructive behaviour in shelters.

Furthermore, the predictability of behavioural problems outlined in the results using the owner information and the behaviour assessment could be due to the timing of the assessment. The assessment was conducted upon arrival, located in a room which was at a considerable distance from the main shelter. The stress of the shelter may cause the normal behavioural repertoire to change in the dog for the purpose of finding the best coping mechanism to deal with acute stress due to changes in the environment. Therefore, the timing of the assessment (currently at a minimum of 3 days after surrender) may cause the predictability of behaviour post adoption to be poorer due to the changes that stress can cause in normal behaviour. If we take human psychology as an example, humans that go into a novel environment which they have never been in before suffer an acute stress response. Humans, like all animals, need to adapt to a new environment; they can find positive and negative coping mechanisms to help with this which is then reflected in their behaviour [39]. If positive coping mechanisms are not found, then negative coping mechanisms are used, causing problem behaviours and sometimes addiction. Dogs that have never been in the novel environment before, such as the shelter, respond with an acute stress response due to social isolation from previous family, daily routine changes, disturbed feeding, walking, socialising, lack of handling and attachment figures, and sensory overstimulation. The dog must adjust to the new environment and if unable to cope effectively, behavioural problems start to occur. Once adopted, however, dogs then need to adjust back to home behaviour, which can be easy for most dogs but other dogs with behavioural problems may find this difficult. This is consistent with the findings of Mornement and co-authors [14] who indicated a high number of new adopters reporting signs of growling, snapping, and attempting to bite a person.

Not all instances of behaviour seen in the behavioural assessment-reflected responses to the C-BARQ questionnaire, including certain categories of aggression (dog-directed, stranger-directed), separation-related behaviours and possessive behaviours. Only one category of the C-BARQ, owner-directed aggression, showed consistency with the behaviour assessment stranger and toddler doll tests.

One might expect that stranger-directed aggression in these tests would be reported in the C-BARQ but this was not the case. A study by Dalla Villa et al. [25] outlined the use of the Socially acceptable behaviour (SAB) protocol for identifying categories of aggression. The results indicate that only categories of C-BARQ predictive of the SABS were associated with owner-reported aggression towards familiar people and familiar dogs, however, these were not directly measured by any of the SAB subtests. The identification of the category of aggression is difficult as there are numerous such categories [40] and aggression can be multifactorial. Therefore, this could explain the lack of results in the predictability of aggression towards another stimulus e.g., dog-directed and stranger directed. Without thorough examination of the context of aggression, the environment, and a comprehensive understanding of all factors at play, it is very difficult for assessments to correctly identify, let alone predict, categories of aggression.

Separation-related behaviours are difficult for assessments to identify predictably due to the multifactorial nature of the issue. The issue can be easily misclassified due to other underlying problems like attachment-seeking, general anxiety, fears, or phobias [41]. Furthermore, differential diagnosis should always be taken into account before outlining that the individual has separation anxiety. Storengen and co-authors' [42] study of 215 dogs diagnosed with separation anxiety reported that only 18.5% of animals actually had only separation anxiety with no other behavioural problems, whereas 82.8% of the animals had other underlying behavioural problems in addition to separation anxiety, with the most common comorbidity being related to noise sensitivity (43.7%) [42].

Possessive behaviour has been reported in the literature to have a low predictability [13,14,31]. This may be due to the manifestation of the problem being environmentally based [13,31]. Possessive aggression is associated with a need to protect a resource from surrounding threats, however, once a threat is no longer present, the behaviour ceases, therefore it is not often seen in post-adoption environments. The study by Marder and co-authors [13] found that a little over half of the dogs with possessive behaviour in the shelter displayed these issues post adoption, whereas 22% of dogs identified in a shelter with no signs of possessive behaviours exhibited the behaviour post adoption. Furthermore, a study by Mohan-Gibbons [31] into the removal of the test, identified that there was a low risk of injury to handlers, volunteers, staff or adopters and no significant difference in the rate of returns. However, even though it was a low relative risk of occurrence in the home it is predictive, just not perfectly predictive. Possession aggression, however, can be stimulated by environmental or competition in the environment, therefore, if in a stable environment, such behaviours will decrease or cease. Therefore, in the current study, this could explain the low occurrence of possessive aggression, especially in the home environment.

Numerous possibilities exist that consider discrepancies between the behavioural assessment results and owner reports. A possibility is that the current standardised behaviour assessment may be adequate at identifying overall behaviours, however, unable to correctly identify certain behavioural problems. However, behavioural problems, such as dog-directed aggression or separation-related behaviours, may be inaccurately identified due to the misinterpretation of the behaviour by the owner in the home. For example, dogs that are reactive to other dogs at a distance could be misclassified as dog-aggressive or offensive aggressive, when what is being displayed is built-up frustration and hyperactivity towards other dogs. A study that assessed the behaviour of privately owned dogs using the Dutch socially acceptable behaviour test, found that a large portion of aggressive dogs remain undetected and the test was unsuitable for assessing types of aggression apart from fear [23]. The current results agree with this, outlining the high degree of detectability of fear.

There are limitations to this study. One limitation is that all dogs in this study had been in a home environment for over 6 months, and therefore, had an attachment figure. Attachment figures have previously been seen to have a significant impact on inhibitory control, problem-solving tasks and social interactions in comparison to dogs that were in shelters with no attachment figure [43–45]. Another limitation includes that the study population may not be representative of dogs that end up in shelters.

The results from this novel study suggest the benefit of an upon surrender assessment to increase the understanding of behaviour from the previous home environment. Early recognition of behavioural problems that include fear, anxiousness, arousal, and aggression can help dogs cope in the environment and allows behaviour modification to be implemented before the stressors of the shelters begin to have an effect [9].

5. Conclusions

This study suggested that the standardised behaviour assessment protocol used at an Australian shelter is a useful tool to reflect home behaviour when conducted upon entry to the shelter as mimicked in this study methodology, with friendliness, fearfulness, anxiousness, high arousal and certain categories of aggression measured by the C-BARQ being reflected in the assessment. The identification of behaviours of dogs upon entry can help to create a more comprehensive understanding of the dog's behaviours in the home environment and further identify any behavioural issues/monitored throughout the stay in the shelter plus allow behaviour modification to start upon entry. Information can give a base line for the dogs before entry, thus allowing the longitudinal monitoring of behaviours and behavioural issues. Investigations into longitudinal monitoring from surrender to adoption, and the relationship of individual behavioural change over time, needs to be conducted.

Author Contributions: L.C., M.B.A.P., P.B., G.P., and C.C.J.P. conceived the project. L.C. drafted the paper and all authors had input into modifying it into the present format. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors acknowledge the assistance of the RSPCA. We would like to thank James Serpell for his permission to use the C-BARQ for this study.

Conflicts of Interest: Mandy Paterson declares that she works for the RSCPA Queensland. Liam Clay declares that RSPCA Queensland funded his studentship. Apart from this, no other author has any conflict.

Appendix A. RSPCA Standardised Behavioural Assessment

Appendix A.1. Test 1: Exploration of Room

Appendix A.1.1. Exploring the Room

The assessor entered the room, dropped the lead attached to the dog, and sat in the centre on a chair. Then, the observer started a timer and waited for 1 min without any interaction with the dog by either person.

Appendix A.1.2. Sociability to Assessor

At the end of exploring the room, the assessor called the dog to them in a friendly voice, remaining in the chair with no other body movement. If there was no response, a second attempt was made, and if still no response the assessor clapped their hands on their lap and said 'come here' in the direction of the dog, trying at least three times to call the dog to them. When the dog came (at the first, second, or third call), the assessor picked up the leash and then stroked the dog from the base of the neck to the tail three times. If the dog did not respond to the first, second, or third call, the assessor approached the dog, picked up the leash, and gave the dog three strokes from the base of the neck to the tail. Following each stroke, the observer and assessor counted 10 s, with the behaviours exhibited noted.

Appendix A.2. Test 2: Tolerance to Handling

There were three components to the test, namely touch sensitivity to collar, stroke, and feet. The assessor dropped the leash and held the dog's collar. After 3 s, the handler stroked the dog from head to tail. With the dog standing, the other assessor (in the standing position, or crouching if a small breed of dog) picked up the dog's rear inside foot, then the front inside foot, then reached over its back to pick up its rear outside foot, and finally the front outside foot. Each foot was held for 2 s. After picking up all four paws in this manner, the assessor stood for 10 s with no dog interaction and finally removed the dog's leash.

Appendix A.3. Test 3: Startle Response

There were two components: startle response and recovery to stimulus. At the end of Test 2, the assessor created a loud sound using a book on a bench or a desk (startle response). The assessors recorded recovery.

Appendix A.4. Test 4: Toy Interactions

Three toys were used in this testing procedure: tennis ball, squeaky toy, and tugging rope. A tennis ball was shown to the dog and gently thrown across the room, and the assessor verbally engaged the dog in play. If the dog picked up the ball, the assessor waited to see if it returned to the assessor without encouragement. If it did not, the assessor encouraged the dog to bring the ball back by calling his/her name and saying "come". If the dog still did not return, the assessor went to the dog.

In both situations, the assessor waited 10 s to see if the dog dropped the ball. If it did not, they asked the dog to "drop it". If the dog did not respond, then a second command was given, "give", and if necessary, a third attempt, "out", was tried. If the dog did not respond to these commands, the assessor approached the dog carefully and removed the ball from the dog's mouth. These steps were repeated for a second throw and after completion, the assessor waited 10 s with no interaction before moving on to the next toy, the squeaky toy, and after that, the tugging rope. The same sequence was used for each toy. After completing all three toys, the assessor moved on to the next test.

Appendix A.5. Test 5: Response to Unusual/Unpredictable Stimulus

The assessor gently moved the dog to the opposite end of the room and left it standing against the wall. Then, they gently moved one hand over its head, down toward the back to gently tap the rump area, and then ran across the room, laughing and waving arms, followed by suddenly stopping, folding their arms, and ignoring the dog. The tap, run, and freeze series was repeated a second time. The assessor waited for 10 s after the run and freeze, ignoring the dog, before moving onto the next test. The dog was then placed back on the leash.

Appendix A.6. Test 6: Resource Guarding

There were four components to the test: wet food, dry kibble/biscuits, pig's ear and bone. The assessor tethered the dog to the wall for safety reasons, and proceeded to show the dog wet canned food, smeared in a bowl. The bowl was then placed near the dog at the end of the leash perimeter, allowing the dog to begin eating for 2 s. The assessor then proceeded with a plastic hand, walking to the side of the dog while it was eating. Using the fake hand, the assessor patted the dog on the head, continuing to stroke down its back and body twice. The fake hand was then placed 5 cm in front of the bowl and moved around in a semi-circle. The hand was then placed on the inside edge of the bowl and moved around the edge of the bowl next to the dog's face, without touching it. Finally, the bowl was pulled away from the dog using the fake hand. The bowl was then returned to the dog, which was observed for 10 s.

The assessor then gave the dog a pig's ear or bone, depending on the dog's food interest, and it was allowed to chew it for 30 s. The steps above with wet food were repeated; then, the assessor attempted to retrieve the food, asking the dog to "drop it", "leave it", or "give" before attempting to retrieve it by offering a new food that is novel.

Appendix A.7. Test 7: Stranger Interaction

There were three components to the test: the entry, approach and exit of a stranger. The assessor placed the dog on a leash as the observer exited the room and returned dressed in a reflective vest, large brimmed hat and using a walking stick. The observer entered the room, and bent down to extend an open flat hand as if to pat the dog on the head. The observer then talked to the dog normally and stopped for 3 s, allowing the dog to approach. If the dog approached, the observer patted the dog on the top of its head for 3 s. If the dog did not approach, it was observed for 10 s, with an emphasis on any interaction between the assessor and/or the observer.

Appendix A.8. Test 8: Fake Toddler Interaction

There were two components of the test: the approach of the toddler doll and the exit/removal of the toddler doll. The assessor stood and held the dog's leash while the observer exited the area and returned carrying a toddler doll simulating a small child. Once the toddler was within the leash perimeter from the dog, the observer placed the doll on the floor facing the dog, with the doll's arm extended toward the dog. The assessor allowed the dog to approach if it desired. If the dog did not approach the observer, it was observed for 20 s. After this, the assessor picked up the toddler doll and walked back out of the room. The assessor allowed the dog to follow to the door or move away from stimulus.

Appendix A.9. Test 9: Fake Cat

The assessor stood and held the dog's leash while the observer exited the area and returned carrying a fake cat as if it were a "real" cat. Once the fake cake was within the leash perimeter from the dog, the observer placed the fake cate on the floor facing the dog. The assessor allowed the dog to approach if he/she wanted to. However, if the dog did not approach the observer, the dog was observed for 20 s with the fake cat present.

Appendix A.10. Test 10: Time Alone

The assessor and observer removed the leash from the dog and left the room for 2 min, with a video camera in the front of the room monitoring behaviour and vocalisations. Then, the assessor and observer re-entered through the same door.

Appendix A.11. Test 11: Behaviour with Another Dog

There were three components to the test: walking parellel, circling activity, and nose-to-nose interaction. This test was conducted in a yard (10−20 m), allowing adequate space between the test dog and another dog. Each dog had an assessor, who interacted with their dog by giving treats and ignoring the other assessor and dog. The assessor had a short, 1 m leash, so that the dog walked close to the assessor. At the start, both assessors walked parallel to each other, 5 m apart, with the dogs on the outside. If one or both dogs were reactive and pulled toward each other, the distance between the assessors was increased. If both dogs were relaxed and focused on their assessor, the assessors moved the dogs to an exercise circle. If the dogs did not breach a minimum distance of 5 m between them, they were introduced on opposite sides of a fence. Then followed a circling activity, which required one assessor to stand still with their dog on no more than 1.5 m of leash while the other assessor and their dog completed a circle around the assessor. The assessors then swapped places and repeated the circling activity. If no adverse behaviours were displayed, the assessor in the middle of the circle remained at that location, ensuring that the only tension on the leash was from the dog. The other assessor identified the leash threshold of the dog in the centre and moved close enough to allow the dogs to be nose to nose, also ensuring that the only tension on their leads was caused by the dog pulling, not them pulling against the dog. Once the leads became loose, and the dogs stopped pulling against the assessor, the assessors took a step closer to each other, allowing the dogs to interact if they chose. Leashes remained loose. If there were signs of adverse reactions or aggression, the dogs were separated by increasing the threshold.

Appendix B

Table A1. C-BARQ Categories and Descriptions.

C-BARQ Categories		Description
Stranger-directed aggression	Dog acts aggressively	When approached directly by an unfamiliar male adult while being walked or exercised on a leash When approached directly by an unfamiliar female adult while being walked or exercised on a leash When approached directly by an unfamiliar child while being walked or exercised on a leash Toward unfamiliar persons approaching the dog while it is in the owner's car When an unfamiliar person approaches the owner or a member of the owner's family at home When an unfamiliar person approaches the owner or a member of the owner's family away from home When mailmen or other delivery workers approach the home When strangers walk past the home while the dog is in the yard When joggers, cyclists, roller skaters, or skateboarders pass the home while the dog is in the yard Toward unfamiliar persons visiting the home
Owner-directed aggression	Dog acts aggressively	When verbally corrected or punished by a member of the household When toys, bones, or other objects are taken away by a member of the household When bathed or groomed by a member of the household When approached directly by a member of the household while it is eating When food is taken away by a member of the household When stared at directly by a member of the household When stepped over by a member of the household When a member of the household retrieves food or objects stolen by the dog
Stranger-directed fear	Dog acts anxious or fearful	When approached directly by an unfamiliar male adult while away from the home When approached directly by an unfamiliar female adult while away from the home When approached directly by an unfamiliar child while away from the home When unfamiliar persons visit the home
Non social fear	Dog acts anxious or fearful	In response to sudden or loud noises In heavy traffic In response to strange or unfamiliar objects on or near the sidewalk During thunderstorms firework displays, or similar When first exposed to unfamiliar situations In response to wind or wind-blown objects

Table A1. *Cont.*

C-BARQ Categories		Description
Dog Rivalry	Dog acts aggressively	Towards another (familiar) dog in your household. When approached at a favorite resting/sleeping place by another household dog When approached while eating by another household dog When approached while playing with/chewing a favorite toy, bone, object by another household dog
Dog-directed aggression	Dog acts aggressively	When approached directly by an unfamiliar male dog while being walked or exercised on a leash When approached directly by an unfamiliar female dog while being walked or exercised on a leash Toward unfamiliar dogs visiting the home When barked, growled or lunged at by an unfamiliar dog
Dog-directed fear	Dog acts anxious or fearful	When unfamiliar dogs visit the home When barked, growled or lunged at by an unfamiliar dog When approached directly by an unfamiliar dog of the same or larger size When approached directly by an unfamiliar dog of a smaller size
Separation-related behavior	Dog displays	Shaking, shivering, or trembling when left or about to be left on its own Excessive salivation when left or about to be left on its own Restlessness, agitation, or pacing when left or about to be left on its own Whining when left or about to be left on its own Barking when left or about to be left on its own Howling when left or about to be left on its own Chewing or scratching at doors, floor, windows, and curtains when left or about to be left on its own Loss of appetite when left or about to be left on its own
Attachment or attention-seeking behavior	Dog	Displays a strong attachment for a particular member of the household Tends to follow a member of household from room to room about the house. Tends to sit close to or in contact with a member of the household when that individual is sitting down Tends to nudge, nuzzle, or paw a member of the household for attention when that individual is sitting down Becomes agitated when a member of the household shows affection for another person Becomes agitated when a member of the household shows affection for another dog or animal

Table A1. *Cont.*

C-BARQ Categories		Description
Trainability	Dog	Returns immediately when called while off leash Obeys a sit command immediately Obeys a stay command immediately Will fetch or attempt to fetch sticks, balls, and other objects Seems to attend to or listen closely to everything the owner says or does Is slow to respond to correction or punishment Is slow to learn new tricks or tasks Is easily distracted by interesting sights, sounds, or smells
Chasing	Dog	Acts aggressively toward cats, squirrels, and other animals entering its yard Chases cats if given the chance Chases birds if given the chance Chases squirrels and other small animals if given the chance
Excitability	Dog overreacts or is excitable	When a member of the household returns home after a brief absence When playing with a member of the household When the doorbell rings Just before being taken for a walk Just before being taken on a car trip When visitors arrive at its home
Touch sensitivity	Dog acts anxious or fearful	When examined or treated by a veterinarian When having its claws clipped by a household member When having feet toweled by a household member When groomed or bathed by a household member
Energy	Dog	Dog is playful, puppyish, and boisterous Dog is active, energetic, and always on the go

Appendix C

Table A2. Number (and %) of respondents (n:107) classifying their dogs in each of five levels on a scale of increasing intensity of behaviour exhibited at home, using the C-BARQ Categories.

Behaviour	Target of Behaviour	Scale †									
		0		1		2		3		4	
Fear	Stranger-direct	73	(68.2)	25	(23.4)	5	(4.67)	2	(1.86)	2	(1.86)
	Non Social	60	(56.1)	33	(30.8)	12	(11.2)	1	(0.93)	1	(0.93)
	Dog directed	49	(45.8)	36	(33.6)	13	(12.1)	8	(7.47)	1	(0.93)
Aggression	Stranger-directed	77	(72.0)	24	(22.4)	5	(4.67)	1	(0.93)	0	(0.00)
	Owner-directed	101	(94.4)	2	(1.87)	4	(3.73)	0	(0.00)	0	(0.00)
	Dog directed	36	(33.6)	25	(23.0)	27	(25.2)	11	(10.3)	2	(1.86)
	Familiar dog	71	(66.3)	24	(22.4)	8	(7.47)	4	(3.73)	0	(0.00)
Separation related problems		82	(76.6)	21	(19.6)	3	(2.80)	1	(0.93)	0	(0.00)
Attention-seeking		1	(0.93)	33	(30.8)	52	(48.6)	18	(16.8)	2	(1.86)
Touch sensitivity		60	(56.1)	33	(30.8)	12	(11.2)	1	(0.93)	1	(0.93)
Chasing behaviour		27	(25.2)	16	(15.0)	28	(26.2)	32	(29.9)	4	(3.73)
Excitability		1	(0.93)	33	(30.8)	46	(43.0)	23	(21.5)	4	(3.73)
Energetic		9	(8.41)	32	(29.9)	45	(42.1)	17	(15.9)	4	(3.73)
Trainability		1	(0.93)	7	(6.54)	68	(63.6)	31	(29.0)	0	(0.00)

† Fear, 0 no fear or anxiety—4 extreme fear, both stranger, non-social and dog fear; aggression, 0 none—4 serious, separately scored for stranger-, owner-, dog and familiar dog-directed; separation-related problems, from 0 never—4 always; attachment/attention-seeking, from 0 never—4 always; touch sensitivity, from 0 never—4 always; excitability, from 0 calm to 4 extremely excitable; chasing, energy, and trainability, from 0 never—4 always.

Appendix D

Table A3. Percentage of coded durations and frequencies of the five behavioural categories (friendliness, fear, anxiety, arousal and aggression) during each subtest in the standardised behaviour assessment.

Test	Friendliness		Fear		Anxiety		Arousal		Aggression	
	F	*D*	*F*	*D*	*F*	*D*	*F*	*D*	*F*	*D*
Exploration	30.6	38.5	19.8	32.5	24.8	15.7	21.0	11.8	3.8	1.5
Tolerance to Handling	31.8	37.5	30.7	39.4	19.1	13.8	9.6	6.8	8.9	2.5
Toy interaction	46.6	44.3	16.3	18.8	16.3	14.8	16.9	19.9	3.9	2.3
Response to stimulus	35.2	37.1	22.3	27.4	20.5	16.9	18.2	15.9	3.8	2.7
Resource guarding	41.0	45.6	26.1	30.3	15.7	11.0	12.9	11.7	4.3	1.5
Stranger	37.0	40.9	25.0	27.1	16.4	13.6	15.4	15.4	6.1	3.0
Toddler doll	38.2	40.8	25.8	27.1	14.4	13.0	14.4	15.3	7.2	3.8
Time alone	26.3	39.3	13.8	29.6	28.8	16.6	28.6	12.5	2.4	2.0
Dog to Dog	35.5	47.2	21.2	25.1	19.2	12.6	17.4	11.5	6.6	3.5

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Article

Do Canine Behavioural Assessments and Characteristics Predict the Human-Dog Interaction When Walking on a Leash in a Shelter Setting?

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Received: 18 November 2020; Accepted: 24 December 2020; Published: 25 December 2020

Simple Summary: We explored 370 human-dog interactions in an animal shelter when volunteers walked shelter dogs on a leash, considering the effects of canine demographics and the results of the shelter's canine behavioural assessments. Results showed that dogs that were more relaxed during the shelter assessment (i.e., when socialising with humans or being left alone in a new environment) were less reactive on the leash, with lower tension and pulling frequency. Moreover, socialised and relaxed dogs displayed more positive body language, such as tail in a high position, gazing at the handler, and exploring the environment. When walking with these dogs, volunteers utilised fewer verbal guidance cues and body language during the walk. In addition to the canine behaviour assessment, there were correlations between canine demographics (i.e., age, skull shape, body size, and previous ownership history) and the behavioural interaction and humans' perception. Finally, volunteers perceived the walk as less satisfactory when they needed to pull the leash harder during the walk. This research suggests that the RSPCA behavioural assessment may be useful in predicting the behaviour of shelter dogs when walked by volunteers.

Abstract: Inappropriate leash reactivity is one of the most common problems in shelter dogs, which negatively affects the health of dogs and reduces their adoptability. We explored 370 human-dog interactions, involving 74 volunteers and 111 dogs, in an animal shelter when volunteers walked shelter dogs on a leash, considering the effects of canine demographics and the results of the shelter's canine behavioural assessments. The interaction was video recorded and coded using ethograms, and a leash tension meter was used to measure the pull strength of dogs and handlers. Results showed that dogs that were more relaxed during the shelter assessment (i.e., when socialising with humans or being left alone in a new environment) were less reactive on the leash, with lower tension and pulling frequency. Moreover, socialised and relaxed dogs displayed more positive body language, such as tail in a high position, gazing at the handler, and exploring the environment. When walking with these dogs, volunteers utilised fewer verbal cues and body language during the walk. In addition to the canine behaviour assessment, there were correlations between canine demographics and the behavioural interaction and humans' perception. Finally, volunteers perceived the walk as less satisfactory when they needed to pull the leash harder during the walk. This research suggests that the RSPCA behavioural assessment may be useful in predicting the behaviour of shelter dogs when walked by volunteers.

Keywords: on-leash walk; canine behavioural assessment; leash tension; behaviour; verbal cue; body gesture; human-dog interaction; shelter

1. Introduction

An animal shelter is a challenging environment for dogs, causing both short-term and long-term stress, with acutely elevated cortisol levels within five days of dogs being transferred into a shelter [1]. Moreover, prolonged confinement leads to more problematic behaviours (e.g., decreased activity or excessive auto-grooming [2]), which compromises the animal's welfare and negatively influences their adoptability [3]. Therefore, positive human-dog interactions that help alleviate the stress of dogs are important in an animal shelter [4]. There are a variety of human-dog interactions that occur routinely in shelters, including petting [4], training [5] and on-leash dog walking [6]. Despite the extended literature on human-dog interactions, limited research has explored the role of human-dog interactions in the shelter context.

Physical characteristics of dogs influence the human-dog interaction. Compared to adult dogs, puppies are more likely to evoke our nurturing instinct and more quickly and easily form a stronger attachment with humans [7,8]. Smaller dogs are generally perceived as less obedient, more anxious, fearful, excitable and aggressive [9], while larger dogs are thought to be more cooperative and playful [10]. Dogs with wider heads are more likely to display self-grooming but less likely to chase [11]. Nevertheless, small-sized dogs are generally preferred by the general public [3], and there is a trend of people favouring breeds with shorter and wider heads [12–14].

To evaluate the behaviour of incoming dogs and their adoptability [15,16], many animal shelters have implemented canine behavioural assessments (e.g., SAFER® [16] and Assess-A-Pet™ [17]). In the Royal Society for the Prevention of Cruelty to Animals Queensland (RSPCA Qld), a similar test is conducted on adult dogs around day 5 after arrival to the shelter [18,19]. The canine behavioural assessments measure how dogs react to different stimuli in different situations [15,20]. Controversies have arisen about whether the assessments conducted in animal shelters do indeed predict future household behaviours after adoption [21–23]. One argument is that certain behaviours, such as aggression [24] and separation anxiety [25], are context- and stimulus-specific. Since the behavioural assessment is usually done by an examiner who may be perceived as unfamiliar and intimidating to the dog, the result may not be transferable to a situation where an owner interacts with the dog [23,24]. Although such arguments have been raised, behavioural assessments still reliably describe animals' behaviours within the shelter [19,26]. For example, fear, anxiety and arousal-related behaviours found in the assessment were also observed when dogs were in the shelter kennels [19].

Behaviours of dogs are also related to how we perceive and interact with them. For instance, owners of aggressive English cocker spaniels were reported to be shy, undisciplined, less emotionally stable and tense [27]. Owners are also less likely to engage in shared activities and training with dogs if their dogs are disobedient, aggressive or bark excessively [28]. When it comes to shelters, a less timid dog prefers to play with humans rather than engaging in independent play [29], and dogs spending more time in front of the kennel, laying proximal to the adopter and reacting to humans' play behaviours attract more preference from potential adopters [30,31]. Problematic behaviours, such as vocalising, house soiling and aggression, negatively affect the owner–dog attachment and significantly increase the risk of unsuccessful dog ownerships, such as owners surrendering dogs to shelters or dogs being returned after adoption [32–34].

In many dog shelters, on-leash walking is an important part of the human-dog interactions that improves the health of shelter dogs, reduces their in-kennel stress level and facilitates their socialisation [35,36]. However, leash reactivity is one of the most common problems in shelter dogs [37]. Dogs often lunge forwards during on-leash walks, which may damage the soft tissue around the neck and trachea [38] and contribute to increased intraocular pressure [39]. In addition, leash reactivity is reported to be a common problem after adoption [37] and is related to the failure of dog ownerships [40].

An equine rein tension meter measures the force exerted on the rein, which enables researchers and trainers to monitor how a rider communicates with the horse [41,42]. A similar concept is adopted in this study using a custom-made leash tension meter to capture the leash tension when walking a dog on a leash. Moreover, this canine leash tension meter includes an accelerometer in the device which differentiates between human and dog pulling during the walk [43].

Apart from the leash tension meter, behavioural observation using video recording was also used [30,44]. For dogs, the position of the tail, facial expression and ongoing behaviours were recorded, which are directly related to the animal's welfare [45,46]. In humans, body gestures and verbal cues were measured as they influence the response of the dogs [47,48]. It was hypothesised that dog tolerance to physical contact would be related to higher leash tension, while dogs that were more relaxed in an unfamiliar environment would have lower leash tension [19]. We also hypothesised that dogs would show more friendly signs and have a lower leash tension if the dogs were more accustomed to human interaction. Finally, we hypothesised that handlers would use fewer verbal and gestural cues when dogs were less easily aroused as scored in the behavioural assessment [19] and were older, presumably because they were generally calmer and more obedient [28]. Additionally, handler satisfaction would be negatively associated with leash tension [28].

This article describes the relationship between the results of canine behavioural assessment, canine demographics and human-dog interactions when the dog is walked on a leash, with the focus mainly on canine behavioural assessment. Therefore, results regarding the behavioural assessment will be presented in the main body of the manuscript, followed by relevant demographics. More details on demographic characteristics are presented in the appendices.

This article is a part of a larger research project that explores the behavioural interaction between shelter dogs and volunteers during walks. In this paper, emphasis will be placed on how canine demographics and behavioural assessment results influenced the behavioural interaction while shelter dogs were being walked on leash by volunteers. The effect of human gender has been reported [49] and the effects of body size, body weight, age and the behavioural level of dogs on the leash tension, and the relationship between canine sex and behaviour and leash tension were also reported [43,49]. The influence of other human demographics and personality will be reported in a future publication.

2. Materials and Methods

This study was approved by the Human Research Ethics and Animal Ethics Committees (approval numbers: 2018001570 and SVS/400/18, respectively) of the University of Queensland.

2.1. Study Site

The research was conducted at the Royal Society for the Prevention of Cruelty to Animals, Queensland (RSPCA, Qld) shelter. The housing schedule and shelter environment have been fully described in a previous paper [49]. This study focused on the human-dog interaction during the walks as described in Shih et al. [49].

2.2. Subjects

This study investigated 370 walks (370 unique dog-walker pairs), involving 111 shelter dogs and 74 volunteer walkers, with each walker walking 5 different dogs. The classification of the dog behavioural level was based on their performance during the daily walk. Level 1 dogs walked on a loose leash most of the time. Level 2 dogs pulled the leash occasionally. Level 3 dogs tended to pull the leash fiercely. Level 3+ dogs had severe behavioural issues (e.g., aggressiveness or fearfulness) and they might or might not pull the leash harder than level 3 dogs. More details about the behavioural level are described in Shih et al. [49].

2.3. RSPCA Canine Behavioural Assessment

Each dog entering RSPCA Qld is behaviourally assessed within 5 days of entry using a standardised assessment. The assessment used is fully described in Clay et al. [19] and comprises the following subtests: Socialisation, Tolerance, Toys, Run and Freeze, Resource Guarding, Toddler Doll, Time Alone and Dog to Dog. In the Socialisation subtest, a dog was allowed to freely explore the room and interact with the handler. Its behaviour and body tension were evaluated. In the Tolerance subtest, the dog's behaviour, body position and tension were assessed when it was stroked and touched on the feet by the handler. In the Toys subtest, the dog was allowed to play with the toys by itself and with the handler; its behaviour during the interaction was recorded. In the Run and Freeze subtest, the handler ran around in the room and suddenly froze in the corner. The response of the dog was recorded. In the Resource Guarding subtest, the dog was given wet food, dry food and a real bone, consecutively. The examiner assessed its behaviour, body position and tension when the handler was trying to remove the food with a fake hand. In the Toddler Doll subtest, the dog was presented with a fake toddler which mimicked the physical appearance and behaviour of a real child, and its reaction to the toddler was assessed. In the Time Alone subtest, the dog was left alone in the room without any room light (but some natural light through a window) while being videoed. Its behaviour was later evaluated by reviewing the digital recording. Finally, in the Dog to Dog test, the assessed dog was introduced to another dog, and the behaviour of the assessed dog was recorded. This research focused on the human-dog dyad and thus the Dog to Dog subtest was not discussed here.

2.4. Canine Leash Tension Meter

The customised canine leash tension (sampling rate: 10 Hz; measuring range: 0–100 kg-force; resolution: 100 g-force) meter was commissioned for this project (RobacScience Australia). The device measures the force exerted on the leash and detects the direction of the pulling (handler versus dog). [43].

2.5. Measurement of the Dog's Body

Body size was determined using the body height (cm), body length (cm), body weight (kg) and body condition score (BCS). Body height and length were measured from the ground to the dorsal scapular rim and from the cranial aspect of the shoulder joint to the caudal aspect of the sciatic tuberosity, respectively, using a tape measure [50]. Body weight was obtained from the RSPCA database. The BCS was determined according to the 9-point scale BCS system [51]. Cephalic index (CI) was calculated as the ratio of skull width to skull length. The skull length was measured from the occipital crest to the tip of the nose, and the width was the widest part of the dog's head (distance between two zygomatic arches) [11].

2.6. Study Design

The study design is explained in Shih et al. [49]. Participants were instructed to walk the dog with one hand and only to touch the leash when the dog got tangled. This represented the most common practice in the real world, even though the official advice is to use two hands [52]. After each walk, participants completed a questionnaire about their perspective of the walk (Table 1).

Table 1. Exit questionnaire used for volunteer walkers (reprinted from Shih et al. [49] for convenience).

1. The dog's behaviour was good.
2. I could not handle the dog well.
3. I felt comfortable when interacting with the dog.
4. I was physically tense.
5. Overall, this was a good experience.
6. The interaction was challenging for me.
7. The dog did not understand me well.
8. I did not feel that I was helping the dog.
9. I felt supported by the dog.
10. I did not enjoy its company.
11. I would love to walk this dog again on another day.
12. I don't think this dog is suitable for a non-experienced adopter.
13. I think the dog is ready for adoption.

Each description was rated on a 5-point Likert scale from 1 (strongly disagree) to 5 (strongly agree). Reverse scores were used for negative wording for the calculation of mean scores. Human satisfaction factor (Factor H) utilised responses to questions 2, 3, 4, 5, 6, 10, 11. A higher score of factor H represented a higher satisfaction of the walk. Walker's perception of dog factor (Factor D) utilised responses to questions 1, 7, 8, 9, 12, 13. A higher score of factor D indicated that the dog was considered more supportive and better behaved. Details about the statistical justification of the questionnaire are described in [49].

2.7. Data Analysis

2.7.1. Video Recordings of Dog and Human Behaviours

To record the interaction during the walk, a camera (GoPro Hero 7 Silver, GoPro®, San Mateo, CA, USA) was mounted on the walker's head, and, at the same time, the researcher recorded the walk from 10 m behind with an iPhone 7 (Apple Inc., Cupertino, CA, USA). Videos were coded in their entirety by the researcher, who is a veterinarian and a certified dog trainer, with Boris© behaviour observation software [53], using the same method described in Shih et al. [49]. To blind the coder, video coding was completed prior to any analysis of human and canine characteristics. Ten randomly selected videos were recoded to check intra-observer reliability (Cohen's Kappa = 0.76). Canine behaviours, human verbal cues and human body language were coded using ethograms developed based on previous research [4,45,54–57], as previously described [49] and modified during practice sessions (tables 2 to 4). These tables are reproduced from Shih et al. [49] to help with the understanding of this paper.

Table 2. Ethogram of canine behaviour [49].

Behaviour	Description	Behaviour Type	Reference
Track	Dog uses nose to follow a scent along the ground.	State event	[54]
Sniff	Dog explores or expresses stress or appeasement by orientating its nose to an object, wall or ground, and the dog stands still.	State event	[54]
Eliminate-mark	Dog defecates or urinates in sitting, squatting or standing positions.	Point event	[45]
Shake	Dog shakes its body or head.	Point event	
Pant	Dog breathes vigorously with its mouth wide open.	State event	[54]
Gaze	Dog looks toward the handler.	Point event	[54]
Lip-lick	Dog shows its tongue and moves its tongue along the upper lip or snout.	Point event	[54]
Tail wag	Dog moves its tail from side to side.	State event	[4]
Tail high	Dog holds its tail upright.	State event	[55]

Point event: the number of times the event was observed. State event: the duration of the observed event.

Table 3. Ethogram of human verbal cues [49].

Behaviour	Description	Behaviour Type	Reference
Sit	Volunteer asks the dog to sit.	Point event	
Command	Volunteer talks to the dog with an utterance containing a single command, exclusive of the "sit" command (e.g., "Stay!" "Come!" "Let's go!").	Point event	[56]
Attention seeking	Volunteer tries to get the attention of the dog by calling the name of the dog and/or using the utterance of "Look!" and/or clicking the tongue.	Point event	[56]
High-pitched voice	Volunteer talks to the dog using a high-pitched voice or baby-talk expressions.	Point event	[4]
Praise	Volunteer talks to the dog with a positive utterance (e.g., "Great!" "Well done!" "Good dog!").	Point event	[4,56]
Negative verbal cue	Volunteer talks to the dog with a negative utterance (e.g., "No!" "Bad dog!" "Don't . . . " "Stop . . . " "Let the lead (it) go").	Point event	
Communication	Volunteer communicates with the dog by asking the dog some questions. (e.g., "Which way do you want to go?" "What are you sniffing at?" "Do you want to fetch?" "Do you want to drink?")	Point event	[57]

Point event: the number of times the event was observed. State event: the duration of the observed event.

Table 4. Ethogram of human body language [49].

Behaviour	Description	Behaviour Type	Reference
Gestural	Volunteer displays voluntary hand movement directed towards the dog (e.g., referential point, patting his/her own thigh, luring the dog with a hand or food).	Point event	[4,56]
Physical contacts	Physical contacts initiated by the volunteer. Including contacts when treats were given.	Point event	
Food reward	Food is given to the dog including directly giving it, tossing it or placing it on the ground or an object.	Point event	

Point event: the number of times the event was observed.

2.7.2. Leash Tension Analysis

Leash tension and pulling frequency were analysed with MATLAB® (MATLAB® and Statistics Toolbox Release 2018b, The MathWorks, Inc., Natick, MA, USA) using the same approach as described in Shih et al. [49].

Every pulling episode can be divided into three phases. Phase one is the “initiation phase”, when either the dog or the handler initiates the pulling, which is marked by an increase in leash tension and acceleration of the device towards the initiator of the pull. Phase two is the “contest phase”, when the other party counteracts the pulling, which is marked by an acceleration in the opposite direction to the initiator and a sharp increase in the leash tension. Finally, phase three is the “losing or winning phase”, when either the party that initiated the pulling wins the contest or the opposite party wins (the party that initiated the pulling loses) the contest. This phase is characterised by a decrease in the leash tension; there should be acceleration towards the “winner”. The leash tension then either returns to the baseline or a new pulling episode characterised by a change in the gradient of the leash tension occurs.

Net maximal tension (NT_{max}), maximal tension by dog (DT_{max}) and handler (HT_{max}) represent the maximal tension throughout the walk, recorded for the dog and handler, respectively. Net mean tension (NT_{mean}), mean tension by dog (DT_{mean}) and mean tension by handler (HT_{mean}) represent the mean tension throughout the walk, recorded for the dog and handler, respectively. Dog pulling frequency (DPF) and handler pulling frequency (HPF) were the frequency of pulling initiated by the dog and handler, respectively.

2.8. Statistical Analysis

Statistical analysis was conducted using RStudio Version 1.2.1335 [58] with packages leaps [59], MASS [60], car [61], carData [62], Matrix [63], polycor [64], plyr [65], psych [66], ggpubr [67] and nlme [68].

The canine behavioural assessment was transferred into the scoring system based on the effect of each behavioural presentation on the human-dog interaction and safety (Table S1). A higher score in the subtest indicates that dogs displayed more behaviours that would favour the human-dog interaction and safety. One dog had two behavioural assessment results because the shelter staff wanted to reconfirm or monitor the result. For that dog, an average score was calculated.

This study used the same statistical analysis methods as described in [49]. Bivariate generalised linear models were used for the analysis of each combination of dependent (leash tension, pulling frequency, behaviour and the score of the exit questionnaire) and independent (human and dog demographics, human personality, canine behavioural assessment) variables, followed by generalised linear mixed models for multivariate analyses and repetitions of dogs and walkers. According to the results of bivariate generalised linear models, independent variables with p values less than 0.2 and those logically expected to affect the dependent variable, regardless of the p value, were included in the generalised linear mixed model. Dependent variables were manually transformed for statistical analysis to meet the assumptions of generalised linear mixed models, including the normality of residual and random effects and homogeneity of variance of residual [49,69].

3. Results

3.1. Demographics

This study involved 111 shelter dogs, with 58 (52.3%) females and 53 (47.7%) males and all were gonadectomised. The mean age of dogs was 3.74 (±2.45) years (44.82 ± 29.37 months) old. The mean body height and length were 52.04 (±6.29) cm and 56.05 (±5.93) cm, respectively. The mean body weight was 24.87 (±6.65) kg and the mean body condition score was 4.59 (±1.07). Finally, the mean cephalic index was 0.58 (±0.058).

There were 43 (38.74%) stray dogs, 31 (27.93%) surrendered to the RSPCA by owners, 19 (17.12%) returned to the RSPCA by previous adopters and 18 (16.22%) having other or unknown sources.

3.2. Canine Behavioural Assessment, Demographics and Leash Tension/Frequency

The median score and interquartile range (IQR) of each behavioural assessment subtest was as follows: socialisation (median = 3.00, IQR= 1.00), tolerance (median = 4.00, IQR = 3.00), toy (median = 1.00, IQR = 3.00), run and freeze (median = 3.50, IQR = 1.00), resource guarding (median = −3.00, IQR = 1.00), toddler (median = 4.00, IQR = 1.00) and time alone (median = −1.00, IQR = 1.00).

Dogs that were more socialised when exploring the room and interacting with humans were correlated with lower maximal net leash tension (p = 0.026). However, dogs that were more tolerant of the human's physical contact were associated with higher maximal net leash tension (p = 0.048). Dogs that were more engaged in playing with toys by themselves or with humans had less pulling frequency (p = 0.043). Dogs being more friendly to a model toddler were related to higher maximal net leash tension (p = 0.018) and higher pulling frequency created by dogs (p = 0.016) and humans (p = 0.0003). Dogs exhibiting more reactions (lower score) to time spent alone were correlated with higher mean net leash tension (p = 0.018), higher mean (p = 0.039) leash tension and pulling frequency (p = 0.038) created by dogs and higher maximal (p = 0.026) and mean (0.025) leash tension created by humans (Table 5).

Table 5. Generalised linear mixed model of the effect of canine behavioural assessment on leash tension and pulling frequency.

Behavioural Assessment	$Log_{10}NT_{max}$	$Log_{10}NT_{mean}$	$Log_{10}DT_{max}$	$Log_{10}DT_{mean}$	$Log_{10}DPF$ [1]	$Log_{10}HT_{max}$	$Log_{10}HT_{mean}$	$Log_{10}HPF$ [1]
Socialisation	β −0.071 SE 0.032 p 0.026	β −0.0038 SE 0.024 p 0.87	β −0.049 SE 0.035 p 0.16	β −0.04 SE 0.024 p 0.092	β 0.078 SE 0.055 p 0.16	β −0.055 SE 0.034 p 0.11	β −0.032 SE 0.025 p 0.19	β 0.022 SE 0.05 p 0.65
Tolerance	β 0.031 SE 0.015 p 0.048	β 0.011 SE 0.011 p 0.32	β 0.03 SE 0.017 p 0.076	β 0.016 SE 0.011 p 0.18	β 0.029 SE 0.027 p 0.29	β 0.032 SE 0.016 p 0.052	β 0.02 SE 0.012 p 0.1	β 0.0084 SE 0.026 p 0.74
Toy	β 0.022 SE 0.028 p 0.43	β −0.0059 SE 0.021 p 0.78	β 0.011 SE 0.031 p 0.72	β −0.0036 SE 0.021 p 0.86	β −0.1 SE 0.05 p 0.043	β 0.011 SE 0.03 p 0.72	β −0.0035 SE 0.022 p 0.87	β −0.041 SE 0.046 p 0.38
Run and Freeze	β −0.0055 SE 0.025 p 0.82	β −0.019 SE 0.02 p 0.34	β −0.015 SE 0.027 p 0.59	β −0.0038 SE 0.019 p 0.84	β −0.068 SE 0.042 p 0.11	β −0.0095 SE 0.027 p 0.72	β −0.003 SE 0.019 p 0.86	β −0.067 SE 0.037 p 0.075
Resource Guarding	β −0.023 SE 0.026 p 0.37	β −0.01 SE 0.019 p 0.6	β −0.029 SE 0.028 p 0.31	β −0.027 SE 0.019 p 0.17	β 0.044 SE 0.045 p 0.32	β −0.026 SE 0.028 p 0.35	β −0.02 SE 0.02 p 0.32	β 0.059 SE 0.041 p 0.15
Toddler	β 0.055 SE 0.023 p 0.018	β 0.0081 SE 0.017 p 0.64	β 0.049 SE 0.025 p 0.053	β 0.014 SE 0.017 p 0.42	β 0.098 SE 0.041 p 0.016	β 0.041 SE 0.025 p 0.11	β 0.0088 SE 0.018 p 0.62	β 0.13 SE 0.037 p 0.0003
Time Alone	β −0.11 SE 0.047 p 0.018	β −0.041 SE 0.038 p 0.28	β −0.09 SE 0.051 p 0.078	β −0.073 SE 0.035 p 0.039	β −0.17 SE 0.08 p 0.038	β −0.11 S 0.05 p 0.026	β −0.081 SE 0.036 p 0.025	β −0.05 SE 0.072 p 0.49

Tension and pulling frequency were analysed in log_{10} transformation. NT_{max}: maximal net leash tension. NT_{mean}: mean net leash tension. DT_{max}: maximal leash tension caused by dog. DT_{mean}: mean leash tension caused by dog. HT_{max}: maximal leash tension caused by handler. HT_{mean}: mean leash tension caused by handler. DPF: dog pulling frequency. HPF: handler pulling frequency. β: regression coefficient. SE: standard error of β. p: p value of the model. [1] Pulling frequency = (Number of pulls) / (walking duration). A pull was defined as a bout of force greater than 0.1% of the dog's body weight force.

Compared to dogs classified as strays, those surrendered by their owners had lower net maximal (p = 0.0084) and mean (p = 0.0008) leash tension, maximal (p = 0.022) and mean (p = 0.0003) leash tension created by dogs and maximal (p = 0.041) and mean (p = 0.0029) leash tension created by humans; dogs returned after a failed adoption were associated with lower maximal leash tension (p = 0.047) and pulling frequency by humans (p = 0.013) (Table S2).

3.3. Canine Behavioural Assessment, Demographics and Canine Behaviour

Dogs that were more socialised when exploring the room and interacting with humans spent a higher percentage of time tracking (p = 0.0085) and keeping their tails in a high position (p = 0.049) and gazed at handlers more frequently (p = 0.036). Dogs that were calmer when seeing a person running and suddenly freezing less frequently displayed gazing (p = 0.038) and lip-licking (0.0013) behaviours, but they spent a greater percentage of time sniffing (p = 0.013). For dogs showing less resource guarding potential, gazing (p = 0.0006) and lip-licking (p = 0.0001) were less commonly observed, but these dogs eliminated more often (p = 0.036). Finally, dogs being calmer and friendlier toward the fake toddler gazed at handlers less frequently (p = 0.045) (Table 6).

Table 6. Generalised linear mixed model of the effect of canine behavioural assessment on canine behaviour.

Behavioural Assessment	Track (%)	Tail High (%)	Tail Wag (%)	Gaze (no./sec)	Lip-Lick (no./sec)	Eliminate-Mark (no./sec)	Shake (no./sec)	Pant (%)	Sniff (%)
Socialisation	β 0.023 SE 0.0086 p 0.0085	β 0.053 SE 0.027 p 0.049	β 0.015 SE 0.013 p 0.27	β 0.014 SE 0.0067 p 0.036	β 0.011 SE 0.0064 p 0.091	β 0.0042 SE 0.002 p 0.059	β 0.0004 SE 0.00043 p 0.35	β 0.0053 SE 0.013 p 0.68	β −0.0057 SE 0.0074 p 0.44
Tolerance	β −0.00097 SE 0.004 p 0.81	β 0.021 SE 0.012 p 0.09	β 0.0081 SE 0.0062 p 0.19	β −0.0026 SE 0.0033 p 0.42	β 0.0035 SE 0.0029 p 0.24	β 0.00031 SE 0.0011 p 0.77	β 0.000053 SE 0.00019 p 0.78	β 0.0066 SE 0.0062 p 0.29	β 0.00082 SE 0.0037 p 0.83
Toy	β 0.0025 SE 0.0075 p 0.74	β 0.0045 SE 0.023 p 0.84	β −0.014 SE 0.012 p 0.24	β 0.0 SE 0.006 p 0.099	β −0.0026 SE 0.0057 p 0.65	β −0.0032 SE 0.0019 p 0.099	β −0.00017 SE 0.00036 p 0.64	β 0.011 SE 0.012 p 0.35	β 0.0065 SE 0.0066 p 0.33
Run and Freeze	β −0.004 SE 0.0062 p 0.53	β 0.00627 SE 0.019 p 0.74	β −0.018 SE 0.01 p 0.08	β −0.01 SE 0.0052 p 0.038	β −0.016 SE 0.0049 p 0.0013	β 0.0028 SE 0.0015 p 0.072	β −0.00043 SE 0.00032 p 0.19	β −0.0086 SE 0.009 p 0.34	β 0.015 SE 0.0061 p 0.013
Resource Guarding	β 0.0034 SE 0.0067 p 0.61	β 0.033 SE 0.021 p 0.12	β −0.0017 SE 0.011 p 0.88	β −0.02 SE 0.0056 p 0.0006	β −0.022 SE 0.0054 p 0.0001	β 0.0036 SE 0.0017 p 0.036	β 0.0002 SE 0.00035 p 0.57	β −0.016 SE 0.01 p 0.14	β −0.0018 SE 0.0068 p 0.79
Toddler	β −0.0016 SE 0.0064 p 0.8	β 0.0097 SE 0.02 p 0.62	β 0.0087 SE 0.011 p 0.42	β −0.011 SE 0.0053 p 0.045	β −0.0072 SE 0.0052 p 0.17	β 0.0021 SE 0.0016 p 0.21	β −0.00004 SE 0.00033 p 0.9	β −0.0026 SE 0.0097 p 0.79	β −0.0037 SE 0.0066 p 0.57
Time Alone	β −0.0042 SE 0.013 p 0.74	β −0.015 SE 0.037 p 0.69	β 0.0059 SE 0.021 p 0.77	β −0.012 SE 0.011 p 0.27	β −0.0067 SE 0.01 p 0.5	β 0.0024 SE 0.0033 p 0.45	β −0.00077 SE 0.00062 p 0.22	β −0.026 SE 0.02 p 0.19	β 0.0017 SE 0.013 p 0.9

Track (%): tracking time (s)/total walking time (s) × 100%. Tail high (%): tail high time (s)/total walking time (s) × 100%, analysed in power of 7. Tail wag (%): tail wagging time (s)/total walking time (s) × 100%, analysed in power of 0.3. Gaze (no./sec): number of gazes / time when the dog's head was visible in the Gopro video (s), analysed in power of 0.4. Lip-lick (no./sec): number of lip-licks/time when the dog's head was visible in the Gopro video (s), analysed in power of 0.4. Eliminate-mark (no./sec): number of eliminate-marks/total walking time (s), analysed in power of 0.6. Shake (no./sec): number of shakes/total walking time (s), analysed in power of 0.8. Pant (%): painting time (s)/time when the dog's head was visible in the Gopro video (s) × 100%, analysed in power of 0.5. Sniff (%): sniffing time (s)/total walking time (s) × 100%, analysed in power of 0.5.β: regression coefficient. SE: standard error of β. p: p value of the model.

The age of the dog was negatively correlated with the percentage of time dogs spent tracking (p = 0.012) and the frequency of shaking behaviour (p = 0.0021). The cephalic index of the dog was positively related to the percentage of time dogs spent tracking (p = 0.0025) but negatively associated with the percentage of time dogs spent panting (p = 0.0009). Compared to level 3 dogs, level 2 dogs wagged their tails more often (p = 0.038); level 3+ dogs displayed higher percentages of tracking (p = 0.0015) and sniffing behaviours (p = 0.039) but a lower percentage of panting behaviours (p = 0.027) (Table S3).

3.4. Canine Behavioural Assessment, Demographics and Human Behaviour

Less frequent communication (p = 0.029), commands (p = 0.0079) and giving food treats (p = 0.034) were observed when handlers walked dogs that were calmer when seeing a person running and suddenly freezing. When walking dogs with less resource guarding potential, handlers were less likely to communicate (p = 0.0025) with them and displayed less body language (p = 0.0017) and were less likely to give food treats (p = 0.0002) and initiate physical contact (p = 0.0008) (tables 7 and 8).

Table 7. Generalised linear mixed model of the effect of canine behavioural assessment on human verbal cue.

Behavioural Assessment	Total Verbal Cue (no./sec) [1]	Attention Getter (no./sec) [2]	Communication (no./sec) [2]	Negative Verbal cue (no./sec) [2]	Praise (no./sec) [1]	High-Pitched Voice (no./sec) [1]	Command (no./sec) [1]
Socialisation	β 0.00049 SE 0.0077 p 0.95	β 0.0033 SE 0.0058 p 0.57	β 0.00021 SE 0.0038 p 0.96	β 0.0007 SE 0.0033 p 0.83	β −0.006 SE 0.0045 p 0.18	β 0.0026 SE 0.0042 p 0.54	β 0.0018 SE 0.0052 p 0.74
Tolerance	β −0.0012 SE 0.0038 p 0.76	β −0.00042 SE 0.0029 p 0.88	β −0.0029 SE 0.0019 p 0.13	β −0.00073 SE 0.0017 p 0.66	β 0.00024 SE 0.002 p 0.91	β −0.001 SE 0.0019 p 0.59	β −0.00047 SE 0.0024 p 0.85
Toy	β −0.002 SE 0.007 p 0.78	β 0.0051 SE 0.0054 p 0.34	β 0.0019 SE 0.0036 p 0.6	β 0.003 SE 0.003 p 0.33	β 0.00068 SE 0.0039 p 0.86	β 0.0041 SE 0.0034 p 0.23	β 0.0019 SE 0.0044 p 0.67
Run and Freeze	β −0.012 SE 0.0059 p 0.052	β −0.0096 SE 0.005 p 0.057	β −0.0076 SE 0.0034 p 0.029	β −0.003 SE 0.003 p 0.31	β −0.005 SE 0.0037 p 0.18	β −0.0052 SE 0.0034 p 0.13	β −0.011 SE 0.004 p 0.0079
Resource Guarding	β −0.013 SE 0.0067 p 0.052	β −0.0024 SE 0.0051 p 0.64	β −0.011 SE 0.0036 p 0.0025	β −0.0027 SE 0.0032 p 0.41	β −0.003 SE 0.0038 p 0.43	β −0.0036 SE 0.0033 p 0.28	β −0.0066 SE 0.0042 p 0.12
Toddler	β −0.0013 SE 0.0064 p 0.84	β 0.0047 SE 0.0053 p 0.38	β −0.0044 SE 0.0037 p 0.23	β −0.0022 SE 0.0031 p 0.48	β 0.0016 SE 0.0038 p 0.68	β 0.0021 SE 0.0033 p 0.53	β −0.0036 SE 0.0042 p 0.4
Time Alone	β 0.023 SE 0.012 p 0.056	β 0.0169400 SE 0.01 p 0.098	β 0.0045 SE 0.007 p 0.52	β −0.0055 SE 0.006 p 0.37	β 0.012 SE 0.007 p 0.088	β 0.0034 SE 0.006 p 0.57	β 0.0089 SE 0.0077 p 0.25

[1] Analysed after transformation to the power of 0.5. [2] Analysed after transformation to the power of 0.4. β: regression coefficient. SE: standard error of β. p: p value of the model.

The age of the dog was negatively correlated with the frequency of human communication (p = 0.043). Compared to level 3 dogs, handlers used more negative verbal cues when walking level 2 dogs (p = 0.042). Compared to stray dogs, handlers were more likely to talk to surrendered dogs using a high-pitched voice (p = 0.028). The size of the dog was positively related to the frequency of communication (p = 0.011), total body language (p = 0.02) and physical contact initiated by the handler (p = 0.0058) (Tables S4 and S5).

3.5. Canine Behavioural Assessment, Demographics and Walking Experience

No significant relationship was observed between the canine behavioural assessment and the walking experience (p > 0.05) (Table 9). The age of the dog was positively associated with the score of factor H (p = 0.041) and factor D (p = 0.0096). Compared to level 3 dogs, level 3+ dogs were correlated with lower scores of factor H (p = 0.016) and factor D (p = 0.026). Mean leash tension created by humans was negatively related to the factor H score (p = 0.0062) (Table S6).

Table 8. Generalised linear mixed model of the effect of canine behavioural assessment on human body language.

Behavioural Assessment	Total Body Language (no./sec) [1]	Food Reward (no./sec)	Hand Gesture (no./sec) [2]	Physical Contact (no./sec) [1]
Socialisation	β 0.002 SE 0.0085 p 0.81	β 0.000025 SE 0.00026 p 0.92	β 0.0024 SE 0.0044 p 0.58	β 0.00048 SE 0.0069 p 0.94
Tolerance	β −0.0013 SE 0.0041 p 0.75	β 0.000091 SE 0.00012 p 0.46	β −0.000036 SE 0.0021 p 0.98	β −0.0017 SE 0.0033 p 0.6
Toy	β 0.0013 SE 0.0077 p 0.87	β −0.00027 SE 0.00023 p 0.25	β −0.0015 SE 0.004 p 0.7	β 0.003 SE 0.0062 p 0.63
Run and Freeze	β −0.012 SE 0.0073 p 0.1	β −0.00048 SE 0.00022 p 0.034	β −0.0074 SE 0.0038 p 0.051	β −0.0026 SE 0.0058 p 0.65
Resource Guarding	β −0.026 SE 0.008 p 0.0017	β −0.00084 SE 0.00022 p 0.0002	β −0.0069 SE 0.0039 p 0.078	β −0.022 SE 0.0064 p 0.0008
Toddler	β −0.011 SE 0.0077 p 0.16	β −0.00023 SE 0.00024 p 0.33	β −0.0026 SE 0.004 p 0.52	β −0.0099 SE 0.0061 p 0.11
Time Alone	β 0.0074 SE 0.014 p 0.61	β 0.00053 SE 0.00043 p 0.22	β 0.00056 SE 0.0073 p 0.94	β 0.016 SE 0.011 p 0.17

[1] Analysed after transformation to the power of 0.5. [2] Analysed after transformation to the power of 0.4. β: regression coefficient. SE: standard error of β. *p*: *p* value of the model.

Table 9. Generalised linear mixed model of the effect of canine behavioural assessment on volunteers' walking experience. Factor H represented human satisfaction factor and factor D represented walker's perception of dog factor.

Behavioural Assessment	Factor H [1]	Factor D
Socialisation	β 281,928 SE 222,792 p 0.21	β 0.0053 SE 0.032 p 0.87
Tolerance	β −20,161 SE 103,723 p 0.85	β 0.0057 SE 0.016 p 0.72
Toy	β 218,989 SE 186,487 p 0.24	β −0.0082 SE 0.027 p 0.76
Run and Freeze	β −185,587 SE 179,140 p 0.3	β −0.0026 SE 0.028 p 0.93
Resource Guarding	β −144,747 SE 178,912 p 0.42	β −0.014 SE 0.029 p 0.64
Toddler	β −112,153 SE 183,151 p 0.54	β 0.02 SE 0.029 p 0.5
Time Alone	β 98,600 SE 357,493 p 0.78	β 0.014 SE 0.052 p 0.79

[1] Analysed after transformation to the power of 10. β: regression coefficient. SE: standard error of β. *p*: *p* value of the model.

4. Discussion

4.1. Canine Behavioural Assessment, Demographics and Leash Tension/Frequency

Dogs being more engaged in playing with toys by themselves or with humans and releasing the toy on command or when traded with treats were less likely to pull on the leash. This finding may indicate that these dogs were more relaxed when interacting with humans and they were more likely to understand and obey human signals when walking on the leash. Dogs that were more friendly and relaxed when facing the fake toddler had higher maximal net leash tension. In addition, these dogs pulled on the leash more frequently and so did the handlers. Since dogs did not encounter any other human being, except the handler, during the walk, it was unlikely that these dogs were attracted by a person or a toddler. Moreover, a previous study on the canine behavioural assessment using a fake toddler or fake cat has shown that instead of testing the dog's response to a real toddler or a cat, the test is likely testing its response to a foreign object [70–72]. Therefore, in our result, it was more likely that these dogs pulled more frequently on the leash because they were more interested in exploring objects around them and handlers were simply responding to the higher pulling frequency by pulling on the leash more frequently too.

Finally, dogs that were more anxious and reactive when left alone in an unfamiliar environment pulled harder and more frequently, which supports our hypothesis about the negative correlation between the relaxation of dogs in a new environment and leash tension. It might also suggest that these dogs were anxious and attempted to escape from the environment. A better image quality is needed to allow a better examination of the dogs and therefore permit a better understanding of the underlying emotion or motivation of the dog. For handlers, during such interactions, handlers also pulled harder on the leash. This subtest was intended to identify the dog's potential to develop separation-related issues. Nevertheless, it has been shown that such tests fail to reliably predict the future development of separation-related behaviours after adoption [23]. It might be, however, that, in the subtest, dogs were demonstrating behaviours responding to the foreign environment, which explains why dogs showing more anxious and stress-related signs in the subtest were less relaxed during the walk, even though handlers were around them.

Compared to dogs found as strays, those relinquished by owners had lower leash tension and those returned by adopters had lower pulling frequency created by handlers. This result satisfies the hypothesis that owner relinquished and returned dogs are more familiar with human interaction and thus are more manageable on the leash. However, this result differs from a previous study showing that the prevalence of unruly behaviour (e.g., jumping up, pulling the lead, poor command responding, lack of concentration) between stray and relinquished dogs was not significantly different [73].

Dogs that were more socialised when exploring the room and interacting with humans had lower maximal net leash tension, potentially because these dogs were generally more relaxed. In line with our hypothesis, dogs that were more tolerant of human physical contact were associated with higher maximal net leash tension, probably because they tended to ignore the pressure exerted on their bodies when pulling on the leash or were simply less concerned about the human and just eager to go for their walk. However, these results should be interpreted with caution because only net tension was measured.

4.2. Canine Behavioural Assessment, Demographics and Canine Behaviour

Dogs that were more socialised when exploring the room and interacting with humans spent a higher percentage of time tracking and keeping their tails high and they more frequently gazed at the handler. Such results satisfy the hypothesis regarding the positive association between canine socialisation and positive body language of dogs. Dogs that explored the exam room were also more likely to explore in other environments, supporting the finding that the RSPCA Qld socialisation test predicts their friendless and sociability in a new environment after adoption [23]. The high tail position and frequent gazing behaviour show that socialised dogs were more confident and more engaged in their interactions with handlers [74,75]. Dogs that were calmer and more relaxed when seeing a person running and freezing less frequently gazed at the handler and displayed lip-licking behaviours, but they spent a higher percentage of time sniffing. Since human-directed gazing and lip-licking can also be interpreted as signs of anxiety or anticipation [76,77], it was possible that these dogs were generally less stressed or aroused when walking on a leash and preferred spending more time exploring the environment through sniffing [76,77]. The sniffing behaviour may also be a displacement signal, indicating conflicting emotions during environmental exploration and mild stress resulting from the novel environment or interacting with the handler [78]. Dogs that were more relaxed in the resource guarding subtest less frequently licked their lips and gazed at the handler but eliminated more frequently. Dogs that were calmer and friendlier toward the fake toddler also gazed at handlers less often. These results might indicate that dogs that were more relaxed when approached during eating and when encountering a foreign object were less defensive and stressed during the walk [76,77]. However, again, results should be interpreted with care as this result might only represent dogs' in-shelter behaviours [19] because shelter assessment of resource guarding has been shown to unreliably predict their behaviours outside of the shelter post-adoption [79].

The age of the dog was negatively correlated with the time spent tracking during the walk, which supports the previous assumption that older dogs accumulate more experiences and thus they naturally become less engaged in their surroundings, showing less interest in exploration and a reduction in excitement [80]. Older dogs also shook their bodies less frequently when on leash. Body shaking is a recognised displacement behaviour linked to stressful situations involving anxiety and excitement, which is believed to be an attempt to relieve the accumulated stress [81]. In addition, older dogs were found to pull less frequently on the leash [43]; therefore, it might be concluded that compared to younger dogs, older dogs were generally calmer and less stressed and excited, showing less interest in the environment during the walk.

Dogs with a wider head (higher cephalic index) spent more time tracking but less time panting, which seems to contradict the fact that brachycephalic breeds are more likely to pant due to brachycephalic obstructive airway syndrome [82]. The result should be interpreted cautiously because most of the dogs in this study were mesocephalic [83]. Therefore, it is more reasonable to conclude that within mesocephalic breeds, wider-headed dogs may spend more time tracking but less time panting when walking on the leash. The cephalic index of dogs is fully explained by neither the breed groups nor the genetic clusters [84]. Consequently, the finding is less likely due to the breed effect but more likely due to the structural differences, which requires further investigation.

Compared to level 3 dogs, level 2 dogs wagged their tails more often, which was more commonly seen when interacting with handlers or waiting for handlers to open the gate to enter the next walking section. This may indicate that level 2 dogs enjoyed being around humans more, which is supported by the lower leash tension created by level 2 dogs [43]. Level 3+ dogs showed more tracking and sniffing but less panting behaviours, potentially because they were actively searching for stimuli.

4.3. Canine Behavioural Assessment, Demographics and Human Behaviour

Less frequent communication, commands and giving food treats were observed when handlers walked dogs that were calmer upon seeing a person running and freezing. Similarly, handlers were less likely to communicate and displayed less body language, including giving food treats and initiating physical contact when walking dogs that were less defensive and anxious in the resource guarding test. These findings support the hypothesis that handlers would use fewer verbal and gestural cues if dogs were less aroused in the shelter assessment. A possible explanation may be that fewer verbal and physical guides are needed during the walk, when dogs are calm and relaxed, and also such results support previous findings that behaviours associated with fear, anxiety and arousal in the RSPCA Qld assessment predict dogs' in-shelter behavioural presentations [19].

Our hypothesis that fewer human verbal or physical signals would be observed when walking older dogs was supported. Handlers less frequently used communication and negative verbal cues when interacting with older dogs, showing that older dogs were generally more stable [85], which aligns with the lower pulling frequency observed in older dogs [43]. Handlers were more likely to use negative verbal cues when talking to level 2 dogs compared to level 3 dogs. A possible explanation may be that level 2 dogs were more often involved in interactions with humans and thus were more likely to get excited and needed to be stopped by handlers. Voice pitch was a key factor modulating the behaviour of younger dogs, and humans often communicate with them using a high-pitched voice [86]. In this study, dog age was not found to influence the tendency of humans to use a high-pitched voice for communication. One possible explanation is that most of the dogs in our study were older than 6 months of age. Therefore, these dogs were less likely to be viewed as puppies. Handlers verbally communicated with larger dogs and initiated physical contact more frequently, which may be because handlers could more easily interact and have physical contact with larger dogs due to the shorter physical distance between them.

4.4. Canine Demographics and Walking Experience

Handlers were less satisfied with the interaction and dogs were perceived as less obedient when dogs were younger [87,88], supporting the hypothesis that higher satisfaction would be found when the walk involved an older dog. The adolescent-phase disobedient behaviour has been reported in younger dogs, which corresponds with the peak age at which dogs are relinquished to shelters [88,89]. Interestingly, the reduced obedience of dogs observed in the adolescent phase is only found with respect to the carer who has developed attachment with the dog [88]. The development of disinhibited attachment has been reported in shelter dogs, which characterises quickly forming bonds to new humans after short interactions [90,91]. Future study is encouraged to explore the relationship between adolescent-phase disobedient behaviour and human-dog attachment in the animal shelter setting.

Compared to level 3 dogs, handlers reported a lower level of satisfaction and rated the dogs as less obedient and supportive when walking level 3+ dogs. However, there was no difference between level 3 and level 2 and 1 dogs, and no significant correlation was observed between the canine behavioural assessment and walking experience. In this study, volunteers were matched with dogs based on training experience and the behaviour of the dog due to safety and welfare concerns, and thus the exit questionnaire might fail to reflect the differences. In addition, the level of each dog was rated by RSPCA Qld staff during the daily walk, which included both walk inside and outside of the research area. To standardise the research, in this study, volunteers were asked to answer the exit questionnaire based on their experiences inside the research area. Differences between level 3 and level 2 and 1 dogs might be more detectable when considering the behavioural presentation of the dog outside of the research area, where more stimuli (e.g., other dogs and humans) are present. Another possibility may be that volunteers were generally more tolerant or preferred not to reveal their true thoughts in the questionnaire despite being told that the survey would be de-identified. In spite of this, the satisfaction score was negatively correlated with the mean leash tension created by the handler, supporting the hypothesis that handlers would be less satisfied with the interaction when they needed to pull the leash harder during the walk.

4.5. Limitations

In this study, there were difficulties in accurately identifying the underlying emotions and motivation of the dog. Similar to the cortisol level and heart rate variability, our leash tension meter may only be able to differentiate the relaxed and aroused states of the dog but cannot specify whether the arousal is due to excitement, anxiety or fear [81,92]. To precisely interpret the underlying emotion and motivation of the dog, a better video quality that records the face and the entire body of the dog, environment and the context of the interaction is needed. Other limitations for this study are described in [49].

5. Conclusions

This research explored human-dog interactions with two features. Different from many human-dog interaction studies with participants being owners and dogs being pets [28,56], this study was conducted in a shelter setting, where volunteers generally shared a short-term relationship and a weaker bond with shelter dogs. Additionally, since on-leash walking is an important activity for dogs in a shelter and postadoption, the other feature of this study is that volunteers interacted with dogs when on a leash.

RSPCA canine behavioural assessment may be useful in predicting the behaviour of shelter dogs when walking on-leash with volunteers. Dogs that were more relaxed during the assessment (e.g., when socialising with humans or being left alone in a new environment) were less reactive on the leash, with lower tension and pulling frequency. Additionally, socialised and relaxed dogs displayed more positive body languages, such as tail in a high position, gazing and exploring the environment, and humans showed fewer verbal cues and body languages during the walk with these dogs.

In addition, we found correlations between canine demographics and the behavioural interaction and human perception. Demographics included age, skull shape, body size and previous ownership history. Finally, the tension of the leash was related to human perception, with the walk perceived as less satisfactory when volunteers needed to pull the leash harder during the walk. This study may help to enhance volunteers' experiences when walking shelter dogs on a leash and improve canine welfare in shelters. Matching shelter dogs with potential adopters, considering the demographics of dogs and humans and canine behaviour to achieve a higher rate of successful adoption, may be another possibility [43,49,93–95].

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2615/11/1/26/s1, Table S1: Scoring of canine behavioural assessment. Details of each subtest are described in [19], Table S2: Generalised linear mixed model of the effect of canine demographics on leash tension and pulling frequency, Table S3: Generalised linear mixed model of the effect of canine demographics on canine behaviour, Table S4: Generalised linear mixed model of the effect of canine demographics on human verbal cue, Table S5: Generalised linear mixed model of the effect of canine demographics on human body language, Table S6: Generalised linear mixed model of the effect of canine demographics and leash tension caused by humans on volunteers' walking experience.

Author Contributions: Conceptualization, H.-Y.S., C.J.C.P. and M.B.A.P.; methodology, H.-Y.S., C.J.C.P., F.G. and M.B.A.P.; software, F.G. and H.-Y.S.; validation, H.-Y.S., F.G. and C.J.C.P.; formal analysis, H.-Y.S., F.G. and C.J.C.P.; investigation, H.-Y.S.; resources, M.B.A.P. and C.J.C.P.; data curation, H.-Y.S., F.G. and C.J.C.P.; writing—original draft preparation, H.-Y.S. and F.G.; writing—review and editing, H.-Y.S., C.J.C.P. and M.B.A.P.; visualization, H.-Y.S. and C.J.C.P.; supervision, C.J.C.P. and M.B.A.P.; project administration, H.-Y.S., C.J.C.P. and M.B.A.P.; funding acquisition, C.J.C.P. and H.-Y.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Higher Degree Research Fund of the University of Queensland and the RSPCA Fund donated by philanthropists.

Acknowledgments: We thank RSPCA, Qld for allowing us to conduct this research at the shelter. We also appreciate all dogs, volunteers and all RSPCA staff for playing important roles in this study. Finally, we acknowledge Solomon Woldeyohannes for the statistical advice.

Conflicts of Interest: Mandy B.A. Paterson is employed as the principal scientist by RSPCA, Qld. None of the authors receive any interest or financial support from people or organisations who can bias the research.

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animals

Article

Do Behaviour Assessments in a Shelter Predict the Behaviour of Dogs Post-Adoption?

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Received: 11 June 2020; Accepted: 16 July 2020; Published: 18 July 2020

Simple Summary: In shelters it is usual to conduct standardised behaviour assessments on all incoming dogs. The information gathered from the assessment is used to identify dogs that are suitable for adoption and assist in matching dogs with suitable adopters. We investigated the predictive value of the standardised behaviour assessment protocol currently used in an Australian shelter for dog behaviour post-adoption. A total of 123 dogs, aged 1–10 years and housed in an animal care shelter, were assessed before they were adopted. The new owners of the dogs took part in a post-adoption survey conducted 1 month after adoption, which explored the behaviour of their dog in its new home. Regression analyses identified that friendly/social, fear and anxiousness identified in the shelter assessment significantly predicted corresponding behaviours post-adoption. However, behaviour problems, such as aggression, food guarding and separation-related behaviours, were not reliably predicted by the standardised behaviour assessment. We recommend that dog behaviour assessments in shelters are used only in conjunction with other monitoring tools to assess behaviour over the whole shelter stay, thus facilitating increased safety/welfare standards for dogs, shelters and the wider community.

Abstract: In shelters it is usual to conduct standardised behaviour assessments on admitted dogs. The information gathered from the assessment is used to identify dogs that are suitable for adoption and assist in matching the dog with suitable adopters. These assessments are also used to guide behaviour modification programs for dogs that display some unwanted behaviours. For some dogs, the results may indicate that they are unsuitable either for re-training or for adoption. In these circumstances the dogs may be euthanised. We investigated the predictive value of a standardised behaviour assessment protocol currently used in an Australian shelter for dog behaviour post-adoption. A total of 123 dogs, aged 1–10 years and housed in an animal care shelter, were assessed before they were adopted. The new owners of the dogs took part in a post-adoption survey conducted 1 month after adoption, which explored the behaviour of their dog after adoption. Ordinal regression analyses identified that friendly/social, fear and anxiousness identified in the shelter assessment significantly predicted corresponding behaviours post-adoption. However, behaviour problems, such as aggression, food guarding and separation-related behaviours, were not reliably predicted by the standardised behaviour assessment. The results suggest that further research is required to improve the predictability of behaviour assessment protocols for more specific behaviour problems, including different categories of aggression and separation-related problems. We recommend that dog behaviour assessments in shelters are used only in conjunction with other monitoring tools to assess behaviour over the whole shelter stay, thus facilitating increased safety/welfare standards for dogs, shelters and the wider community.

Keywords: dog behaviour prediction; dog behaviour problems; dog behaviour assessment; canines; animal shelters; dog post-adoption behaviour

1. Introduction

In Australia, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) is a National, not-for-profit organisation that accepts approximately 46,000 dogs per year [1]. A 2014 study [2] found that these dogs, most of which were adult, were most commonly admitted after being collected by local council officers as strays (34%). Others were presented by members of the public as strays (24%), owner surrenders (19%), or euthanasia requests (4%), with a small number being brought in by Humane Officers, employees of the RSPCA tasked with rescuing animals from situations where their welfare may be compromised (6%). Other studies have shown that relinquishment reasons are usually human-related (unwanted, changed circumstances, financial, owner's health, household problems) but medical issues and behavioural problems also lead people to relinquish their dog [3–10]. In the Australian study [2] most dogs were either reclaimed (32%) or adopted (43%), with 14% euthanised. Reasons for euthanasia were dog behaviour (53%), dog health (23%), and owner requested (20%). If euthanased for behavioural reasons, it is likely that the dog displayed severe aggression, fearfulness and/or escaping behaviour.

Many shelters attempt to identify behavioural problems by continually monitoring behaviour and by formal behaviour assessments (BAs) while dogs are in care [11–13]. The behaviour assessments aim to identify behaviours that may cause problems in the dog's future home, and to give an overview of the dog for potential adopters [14]. However, their ability to predict future behaviour or behavioural issues is questioned [15]. There is a concern that dogs that appear aggressive during a BA are being unnecessarily euthanased because they would not necessarily be aggressive in a home environment, and that non-aggressive dogs may be adopted out only to become aggressive at a later stage in the new home.

Life in a shelter is stressful and traumatic for dogs due to sensory overstimulation, social isolation, change/loss of control of daily routines and the novelty of the environment [14,16,17]. Stress has wide-ranging impacts, including on cognitive ability, behaviour and the dogs' emotional state [18–20]. Therefore, a standardised BA conducted in shelters may not provide an accurate representation of the normal behaviour of the dog in a more stable and settled home environment.

Research conducted by Mornement et al. [14] in Australia compared the results of a Behaviour Assessment for Re-homing, K9's (B.A.R.K.), administered in shelters, with results of a post-adoption survey. They reported that the only predictable outcomes were friendliness and fear-related behaviours. However, other behaviours, in particular aggression and food guarding, are rare post-adoption; Mohan-Gibbons [21] found that only six out of 96 adopted dogs were reported to display at least one incident of food guarding in the first 3 weeks, and at 3 months the adopters reported no food guarding behaviours at all. There was no evidence in this study, or a subsequent study [22], that food guarding increased return of the dogs to the shelter. In addition, injuries to staff, volunteers and adopters were rare and did not change if the food guarding test was omitted from the assessment.

'Time alone' tests have been used to identify dogs with separation-related behaviours [23]. Separation causes dogs to exhibit anxiety when away from owners or people in general; it is expressed as vocalisation, destruction of their environment, excretion, drooling, attempting to escape and depression-like responses [24,25]. Most shelters include a time alone test in their BA, during which the dog is placed alone in an unfamiliar room and observed for up to 10 min [23]. Dogs with separation-related anxiety spend the majority of the time vocalising, orienting to escape, panting and engaging in destructive behaviour.

Despite the current controversy about the use of BAs in shelters to gain an understanding of a dog's behaviour and to identify any major or minor behavioural problems, we consider that assessments still have a role to play [26]. They can be used to identify stable behaviours. To further our understanding of how well BAs can predict dog behaviour in adoptees' homes, we aimed to identify whether the standard BA protocol conducted at a Queensland shelter 5 days after admission predicted behaviour in adopters' home environment, as assessed 1 month post-adoption.

2. Materials and Methods

2.1. Ethical Approval

This study was conducted with the approval of The University of Queensland Human Ethics Committee (2017000044). The RSPCA Animal Welfare and Ethics committee approved the use of data from the RSPCA Queensland survey of adoptees and behaviour assessment data.

2.2. Subjects

The dogs used in the study were housed at the RSPCA Queensland Animal Shelter at Wacol. Before inclusion in the experiment, dogs were assessed by a veterinarian and identified as having no apparent medical problems. Upon admission to the RSPCA, behaviour profiles were completed by the owners for owner-surrendered dogs (these were not available for stray dogs). Each dog was then evaluated by an RSPCA behaviour assessor using the RSPCA Qld. behaviour assessment 5 days after admission [13]. Data were collected from 955 dogs. Of the 955 owners that adopted these dogs, 125 were successfully contacted later and completed a post-adoption survey (14% response rate). Two owners initially agreed to participate in the study when contacted but later declined to take part.

2.3. Behaviour Assessment

A standardised behavioural assessment (Supplementary Materials) was conducted on all dogs during their stay at RSPCA Queensland by two staff (one Handler and one Observer/Rater) responsible for evaluating the dogs' suitability for re-homing. These assessments were not able to be repeated due to staffing changes, therefore intra-rater and inter-rater reliability assessments were not possible. The assessments monitored the following behaviours: room exploration, behaviour when on a leash, sociability, tolerance, play behaviour with toys, tag (run and freeze), possessive behaviours, toddler and stranger interaction, time alone and social interactions with other dogs (RSPCA, 2012) [13]. The assessment comprised 11 different tests performed over a 15 min period, 10 have previously been described in detail [13]. The additional test 'Response to a fake cat" is outlined in Supplementary Materials. The equipment used followed RSPCA Queensland's protocol and included a 1.8 m leash, tennis ball, squeaky toy, rope, plastic hand on an extended pole, bowl, raw hide or bone, and combination of wet and dry dog food. At the conclusion of the behavioural assessment, animals were either deemed suitable for re-homing (n = 772), enrolled in a behaviour modification program (n = 133) or scheduled for euthanasia (n = 50). Decisions for behaviour modification and/or euthanasia were made by a professional review panel.

2.4. Behaviour Scoring by RSPCA Assessors

In each test, one RSPCA assessor rated the behaviour of the dog using binary occurrence of behavioural states (present or absent), except for the resource guarding test, which relied on a score by the assessor on an 8 point scale (Table 1). An overall score using the 11 tests was determined. All behaviours were assessed in each test using binary scoring (present or not) (Table 2)

Table 1. Resource guarding scoring system aimed at identifying possessive aggression by the dogs in defence of food.

Possession Level	Description
Level 1	Stops eating, wags tail loosely, and sniffs hand and looks to handler with soft eyes and relaxed body. Body language indicates no distancing behaviours.
Level 2	Continues eating, soft eyes, wags tail loosely, and body language indicates no distancing behaviours; typically a relaxed body stance/carriage.
Level 3	Continues eating but at a faster rate of intake. Body is slightly tense, particularly on human approaching the dog; tail wagging with an increased speed, especially on interaction with the dog and/or the food/treat. The dog blocks access to the food with their body (head and shoulder over the food and treat).
Level 4	The dog's discomfort and behaviour starts to escalate. The dog glares, lifts its lip in a snarl, and/or produces a low growl. Increases eating speed, or with a treat the dog will whip its head away in an attempt to move it away from handler.
Level 5	Dog will carry the food item under a chair, bed, or into its crate, then growl on approach. If it cannot pick the food/treat up, it pushes the food bowl farther away. Dog freezes (stops eating or chewing), with whale eyes (exhibiting sclera) or direct stare, with or without lifting the lip in a snarl or other type of growl.
Level 6	Dog snaps but with no contact with fake hand. Level 5 behaviour usually continued but dogs move through the behaviours rapidly.
Level 7	Dog's protectiveness increases with one or more rapid bites that touch the fake hand with quick and hard contact.
Level 8	Dog freezes with whale eyes or direct eye contact and biting aimed at the intruder even if they are at the perimeter of the room. At this level, it may be too dangerous to step into the perimeter to determine if the dog will bite or not.

Table 2. Behaviours evaluated in the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Queensland canine behaviour assessment.

Behaviours	Definition
Play	Interacting with toys in social manner, may interact with handlers.
Friendly	May jump up on the person/dog licks person, dog nudges hand; play bow.
Social	Approaches and looks at assessor; stays with assessor making regular soft eye contact; low tail wagging, body relaxed, when assessor interacts may lower body.
Fearful	Cowers; runs away or avoids interaction, may tremble; tail tucked tightly, attempts to hide; at end of taut leash; mouth closed or panting excessively.
Anxious	Inability to settle and relax, distressed vocalisation, wide eyes, dilated pupils, excessive panting and licking, yawning and proximity seeking behaviour.
Arousal	Medium to hard mouthing of person; jump up and grab person's clothing or body part; may mount person; inability to calm down; takes little to escalate the arousal levels.
Predatory behaviour	Sequence of behaviours that are associated with the catching and killing of another 'animal' for consumption, in this case a fake cat.
Reorienting	Changes direction away from stimulus.
Avoiding stimulus	Moves away from the stimulus.
Unresponsive	No behaviours change due to stimulus.
Aggression	Growls; shows teeth; snaps; directed stare; dilated pupils; attacks; bites
Displacement	The transfer of feelings or behaviour from their original object to a person or thing. Displacement behaviours include self-grooming, touching, stretching, yawning, displayed when an animal has a conflict between two motivations, such as the desire to approach an object while at the same time being fearful of that object.
Attracted to stimulus	Moving all the way to the end of the lead towards a stimulus until it is in full tension.
Appeasement	Individual attempts through appeasement displays to avoid injury by a dominant dog or human.
Reactive	Dogs respond with excessive reactions to a stimulus.
Separation related behaviours	Behaviours that are associated with being left alone; behaviours can include panting, pacing, excessive vocalisation, scratching at doors, excessive jumping, and damage.
Possessive behaviour	Aggression whilst guarding things (food bowls, rawhides, stolen, or found items, toys).

2.5. Post-Adoption Phone Interview

Participants were asked when adopting a dog if they would agree to be included in a post-adoption phone survey. The survey was conducted by RSPCA customer service staff 1 month after adoption of the dog. The phone survey asked about the dog's behaviour in the home environment and in different everyday situations (Supplementary Materials). It took approximately 10 min to complete and consisted of 36 multi-choice questions with the option to add additional information.

Participants rated the frequency of socialisation to owners and children, and behaviour with run and freeze play, an unfamiliar person, unfamiliar children, an existing dog, an unfamiliar dog, and interactions with cats, on a 5 point scale (1: moves towards you in a playful manner, 2: moves, leans, or looks away, 3: no response, 4: moves or leans away in a manner that concerns you, 5: moves towards you in a way that concerns you).

2.6. Statistical Analysis

Statistical analysis was conducted using Minitab 18. Behaviour data were first screened for errors and then transposed into percentage of occurrence in tests for descriptive analyses. Ordinal logistic regression analysis using a logit model was used to identify behaviours in the assessment that best predicted dog behaviour post-adoption.

3. Results

3.1. Descriptive Details

The sample included 123 companion dogs (males: 61, females: 62) over the age of 1 year and under 10 years. The sources for the 125 dogs were as follows: owner surrender (45%); transfer (17%); RSPCA officer intake (13%); stray (12%); return (6%); lost (5%); emergency ambulance intake (3%); and pound (1%). The majority of dogs in the study were mixed breeds (45%). Median time of stay in shelter was 55.5 days (range 3–114 days).

3.2. Behaviour Assessment (Table 3)

The number of dogs displaying the different behaviours during each test is presented in Table 3.

In Test 1, "Exploring the Room", in the Exploration and Upon Call phases, dogs had a high occurrence of Friendly behaviour, with low occurrences of Anxious, Fear, and Arousal behaviours (Table 3). In Test 2, "Tolerance to Handling", in all components the majority of dogs displayed friendly interactions with the assessor, with increases in Anxious behaviours in Stroke and Foot Sensitivity (Table 3). In Test 3, "Startle Response", there was higher Avoidance, Fear, and Arousal in the Startle component, compared to the Recovery period, with a high occurrence of dogs displaying Friendly behaviours (Table 3). Recovery times varied between dogs, with 68% recovering within 5 s, 22% within 6–10 s and 3% taking over 10 s (7% of dogs did not exhibit as startle response).

In Test 4, "Toy Interactions", there was a high occurrence of Play in all components of the test, with low instances of Fear and Anxious behaviour (Table 3). The component with the greatest number of dogs exhibiting Arousal was Rope interactions. In Test 5, "Response to Unusual/Predictable Stimulus", there were high occurrences of Friendly behaviour in the Run and Freeze components but low levels of Anxious, Arousal and Fear behaviours (Table 3). In Test 6 (data not shown), "Resource Guarding", dogs displayed a high occurrence of levels between 2 and 3 with wet (68.2%) and dry food (80%). There were low occurrences of levels 4–6 with bone (9.9%) or pig's ear (7.43%).

Table 3. Number of dogs (and %) exhibiting behaviour's in the various test components in the behavioural assessment of shelter dogs (n = 123).

Test	Component	Behaviour															
		Friendly	Anxious	Fearful	Arousal	Appeasement	Aggression	Avoided	No Response	Displacement	Reorientated Away	Predation	Attraction to Stimulus	Reactive	Play	Possession	Separation Related Behaviours
1	**Exploring the room** Exploration	111 (85)	12 (9)	3 (2)	1 (1)	0	0	0	0	0	0	0	0	0	0	0	0
	Upon Call	91 (70)	13 (10)	23 (18)	3 (2)	0	0	0	0	0	0	0	0	0	0	0	0
2	**Tolerance to Handling** Collar	73 (58)	19 (15)	10 (7)	2 (1)	21 (17)	0	0	0	0	0	0	0	0	0	0	0
	Stroke	70 (56.5)	20 (15.6)	7 (5.6)	6 (5)	21 (16.7)	1 (0.6)	0	0	0	0	0	0	0	0	0	0
	Foot	68 (54.8)	15 (11.63)	6 (5.35)	12 (9.23)	23 (18.49)	1 (0.5)	0	0	0	0	0	0	0	0	0	0
3	**Startle response** Startle	29 (24)	13 (10)	24 (19)	24 (19)	1 (1)	0	34 (27)	0	0	0	0	0	0	0	0	0
	Recovery	102 (82)	14 (11)	9 (7)	0	0	0	0	0	0	0	0	0	0	0	0	0
4	**Toy interactions** Tennis ball	0	0	5 (4)	11 (8.5)	0	0	0	16 (13)	0	0	0	15 (12)	0	75 (60)	3 (2)	0
	Squeaky toy	0	0	3 (2.2)	10 (7.7)	0	0	0	14 (11.3)	0	0	0	28 (22.5)	0	68 (54.5)	3 (2)	0
	Rope	0	9 (7.5)	9 (7)	19 (15.1)	0	0	0	4 (3)	0	0	0	8 (6)	0	75 (60)	0	0
5	**Response to unusual/unpredictable stimulus** Run	87 (69.35)	16 (12.9)	12 (10)	0	0	0	0	3 (2.3)	1 (1)	0	0	0	0	0	0	0
	Freeze	73 (58.25)	15 (11.7)	1 (1)	18 (14)	0	0	0	3 (2.6)	6 (4.5)	17 (14.3)	0	0	0	0	0	0
7	**Stranger interaction** Entry	105 (84)	9 (7.7)	3 (2)	0	0	0	0	5 (3.6)	3 (2.4)	0	0	0	0	0	0	0
	Approach	98 (78.65)	7 (5.74)	8 (6)	1 (1)	0	0	0	1 (1)	9 (7.3)	10 (14.3)	0	0	0	0	0	0
	Leaving	68 (54)	0	0	0	0	1 (0.84)	0	0	15 (12.1)	41 (33.06)	0	0	0	0	0	0
8	**Fake toddler interaction** Approach	93 (74)	8 (6.34)	9 (6.9)	4 (3.54)	0	1 (1)	0	0	11 (8.7)	0	0	0	0	0	0	0
	Leaving	71 (56.41)	0	0	0	0	1 (1)	0	0	9 (6.84)	43 (34.19)	0	0	0	0	0	0
9	**Fake Cat** Approach	101 (81)	0	7 (5.36)	2 (1.7)	0	2 (1.7)	0	5 (4)	5 (4.34)	0	2 (1.7)	0	0	0	0	0
10	**Time alone** 2 min	0	22 (18)	0	0	0	0	0	39 (31.4)	0	0	0	0	0	0	0	64 (51)
11	**Behaviour with another Dog** Walking	100 (79.84)	0	0	0	0	3 (2.48)	0	0	7 (5.52)	0	0	0	15 (12.16)	0	0	0
	Circling	88 (70.07)	6 (4.71)	2 (1.39)	8 (6.5)	0	9 (7.12)	0	2 (1.39)	4 (3)	0	0	7 (5.81)	0	0	0	0
	Nose-Nose	82 (65.93)	5 (3.9)	8 (6.45)	8 (6.23)	0	4 (3.15)	0	0	10 (14.33)	0	0	8 (6.23)	0	0	0	0

Test 6 resource guarding was not included in the table due to the different method of scoring of the behaviour.

In Tests 7 and 8 "Stranger Interactions" and "Toddler Interactions", there were high occurrences of dogs displaying Friendly behaviour, with under 10% displaying Anxious or Displacement behaviours, Fear, or No Response towards the stranger (Table 3). Furthermore, there was only one dog that displayed Aggressive behaviour in each test. In Test 9, "Fake Cat", there were high occurrences of Friendly behaviour towards the fake cat, with minimal dogs displaying other behaviours (Table 3). In Time Alone (Test 10), 51% of dogs displayed Separation-Related behaviours, 31.4% displayed no problematic behaviours and 18% displayed Anxious behaviours.

Finally, in Test 11, "Behaviour with Another Dog", Friendly behaviours had the highest occurrence in dogs in all components of the test, with low levels of all other behaviours (Table 3). One interesting finding was the higher instance of Reactivity towards the opposing dog during the Walking component, which did not occur in the Circling or Nose to Nose components (Table 3).

3.3. Post-Adoption Behaviour

Only three participants no longer had the dog they had adopted. The remaining 120 participants still had their dog. With regard to the dogs' living arrangements, 49% were indoor/outdoor dogs, 29% mainly indoors and 23% mainly outdoors.

Participants were asked how the dog responded to different situations (Table 4) with most owners outlining that the dog "moves towards the stimulus in a playful manner" and a low occurrence of the opposite response. In situations related to unfamiliar visitors and unfamiliar dogs, there were higher levels of "moves, leans or looks away", "moves or leans away in a manner that concerns you", and "moves towards in a way that concerns you" (Table 4).

Table 4. The percentage (%) of dogs (n = 120) displaying specific behaviours post-adoption.

Question	Moves towards in a Playful Manner (1)	Moves, Leans or Looks Away (2)	No Response (3)	Moves or Leans away in a Manner that Concerns you (4)	Moves towards in a Way that Concerns You (5)
Attention (Q5)	91.87	0.82	3.25	0.82	3.25
Children (Q7)	88.73	1.41	2.82	1.41	5.63
Run and freeze (Q8)	91.89	1.00	4.50	1.00	2.70
Unfamiliar visitors (Q9)	73.17	9.76	4.88	6.50	5.69
Unfamiliar children (Q10)	85.58	3.85	5.77	1.92	2.88
Existing dog (Q14)	84.62	5.13	0.00	2.56	7.69
Unfamiliar dog (Q16)	60.16	6.50	11.38	2.44	7.32

In terms of interactions with cats, 93 (74%) participants did not answer, with 32 participants answering that their dogs interact with cats with 19% of dogs moving towards them in a playful/friendly manner, and under 3% displaying other behaviours. With respect to resource guarding, participants were asked whether they were concerned about their dog's behaviour around food, treats, toys, and human food; over 90% reporting that there were no issues and under 10% saying there were issues (Table 5).

Table 5. The percentage (%) of dogs (n = 120) displaying possessive behaviour post-adoption.

Concern about Behaviour around Food, Treats, Toys and Human Food	No	Yes
Dog food	90.8	9.2
Treats	95.0	5.0
Toys	95.8	4.2
Human food	93.3	6.7

Participants were asked how their dog reacts to a loud noise or something else startling the dog. 37% ignored the question, 25% reported a mild startle response from their dog, 9% of dogs ran and hid, and 4% displayed a pronounced startle response. With dogs that were startled, participants were asked how long it took them to recover; 45% recovered immediately, 29% recovered within a few seconds, 15% recovered between 5 and 10 s, and 11% took longer than 10 s, avoided the situation and did not settle.

Participants were asked if they had ever left the dog alone, with 114 saying yes, and only nine saying no. Of the 114 participants that responded yes, 59% of dogs were left outside, 24% were left inside, 14% were allowed a combination of inside and outside, and 3% were left in a laundry or garage. Time spent alone ranged from 5 to 12 h (55%), 1–4 h (36%) and less than an hour (9%). Participants were asked whether their dog's behaviour changed when they were preparing to leave, with 72% reporting no change and 28% some changes in behaviour. Participants were asked if any behaviours were of concern, with 80% saying no, and 21% saying yes.

3.4. Standardised Assessment Scores Verses Owner Surveys

Ordinal regression analyses were conducted to determine whether scores derived from the behaviour scores in assessment tests could predict behavioural traits in the new home using reported behaviour in the home environment as the dependent variable. Questions from the survey that called for a response along a 5-point scale were related to relevant tests in the assessment that measured interactions with the handler, children, strangers and dogs, as well as the startle response, response to usual stimulus, food items and time alone situations. The regression analyses found that friendly/social behaviours (scored in tests: Interaction with Assessor in exploration of room, Response to unusual/unpredictable stimulus, Stranger interactions, Behaviour with another dog) significantly predicted 'playful/friendly manner' behaviour post-adoption in interactions with owners, children, strangers, existing dogs and unfamiliar dogs (Table 6). Anxious behaviour (scored in the tests: Assessor in exploration of room, Response to unusual/unpredictable stimulus, Fake toddler doll and Behaviour with another dog) significantly predicted 'Moving towards owner/children/stranger in a way that concerns you' behaviour post-adoption with interactions with owners, unfamiliar child, running and freezing, and unfamiliar dog (Table 6). Fear (scored in the tests: Assessor in exploration of room, and Fake toddler doll) significantly predicted 'Moves or leans away in a manner that concerns you' post-adoption with interactions with owners, and children (Table 6). The remaining 13 post-adoption behaviours were not predicted by the standardised behaviour assessment protocol conducted at the shelter.

Table 6. Significant or trend level ($p < 0.10$) relationships between behaviours scored from the shelter behaviour assessment and responses in the post-adoption survey, analysed by ordinal logistic regression.

Behaviour	Test	Proportion Showing Behaviour in each Survey Category	Post Adoption	Coef	SE Coef	Z	*p*	Ratio	Lower	Upper
Friendly/social	1	0.91	Owners	2.50	1.45	1.73	0.05	12.21	0.71	208.88
	8	0.88	Children	2.68	1.20	2.23	0.02	14.65	1.39	154.41
	7	0.73	Stranger	1.06	0.55	1.94	0.05	2.89	0.99	8.46
	11	0.84	Existing dog	1.23	0.63	1.94	0.05	3.42	0.99	11.83
	11	0.60	Unfamiliar dog	1.42	0.63	2.27	0.02	4.14	1.21	14.16
Anxious	1	0.03	Owners	−1.43	0.79	−1.80	0.07	0.24	0.05	1.14
	11	0.07	Unfamiliar dog	−1.40	0.53	−2.62	0.01	0.25	0.09	0.70
	8	0.03	Unfamiliar child	2.38	1.02	2.34	0.02	10.83	1.47	79.46
	5	0.03	Run and freeze	−1.40	0.53	−2.62	0.00	0.25	0.09	0.70
Fearful	1	0.01	Owners	2.20	1.10	2.00	0.04	9.00	1.05	77.36
	8	0.01	Children	1.50	0.81	1.86	0.05	4.49	0.92	21.85

4. Discussion

The aim of this paper was to evaluate how well the standardised behaviour assessment (BA) protocol currently used in a Queensland RSPCA shelter predicted post-adoption behaviours. In general, the ability of the standardised BA protocol to predict specific behaviours post-adoption was only somewhat effective. It appears, then, that the standardised BA may, as previous authors have outlined [16], be useful as a tool for providing an overall measure of dog behaviour, particularly with respect to friendly, fearful, and anxious behaviour, but that it requires supplementation with other sources of information. However, our study was unable to adequately assess whether behavioural problems, specifically the identification of different categories of aggression, possessive behaviour (resource guarding), or separation anxiety, can be predicted from shelter assessments, since dogs displaying these behaviours were not rehomed.

There are several possible explanations for why the assessment was not more strongly predictive of our outcome measures. One constraint is that we cannot predict how an owner's behaviour or personality, and other animals/individuals in the household, can influence/affect the dog's behaviour post-adoption. Such effects may be substantial. Due to this, it may not be realistic to expect to be able to predict with accuracy behaviour over time.

A further explanation is that the standardised protocol may be inadequate as a tool to assess complex canine behaviours and behavioural problems either because of the structure of the assessment and/or its administration or due to the complex nature of such behavioural problems. We argue that the instrument is unlikely to be inadequately designed as it draws upon countless research studies and has been used and modified over many years [14,27–30]. The administration is also unlikely to have been inadequate, due to the standardised nature of the tests. Staff were trained and evaluated in the shelter, with the majority of the dogs in the large sample being assessed by the same individuals.

Another possible explanation is that due to the nature of canine behaviour, only some aspects of behaviour are stable [31,32]. Some aspects of canine behaviour may not be predictive in a single test, including aggression or other behaviour problems. Consistent with this idea was the number of new owners who reported their dog moving towards an individual in a way that concerned them, even though these dogs did not show these behaviours in the shelter assessment, or were not identified by shelter staff as displaying aggressive tendencies outside of the assessment. Dogs that displayed aggressive tendencies in the BA, or at other times during their stay at the shelter in the Queensland facility, were reviewed by a consultant for further testing. Such dogs were either then enrolled in a behaviour modification program or deemed to be unsuitable for adoption. Indeed, this study is similar to other studies in the area of canine behaviour assessment in shelters [12,21,22], where only dogs that did not show signs of aggression were made available for adoption and therefore included in the sample.

This suggests that there is a high possibility of a number of false negatives in the initial BA, which therefore is not offering a valid index of aggression. As seen in numerous studies, to reliably identify aggression and diagnose its causation is difficult, due to its infrequency and the nature of behavioural problems. Canine aggression is complex, and may be context specific [33]. The belief that one can assess a dog and diagnose it as aggressive is incorrect and should not be done. A specialist trained to identify and classify canine aggression would be in a better position to have a comprehensive understanding of physiology, behaviour and neurology, thus allowing a more nuanced diagnosis to be drawn [34]. Even in an assessment used primarily for identification of aggression, for example, the Dutch Socially Acceptable Behaviour (SAB) test, a portion of aggressive dogs remained undetected and the test was substandard for the assessment of types of aggression unrelated to fear [35]. This leads to the idea that fearful and anxious behaviours may be more stable and easier to detect than forms of aggression that can be motivated by numerous factors [17].

The final possibility is that canine behaviour may be predictable and the standard BA protocol used may be adequate at measuring certain categories of common/prominent canine behaviours (Friendly, Fearful, Arousal, Anxious), due to the common occurrence of these behaviour in everyday populations. However, due to the administration of the assessment after 5 days in the new environment, the tests may produce deceptive results. While many shelters maintain the highest standards of animal welfare, dogs still suffer from social isolation, abnormal sleep patterns, auditory pollution, olfactory overstimulation, and emotional stress, especially if individuals have no prior experience in shelters and do not habituate using positive coping mechanisms. The stressors that are inherent in any shelter may force some dogs to employ negative coping mechanisms (avoidance, inhibition or appeasement) as an outlet rather than displaying aggression [36,37]. This may especially be the case after surrender and over the first few days of entering the shelter, with some dogs likely to experience acute stress and social isolation [17]. Research into this area has found that shelter dogs showed more aggression when tested 2 weeks after being admitted to a shelter in comparison to 1–2 days after surrender [38]. Furthermore, only a few studies have studied the relationship of aggression with welfare standards for dogs [17,20] and whether the behaviour is due to environment stressors. Evidence in the literature suggests that stress can have an effect on cognitive function, negative emotional state and behaviour [18–20]. This implies that standardised canine BAs, timed incorrectly and used to make decisions about dogs (rehomed, trained or euthanised), may give false information to shelter staff.

Consistent with this possibility, recent studies into the test used to identify food resource guarding found the prevalence of issues post-adoption were low and that removal of the test did not increase the likelihood of food guarding in the new home [21,22]. The reason for this result can be identified in the complex aetiology behind food resource guarding. It is defined as the use of avoidance, threatening or aggressive behaviours by a dog to retain control of food or non-food items in the presence of a person or other animal [39]. It is not surprising that many dogs are so labelled in a shelter environment, due to the high occurrence of acute stress from sensory overload causing dogs to feel threatened and in turn aggressive. However, outside of the shelter environment, in a non-threatening and predictable environment, this reaction decreases. In addition, other types of aggression, such as territorial and maternal, remain very difficult to assess in shelters [33,40].

We advocate that shelters must look for a new approach that allows an improved ability to identify behaviour problems in a more stable environment. One such solution currently implemented in RSPCA Queensland shelters is the use of a foster care system, in which dogs that are unable to cope in the shelter are housed with foster carers until they are able to be adopted. This solution allows dogs to live in a stable environment with minimal exposure to stressors that may otherwise lead to the deterioration of the dog's behaviour thus leading to behaviour problems. Furthermore, it allows shelters to house more dogs able to cope in the shelter environment, as well as individuals requiring behaviour modification and further testing of behaviour problems. In addition, RSPCA Queensland uses a qualified behaviourist to help to understand dogs that are identified in the behaviour assessment as having behavioural issues. The consultant conducts further tests to better identify the behavioural problems and implement behaviour modification programs with the use of qualified dog trainers. The dogs are constantly reviewed and evaluated to monitor progress over time.

However, implementing these solutions requires resources that most shelters do not have. Most shelters have financial, time and staff constraints that hinder them utilising such techniques. The authors understand that no one BA protocol has the ability to accurately predict every future behaviour, but these assessments can be used as one tool in conjunction with continual monitoring of behaviour and health of dogs in shelters, to gain an overview of the dog's behaviour and identify dogs that require further testing or behaviour modification. Additionally, BAs can be used as monitoring tools to identify dogs not coping in the novel shelter environment. This, in conjunction with surrender information, veterinary monitoring and evaluations, in-kennel scoring from staff and volunteers, and behaviour modification should help develop a better system for shelters. To achieve this, continuous improvement and studies into dog behaviour in shelters are required.

5. Conclusions

Findings from this study suggest that a standardised behaviour assessment protocol used at an Australia shelter is a useful tool to predict some behaviours, mainly, friendly, fearful, arousal and anxious behaviours. However, in the predictability of behaviour problems, such as different categories of aggression or separation anxiety, it appears largely ineffective. This may be a result of the assessments being conducted in a highly stressful/novel environment where dogs experience many stressors in addition to lack of a human–animal bond, and then trying to use that information to predict home behaviour in a stable environment where supportive social bonds have formed. A thorough review of the protocol is recommended to identify any possible improvements, and care should be taken if the BA is the only tool used to identify a dog's adoption suitability. However, using the BA as one tool in a toolbox of many others, including pre-surrender information, veterinary clinical assessments, monitoring in kennel and responses to training, may provide a more comprehensive picture of behaviour. Behaviour is multifactorial, requiring an in-depth understanding of multiple neurological and physiological processes. Therefore, continuous research and training in shelters together with ongoing support may help gain a better understanding of canine behaviour.

Supplementary Materials: Supplementary Materials are available online at http://www.mdpi.com/2076-2615/10/7/1225/s1.

Author Contributions: L.C., M.B.A.P., P.B., G.P., and C.C.J.P. conceived the project. L.C. drafted the paper and all authors had input into modifying it into the present format. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors acknowledge the assistance of RSPCA Queensland.

Conflicts of Interest: Mandy Paterson declares that she works for the RSCPA Qld. Liam Clay declares that RSPCA Qld funded his studentship. Apart from this, no other author has any conflict.

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Characteristics and Adoption Success of Shelter Dogs Assessed as Resource Guarders

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Received: 16 October 2019; Accepted: 14 November 2019; Published: 17 November 2019

Simple Summary: Dogs that aggressively guard resources, such as food, toys, and sleeping sites, can pose risk to people unfamiliar with canine communication. Such dogs also present challenges to animal shelters, which typically screen for food-related guarding during behavioral evaluations. Some shelters euthanize dogs that aggressively guard food, whereas others restrict adoptions. However, few studies have examined the characteristics and adoption success of dogs that guard food in shelters. I analyzed demographic data and adoption success of dogs assessed as resource guarders at a shelter in New York (NY) over a nearly five-year period. Fifteen percent of the dog population was identified as resource guarders during shelter behavioral evaluations. Resource guarding was more common in adults and seniors than in juveniles, and it was more common in small and large dogs than medium-sized dogs. While spayed females were more likely than intact females to guard food, neutered males and intact males did not differ in their propensity to guard food. Dogs that showed severe guarding were more likely to be returned by adopters, but almost all were successfully re-adopted. These findings provide a detailed description of food guarders in a shelter dog population and show that most such dogs were successfully re-homed.

Abstract: Some domestic dogs aggressively guard resources. Canine resource guarding impacts public health through dog bites and affects dog welfare through adoption and euthanasia policies at animal shelters. However, little is known about the demographic characteristics and adoption success of dogs assessed as resource guarders during shelter behavioral evaluations. I reviewed nearly five years of records from a New York (NY) SPCA and categorized 1016 dogs by sex; age; size; reproductive status; and resource guarding. I then examined how these characteristics influenced the returns of dogs by adopters. The prevalence of resource guarding in this shelter dog population was 15%. Resource guarding was more common in adult and senior dogs than in juvenile dogs; and it was more common in small and large dogs than medium-sized dogs. Spayed females were more likely than intact females to guard food; neutered males and intact males did not differ in their likelihood of food guarding. Most dogs identified as resource guarders showed mild to moderate guarding. Severe guarders were more likely to be returned by adopters; although almost all were eventually re-adopted and not returned to the shelter. Data presented here provide the most comprehensive description of resource guarders in a shelter dog population and show the successful re-homing of most.

Keywords: dog; food aggression; food guarding; resource guarding; shelter; behavior; adoption; return rate

1. Introduction

Some domestic dogs are possessive of resources such as food, toys, and sleeping sites, and they display threatening or aggressive behavior when a person approaches or attempts to take control of the resource. Such resource guarding occurs in homes and in animal shelters. One survey of animal shelters in the United States found that most shelters test for food guarding as part of their canine behavioral evaluations, and about half do not make available for adoption dogs assessed as food aggressive [1]. Shelters that make food guarding dogs available for adoption often place restrictions on who can adopt them (e.g., experienced dog owners with no young children in the household), which can prolong the time these dogs remain in shelters [2,3]. Thus, canine resource guarding can impact not only public health through dog bites, which are the most extreme form of guarding behavior [4], but also dog welfare via shelter policies on adoption and euthanasia [1–3]. The need for informed re-evaluation of shelter policies whereby all dogs classified as food aggressive are euthanized is especially critical given evidence that dogs assessed as food aggressive in a shelter do not necessarily guard food in their adoptive home [1,3]. In addition to these findings specific to food aggression and the predictive utility of behavioral evaluations [1,3], other research has more generally revealed the inadequacies of behavioral evaluations [5–7].

Few studies have examined characteristics of dogs that guard food. Most such studies have been based on owner reports of food guarding by dogs in the home [4,8,9] rather than observations of dogs during behavioral evaluations at shelters; one study used both shelter evaluations and reports from adopters [3]. For studies based on owner reports, two found that mixed breed dogs were more likely than purebred dogs to guard food [4,8]. Owner reports also identified increasing age of dog at acquisition as a predictor of food guarding [8]. A dog's body size, as estimated by height at withers, was found to be negatively correlated with owner-directed aggression, a category that included resource guarding [9]. Conflicting results have been obtained regarding the influence of the sex of dogs on the likelihood of resource guarding. One study, based on owner reports, indicated that males were more likely than females to guard resources, and this was particularly true for neutered males [4]. In contrast, Marder et al. [3] found no sex difference in incidence of food aggression based on either shelter behavioral evaluations or subsequent reports by adopters. To date, no study has examined multiple demographic characteristics of dogs assessed as displaying food aggression during behavioral evaluations at shelters; current information is limited to one study that examined the influence of a single characteristic, the sex of shelter dogs, on the likelihood of food guarding during behavioral testing [3]. Understanding the characteristics associated with the expression of food guarding could serve as the basis for future research on the causation of guarding behavior [4]. Additionally, given that some shelters do not behaviorally evaluate all dogs made available for adoption [2], information on additional characteristics that might be associated with food aggression, such as age, reproductive status, and body size, could be useful to staff making decisions concerning which dogs to evaluate.

Two studies have examined the adoption success of dogs assessed as food aggressive in shelters. Mohan-Gibbons et al. [1] identified 96 food aggressive dogs at one shelter, placed them on a behavior modification program, and contacted their adopters three times in the months following adoption (adopters were asked to continue the behavior modification program that had begun in the shelter). Marder et al. [3] followed 97 shelter dogs, some of which were food aggressive and others not, and contacted adopters at least three months after adoption. Both studies found that dogs assessed as food aggressive during shelter behavioral evaluations did not necessarily guard food in their new homes, although the percentages of adopted dogs that continued food guarding in the home varied considerably, ranging from less than 10% [1] to 55% [3]. Results from both studies indicated that even if dogs displayed food aggression in the home, adopters did not consider the behavior to be a major challenge [1,3]. Mohan-Gibbons et al. [1] also found that the return rate for dogs assessed as food aggressive at their study shelter was lower than that for dogs assessed as not food aggressive; Marder et al. [3] did not provide return rates. No study has examined how food guarding, when considered with demographic characteristics such as sex, age, and body size, influences return rates. Additionally, no study has examined how the severity of food aggression (mild to moderate versus severe) influences return rates.

To further inform shelter policies regarding resource guarding dogs, additional information is needed on the demographic characteristics and adoption success of dogs identified as resource guarders during shelter behavioral evaluations. This paper considers food-related guarding; it does not consider other forms of resource guarding, such as the guarding of toys or sleeping sites. I reviewed nearly five years of records from a New York (NY) SPCA to develop a demographic profile for dogs assessed as resource guarders at the shelter and to determine the success of these dogs once adopted. I first examined whether sex, age class, reproductive status (intact versus spayed or neutered), or body size could be used to predict resource guarding, and then I assessed how these demographic characteristics, along with resource guarding, influenced the returns of dogs by adopters.

I predicted that likelihood of resource guarding would increase with the age of dogs, given the association found between food guarding and increasing age of dogs at acquisition [8]. Based on data indicating that behavioral problems are more common in small dogs than in large dogs [9], I predicted that small dogs would be more likely than medium and large dogs to display guarding behavior. I did not expect likelihood of resource guarding to vary by sex or reproductive status, given findings of no sex difference in the incidence of food guarding during shelter evaluations [3] and little or no effect of gonadectomy on aggression directed by dogs to people [10]. Adopters often cite behavioral problems as their reason for returning dogs to shelters [11–14], so I predicted that dogs assessed as resource guarders in the shelter would have higher return rates than dogs assessed as non-resource guarders and that severe resource guarders would be returned more frequently than dogs that showed mild to moderate guarding or no guarding.

2. Materials and Methods

I analyzed records of dogs at the Tompkins County SPCA in Ithaca, NY, USA. These records included data input by shelter staff into the PetPoint data management system from 1 September 2014 through 31 May 2019. Records included information on dog intakes (including returns), behavioral evaluations, and adoptions (n = 1016 adopted dogs; puppies excluded, see Section 2.2). Tompkins County SPCA is a no-kill, open-admission shelter with scheduled intake. The shelter has a small set of dog foster parents and allows for overnight fostering with dog volunteers. Additional programs to promote dog adoptability include: volunteer dog walking, volunteer in-kennel companionship, volunteer day-trips with dogs, playgroups for suitable pairs of dogs, nightly stuffed Kong enrichment, adoption promotion in local print and social media, off-site events to advertise dogs, and a volunteer group independently promoting dogs that are hard-to-place or have been at the shelter a long time. All procedures were carried out under protocol 2012-0150, which was approved by Cornell University's Institutional Animal Care and Use Committee.

2.1. Dogs and Housing

Original sources were available for 1015 of the 1016 adopted dogs whose records I reviewed: owner surrendered, 473 (46.6%); transferred from other shelters, 343 (33.8%); picked up as strays, 166 (16.4%); and seized by animal control officers, 33 (3.2%). Most dogs at the Tompkins shelter were mixed breeds; the number of purebred dogs was unknown due to lack of pedigrees and DNA analyses. A brief description of housing and care of dogs is provided here because details have been presented elsewhere [15].

At intake, dogs were housed in the rescue building in chain link cages with an indoor space (2.2 m^2) and outdoor run (3.5 m^2). Veterinary staff examined each dog at intake or within a few hours of intake and performed vaccinations, flea control, fecal exam, deworming, and a heartworm test. Following the veterinary exam, each dog was scheduled for behavioral evaluation (see Section 2.2). Within a few days of the completion of the behavioral evaluation, dogs were moved to the pet adoption center, adjacent to the rescue building. Once on the adoption floor, dogs were housed in one of 13 cubicles, which ranged in size from 5.2 to 7.3 m^2. Almost all dogs were housed individually; only dogs that came in together and staff deemed needed to stay together shared the same cubicle. Each cubicle contained a water bowl, a raised bed, a blanket, and a toy. Volunteers or staff either walked the dogs or brought them to an outdoor enclosure several times a day. Staff fed the dogs each day between 08:00

and 09:00 h and again between 15:00 and 16:00 h. Intact dogs were spayed or neutered when housed in either the rescue building or the pet adoption center; all dogs were spayed or neutered before adoption.

2.2. Behavioral Evaluations

Shelter staff evaluated each dog's behavior using a series of tests based on Sternberg's Assess-a-Pet [16], with modifications described by Bollen and Horowitz [17]; these modifications were made as part of the shelter's standard operating procedures and were in place well in advance of the present study. Behavioral evaluations were performed approximately 3 days after intake and included nine tests in the following sequence: cage presentation; sociability; teeth exam; handling; arousal; food bowl (using a mix of kibble and canned food); possession (using a raw hide chew, pig ear, etc.); human stranger; and dog-to-dog. Behavioral responses on the food bowl test were organized into seven levels, listed in order of increasing intensity of response: (1) stopped eating and backed away from the dish; (2) continued eating without showing any signs of uneasiness; (3) moved muzzle deeper into the dish and ate faster; (4) stiffened slightly; (5) moved muzzle toward the Assess-a-Hand; (6) stiffened, exhibited whale eye, and snarled; and (7) froze, growled, lunged, snapped, and bit the Assess-a-Hand. Behavioral responses on the possession test were organized into five levels, also listed in order of increasing intensity of response: (1) readily dropped the item; (2) allowed the Assess-a-Hand to take the item; (3) resisted letting go of the item but did not show outward aggression; (4) stiffened, exhibited whale eye, and snarled; and (5) froze, growled, lunged, snapped, and bit the Assess-a-Hand. When a dog was very uncomfortable with the Assess-a-Hand, the evaluator used her own hand to remove the food bowl and chew. Dogs were assessed as resource guarders if they exhibited at least one of the following behaviors during either the food bowl test, possession test, or both tests: stiffened, exhibited whale eye, snarled, froze, growled, lunged, snapped, or bit the Assess-a-Hand. For one analysis, I classified resource guarding dogs as exhibiting either mild to moderate resource guarding (stiffened, exhibited whale eye, snarled, froze, or growled) or severe resource guarding (lunged, snapped, or bit the Assess-a-Hand) during either the food bowl test, possession test, or both tests. This categorization was based on that described by Mohan-Gibbons et al. [2].

Though the behavior of puppies was formally evaluated by staff, the tests differed somewhat from those of older dogs (e.g., recent tests were conducted in the cubicle in which puppies were housed on the adoption floor rather than in the conference room where tests were conducted for dogs in older age classes). Additionally, puppy results were not input into the PetPoint database. For these reasons, puppies were not included in the present study.

2.3. Statistical Analyses

I classified dogs by sex, age class, body size, and reproductive status. The ages of dogs were estimated by shelter veterinarians. For the purpose of this study, the following age classes were defined based on those used in previous studies [18,19]: juveniles, from 4 months to <1 year; adults, from 1 year to <8 years; and seniors, ≥8 years. The number of dogs in each sex and age class during the study period was as follows: males, 100 juveniles, 348 adults, 66 seniors; females, 99 juveniles, 340 adults, and 63 seniors. I used the body mass recorded at veterinary intake exams to classify adult and senior dogs into the following size classes: small, <11 kg; medium, 11–24 kg, and large, ≥25 kg (categories modified from those used by Taylor et al. [20]). I did not assign juveniles a size class because they were still growing; thus, juveniles were excluded from data analyses in which body size was a variable. Mature dogs (adults and seniors) fell into the following size classes: small, 32.1%; medium, 37.7%; and large, 30.2% (body mass was not available for one adult female out of the combined 817 adults and seniors). The following percentages of dogs by sex and age class were intact at the time of behavioral evaluation: males, 83.0% of juveniles, 53.7% of adults, 30.3% of seniors; females, 85.9% of juveniles, 54.4% of adults, and 25.4% of seniors. The final dispositions of returned dogs were classified as adopted again and not returned, euthanized for either behavioral or medical reasons, transferred to a rescue group, or returned to the original owner (i.e., the person who originally surrendered the dog to the shelter experienced a change in living situation such that he or she was able to take the dog back). The final dispositions of dogs returned toward the end of the study period (May 2019) were followed for an additional 4 months.

I used logistic regression to determine significant predictors of resource guarding. Fixed factors in the first model for resource guarding were sex, reproductive status, and age class (juveniles, adults, and seniors). I then excluded juveniles from the data set so that body size could be added as a fixed factor in the second model for resource guarding. I also used logistic regression to determine significant predictors of a dog being returned to the shelter by adopters. Fixed factors in the first model for likelihood of return were sex, age class (juveniles, adults, and seniors), and resource guarding status. In the second model for likelihood of return, I excluded juveniles from the data set so that body size could be added as a fixed factor. Finally, in the third model for likelihood of return, I considered the level of resource guarding and categorized dogs as non-resource guarders, mild to moderate resource guarders, or severe resource guarders, as defined in Section 2.2. For all models, I examined the main effects and two-way interactions; I dropped two-way interactions that were not significant from final models. All dogs were spayed or neutered prior to adoption, so reproductive status was not a fixed factor in any of the models for likelihood of return. Statistical analyses were completed in JMP Pro (version 13.1.0).

3. Results

3.1. Resource Guarding

Over the nearly five-year study period, staff evaluated the behavior of 1051 individual dogs (juveniles, adults, and seniors); 161 dogs were assessed as resource guarders, resulting in a prevalence of 15.3% of dogs evaluated. Fifteen of the resource guarding dogs were not made available for adoption: 10 were euthanized for behavioral reasons and one for medical reasons; three were transferred to rescue groups; and one was returned to her original owner. All of the results that follow pertain to the 1016 dogs that were behaviorally evaluated and made available for adoption.

Overall, 14.4% of dogs moved to the adoption floor were classified as resource guarders based on behavioral evaluations (146/1016; juveniles, adults, and seniors). Of the dogs assessed as resource guarders, 30.8% (45/146) guarded on the food bowl test, 83.6% (122/146) guarded on the possession test, and 17.1% (25/146) guarded on both tests. On both the food bowl test and the possession test, freezing was the most common behavior displayed by resource guarding dogs, and lunging was the least common (Table 1). The two most extreme behaviors, snapping and biting the Assess-a-Hand, occurred in less than 14% of resource guarding dogs (Table 1).

Table 1. The percentages of resource guarding dogs that displayed specific behaviors during the food bowl test and possession test. The number of dogs that displayed the behavior/number of dogs assessed as resource guarding on the particular test are in parentheses.

Behavior Shown [1]	Food Bowl Test	Possession Test
Stiffened	20.0 (9/45)	32.8 (40/122)
Exhibited whale eye	20.0 (9/45)	9.8 (12/122)
Snarled	17.8 (8/45)	18.9 (23/122)
Froze	57.8 (26/45)	53.3 (65/122)
Growled	35.6 (16/45)	24.6 (30/122)
Lunged	0.0 (0/45)	4.9 (6/122)
Snapped	11.1 (5/45)	11.5 (14/122)
Bit Assess-a-Hand	13.3 (6/45)	8.2 (10/122)

[1] Mild to moderate resource guarding included the behaviors from stiffened through growled; severe resource guarding included the behaviors lunged, snapped, and bit the Assess-a-Hand.

The percentages of dogs assessed as resource guarders in relation to main effects of sex, age class, reproductive status, and body size are shown in Table 2. Age class was a significant predictor of resource guarding (X^2 = 13.53, *d.f.* = 2, p = 0.001), with adults and seniors more likely than juveniles to show guarding behavior (Table 2, second column). Seniors tended to be more likely than adults to guard resources (p = 0.08; Table 2, second column). There was a significant sex by reproductive status interaction for likelihood of resource guarding (X^2 = 5.24, *d.f.* = 1, p = 0.022). While spayed females (17.1%; 37/216) were more likely than intact females (9.1%; 26/286) to guard food, neutered males (15.2%; 34/224) and intact males (16.9%; 49/290) did not differ in their propensity to guard food. Neutered males and intact males also were more likely than intact females to guard food.

Table 2. The percentages of dogs assessed as resource guarders in relation to sex, age class, reproductive status, and body size. The number of dogs assessed as resource guarders/number of dogs evaluated and made available for adoption shown in parentheses. Within columns and specific variables, values with different superscript letters are significantly different ($p \leq 0.05$).

Variable	Juveniles, Adults, and Seniors [1]	Adults and Seniors [1]
Sex		
Male	16.1 (83/514)	17.9 (74/414)
Female	12.6 (63/502)	14.4 (58/403)
Age class		
Juvenile	7.0 (14/199) ^{a}	
Adult	15.1 (104/688) ^{b}	15.1 (104/688)
Senior	21.7 (28/129) ^{b}	21.7 (28/129)
Reproductive status		
Intact	12.4 (71/574)	15.4 (63/408)
Spayed/neutered	16.1 (71/440)	16.9 (69/409)
Body size		
Small		19.8 (52/262) ^{d}
Medium		11.0 (34/308) ^{c}
Large		18.7 (46/246) ^{d}

[1] Age classes included in analyses.

When juveniles were excluded from the data set to allow for the inclusion of body size as a fixed factor in the model, body size was a significant predictor of resource guarding (X^2 = 7.05, *d.f.* = 2, p = 0.03), with small dogs and large dogs more likely than medium dogs to display guarding (Table 2, third column). Small and large dogs did not differ from one another in propensity to guard. With juveniles excluded, age class did not predict food guarding (X^2 = 1.63, *d.f.* = 1, p = 0.20; Table 2, third column). As before, there was a significant sex by reproductive status interaction for likelihood of food guarding (X^2 = 5.45, *d.f.* = 1, p = 0.02). While spayed females (18.3%; 37/202) were more likely than intact females (10.4%; 21/201) to guard food, neutered males (15.5%; 32/207) and intact males (20.3%; 42/207) did not differ in their propensity to guard food. Intact males were more likely than intact females to guard food.

3.2. Returns of Dogs by Adopters

Of the 1016 dogs adopted during the nearly five-year study period (juveniles, adults, and seniors), 181 (17.8%) were returned to the shelter at least once. The number of returns per dog ranged from one to six, with one being most common: one return, 80.7% (146/181); two returns, 17.1% (31/181); three returns, 1.7% (3/181); and six returns, 0.5% (1/181). The percentages of adopted dogs returned to the shelter in relation to main effects of sex, age class, resource guarding status, and body size are shown in Table 3. Age class did not predict likelihood of return (X^2 = 2.94, *d.f.* = 2, p = 0.23; Table 3, second column). There was a borderline significant sex by resource guarding status interaction for likelihood of return (X^2 = 3.80, *d.f.* = 1, p = 0.0514). While food aggressive males (27.7%; 23/83) were more likely than non-food aggressive males (17.2%; 74/431) to be returned, food aggressive females (14.3%; 9/63) and non-food aggressive females (17.5%; 77/439) did not differ in their likelihood of return. Food aggressive males also were more likely to be returned than food aggressive females and non-food aggressive females.

Table 3. The percentages of adopted dogs returned to the shelter in relation to sex, age class, resource guarding status, and body size. The number of dogs returned/number of dogs adopted shown in parentheses. Within columns and specific variables, values with different superscript letters are significantly different ($p \leq 0.05$).

Variable	Juveniles, Adults, and Seniors [1]	Adults and Seniors [1]
Sex		
Male	18.7 (96/514)	19.3 (80/414)
Female	17.1 (86/502)	18.4 (74/403)
Age class		
Juvenile	14.1 (28/199)	
Adult	18.5 (127/688)	18.5 (127/688)
Senior	20.9 (27/129)	20.9 (27/129)
Resource guarding		
Yes	21.2 (31/146)	20.5 (27/132)
No	17.4 (151/870)	18.5 (127/685)
Body size		
Small		13.7 (36/262) [b]
Medium		16.9 (52/308) [b]
Large		26.8 (66/246) [a]

[1] Age classes included in analyses.

Body size was a significant predictor of a dog being returned ($X^2 = 15.38$, *d.f.* = 2, $p = 0.0005$), with large dogs more likely than small and medium dogs to be returned (Table 3, third column; juveniles excluded). Small and medium dogs did not differ from one another in their likelihood of return. Age class did not predict likelihood of return when the data set was restricted to adults and seniors ($X^2 = 0.76$, *d.f.* = 1, $p = 0.38$; Table 3, third column). As before, logistic regression revealed a sex by resource guarding status interaction for likelihood of return ($X^2 = 4.47$, *d.f.* = 1, $p = 0.034$). While food aggressive males (27.0%; 20/74) were more likely than non-food aggressive males (17.9%; 61/340) to be returned, food aggressive females (13.8%; 8/58) and non-food aggressive females (19.1%; 66/345) did not differ in their likelihood of return. Note, however, that the subsequent pairwise comparison between percentages of food aggressive males and non-food aggressive males returned by adopters fell short of statistical significance ($p = 0.08$). There was a tendency for food aggressive males to be more likely than food aggressive females to be returned ($p = 0.06$). The interaction between body size and resource guarding status was not significant ($X^2 = 0.74$, *d.f.* = 2, $p = 0.69$), indicating that returns of food aggressive dogs did not vary by size of dog.

Of the 146 dogs assessed as resource guarders at the shelter, 121 (82.9%) showed mild to moderate guarding, and 25 (17.1%) showed severe guarding. (Note: 25 does not equal the sum of number of dogs shown in Table 1 that lunged, snapped, and bit the Assess-a-Hand, because some dogs exhibited more than one of these behaviors during either the food bowl test, possession test, or both tests). The 25 dogs that showed severe guarding included two juveniles, 18 adults, and five seniors. Given the small numbers of juveniles and seniors in the severe guarding group, I did not include age class as a fixed factor in the third model for likelihood of return. When resource guarding was differentiated by level, guarding was a significant predictor of a dog being returned ($X^2 = 6.72$, *d.f.* = 2, $p = 0.035$), with severe guarders more likely to be returned (40.0%; 10/25) than mild to moderate guarders (18.2%; 22/121) and dogs classified as non-resource guarders (17.5%; 152/870). Dogs showing mild to moderate guarding and dogs classified as non-resource guarders did not differ from one another in likelihood of return. Sex did not predict likelihood of return when resource guarding was differentiated by level ($X^2 = 0.40$, *d.f.* = 1, $p = 0.53$).

Fifteen of the 25 dogs that exhibited severe guarding during behavioral evaluations were adopted and not returned to the shelter. For the remaining 10 dogs in the severe group, seven were returned once and then adopted without return; one was returned twice and then adopted without return; one was returned three times and then adopted without return; and one was returned twice and euthanized (this dog bit an adult in its second adoptive home). Thus, of the 25 dogs classified as severe resource guarders at the shelter, 24 (96%) were eventually placed in a home and not returned to the shelter.

The canine surrender profile form of the Tompkins shelter includes the statement, "Please explain why you need to relinquish your dog in your own words." Reasons given for returns of the nine severe guarders that were eventually successfully re-homed included elimination in the house, owner allergies, unforeseen personal reasons, moving, aggression directed at another dog in the home, and over-arousal; one small dog bit the adopter's grandson. None of the adopters completing the form described aggression around food; one adopter, who chose to provide a lengthy written explanation rather than completing the surrender form, described over-excitement around food, but stated the reason for surrender was unforeseen personal reasons. The surrender form also includes the statement, "Please check all that apply to your dog's personality" and lists the following options: friendly, shy, independent, fearful, playful, affectionate, aloof, aggressive, and overly reactive. Two adopters listed shy, fearful, and overly reactive; one listed aggressive but to another dog; some combination of friendly, independent, playful, and affectionate were checked by remaining adopters.

Of the 181 dogs returned during the study period, one was brought to a shelter located in a different state, and his final disposition was unknown. The final dispositions for the remaining 180 dogs returned at least once to the Tompkins shelter were as follows: re-adopted and not returned to the shelter, 87.2% (157/180); euthanized for either behavioral or medical reasons, 8.9% (16/180); transferred to a rescue group, 2.2% (4/180); and returned to the original owner, 1.7% (3/180).

4. Discussion

Measures of prevalence and severity of resource guarding in dogs at the Tompkins County SPCA, as well as overall return rate, are similar to those reported previously for dogs at other shelters. Fifteen percent of dogs behaviorally evaluated at the Tompkins shelter were assessed as resource guarders. This measure of guarding prevalence is similar to those reported by Mohan-Gibbons et al. [1], who surveyed 77 shelters in the United States and found that percent of dog populations exhibiting food guarding ranged from 7–30%, with an average of 14%. In a later study involving nine shelters, Mohan-Gibbons et al. [2] reported that 17% of behaviorally evaluated dogs were classified as food guarders, and Marder et al. [3] found that 21% of dogs at one shelter exhibited aggression around food. Additionally, 83% of the dogs assessed as resource guarders at the Tompkins shelter showed mild to moderate guarding, and 17% showed severe guarding; these same percentages were obtained by Mohan-Gibbons et al. [2] for their sample of shelter dogs assessed as food guarders. Finally, the overall return rate at the Tompkins shelter (18%) was similar to the average return rate reported for shelters in the United States, United Kingdom, and Italy (15%; see review by Protopopova and Gunther [21]). The consistency in both the prevalence and degree of severity of resource guarding across shelters despite the use of different behavioral assessments (e.g., Assess-a-Pet, SAFER^{TM}, blends and modifications of these and other assessments, as well as assessments developed by individual shelters) and the similarity in overall return rates suggest that the present findings on characteristics and returns of resource guarding dogs at the Tompkins shelter might generalize to other shelters.

Age class was a significant predictor of resource guarding in dogs at the Tompkins shelter, with adults and seniors more likely than juveniles to show food-related guarding during behavioral evaluations. Additionally, there was a tendency for seniors to be more likely than adults to guard food. This is the first study based on direct observations of dogs during behavioral evaluations at a shelter to examine the relationship between resource guarding and age. Using owner responses to a questionnaire distributed by an Australian dog magazine, McGreevy and Masters [8] reported that food-related aggression was associated with increasing age of dogs at acquisition (485 respondent households and a total of 690 dogs obtained from a variety of sources, including pet shops, breeders, pounds, shelters, friends, and family). Dogs included in the survey ranged from eight weeks to over 11 years old [22]; thus, this study differed from the present study not only in its method of obtaining information on dogs with respect to resource guarding but also in its inclusion of data on puppies (results from evaluations of puppies were not available in the present study). Guarding behaviors have been described in puppies only a few weeks old [23]. It would be useful in future studies with shelter dogs to include data from puppies to provide a more complete picture of age-related patterns in resource guarding.

Body size, based on body mass, was a significant predictor of resource guarding in the dog population at the Tompkins shelter, with small dogs and large dogs more likely than medium dogs to display guarding behavior. Small and large dogs did not differ from one another in propensity to guard resources. Using behavioral and body mass data collected from dog owners who completed the Canine Behavioral Assessment and Research Questionnaire (C-BARQ; 49 common breeds were represented in the study sample) and height data drawn from breed standards, McGreevy et al. [9] found that height was negatively correlated with owner-directed aggression, a category that included resource guarding. More specifically, McGreevy et al. [9] found that shorter dogs were more likely than taller dogs to display threatening or aggressive responses to household members in a variety of situations, which included being roughly handled, stared at, challenged, stepped over, or approached when possessing food or objects; body mass, however, did not predict owner-directed aggression. It is possible that absence of a relationship between body mass and owner-directed aggression in the study by McGreevy et al. [9] reflected the same pattern found here, i.e., despite differing from medium dogs, small (light) and large (heavy) dogs did not differ from one another in their tendency to guard resources. However, direct comparison of the present results with those of McGreevy et al. [9] are difficult given major differences between the two studies in methods of classifying dogs with respect to guarding behavior (direct observations by shelter staff during behavioral evaluations versus owner reports), dog populations (primarily mixed breed dogs at a shelter versus purebred dogs in homes), and scope of behavioral categories (restricted to food-related guarding versus owner-directed aggression, which included resource guarding and several other situations involving dogs and household members). Nevertheless, the present finding that small dogs were more likely than medium dogs to show resource guarding is consistent with the general pattern that problem behaviors are more common in small dogs [9]. Factors underlying the present finding that large dogs were more likely than medium dogs to guard resources remain to be determined.

The effect of reproductive status on propensity to guard resources varied by sex at the Tompkins shelter, with spayed females more likely to guard than intact females, and no difference in guarding propensity between neutered males and intact males. Neutered males and intact males were more likely to guard than intact females and did not differ from spayed females. To my knowledge, the present study is the first based on shelter behavioral evaluations to examine the relationship between resource guarding and both sex and reproductive status in dogs. The present findings differ from those of Jacobs et al. [4], who surveyed dog owners and found that dogs showing aggressive resource guarding in the home were more likely to be male and neutered. Jacobs et al. [4] acknowledged that dogs in their study might have been neutered after showing resource guarding aggression, in which case neutering might be considered a consequence of aggression rather than a cause (age at castration and age at first display of resource guarding aggression were not obtained from owners). The present findings are consistent with the general conclusions of Farhoody et al. [10] that gonadectomy does not result in predictable decreases in aggression in all male and female dogs.

Initial analyses that coded dogs at the Tompkins shelter as either resource guarders or non-resource guarders indicated that the effect of resource guarding on likelihood of a dog being returned to the shelter varied by sex. More specifically, whereas food aggressive males were more likely to be returned than non-food aggressive males, food aggressive females and non-food aggressive females did not differ in their likelihood of return. In other words, food aggression either increased returns (in the case of males) or had no effect on returns (in the case of females). The only other data available comparing return rates of food aggressive and non-food aggressive dogs to shelters are those of Mohan-Gibbons et al. [1], who reported slightly lower return rates for dogs identified as food aggressive (5%) when compared to dogs assessed as not food aggressive at one shelter (9%); the sex of dogs was not considered with resource guarding. Possible explanations for these different patterns include the following aspects of the study design used by Mohan-Gibbons et al. [1], which differ from the present study: pit bulls and Rottweilers were excluded, the inclusion criteria focused on dogs showing highly adoptable behavior except on the food bowl test, and food aggressive dogs were in a behavior modification program while in the shelter and later in their adoptive home (although many adopters did not comply). Finally, the study by Mohan-Gibbons et al. [1] used results from the food bowl test, whereas results from the food bowl test and possession test were used here.

When resource guarding was differentiated by level of severity in the present study, guarding was a significant predictor of a dog being returned, with severe resource guarders more likely to be returned than mild to moderate guarders and dogs classified as non-guarders. Dogs showing mild to moderate guarding did not differ from dogs classified as non-guarders in their likelihood of return. The reasons for return of dogs identified in the shelter as severe guarders typically did not involve aggression to humans; instead, the reasons given were those commonly provided by adopters returning dogs to shelters (e.g., allergies, moving, personal reasons, not getting along with other pets, and behavioral problems such as elimination in the house and over-arousal; [13,24]). Importantly, despite the greater likelihood of return of severe resource guarding dogs to the shelter, almost all of these dogs (24 of 25) were eventually placed in a home. Adopter surveys have revealed that many dogs assessed as food aggressive in shelters do not guard food in their adoptive homes, and, even when dogs continue to display food guarding in the home, adopters do not consider it to be a major problem [1,3]. Taken together, the present results on adoption success and published results from adopter surveys [1,3] strongly suggest that shelter staff consider adoption rather than euthanasia for most dogs identified as resource guarders during behavioral evaluations in shelters.

Body size also influenced likelihood of return at the Tompkins shelter. Using body mass as the measure of body size, I found that large dogs were more likely to be returned than small and medium dogs. Similar results have been obtained by Marston et al. [12], Diesel et al. [14], and Posage et al. [25]; suggested explanations for the observed pattern include the greater costs, space needs, and exercise requirements of large dogs, as well as the increased challenges of managing any behavioral issues. Interestingly, in the present study, the body size by resource guarding status interaction was not significant in the analysis of factors affecting likelihood of return, indicating that returns of food aggressive dogs to the Tompkins shelter did not vary by size of dog (e.g., adopters were not more likely to return large food aggressive dogs than small food aggressive dogs).

Most dogs (87%) returned to the Tompkins shelter were subsequently re-adopted and not returned to the shelter; 9% of returned dogs were euthanized, and the remaining 4% of dogs were either transferred to a rescue organization or returned to the original owner. Lower rates of re-adoption and higher rates of euthanasia have been noted for returned dogs at other shelters. Patronek et al. [26] reported that 50% of returned dogs were subsequently adopted; these authors also found a 33% euthanasia rate for all potentially adoptable dogs at the study shelter, although this value likely represented an upper limit (the percentage of returned dogs euthanized was not described). Across three Australian shelters, Marston et al. [12] reported 57% of returned dogs were subsequently re-adopted, 38% were euthanized, and fates were unknown for the remaining 5%. I cannot definitively state that all re-adopted dogs remained in the home; I can only state that the dogs were not returned to the Tompkins shelter. However, several policies at the Tompkins shelter encourage people who do not wish to keep their adopted dog to return it to the shelter rather than give the dog to someone else. First, all adopters must sign a contract stating that they will return the dog to the Tompkins shelter if the dog is not a good fit for their household. Second, all dogs receive a microchip, which is registered before leaving the shelter, so dogs can be identified if brought elsewhere, such as to a different shelter. Finally, if a dog is returned within two weeks of adoption, then the shelter refunds 75% of the adoption fee. For these reasons, I expect that most, if not all, re-adopted dogs remained in their new homes.

5. Conclusions

The prevalence of resource guarding during behavioral evaluations was 15% in the population of dogs at the Tompkins shelter, which is comparable to that observed at other shelters in the United States [1]. The demographic profile developed for dogs identified as resource guarders at the Tompkins shelter indicated they were more likely to be adults and seniors than juveniles, and when fully grown, more likely to be either small or large than medium with respect to body size based on body mass. Spayed females, intact males, and neutered males were more likely than intact females to guard resources. Ideally, shelters should conduct behavioral evaluations of all dogs made available for adoption. However, some shelters do not follow this procedure, especially with dogs considered highly adoptable at intake [2]. The profile provided here may help such shelters make informed decisions about which dogs should be evaluated for resource guarding. For example, shelters might be less likely to assess small dogs than large dogs, but the data presented here show that small dogs are just as likely as large dogs to display food-related guarding during behavioral evaluations, and those that do are just as likely as large dogs assessed as food guarders to be returned by adopters. The ability to generalize results presented here to other shelters will depend on how similar other shelters are to the Tompkins shelter with respect to dog populations and shelter policies.

Most dogs assessed as resource guarders at the Tompkins shelter showed mild to moderate guarding. Dogs assessed as severe guarders were more likely to be returned by adopters than dogs assessed as mild to moderate guarders or non-guarders. However, almost all severe guarders that were returned to the shelter were eventually re-adopted and not returned. Thus, results from this population of shelter dogs indicate that most dogs identified as resource guarders during behavioral evaluations can be successfully re-homed, although it might take more than one effort at adoption. These data on adoption success, together with data showing that dogs assessed as food aggressive at shelters do not necessarily display food-related guarding in their adoptive homes [1,3], strongly suggest that shelter staff consider adoption rather than euthanasia for most dogs identified as resource guarders during behavioral evaluations in shelters.

Funding: This research received no external funding.

Acknowledgments: I thank Jim Bouderau, Executive Director of the Tompkins County SPCA, for permission to analyze dog records. Emme Hones, Behavior Program Manager at the shelter, provided PetPoint files and body mass data and shared her knowledge and expertise concerning behavioral evaluations and resource guarding. Samantha Rubio was instrumental in initiating this project. Willy Bemis and Kate Bemis read an earlier version of this manuscript and three anonymous reviewers also provided helpful comments.

Conflicts of Interest: The author declares no conflict of interest.

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animals

Article

Evidence for Individual Differences in Behaviour and for Behavioural Syndromes in Adult Shelter Cats

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Received: 1 April 2020; Accepted: 29 May 2020; Published: 1 June 2020

Simple Summary: An important activity of modern animal shelters is the development of successful adoption programmes. In this regard, there is a need for reliable tests of individual differences in behaviour to help match the "personality" of potential adoptees with the lifestyle and needs of prospective owners; a companion animal for an elderly person remaining at home requires a different match than a pet for someone who will be away most of the day; a pet kept exclusively indoors in a small apartment requires a different match than an indoor/outdoor pet. In the present study, we repeatedly tested 31 mixed-breed adult cats of both sexes and a wide range of ages in five behavioural tests at a shelter in Mexico City, Mexico. The tests were designed to be easily implemented by shelter staff, and were short and low cost and intended to simulate common situations in a pet cat's everyday life. We found consistent (stable) individual differences in the cats' behaviour on all five tests, as well as correlations between their behaviour across tests. This suggests that such tests may contribute to reliably characterizing the "personality" of individual cats and so help increase the rate of successful adoptions.

Abstract: Consistent inter-individual differences in behaviour have been previously reported in adult shelter cats. In this study, we aimed to assess whether repeatable individual differences in behaviours exhibited by shelter cats in different situations were interrelated, forming behavioural syndromes. We tested 31 adult cats in five different behavioural tests, repeated three times each: a struggle test where an experimenter restrained the cat, a separation/confinement test where the cat spent 2 min in a pet carrier, a mouse test where the cat was presented with a live mouse in a jar, and two tests where the cat reacted to an unfamiliar human who remained either passive or actively approached the cat. Individual differences in behaviour were consistent (repeatable) across repeated trials for each of the tests. We also found associations between some of the behaviours shown in the different tests, several of which appeared to be due to differences in human-oriented behaviours. This study is the first to assess the presence of behavioural syndromes using repeated behavioural tests in different situations common in the daily life of a cat, and which may prove useful in improving the match between prospective owner and cat in shelter adoption programmes.

Keywords: individual differences; behavioural assays; behavioural syndromes; companion animal; *Felis silvestris catus*; shelter cats; human-cat relation

1. Introduction

For years, the domestic cat (*Felis silvestris catus*) has been among the most popular pets in the world [1,2]. Interest in cat behaviour, and particularly in inter-individual differences (animal personality), is reflected in recent reviews [3–6] and special issues in scientific journals treating such topics [7,8]. The cat is a good candidate for the study of individual differences as it is readily accessible and has a rich behavioural repertoire. It is also by far the most studied feline species in this respect [3]. As with other domestic animals (companion, farm and working animals), taking into account cats' personality differences when rehoming or selecting them for specific tasks can have implications for management, welfare and economy [3,9,10].

Broadly defined, animal personality refers to relatively stable inter-individual differences in behaviour [11–13]. When several of these behaviours correlate across contexts, they can be characterized as a behavioural syndrome [12,14,15]. The most common methods used to study individual differences in behaviour in the cat include observation [16,17], owner surveys [18,19] and behavioural tests [20,21]. The latter have the advantage that they can be used to evaluate and quantify the stability of individual differences across repeated standardised testing. Since an individual's behaviour is expected to be variable to some degree, some behaviours may be inconsistent and therefore less informative of the individual's behaviour at a later time. Therefore, when testing cats, reliable methods are needed, i.e., behavioural tests and measures that have been found to be highly repeatable.

Many studies of cat personality or temperament are based on behavioural observation ([3,4] see reviews), which provide important information about cats' behaviours in their daily environments. However, to explore cats' reactions to specific situations, behavioural tests are necessary. The two most commonly used tests in cat personality research are novel object tests, where the animal is presented with an unfamiliar object, and tests of reaction to either familiar or unfamiliar humans [3]. Novel object tests tend to use stimuli of unclear biological relevance (e.g., a fan with paper streamers, a remote-control car, a metal container with a spring, or a wooden box; [20,22,23]). While these tests have been reported to reveal individual differences, their meaning in daily situations of the life of the cat is unclear. Therefore, in the present study, we decided to test the behavioural responses of cats to situations corresponding to what they would likely encounter in real-life situations.

Given cats' popularity as companion animals, there has been a tendency to study their individuality in terms of their interaction with humans, for example, in their reaction to approach or handling by a familiar or unfamiliar person [20,24–27]. Other behaviours of interest for both companion and working cats (particularly mousers) include their reaction to everyday stressful situations or to prey, respectively. However, we are unaware of any studies that have experimentally addressed the inter-individual consistency of behavioural differences in these situations. Nevertheless, animal shelters have begun to implement personality testing as part of their adoption programmes, favouring a combination of surveys and behavioural testing, as in the Feline Temperament Profile [21] and the Meet Your Match Feline-ality assessment [28].

The present study is the first to incorporate repeated measurements using several behavioural tests and to take a behavioural syndrome approach by evaluating correlations among these measurements in a heterogeneous population of cats (wide age range, different backgrounds) housed in an animal shelter. Animal shelters have a continuing need for reliable personality tests, for example, to better match potential pets with prospective owners and households or to identify cats that may better fit a specific situation, such as working or therapy cats. We used five behavioural assays that we consider to be ethologically and ecologically relevant to the daily life of the domestic cat, repeated three times each (see details below). We previously reported an analysis of data which included a subset of the data presented in the present paper, gathered during the separation/confinement test [29], but here we include further behavioural tests with the aim of identifying a larger range of repeatable individual differences and behavioural syndromes.

2. Materials and Methods

2.1. Study Site and Animals

We collected data from 31 adult cats (14 males and 17 females) from a shelter in Mexico City, Mexico, aged between 1 and 11 years (mean 4.5, SD 2.6, Supplementary Material Table S1). In some cases, the cats' ages were not known with certainty and were estimated by veterinarians. Participants were chosen randomly from among the cats at the shelter, which were in good health and permitted handling. All the cats had been neutered and had received post-operative care by qualified veterinarians within three days of entering the shelter, and all cats participating in the study had been at the shelter for at least six weeks prior to the start of behavioural testing. The shelter was a four-storey house divided into sections; approximately 50 cats were housed in each section according to how well they tolerated each other. All sections consisted of at least two rooms (approx. 2.5 × 3.5 m each) with access to a fenced outdoor area (approx. 2 × 4 m). Each cat was free to roam within its section. The rooms were furnished with cat beds, boxes of assorted sizes with blankets, scratchers and toys. Water, commercial dry cat food and sand boxes were always available.

2.2. Procedures

Tests were performed weekly for 12 sequential weeks; each of the five tests was performed three times across three sequential weeks (the human approach tests were performed on the same days). One test was performed per day on all subjects, tested in randomized order between 13:00 and 18:00. Not all cats were available for all trials, therefore sample sizes differ slightly between the tests (see Supplementary Material Table S1 for information on which cats participated in each test). All tests were video recorded (GoPro© Hero3+, GoPro, Inc., San Mateo, CA, USA) for subsequent behavioural analysis.

2.3. Behavioural Testing

2.3.1. Struggle Test

We proposed the struggle test as a proxy for the handling tests used in different mammalian [30–33] and bird species [34–36]. Since domestic cats are frequently handled by their owners, by other familiar and unfamiliar humans, and by veterinarians, we redesigned this test to evaluate the struggle response when they are picked up and restrained. We tested 30 adult cats (13 males and 17 females; mean age 4.5, SD 2.6 years, min = 1, max = 11). The test was performed in the section of the shelter where the cat normally resided. One of the experimenters (S.M.-B.) approached the cat and stroked it three times from the head to the base of the tail, then picked it up, holding it with both hands around the thorax, under its forelimbs. The test lasted until the cat began to struggle (see Table 1 for behavioural definition) or until 30 s elapsed after picking it up. When this happened, the cat was immediately set down. The experimenter wore gloves as a precaution against scratches.

Table 1. Behavioural variables recorded in each test.

Behaviour Measured	Definition
Struggle test	
Struggle (latency)	Lifting one of the hind paws and touching or kicking the experimenter's forearm
Separation/confinement test	
Vocalization (latency and number)	Meow-type vocalizations
Motor activity (latency and duration)	Displacement of any of the limbs on the floor or sides of the carrier for at least 1 s

Table 1. *Cont.*

Behaviour Measured	Definition
Mouse test	
Near the mouse (latency and duration)	At least the front paws within 50 cm of the jar containing the mouse
Tail swishing (duration)	Any time the cat swished its tail from side to side at least twice
Interaction (latency and duration)	Contact with the jar, either sniffing or pawing
Walking around the jar (duration)	Walking from one side of the jar to the other while near it
Passive human approach test	
Approach score (1–5)	Maximum degree of proximity to the unfamiliar human
Vocalization (latency and number)	Meow-type vocalizations
Finger–nose contact (binary)	If the cat established contact by touching its nose to the human's outstretched finger
Active human approach test	
Stroke (latency)	Latency to the first full stroke from head to tail in a set by the unfamiliar person

2.3.2. Separation/Confinement Test

Separation/confinement tests are used for personality testing in many animals, particularly in social species [37–41]. Despite the fact that cats are considered only facultatively a social species [42,43], in previous studies this type of test has been successfully used for evaluating individual differences in kittens of the domestic cat [44,45] and adult shelter cats [29]. Moreover, this test represents a common situation in a cat's daily life around humans, since cats are often confined in a carrier to take to other places outside their home.

The data from this test combined with other data from additional shelter cats have been previously reported in Urrutia et al. [29]. We tested 28 adult cats (12 males and 16 females; mean age 4.6, SD 2.7 years, min = 1, max = 11). Tests were performed in a small closed room unfamiliar to the cats; the room was 1.5 × 2 m, with flat-finished, unpainted concrete floor, walls and ceiling, and without furnishings. During the test, no other animals or humans were allowed to enter either the test room or the room adjacent to it to limit auditory and olfactory contact. One experimenter approached the cat (either S.M.-B. or A.U.), briefly stroked it and then carried it in her arms into the test room. With the help of a second experimenter, they placed the cat inside a standard commercial pet carrier (42 × 61 × 38 cm), which was a closed plastic box with a steel grill door at one end and ventilation holes along the sides. The carrier, with the cat inside, was then placed on the floor at a previously marked position and the experimenters left the room. The test lasted two minutes. Once this time had elapsed, the cat was removed from the carrier and returned by one of the experimenters to its home room. The video camera was set up 60 cm from the carrier. To improve visibility, a red light was mounted inside the carrier. The carrier was cleaned between trials with isopropyl alcohol. See Table 1 for definitions of the behaviours analysed in this test.

2.3.3. Mouse Test

In our experience, neither kittens nor adult cats show sustained interest in interacting with the types of inanimate objects conventionally used in novel object tests. We therefore chose tame, laboratory-strain (BALB/c) mice as the "novel object" to more closely approximate a biologically relevant stimulus, since small rodents are the most common prey of the domestic cat [46–50] and because of the ease with which they can be maintained and handled (see below for details on how the mouse was presented; see also [51]). In a previous study by Yang et al. [52], the BALB/c mouse strain was found to show the least fearful reactions in response to a predator. In our tests, a total of five mice were used in rotation; three of them were taken to the shelter on test days. The mouse in the jar was switched every two trials (approx. 10 min) to minimize stress. The stimulus animals showed no obvious signs of fear in the presence of the cats; there were no signs of panic (e.g., freezing) or attempted escape or defence (e.g., jumping), they moved around in the jar in apparent calm, sometimes adopting the stretch–attend posture—which according to previous research is indicative of risk assessment rather than a fearful reaction [52]—in apparent curiosity at the presence of the cats. At the end of the study, the mice were adopted by student participants. For more details on the housing of the mice outside the tests, see Supplementary Material File S2. Additionally, during pilot tests, thermal pictures of the mice were taken before and after being in the jar with a cat in the room. Analysis of these images showed that the stress experienced by the mice (as measured by the increase in eye temperature) was comparable to that experienced in routine laboratory tests [53,54].

We tested 23 adult cats (7 males and 16 females; mean age 4.4, SD 2.5 years, min = 1, max = 11). Cats were individually tested in an unfamiliar room (4 × 6 m) which was cleared of all other cats and any objects that could be distracting. Subjects were given a two-minute habituation period before introducing the mouse. During habituation, and throughout the test, an experimenter (S.M.-B.) remained in the room, standing motionless and silent in a corner.

At the end of the habituation period, the experimenter restrained the cat in the middle of the room while a second experimenter brought in a mouse inside a clear, thick glass jar (15 cm in diameter × 20 cm high) with a perforated lid and covered with a cardboard box. At a marked position approximately 1.5 m from the cat and against a wall, the second experimenter fixed the jar to the floor with double-sided tape, removed the cardboard box and left the room. The first experimenter then released the cat and returned to the corner. The cat could see and presumably hear and smell the mouse but could not access it. The cat was free to interact with the jar for two minutes, after which the test ended and the cat was returned to its section of the shelter. The video camera was mounted on the wall 2 m above the jar. See Table 1 for definitions of the behaviours analysed.

2.3.4. Human Approach Tests

Human approach tests have been commonly used to evaluate cat behaviour [20,25,55–57], especially in shelters [27,58]. We modified the test from Adamec et al. [59] and tested the response of 28 adult cats (11 males and 17 females; mean age 4.6, SD 2.7 years, min = 1, max = 11) to an unfamiliar person. This person, a male volunteer, was the same person on a given test day but a different volunteer each week (age 21–25 years). To minimize unintentional odour cues, all were non-cat owners, were asked to wear fresh clothes and were unknown to the cats. Thus, the cats had the opportunity to interact with three different humans, one in each trial.

- Passive human approach test

Tests were performed in the same room as described for the mouse test. Before testing, two concentric circles, 1.5 and 3 m in diameter, were drawn on the floor with chalk to use as references of cat–human distance in the later video analysis, and the male volunteer was asked to sit cross-legged on the floor in the centre of the inner circle. When the volunteer was in position, the cat was carried in arms into the room by a familiar experimenter and placed in a shallow (20 cm deep) open wooden box against the wall next to the door. The experimenter then left the room. The test started when the door closed, leaving the cat alone with the unfamiliar person. The test consisted of two parts. For the first three minutes the unfamiliar volunteer sat cross-legged on the floor, looking at the wall and ignoring the cat however close it got. We used an approach score from 1 to 5 depending on whether the cat did the following: (1) remained outside the large circle; (2) entered at least its forepaws in the large circle; (3) entered at least its forepaws in the small circle; (4) established physical contact with the human (rub, sniff, touch with paw); (5) put at least its forepaws on top of the human. Then, in the second part, the volunteer continuously called the cat by its name for one minute while extending his arm and index finger as a greeting, pointing in the cat's direction, even if the cat had already made physical contact with him. See Table 1 for definitions of the behaviours analysed in this test.

- Active human approach test

This test was performed immediately after the passive human approach test. The volunteer was instructed to slowly rise to his feet, approach the cat and attempt to stroke it six times from the head to the base of the tail. If the cat moved away before it could be stroked six times, the unfamiliar human walked after it and attempted to stroke it again. The test ended after the sixth stroking attempt (whether successful or unsuccessful) or after 1 min. The experimenter then entered the room and returned the cat to its home room.

2.4. Ethical Considerations

Throughout the study, animals were kept and treated according to the guidelines for the use of animals in research as published in Animal Behaviour (ABS, 2016), as well as the relevant legislation for Mexico (National Guide for the Production, Care and Use of Laboratory Animals, Norma Oficial Mexicana NOM-062-200-1999), and approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL, permission ID 6315) of the Institute of Biomedical Research, UNAM, Mexico City, Mexico.

2.5. Video and Statistical Analysis

All behavioural variables were coded using Solomon Coder software for video analysis [60]. Statistical analyses of the data were carried out using the programme R, version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) [61]. Prior to fixed-effects and repeatability analyses, any non-normally distributed continuous variables were normalized using either a Box–Cox or log transformation with the R package MASS [62]. Effects of sex, age and trial number on behavioural variables were analysed using linear mixed-effects models (LMM) for continuous, and generalized linear mixed-effects models (GLMM) for count (i.e., Poisson distributed) or binary (binomially distributed) dependent variables with the R package lme4 [63]. As fixed effects, we included sex, trial number (1 to 3), age (as a covariate), the interaction of sex × age and the interaction of trial number × age. As a random factor, we included individual identity. We applied backwards stepwise reduction of the full models beginning with non-significant interactions followed by non-significant fixed effects when $p > 0.05$. Individual identity as a random factor was retained in all models to account for repeated measures of individuals. p-values were extracted by Wald chi-squared tests (type III).

We then analysed the repeatability of individuals' behaviour across the three trials by intra-class correlations calculated as the proportion of phenotypic variation that can be attributed to between-subject variation [64]. We used GLMM-based calculations for count (Poisson distributed) or binary (binomially distributed) data and LMM-based calculations (Gaussian distributed) for continuous data for testing the repeatability of individual differences using the R package rptR [65,66]. Individual identity was used as a random factor and the fixed effects found to have a significant effect on each behaviour in the previous analysis were included where applicable. For all intra-class correlations, we calculated 95% confidence intervals by 1000 bootstrap steps, and p-values were calculated by 1000 permutations.

To investigate the possible association of the behaviours between the different tests, we first performed principal component analyses (PCAs) independently on each of the following tests: separation/confinement, mouse and passive human approach using spectral decomposition assuming correlation matrices, to reduce the number of dimensions; no rotations were used. In the case of the struggle and active human approach tests, we used the raw behavioural data, since only one behaviour was coded in each of these two tests. Since phenotypic correlations between traits may originate from two sources, that is, (i) from individuals' average levels of two traits (between-individual correlation) or (ii) from individuals' change in behaviour (within-individual correlation) [67–70], we calculated between-individual and within-individual (residual) correlations by using multivariate linear mixed models with the R package sommer [71] to partition possible phenotypic correlations between the traits. p-values were corrected for multiple tests using the Benjamini–Hochberg method.

3. Results

3.1. Repeatability of Individual Differences within Tests

3.1.1. Struggle Test

No effects of age, sex or trial or of the interaction between these were found on the latency to struggle (Supplementary Material Table S3). All cats (n = 30) struggled within the 30-s limit, with only one cat still held at 30 s on one occasion. Individual differences in the latency to struggle were significantly repeatable across the three trials (Table 2).

Table 2. Repeatability of the variables analysed for each of the behavioural tests. Intra-class correlation coefficients (R), 95% confidence intervals (lower bound, upper bound) based on 1000 bootstrap steps and significance values (p) are given. Asterisks indicate significance levels at $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.

Behaviour	R	95% CI (lower bound, upper bound)	p-Value
Struggle test			
Latency to struggle	0.555	(0.314, 0.726)	0.001 ***
Separation/confinement test			
Latency to vocalize	0.761	(0.597, 0.861)	0.001 ***
Number of vocalizations	0.920	(0.766, 0.969)	0.006 **
Latency to motor activity	0.191	(0, 0.442)	0.066
Duration of motor activity	0.323	(0.06, 0.533)	0.001 ***
Mouse test			
Latency to approach	0.515	(0.248, 0.714)	0.001 ***
Duration near	0.498	(0.219, 0.679)	0.001 ***
Duration of tail swishing	0.806	(0.366, 0.944)	0.001 ***
Latency to interact	0.477	(0.201, 0.672)	0.001 ***
Duration interacting	0.501	(0.236, 0.716)	0.001 ***
Duration walking around	0.284	(0.019, 0.542)	0.017 *
Passive human approach test			
Approachscore (1–5)	0.312	(0, 0.507)	0.004 **
Latency to vocalize	0.668	(0.461, 0.806)	0.001 ***
Number of vocalizations	0.844	(0.632, 0.942)	0.008 **
Finger–nose contact (binary)	0.761	(0.376, 0.985)	0.001 ***
Active human approach test			
Stroking (latency)	0.496	(0.229, 0.692)	0.004 **

3.1.2. Separation/Confinement Test

Age and trial number (1–3) were found to have a small, significant effect on the number of vocalizations and the duration of motor activity; older cats vocalized less and moved less in the carrier, and both behaviours diminished in consecutive trials (Supplementary Material Table S3). In the case of latency to initiate motor activity, there was a significant but very small effect of sex, where males began motor activity slightly sooner. There was a small effect of the interaction between age and sex, where the latency to move was slightly higher in older males than in younger males. There was also a small effect of trial number, where latency to begin motor activity began slightly later in consecutive trials (Supplementary Material Table S3). Therefore, these significant fixed effects were included in the respective repeatability analyses. Individual differences in the latency to vocalize and the number of vocalizations emitted by the cats (n = 28) were highly repeatable. Duration of motor activity was also significantly repeatable, although the latency to locomote was not (Table 2).

3.1.3. Mouse Test

The sequence of trials was found to have an effect on the duration of interactions (cats interacted less with the mouse on the third trial than during the first two trials) and was thus added as a fixed effect in the analysis (Supplementary Material Table S3). No other variable showed an effect of age, trial number or sex or the interaction between them. We found highly repeatable individual differences in the latency to approach and the time cats (n = 24) spent near the mouse across trials. Variables associated with proximity to the mouse were likewise repeatable, such as the time spent walking around the jar, the latency to interact and the duration of interaction (Table 2). Even tail swishing, which was coded from any area of the room, showed repeatable individual differences, a possible sign of interest or arousal of the animal even from afar.

3.1.4. Human Approach Tests

None of the behavioural variables measured in these tests was significantly affected by age, trial number or sex or the interaction between them (Supplementary Material Table S3).

- Passive human approach test

We found repeatable individual differences (n = 28) for all behavioural measures in both phases of the test across trials, that is, the distance individual cats kept from the unfamiliar human was consistent even though each of the three trials used a different unfamiliar volunteer. We also found repeatable individual differences for the finger-nose contact measure of phase two. Moreover, individual differences in the latency to vocalize and in the number of vocalizations emitted during the entirety of trials were also highly repeatable (Table 2).

- Active human approach test

Individual differences in the latency for the unfamiliar person to be able to stroke the cat were consistent across trials and even though this involved three different people (Table 2).

3.2. Correlations Between Tests

For dimension reduction purposes, we performed three separate PCAs on the behavioural variables of the following tests: separation/confinement, mouse and passive human approach. For the full results of the PCAs, see Supplementary Material Table S4. In the separation/confinement test, two principal components were extracted. For factor 1 ("confinement/separation vocalization"), the behaviours with the highest loadings were those related to vocalization and, for factor 2 ("confinement/separation motor activity"), the highest loading was the duration of motor activity. In the mouse test, two principal components were extracted. For factor 1 ("interaction with the mouse"), the behaviours with the highest loadings were related to the cats' proximity to and interaction with the mouse jar and, for factor 2 ("tail swishing"), the highest loading was for the duration of tail swishing. In the passive human approach test, two principal components were also extracted. For factor 1 ("approaching the passive human"), the behaviours with the highest loadings involved the human approach score and finger–nose contact; for factor 2 ("passive human approach vocalization"), the behaviour with the highest loading was the number of vocalizations.

In each of the two remaining tests (struggle and active human approach), we measured only one behavioural variable (latency to struggle and latency to be stroked by the human, respectively), hence we did not perform a PCA for these tests. Using the raw data for these variables, along with the six previously described factors obtained from the PCAs, we calculated correlations using multivariate linear mixed models. From a total of 34 correlations (Supplementary Material Table S5), we found eight that were significant after adjusting p-values for multiple comparisons (Benjamini–Hochberg method; Figure 1).

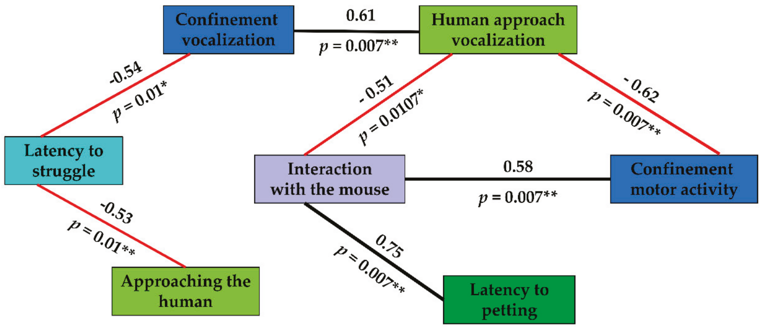

Figure 1. Correlations between behavioural variable scores showing stable individual differences at the between-individual level. Asterisks indicate significance levels at $p < 0.05$ *, $p < 0.01$ **. Black lines correspond to positive correlations, red lines correspond to negative correlations. Line thickness corresponds to the strength of a correlation. Further details are available in Supplementary Material Table S5, including confidence intervals.

4. Discussion

4.1. Consistency Across Time

In this study, we first evaluated the consistency across time of individual differences in behavioural responses of adult shelter cats in five different tests, and for all tests we found measures that showed significant repeatability. Stable individual differences were evident even though the cats were a heterogeneous population that differed in age, sex and (largely unknown) background. Perhaps surprisingly, individual differences in behavioural responses in most of the tests were unrelated to age or sex, suggesting that the behaviours measured here may be useful for evaluating individual differences in adult cats in general. This is supported by previous studies reporting stable individual differences in cats and other mammals in tests similar to those used here, that is, struggle or restraint tests used in cats [26], mice [31], rabbits [32,72,73], North American red squirrels [74,75] and pigs [33,76]; social separation tests used in cats [29,44,45], horses [77] cows [39,78] and dogs [40]; mouse tests used in cats [51,79]; and human approach tests used in cats [21,25,27,56,57,59], dogs [80], pigs and cattle [81,82]. These tests in their various forms are all relevant to the daily life of most cats, and thus provide a promising basis for assessing cat personality across a wide range of populations and conditions, including in shelter cats.

4.2. Behavioural Syndromes

We found seven significant correlations between behavioural scores from the different tests (Figure 1). Most of these seemed to be connected with humans; for example, cats that readily approached the unfamiliar human in the passive human approach test also struggled sooner in the struggle test, which may suggest that these cats were more confident around humans. Cats that struggled sooner also tended to vocalize (meow) more during the confinement test when separated from humans and other cats, suggesting that these individuals may seek the company of humans more, since meowing is considered a human-oriented behaviour ([83,84] our observation). Such correlations may indicate the existence of behavioural syndromes as defined in the Introduction.

We can suppose that while the separation/confinement test was probably a negative experience for all the cats, the human approach test was a positive experience for at least some individuals. A more detailed acoustic analysis of the meows may help disentangle the emotional valence and motivation (e.g., stress, attention-seeking, greeting) underlying them in these tests, since meows emitted during distress have a distinct pattern (low mean fundamental frequency, longer duration; Schötz et al. [85]). Additionally, the cats for which the human approach test was a positive experience may have emitted other vocalizations (e.g., purrs, which Fermo et al. [86] found are exclusively associated with positive experiences). However, we were not able to record them due to their low volume. It is also possible, as Guillette and Sturdy [87] have suggested, that the degree of arousal or readiness of the cat to act (due to activation of the sympathetic nervous system) may contribute to the pattern of vocal emissions in different contexts [88].

Consistent with previous findings, we did not find an association between the number of vocalizations and motor activity within the confinement/separation test, suggesting different underlying mechanisms (motivation) between these variables (see more details in [29]). However, there was a negative correlation between motor activity in the confinement/separation test and the number of vocalizations emitted during the passive human approach test. The only explanation we can presently offer is that the cats for which the passive human approach test was a positive experience may have "carried" this correlation, meaning that possibly only positive meows are correlated with motor activity. Further study into the relationship between meows and motor activity in positive and negative situations may help to disentangle this.

Additionally, interaction with the mouse was significantly correlated with three different variables. It was negatively correlated with vocalization in the human approach passive phase, which can be interpreted as cats that were more focused on the mouse were less demanding of human attention (vocalized less). The latter is supported by the positive correlation between interaction with the mouse and latency to be stroked in the active human approach test, i.e., cats that spent more time with the mouse took longer to allow themselves to be stroked. Taken together, these correlations suggest a syndrome where more prey-oriented individuals are also less human-oriented. Although cats' backgrounds in the present study were unknown, we speculate that such a syndrome may arise as a consequence of experiences prior to their arrival at the shelter, that is, cats that were more independent from humans may have relied more on hunting to obtain food, whereas cats that were more social with humans had relied on them for sustenance. Finally, there was also a positive correlation between interaction with the mouse and motor activity during the confinement/separation test, suggesting that some cats were more "excitable" than others, possibly due to differences in sympathetic nervous system arousal as discussed previously for vocalizations.

4.3. Behavioural Testing in Animal Shelters

All five tests implemented in this study are simple and fast (no more than five minutes each), and any materials used are inexpensive and easily procured. Because of this, they can be reproduced practically anywhere in the world with minimal instruction of shelter personnel. Together, this makes them a suitable option for shelters looking to evaluate personality as part of their adoption programme. While millions of cats enter animal shelters every year, in the United States, for example, only an estimated 11.5% of pet cats come from a shelter [4,28]. Furthermore, even if a cat is adopted, there is still a high chance that it will be returned due to not fulfilling the new owner's expectations, which risks euthanasia. Organizations like the American Society for the Prevention of Cruelty to Animals have managed to decrease the number of returned cats by applying questionnaires and personality tests [28].

However, these protocols are not applied worldwide, due to differences in owner expectations and the way shelters operate in different locations, among others. For example, animal shelter facilities in Mexico and throughout Latin America differ from those in the United States and Europe, something also noted by Fukimoto et al. [89] in their study of shelter cats in Brazil. Although our tests share some similarities with the ASPCA's Feline-ality behavioural assessment, we sought to develop tests that could be a better fit for the shelter conditions and owner expectations we are familiar with. For example, we chose to use the pet carrier as a test within itself to evaluate individual responses to isolation and confinement, as separation anxiety is a common concern for owners who work long hours away from home. We also included a novel test (mouse test) in which the cats are presented with a biologically relevant stimulus. Although we recognize that this test will not be relevant to all cats that are offered for adoption as pets, nor is it feasible for all shelters to keep mice for this test, we would like to note that. In some shelters around the world. there are programmes to adopt out or loan "mouser" or "barn" cats ([c.f. [90] and also see the programs of the following organizations: Battersea Dogs & Cats Home (UK), Dereham Adoption Center (UK), Animal Humane Society (USA), Best Friends Animal Society (USA), Barn Cats Inc. (USA), among others). In recent years, there has been an increase in the demand for mousers by more environmentally friendly businesses and organic farms seeking to avoid rodenticides and to switch to biological pest control. This is an option for cats that are not sociable with people. Those individuals that show a strong interest in potential prey probably have a better chance of being successfully adopted into a working context.

Implementing repeated behavioural testing in the adoption process, whenever possible, could help match prospective owners with an animal that best suits the needs and lifestyle of both parties. For example, a family with small children needs a cat that tolerates handling; a calm person may want a calm cat; and someone who is not home most of the day would do better with a cat that is not stressed by separation.

5. Conclusions

Reliable, economic and easily implemented behavioural tests are needed by animal shelters to improve their adoption programmes by improving the match between the personality of the prospective pet, in this case the cat, and the context of its new home. This can be best achieved by using tests based on the natural, evolved behaviour of the cat relevant to its everyday life and using correlations between more than one behavioural measure to form a more reliable profile of each individual cat's personality. Results of the present study indicate that this is, indeed, feasible.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2615/10/6/962/s1, Table S1: Details of the subjects and test assignment, File S2: Description of the housing of the mice during the experiments, Table S3: Results of the LMMs and GLMMs to evaluate the impact of age, sex and trial number on behaviour, Table S4: Full results of the PCAs performed on the confinement/separation, mouse and passive human approach tests, Table S5: Correlations between behavioural variable scores showing stable individual differences.

Author Contributions: Conceptualization, P.S., R.H. and O.B.; methodology, P.S., O.B., R.H. and S.M.-B.; data collection and video analysis, O.B., P.S., A.U. and S.M.-B.; statistical analysis, P.S. and A.U.; visualization, A.U.; writing—original draft preparation, S.M.-B. and A.U.; writing—review and editing, O.B. and R.H.; supervision, O.B. and P.S.; project administration, S.M.-B.; funding acquisition, R.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México, Mexico City, Mexico, grant number DGAPA-IN212416, by a postdoctoral fellowship to O.B. by the Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México and by a Cátedra grant to P.S. from the Consejo Nacional de Ciencia y Tecnología, México, grant number 691. S.M.-B. and A.U. received a fellowship from CONACYT, number (número de becario) 948973 and 616266, respectively, and they thank the Posgrado en Ciencias Biológicas, UNAM, Mexico City, Mexico, for its support.

Acknowledgments: We are very grateful to Betty McGuire who invited us to participate in this Special Issue and made it possible. We thank Jimena Chacha for help with behavioural testing, and the staff of the Gatos Olvidados shelter for their support and for allowing us repeated access to their cats and facilities. This study was performed in partial fulfilment of the requirements for A.U. to obtain the PhD degree in the Posgrado en Ciencias Biológicas at the Universidad Nacional Autonóma de México, Mexico City, Mexico.

Conflicts of Interest: The authors declare no conflict of interest.

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Characterizing Human–Dog Attachment Relationships in Foster and Shelter Environments as a Potential Mechanism for Achieving Mutual Wellbeing and Success

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Received: 25 November 2019; Accepted: 26 December 2019; Published: 30 December 2019

Simple Summary: The majority of research on attachment behavior in dogs has focused on the bonds between pet dogs and their owners. In this study, we examined attachment relationships between dogs living in animal shelters and foster homes and their temporary caregivers-shelter volunteers or foster volunteers, respectively. We also examined these results in relation to previously published data from pet dogs in order to contextualize our findings. Our findings indicate that the percentage of securely attached shelter dogs was significantly lower than that previously observed in scientific studies of the pet dog population. No differences were found between proportions of securely attached foster dogs and prior research with pets. We did not find significant differences between foster and shelter dogs in terms of attachment style proportions. We also found evidence of disinhibited attachment, which is associated with a lack of appropriate social responses with unfamiliar and familiar individuals in foster and shelter dogs. This is the first study to apply attachment theory to foster and shelter settings.

Abstract: This study aimed to characterize attachment relationships between humans and dogs living in animal shelters or foster homes, and to contextualize these relationships in the broader canine attachment literature. In this study, 21 pairs of foster dogs and foster volunteers and 31 pairs of shelter dogs and shelter volunteers participated. Each volunteer–dog dyad participated in a secure base test and a paired attachment test. All volunteers completed the Lexington Attachment to Pets Scale (LAPS), a survey designed to measure strength of attachment bonds as reported by humans. Although no significant differences were present in terms of proportions of insecure and secure attachments between foster and shelter populations, proportions in the shelter population were significantly lower ($p < 0.05$) than the proportions of attachment styles that would be expected in a population of pet dogs based on the published literature on pet dog attachment styles. Additionally, findings are presented in relation to data from a paired attachment test that demonstrate foster and shelter dogs spend more time in proximity to humans when the human is actively attending to the dog and encouraging interaction, as would be expected based on previous studies. We also present findings related to the presence of disinhibited attachment (previously reported in children who spent a significant portion of time living in institutionalized settings) which is characterized by a lack of preferential proximity seeking with a familiar caregiver and excessive friendliness towards strangers in foster and shelter dogs.

Keywords: attachment behavior; shelter dog; foster dog; disinhibited attachment; attachment style

1. Introduction

Although it is widely agreed that dogs and humans form attachment relationships with one another, the method of applying attachment styles to pet dog research is a fairly recent area of interest. Previous studies have explored attachment relationships in pet dogs [1–3] and in the ability of shelter dogs to form attachment relationships to an unfamiliar human in a shelter setting [4]. More recently, research has shown that dogs' behavior in attachment tests can be used to categorize dogs into attachment styles [5–7] originally described in literature focusing on infant–mother attachments [8]. While additional attachment styles have been described, the three primary attachment styles have commonly been defined in infant research as follows: secure (the infant shows signs of distress when separated from the mother and seeks proximity and contact when reunited), insecure-avoidant (the infant does not show much distress and does not seek proximity when reunited) and insecure-resistant (the infant is very distressed when the mother is absent but is not calmed when the mother returns and resists contact) [8].

While these attachment styles have since been applied to the pet dog–owner relationship, this component of attachment theory has not previously been applied to dogs in foster and shelter settings. Given that dogs living in animal shelters have been found to quickly form bonds to new humans [4] understanding the nature of these bonds, including the degree of attachment security that exists (which could have welfare implications) as well as similarities and differences with respect to the dog–'owner' bond seem especially relevant. Furthermore, although disinhibited attachment has been described among human children placed in homes after early life experiences in institutionalized settings [9], this topic has not previously been explored in dog attachment, and could be of particular interest for dogs in shelter settings. Disinhibited attachment is characterized by a lack of attenuating social responses to adults of varying familiarity, low levels of checking in with a familiar attachment figure in a stressful situation, and inclination to go off with an unfamiliar person. Disinhibited attachment can be mild or severe, and it can occur in individuals with any attachment style, although in humans, it is most pronounced in children with secure attachment styles.

Dogs that are housed in shelters for a prolonged period of time may be more likely to develop new behavior problems [10,11], or experience higher stress levels [12] and socio-cognitive declines [13]. For a review of sheltering's effects on dog behavior, welfare and physiology, see [14]. However, regular interactions with a person have been associated with improved behavioral outcomes [15,16] and decreased cortisol levels [17,18]. Another unexplored benefit of volunteer programs in which dogs are provided with opportunities to regularly interact with familiar people may be the opportunity for transitioning dogs to develop a secure attachment bond with these volunteers, which has been found to promote positive behavioral and cognitive outcomes in both pet dogs [19] and human children [20]. Orphaned children have been shown to have a greater likelihood of thriving and developing secure social bonds later in life if they develop a secure bond with a foster parent [21].

In this study, we used the Lexington Attachment to Pets Scale (LAPS) [22] to examine levels of attachment foster and shelter volunteers report feeling towards partnered dogs. This can allow us to gain a better understanding of how volunteers perceive their relationships to dogs in these settings. In many cases volunteers are responsible for carrying out the majority of enrichment and socialization activities that dogs experience while living in an animal shelter, directly impacting the welfare of animals housed there. However, shelter staff and volunteers—especially those with animal contact—often experience burnout. Conflicted feelings about bonding with dogs under their care is one potential source of stress for these individuals, due to concerns about how the dog will feel when separated from them at the time of adoption. Shelter and foster volunteers may also miss animals they have bonded strongly with, and thus may experience feelings of loss even if they are happy a dog has been adopted. However, potential benefits and costs of the bonding experiences shared between shelter and foster volunteers and dogs in their care are not well understood.

Given that dogs living in animal shelters have been found to quickly form bonds to new humans [4], foster dogs are likely primed to form some kind of attachment to their new caretaker quickly as well. However, the style of attachment developed depends on both foster volunteer and foster dog behavior [3,23]. The existing body of prior research on children and pet dogs suggests that secure attachment formation in the foster home could be beneficial to foster dog welfare, improve behavior outcomes and increase the speed and likelihood of secure bond formation with their new owner in the adoptive home [19–21]. More information about how attachment bonds within the foster home are associated with foster volunteer perception, foster dog welfare and future adoption success could be used to promote optimal fostering practices that take into account both dog and volunteer wellbeing. There is also a great need to evaluate the relative benefits of fostering, including the potential for stable bonding opportunities for stray and relinquished dogs, compared with other in-shelter socialization opportunities. In many cases, it is not feasible for shelters to foster their entire canine population, and therefore there is a critical need for an empirical investigation into how regular interactions with a familiar volunteer affect shelter dog welfare and adoption outcomes. However, to date, the potential benefits of regular interactions with a familiar volunteer on dog welfare, including the potential to positively impact the formation of future bonds with adopters, has not been evaluated in shelter dogs.

The goals of this project included identifying different volunteer–dog attachment profiles using data from a behavioral test and from a scale measuring volunteer-reported attachment levels with shelter and foster dogs. We compared relative preference for an unfamiliar person in a paired attachment test, and also analyzed these data in conjunction with volunteer-reported attachment levels. In addition, we explored whether behaviors associated with disinhibited attachment in humans were also present among the foster and shelter dogs that participated in this study. Given the role of secure attachment formation in terms of positive behavioral and cognitive outcomes in human children, we wanted to explore attachment relationships in foster and shelter dogs. As shelter dogs have been shown to form attachments to unfamiliar people quickly [4], we wanted to discover whether attachment relationships between foster and shelter volunteers and dogs in these settings are secure, and to what extent they are similar to attachments seen in pet dogs living in homes. This is the first study looking at the quality of attachment using attachment styles in foster and shelter dogs.

2. Materials and Methods

2.1. Animal Subjects

Foster dog subjects included 21 dogs living in foster homes with volunteers of Willamette Humane Society in Salem, Oregon and other local rescue groups, including Senior Dog Rescue of Oregon and Greenhill Humane Society in Eugene, Oregon. Shelter dog subjects consisted of 31 shelter dogs at Willamette Humane Society. All dogs were spayed or neutered prior to participation in the study. Shelter dogs were selected by volunteers, and foster dogs were assigned to foster homes by animal shelter and foster staff. All dogs were eligible for adoption at the time of participation in the study. See Table A1 in Appendix A for a description of all subjects. All procedures were approved by Oregon State University's institutional ethical review boards, animal related procedures were covered under OSU ACUP #4837.

2.2. Human Participants

Foster participants included 20 foster parent volunteers. Although all foster parent volunteers were invited to participate in a second round of testing, only one volunteer participated in a second testing session. (In some cases, testing was not possible because foster dogs were returned to the shelter before sessions could be conducted.) Shelter participants included 20 shelter volunteers that interact with dogs regularly as part of their volunteer duties. Twenty shelter-dog pairs took part in the first round of testing, and all volunteers were invited to participate in a second round of testing with a different dog. A total of 11 volunteers from round one participated in round two of testing. All analyses focusing on dog behavioral testing have been pooled. However, survey measures do not include pooled data to avoid partial dependence within the data set. Each participant provided informed written consent to participate in the study. All procedures were approved by Oregon State University's institutional ethical review boards, human related data collection was covered under IRB #7818.

2.3. Behavioral Tests and Surveys

For dogs residing in foster homes, all testing was conducted at least three days after the dog entered the foster home. Participating shelter volunteers were asked to select a dog that they had interacted with for at least three separate ten-minute sessions, as this has previously been established as a sufficient amount of time for shelter dogs to establish attachment relationships with an unfamiliar person [4]. Testing sessions consisted of a Secure Base Test, designed to assess attachment relationships between dogs and familiar humans, immediately followed by a Paired Attachment Test, which aims to assess preference for a familiar vs. unfamiliar human. Tests were always conducted in this order. While both the Strange Situation Test (SST) and Secure Base Test have been validated for use with dogs, we chose to use the secure base test methodology (modeled after the first tests [24] designed to measure secure base and social preferences of this type) because it has several methodological advantages noted in the prior literature including reduced testing time, a reunion phase by the 'caretaker' that directly follows the alone phase, the elimination of order effects and the focus on the alone and reunion phase which have been found to produce the most reliable results in the human literature [25,26]. Following these behavioral tests, volunteers were asked to fill out a series of surveys, including the Lexington Attachment to Pets Scale.

2.3.1. Secure Base Test (SBT)

All SBT sessions were conducted in a testing room unfamiliar to the dog. All testing sessions took place in a location that was unfamiliar to the dogs. In some cases, foster dogs were tested in the Oregon State Human-Animal Interaction Lab's on-campus testing space, but most dogs were tested at the Willamette Humane Society or Greenhill Humane Society in a novel testing room. Two chairs were placed in the room, and a semi-circle of 1 m in radius was taped on the floor around the chair prior to the beginning of the testing session. Three toys of different types were placed on the floor (outside of the 1-m radius circle) before volunteers and dogs entered the testing room. Toys included a tennis ball with a squeaker, a rope toy and a stuffed toy with a squeaker.

Phase one (Baseline, two minutes): The familiar volunteer was asked to sit neutrally in a chair within the 1 m radius circle. The volunteers were permitted to interact freely (petting, talking, etc.) with the dog (without restraining it) each time the dog placed at least two paws in the circle, but were instructed to sit neutrally if the dog exited the circle. Dogs were able to freely explore the room. The volunteer could play with toys with the dog if the dog brought them to the volunteer inside the circle.
Phase two (Alone, two minutes): The familiar volunteer or adopter exited the testing room so that the dog was left alone.
Phase three (Return, two minutes): The familiar volunteer or adopter re-entered the testing room and the instructions were identical to phase 1 (baseline).

2.3.2. Video Analysis of SBT

Two independent coders reviewed the return phase videos for each dog's SBT and categorize dogs' attachment styles based on patterns of behavior seen in the return phase. A holistic analysis was used for these categorizations (see [5–7]). Inter-rater reliability was assessed based on the percentage of independent agreement after this initial round of coding. After the two coders reviewed each video independently, they watched any videos for which they disagreed on attachment style categorization together and reached an agreement. A description of all attachment style classifications can be found in Table 1. Degree of independent agreement among coders for attachment style was 72%, and a consensus was reached for all dogs when coders reviewed videos together.

Table 1. Holistic coding attachment style definitions (adapted from [5]).

Attachment Style	Definition
Secure	Little or no resistance to contact or interaction. Greeting behavior is active, open and positive. Seeks proximity and is comforted upon reunion, returning to exploration or play.
Insecure ambivalent	Shows exaggerated proximity-seeking and clinging behavior, but may struggle if held by familiar volunteer. Mixed persistent distress with efforts to maintain physical contact and/or physically intrusive behavior directed toward the familiar volunteer. (Dogs who the judges agreed seemed essentially secure but with insecure ambivalent tendencies, were included in the secure group).
Insecure avoidant	May show little/no distress on departure. Little/no visible response to return, ignores/turns away but may not resist interaction altogether (e.g., rests or stands without bodily contact, out of reach or at a distance).
Insecure disorganized	Evidence of strong approach avoidance conflict or fear on reunion, for example, circling familiar volunteer, hiding from sight, rapidly dashing away on reunion, "aimless" wandering around the room. May show stereotypies on return (e.g., freezing or compulsive grooming). Lack of coherent strategy shown by contradictory behavior. "Dissociation" may be observed, that is, staring into space without apparent cause; still or frozen posture for at least 20 s (in the nonresting, nonsleeping dog).

2.3.3. Paired Attachment Test

The Paired Attachment test included the following phases:

Phase 1 (two minutes): A two-minute alone period immediately following the SBT.
Phase 2 (Passive, two-minute phase): The dog's caretaker and a stranger sat neutrally for two minutes in chairs opposite each other surrounded by a 1m radius circle. Each individual was instructed to pet the dog twice each time it entered the circle with at least two paws, but were instructed to otherwise remain neutral.
Phase 3 (Active, two minutes): Both humans were asked to call the dog and provide continuous petting and attention if the dog entered their circle with at least two paws.

Video analysis of Paired Attachment Test

All videos were coded across phases for first person approached (unfamiliar or familiar), duration of proximity seeking with each person, and duration of contact with each person. An ethogram can be found in Table 2. Please see Figure 1 for a picture of the Paired Attachment test set-up.

Table 2. Paired Attachment ethogram.

Behavior	Definition
Proximity seeking	Proportion of the episode in which the dog had at least 2 paws (or half their body) within the 1 m radius circle the human was sitting in.
Dog-human contact	Proportion of the episode in which the dog or human engaged in physical contact with the other individual. Contact must be in circle to count. Sniffing and body touches count as contact.

Figure 1. Paired attachment test set-up.

2.3.4. Disinhibited Attachment Coding

Based on the methods used in [9], we developed a scale to assess disinhibited attachment using rankings of different measures that were combined into a composite score. We assigned rankings to each dog based on the proportion of the return phase of the SBT spent seeking proximity to the familiar person. Because severe disinhibited attachment is associated with a lack of proximity seeking with a familiar person, the highest proportion of time spent in proximity to the familiar person received the lowest rankings. Inter-rater reliability for this measure was 75%. In addition, we assigned rankings based on total amount of time spent in proximity (within the 1-m radius circle taped on the floor around the chair) to the unfamiliar person across both phases of the Paired Attachment Test. Inter-rater reliability for total proportion of time spent in proximity to the unfamiliar person was 93.8%. Low proximity seeking with the unfamiliar person received low rankings on the scale; higher proportions of time spent proximity seeking received higher rankings. Across all measures, in the case of a tie, those values were assigned the same rank. For instance, if two dogs spent 98.3% of the session seeking proximity to the familiar person, they would receive the same rank. Both scores were summed to create an overall disinhibition score.

2.4. Statistical Methods

All statistical analyses were conducted using R Studio (version 1.1.463). All statistics were two-tailed with an alpha level of $p < 0.05$.

2.4.1. Attachment Analysis

A Fisher's Exact Test was used to compare proportions of securely and insecurely attached dogs within foster and shelter groups. A Chi Square test was used to compare the proportions of attachment styles seen in the foster and shelter populations to expected frequencies of proportions of pet dogs based on all published literature categorizing attachment styles in pet dogs [5–7]. A McNemar's Test was used to compare dogs' attachment styles for shelter volunteers who participated in two separate rounds of testing.

2.4.2. Paired Attachment Analysis

Normality was assessed using the Shapiro–Wilk Test. For shelter and foster groups, all proximity and contact data were not normally distributed ($p < 0.05$). For both foster and shelter groups, a Kruskal–Wallis test was used to assess whether differences in proximity or contact seeking were present with respect to whether the humans were passively interacting with the dogs (two pets every time the dog enters each person's respective circle) or actively encouraging interaction from the dogs. Post hoc comparisons were made using Wilcoxon Signed-Rank Tests for within-subject comparisons across phases. Fisher's Exact tests were used to determine whether the first person approached (unfamiliar or familiar) varied according to dog source (shelter or foster) or attachment style (insecure or secure) for active and passive phases. Mann–Whitney *U*-tests were used to compare total amount of time spent with the familiar person vs. the unfamiliar person across both passive and active phases for both populations (shelter and foster) and attachment style (secure vs. insecure). A difference score was calculated to assess overall preference for a familiar human vs. an unfamiliar human by subtracting the total proportion of time spent with an unfamiliar person across active and passive phases from the total proportion of time spent with a familiar person across active and passive phases, and these data were not normal, $p < 0.05$. See Table 3 for inter-rater reliability scores for all measures. Percent agreement between two coders was assessed using an 8% tolerance.

Table 3. Inter-rater reliability for all Paired Attachment measures.

Measure	Percent Agreement between Two Coders
Familiar human: proximity seeking (duration)	95.3%
Familiar human: contact (duration)	87.5%
Unfamiliar human: proximity seeking (duration)	100%
Unfamiliar human: contact (duration)	92.2%

2.4.3. Disinhibited Attachment Analysis

Normality of disinhibited attachment scores was assessed using the Shapiro–Wilk test, $p > 0.05$. *T*-tests were used to compare disinhibition scores across four categories: insecure shelter dogs, secure shelter dogs, insecure foster dogs, and secure foster dogs. We also used Mann–Whitney *U*-tests for non-normally distributed data for proportion of time spent in proximity to familiar person for analyses by group and by attachment style.

2.4.4. Lexington Attachment to Pets Scale (LAPS) Analysis

Normality was assessed using the Shapiro–Wilk test and LAPS data were normal, $p > 0.05$. Because assumptions of normality were not violated, parametric statistics were used to compare between groups. A two-way ANOVA with attachment category and environment (foster vs. shelter) and possible interactions was used to analyze LAPS data. A Pearson's Correlation was used to determine whether there was a relationship between LAPS score and overall preference score for a familiar vs. unfamiliar person. Only data from the first round of shelter and foster volunteer participation were used for these comparisons, in order to avoid any confounding effects of volunteers who participated in both rounds. A Pearson's Correlation was used to assess whether scores from shelter volunteers' first participation were related to LAPS scores from shelter volunteers' second participation.

3. Results

3.1. Attachment

Within the foster group, a total of twelve dogs (57.14%) were categorized as secure and nine dogs (42.86%) were categorized as insecure (eight dogs (38.10%) were categorized as insecure-ambivalent, and one dog (4.76%) was categorized as insecure-disorganized). Within the shelter group, a total of twelve dogs (38.71%) were categorized as secure and a total of nineteen dogs (61.29%) were categorized as insecure (sixteen shelter dogs were scored as insecure ambivalent (51.61%), and three insecure shelter dogs (9.68%) were scored as insecure avoidant). The Fisher's Exact test comparing the proportion of insecure and secure dogs in the shelter and foster groups was not significant ($p = 0.26$). See Figure 2 for a comparison of attachment styles in each population.

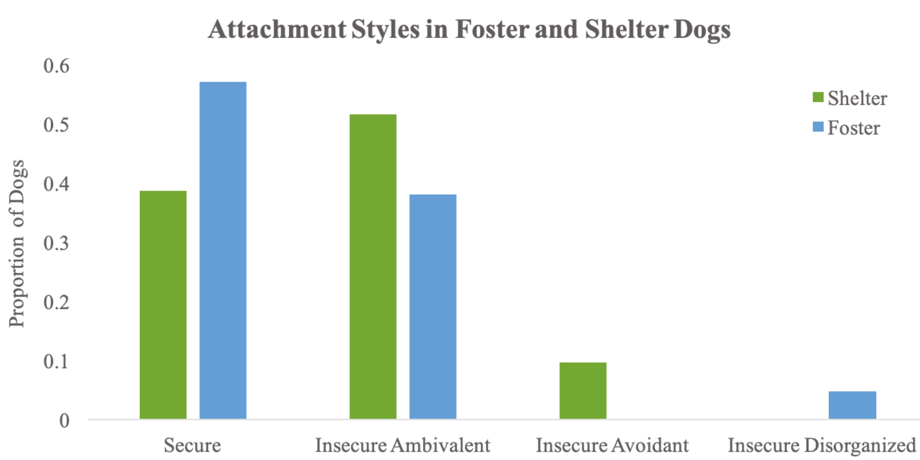

Figure 2. Proportion of dogs categorized into each attachment style for foster and shelter dog populations.

To obtain an overall picture of how each population of foster and shelter dogs, respectively, compared to pet dogs in terms of proportions of attachment styles, we summed data from all published literature involving categorization of attachment styles in pet dogs for each category of attachment styles (secure, insecure-ambivalent, insecure-avoidant, and insecure-disorganized) [5–7]. Only data from dogs in the saline condition were used for [6], a study which included a counterbalanced repeated measures design in which oxytocin was administered during one testing session and saline upon another visit prior to participating in the secure base test, to avoid dependence (i.e., if we did not exclude the oxytocin sessions, we would be using data for the same dogs twice) and any effect of oxytocin on behavior. Across previously published studies, 68% of pet dogs had been categorized as having a secure attachment to their primary caretaker and 32% of pet dogs had been categorized as displaying a type of insecure attachment. The proportion of secure shelter dogs significantly differed from what was expected when compared against previously published attachment outcomes in pet dogs, χ^2 (1, N = 31) = 12.22, p = 0.0005 (Figure 3). No significant differences were found when proportions of attachment styles for the foster dog group were compared to pet dog attachment style proportions, $p > 0.05$ (Figure 3).

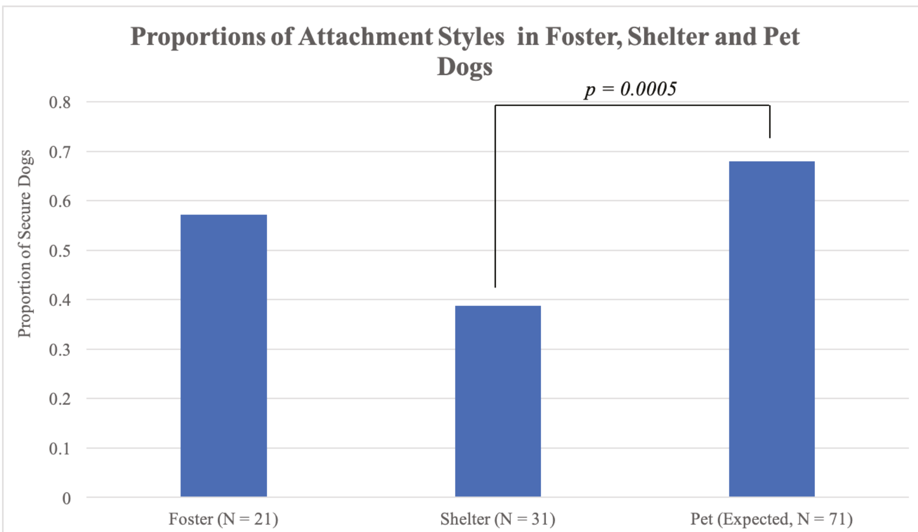

Figure 3. Proportions of observed foster and shelter dogs categorized as secure, compared to expected proportions of pet dogs with secure attachments based on published literature [5–7].

No significant differences were found in terms of comparisons of attachment styles among dog–volunteer dyads for shelter volunteers who participated in two rounds of testing with two different dogs, p = 0.62.

3.2. Paired Attachment

A trend was found for proportion of time shelter dogs spent seeking proximity to both the familiar and unfamiliar person when all conditions (familiar passive, familiar active, unfamiliar passive, unfamiliar active) were compared to each other, H (3) = 7.80, p = 0.05. A significant difference was found with respect to the proportion of time spent in contact across all conditions, H (3) = 23.103, $p = 3.84 \times 10^{-5}$. This difference was driven by the finding that shelter dogs spent significantly more time in contact with both the familiar person (W = 456, $p < 0.001$) and the unfamiliar person (W = 68, p = 0.001) in the active phase of the sociability test when compared to the passive phase. No significant differences were found in terms of proportion of time spent in contact with the unfamiliar person vs. the familiar person ($p > 0.05$ for both the passive and active phases).

For the foster group, a significant effect was found with respect to the proportion of time spent in proximity across all conditions, H (3) = 11.49, p = 0.01. A trend was present with respect to the proportion of time foster dogs spent in proximity to the familiar person compared to the unfamiliar person in the active phase, W = 165, p = 0.09. The median proportion of time foster dogs spent with the familiar person in the active phase was 0.52, and the median proportion of time spent in proximity to the unfamiliar person in the active phase was 0.28. A trend was also found within the foster group with respect to proportion of time spent in contact H (3) = 9.23, $p = 0.03$. After Bonferroni correction, the significance threshold for the Kruskal–Wallis test was 0.0257.

Based on the overall score for time spent with the familiar person vs. time spent with the unfamiliar person across phases, no significant differences were found with respect to group (foster vs. shelter) or attachment style (secure vs. insecure). In addition, we analyzed whether differences were present with respect to overall time spent in proximity to the familiar person compared to the unfamiliar person, and no significant differences were found with respect to group or attachment style, $p > 0.05$. No significant differences were found with respect to first person approached for active or passive phases when shelter and foster dogs were compared across groups, $p > 0.05$.

3.3. Disinhibited Attachment

Insecure foster dogs had the lowest disinhibited attachment rankings, with a mean of 22.0. Insecure shelter dogs had the second lowest scores on average with a mean score of 36.42. The mean score for the secure foster group was 47.17 and the mean score for the secure shelter group was 48.75. Insecure foster dogs displayed significantly lower mean disinhibition scores than secure foster dogs, $t(18.99) = -3.5499$, $p = 0.002$. Insecure foster dogs also displayed significantly lower mean scores of disinhibited attachment compared to secure shelter dogs, $t(16.33) = -4.64$, $p = 0.0003$. Furthermore, insecure foster dogs scored significantly lower on disinhibition than insecure shelter dogs, $t(21.57) = -2.2835$, $p = 0.03$. In addition, insecure shelter dogs displayed significantly lower disinhibited attachment scores than secure shelter dogs, $t(28.97) = -2.184$, $p = 0.04$. No significant differences were present with respect to insecure shelter dogs and secure foster dogs, $p > 0.05$. In addition, we did not find significant differences between secure foster dogs and secure shelter dogs on disinhibition, $p > 0.05$. See Figure 4 for mean scores on disinhibited attachment among foster and shelter dogs with secure and insecure attachments.

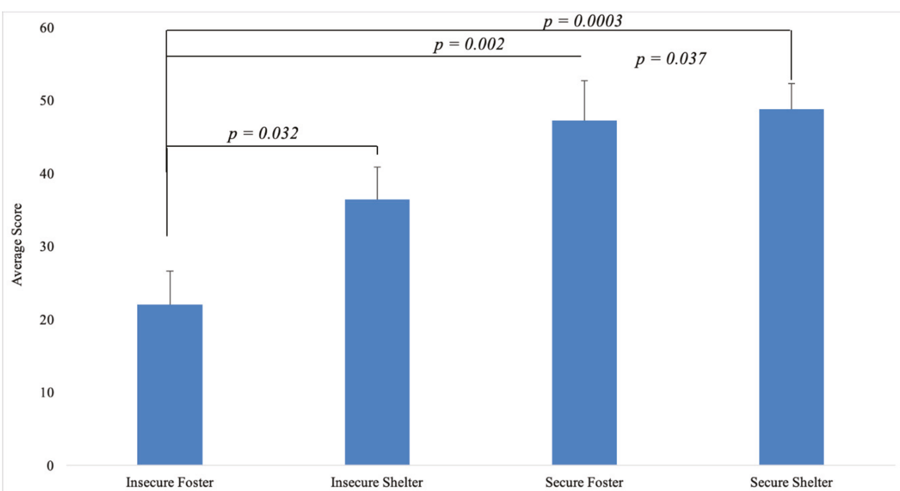

Figure 4. Mean disinhibited attachment scores for foster and shelter dogs with insecure and secure attachment styles. Error bars indicate standard error of the mean.

3.4. Lexington Attachment to Pets Scale (LAPS)

In terms of the results of the two-way ANOVA analysis of LAPS scores, no significant main effect or interaction effect was found, F (1, 36) = 0.20, $p > 0.05$ for attachment style categorization or group (shelter/foster). The average LAPS score for shelter volunteers was 15.45 and the average LAPS score for foster volunteers was 20.40. This difference was not statistically significant, $t(37.96) = 1.11$, $p > 0.05$. The average LAPS score for insecure dogs was 18.60 and the average LAPS score for secure dogs was 17.25. This difference was not statistically significant, $t(37.16) = 1.11$, $p > 0.05$. Based on comparisons between first participation and second participation for the 11 volunteers that participated twice, there was a positive correlation between LAPS scores on each round, $r = 0.62$, $p = 0.04$. With respect to the familiar person in the active phase, there was a significant positive correlation between the amount of time spent proximity seeking and the LAPS score, $r = 0.35$, $p = 0.03$. There was not a significant correlation between LAPS scores and the amount of time spent seeking proximity with the familiar person in the passive phase, $p > 0.05$.

There was a significant positive correlation between a dog's preference score (calculated by subtracting the total proportion of time spent with an unfamiliar person across active and passive phases from the total proportion of time spent with a familiar person across active and passive phases) for the familiar person compared to an unfamiliar person on the Paired Attachment Test and the strength of attachment reported by the caretaker/familiar person on the LAPS survey, $r = 0.34$, $p = 0.03$ (Figure 5).

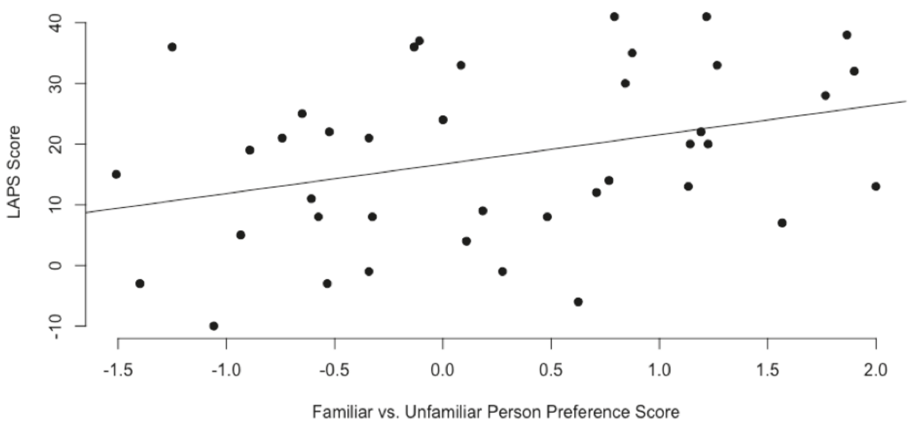

Figure 5. Scatterplot for overall score for preference for a familiar human compared to an unfamiliar human compared to LAPS scores. Positive preference scores indicate a preference for the familiar human; negative preference scores indicate a preference for the unfamiliar human. Higher LAPS scores indicate a stronger degree of attachment; lower LAPS scores indicate a weaker degree of attachment, as reported by familiar volunteers. This suggests that there is a relationship between the strength of attachment human volunteers feel for a dog and the amount of preferential proximity seeking that dog displays in an attachment, however the direction and causality of the relationship is unknown.

4. Discussion

To our knowledge, this is the first application of attachment style categorization in human–dog relationships in foster and shelter settings. We explored the proportions of attachment relationships seen among foster and shelter volunteers and dogs in foster and shelter settings, and also explored disinhibited attachment in these populations. Our findings indicate that the proportion of secure attachment styles in shelter dogs included in this study were significantly lower than the proportion of secure attachment styles previously reported for pet dogs. Conversely, foster dogs formed secure attachments to their caretakers at rates more similar to those reported in dog–owner relationships, although no significant differences were found between proportions of attachment styles when comparing between foster and shelter dogs directly [5–7].

The finding that shelter dogs are significantly more likely than pet dogs (based on data from previously published literature) to form insecure attachments to their temporary caretakers is of note, especially given the high rate of insecure ambivalent attachments observed. This attachment style is associated with excessive proximity seeking upon return, even though this contact is less effective at reducing stress than in secure attachment relationships. Given that previous research has shown that lying in proximity to adopters positively influenced dog adopter decisions in a shelter setting [27], it is possible that attachment styles aligned with greater proximity seeking could have some benefit in this environment. For example, dogs behaving ambivalently might be perceived as more social and therefore be more attractive to potential adopters. However, more research should be done before conclusions can be drawn. Future research could explore whether attachment styles formed with familiar volunteers in the SBT corresponds to behavior in adopter-dog interactions at the shelter. Future studies could also explore attachment relationships between dogs that have been rehomed from foster and shelter settings and their adoptive owners, as this has not been previously explored.

We also found evidence of disinhibited attachment among dogs in foster homes and shelter settings. We expected to see higher levels of disinhibition among dogs displaying secure attachments within these settings (as that would be consistent with the human literature), and indeed, the highest levels of disinhibition were exhibited among secure shelter and foster dogs and the lowest levels among insecure shelter and foster dogs. Previous work has shown that disinhibited attachment was also present in human orphans from Romanian orphanages who had experienced institutional deprivation and children adopted in the UK who had no exposure to institutional deprivation [9]. Thus, disinhibited attachment could be a product of the temporary nature of relationships with caregivers, traumatic experiences early in life associated with becoming an orphan, or other factors beyond the quality of the environment. Shelter and foster dogs with secure attachments may show higher levels of disinhibition because it allows for greater social flexibility within these environments, which could provide advantages in terms of greater likelihood of being taken for walks, greater chances of interaction with potential adopters, or more social attention from volunteers. Experiences prior to placement in a foster home or a shelter could also contribute to the development of disinhibited attachment, and further research is needed in this area to better understand the development and implications of disinhibited attachment in foster and shelter dogs. It should also be noted that the dogs in the shelter environment receive a great deal of enrichment and social interaction, including play groups with conspecifics, training opportunities, and several walks each day. Future studies should assess disinhibited attachment in kenneled dogs that do not receive as many opportunities for enrichment and social exposure to both humans and conspecifics. We also found that insecure dogs spent a greater proportion of time in proximity to the familiar person during the SBT than secure dogs. Although disinhibited attachment has not previously been reported in canine attachment literature, it appears to be relevant for dogs in shelter and foster settings. To date no research has been done on disinhibition in pet dogs, so it is unknown to what degree these results may differ from pet dog populations, but such comparisons merit further investigation. Furthermore, additional aspects of disinhibition could be explored. For example, in the current study, we did not look at the dog's willingness to leave with the stranger. This would be an interesting additional condition for future studies, since it is one trait often noted in human children exhibiting disinhibited attachment disorders.

The results of the paired attachment test suggest that shelter dogs are more likely to seek contact depending on attentional state (i.e., whether the person is actively interacting with the dog/encouraging attention or sitting passively and petting the dog twice each time it enters the 1m radius circle surrounding the person's chair), while foster dogs are more likely to seek proximity regardless of whether the familiar volunteer or unfamiliar person are actively attending to the dog. We found that shelter dogs were attentive to the attentional state of the person to a greater degree than foster dogs. It is possible that although foster homes may allow for an easier transition to an adoptive home environment, that dogs living in foster homes may be less attuned to human attentional state. Shelter dogs, on the other hand, typically spend more time in social isolation than foster dogs, and paying close attention to the attentional state of humans may provide benefits, such as increased socialization, exercise, or interactions with adopters. Furthermore, no significant differences were found with respect to contact or proximity seeking when comparisons were made based on attachment style. It should also be noted that no differences were found with respect to the first person approached by either shelter or foster dogs. Overall, no differences were found between foster and shelter dogs based on preference for an unfamiliar or a familiar person in the paired attachment test. We did not find any significant differences in terms of attachment style and preference for an unfamiliar person compared to a familiar person. This indicates that both shelter and foster dogs show flexibility in terms of social interaction.

We found a significant correlation between LAPS scores, which measured the attachment strength of shelter and foster human volunteers, and a dog's preference for that volunteer versus an unfamiliar human in the Paired Attachment test. This indicates that familiar volunteers whose dogs exhibited a stronger preference for them (i.e., dogs who spent more time in proximity to the familiar person across phases), also reported a stronger attachment to their chosen dog (or vice versa). While the causality of this relationship cannot be determined from the current data, this connection warrants further investigation in the future, although regardless of causality, this finding is interesting as it indicates reciprocity in the attachment relationship.

The reported strength of attachment by volunteers (LAPS scores) did not differ between shelter and foster volunteers. Although we expected that foster volunteers would report a greater degree of attachment to foster dogs than shelter volunteers to shelter dogs, this was not the case. Thus, it is possible that they were primed to think of themselves as having a stronger attachment to the dog that they chose for the study than they would have naturally. Future studies could examine LAPS ratings for shelter volunteers more generally, to determine if the level of attachment reported towards dogs in this study was unique (and possibly due to the experimental context) or if shelter volunteers commonly feel highly bonded to shelter dogs. Another possibility is that foster volunteers may try to avoid forming strong attachment bonds with foster dogs, to protect their own emotions or to protect the dog from perceived feelings of loss when eventually rehomed. Such strategies might also be used to reduce temptation to adopt the foster dog. Future research could measure LAPS scores in relation to several foster dogs, including foster volunteers who did and did not adopt their foster dogs, in order to determine whether strength of attachment, as reported by the person, plays a role in decision-making regarding adoption of foster dogs by their foster parent volunteers. It is also of note that the correlation between LAPS scores and proximity seeking towards volunteers appears to be driven by the active phase of the test, where the volunteer can call and interact with the dog. This suggests that the attachment strength and behavior of the familiar person may be influencing the dogs' attachment behavior, but more studies are needed to be sure. While future research should directly evaluate long term outcomes of foster and shelter dogs who formed secure and insecure attachment bonds to transitional caretakers, in the human literature, orphaned children who develop secure bonds to a foster parent are more likely to form secure social bonds later in life [20]. If this proves to be true of dogs as well, this would suggest that fostering may provide an additional benefit by increasing the likelihood of a dog establishing a secure attachment prior to final adoption.

It should be noted that differences were not found with respect to attachment styles of dogs for volunteers who participated in the first and second round of testing. However, only eleven shelter volunteers participated in the first and second round of testing, resulting in a relatively small sample size. For six of eleven participants, the dogs that they participated in the SBT with in the first round were insecurely attached. Based on these data, we cannot conclude whether the attachment styles dogs presented with in the SBT were due to the nature of their interactions with the volunteers, or due to personality traits of the dogs, or a combination of these factors. It would also be interesting to evaluate whether volunteer personality plays a role in dog selection. For instance, do volunteers with certain personality traits, such as higher anxiety levels, choose more anxious dogs? Research with owners of pet dogs has shown that attachment avoidance in people on the Adult Attachment Scale is associated with increased occurrence of separation anxiety in their dogs [28].

Another finding with respect to LAPS scores was that no differences were present with respect to attachment security. It is possible that volunteers are not attuned to attachment style-related behaviors, and therefore evaluate attachment to shelter and foster dogs based on other observations. It is also possible that some volunteers prefer dogs who are securely attached and others prefer dogs who are insecurely attached. Future research could evaluate the attachment styles of dogs owned by volunteers in conjunction with SBT results between volunteers and dogs in foster and shelter settings, in order to gain a better understanding of the role of the human in forming and maintaining attachment relationships in these contexts. Further research is needed to determine whether LAPS scores correspond to attachment style. Also, of note is that even with a relatively small sample size of 11 volunteers who participated in both rounds of testing in the shelter setting, there was a trend of a significant correlation between LAPS scores in each round. This suggests that volunteer personality and individual framework for attachment relationships may play a role in the formation and maintenance of social bonds between volunteers and dogs in animal shelters. It is also important to note that volunteers have many opportunities to interact with a variety of dogs, as dogs are typically adopted out relatively quickly from the shelter at which this study was conducted, and thus, it is remarkable that volunteers form strong attachment bonds to dogs over a relatively short time period. It is also possible that volunteers with lower LAPS scores may attempt to avoid developing attachment bonds to individual dogs at the shelter, and this could be a coping mechanism to help prevent compassion fatigue.

5. Conclusions

We have shown that shelter dogs differ significantly from a meta-analysis of pet dogs with respect to proportions of attachment styles, while no differences were found between attachment proportions of shelter and foster dogs. This provides evidence that social relationships formed between foster parent volunteers and foster dogs in the foster home may be more similar in nature to those formed in the typical home environment of pet dogs. Therefore, fostering dogs may provide an important additional benefit, by increasing the likelihood that a dog will experience the establishment of a secure attachment to a caretaker before final adoption. In studies with human children, this scenario not only allowed for better coping with stress in the short term, but also increased the likelihood that the child would develop a secure attachment to their caretaker in their final home upon being adopted. More research is needed to evaluate if the same is true for adopted foster dogs. Additionally, disinhibited attachment, characterized by excessive friendliness towards unfamiliar people, lack of discrimination among adults based on social familiarity, and lack of checking in with an attachment figure during a stressful situation in humans, is present in shelter and foster dogs. We also found preliminary evidence that would suggest a relationship between how attached a human volunteer feels towards a dog in their care and the dog's behavior towards that person, however more research is needed to determine if a causal relationship exists, and if so, the direction of that relationship.

Author Contributions: Conceptualization, L.E.T. and M.A.R.U.; methodology L.E.T. and M.A.R.U.; performed experiments and statistical analyses, L.E.T.; writing—original draft preparation, L.E.T.; writing—review and editing L.E.T. and M.A.R.U.; funding acquisition, L.E.T. and M.A.R.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Maddie's Fund.

Acknowledgments: Thank you to Maddie's Fund for funding this study. We are grateful to the volunteers, shelter staff, and dogs who participated in this research. Thank you to Willamette Humane Society, Greenhill Humane Society, and Senior Dog Rescue of Oregon for assistance with participant recruitment and the use of facilities for data collection. Thank you in particular to Betsy Bode and Janine Catalino for their help with coordinating data collection. This project would not have been possible without the help of several research assistants in the Human-Animal Interaction Lab, particularly Holly Duvall and Hadley Schoderbeck. We are grateful to Giovanna Rosenlicht, Alexandra Protopopova and Frank Bernieri for their advice during study design and helpful comments on a previous version of this manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Subjects' demographic information, including names, groups, ages, modes of intake, length of stay (LOS), and breed (as listed by their associated shelter or rescue group). Several of the dogs in the study were returned; length of stay was calculated based on initial intake date and final adoption date. All dogs were spayed and neutered prior to participating in the study. Length of stay data were not available for some dogs that participated, and in a few cases (noted below), dogs were still available for adoption at the completion of the study. All breed determinations were based on visual inspection by the participating shelters and rescue groups, and it is important to note that reliability for this method is low [29].

Dog's Name	Group	Age	Mode of Intake	LOS	Breed
Whisper	Foster	8 years	Transfer	32	Mixed breed, medium
Tilly	Foster	5 years	Transfer	46	Mixed breed, small
Jazzy	Foster	1 year	Unknown	Unknown	Greyhound
Jupiter	Foster	2 years	Transfer	16	Mixed breed, medium
Maddie	Foster	4 months	Surrender	43	Mixed breed, small
Opal	Foster	2 years	Transfer	154	Mixed breed, medium
Ginger	Foster	2 years	Transfer	57	Mixed breed, medium
Hera	Foster	5 years	Transfer	70	Mixed breed, medium
Helios	Foster	2 months	Transfer	62	Mixed breed, medium
Mackenna	Foster	Unknown	Unknown	Unknown	Mixed breed, medium
Sierra	Foster	Unknown	Unknown	Unknown	Mixed breed, medium
Brisa	Foster	8 years	Surrender	Unknown	Mixed breed, large
Skipper	Foster	5 months	Transfer	52	Mixed breed, medium
Remy	Foster	1 year	Unknown	120+ (still available for adoption)	Mixed breed, medium
Lux	Foster	2 years	Unknown	Unknown	Greyhound
Lillie	Foster	13 years	Unknown	Unknown	Mixed breed, small
Dr. Zeuss	Foster	4 years	Stray	107	Mixed breed, large
Panda	Foster	4 years	Unknown	Unknown (still available)	Mixed breed, medium
Bella	Foster	8 years	Unknown	Unknown (still available)	Pomeranian
Nellie	Foster	9 years	Stray	23	Shih Tzu
Stormy	Foster	Unknown	Unknown	unknown	Mixed breed, medium
Bandit	Shelter	2 years	Surrender	87	Mixed breed, large
Biscay	Shelter	2 years	Transfer	147	Mixed breed, medium
Brownie	Shelter	5 years	Stray	30	Mixed breed, large
Charlie	Shelter	5 years	Surrender	134	Mixed breed, large
Cheyenne	Shelter	8 months	Transfer	8	Mixed breed, medium
Chico	Shelter	5 years	Surrender	31	Mixed breed, large
Clooney	Shelter	1.5 years	Stray	19	Mixed breed, large
Dakota	Shelter	5 years	Surrender	83	Mixed breed, large
Floki	Shelter	1 year	Surrender	69	Mixed breed, large
Gemma	Shelter	2 years	Surrender	35	Mixed breed, large
Hoagie	Shelter	3.5 years	Transfer	427	Mixed breed, large
Hunny	Shelter	7 years	Surrender	32	Mixed breed, small
Jordy	Shelter	1 year	Transfer	27	Mixed breed, small
Luna	Shelter	3 years	Surrender	49	Mixed breed, large
Maizie	Shelter	5 years	Transfer	6	Mixed breed, small
Lincoln	Shelter	1 year	Transfer	200	Mixed breed, large
Mari	Shelter	2 years	Surrender	159	Mixed breed, large
Numair	Shelter	7 years	Surrender	64	Mixed breed, small
Smallz	Shelter	10 years	Transfer	23	Mixed breed, small
Jacob	Shelter	6.5 years	Surrender	48	Mixed breed, small
Elfie	Shelter	4 years	Surrender	141	Mixed breed, large
Champ	Shelter	5 years	Surrender	85	Mixed breed, large
Daisy	Shelter	7 years	Surrender	39	Mixed breed, medium
Molly	Shelter	3 years	Surrender	69	Mixed breed, medium
Hans	Shelter	3.5 years	Surrender	40	Mixed breed, large
Heidi	Shelter	1.5 years	Transfer	126	Mixed breed, large
Charlie Boy	Shelter	3.5 years	Surrender	57	Mixed breed, small
Brinx	Shelter	4.5 years	Surrender	177	Mixed breed, medium
Peaches	Shelter	1.5 years	Surrender	38	Mixed breed, large
Carly	Shelter	4 years	Transfer	200	Retriever mix
Starlord	Shelter	1 year	Stray	39	Mixed breed, small

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animals

Article

Sex of Walker Influences Scent-marking Behavior of Shelter Dogs

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Received: 13 March 2020; Accepted: 3 April 2020; Published: 7 April 2020

Simple Summary: In diverse settings, human presence and handling influence the behavior and physiology of other animals, often causing increased vigilance and stress, especially if the human is unfamiliar. Domestic dogs are unusual in that human interaction often reduces stress and behavioral signs of stress. Nevertheless, there is some evidence that the sex of an unfamiliar person can influence canine behavior. To determine whether sex of an unfamiliar walker might influence the behavior of dogs at an animal shelter, we observed 100 dogs during leash walks and recorded all occurrences of scent-marking behaviors. Male dogs urinated at higher rates when walked by unfamiliar women than when walked by unfamiliar men. Female dogs urinated at similar rates when walked by unfamiliar men and unfamiliar women. Sex of walker also influenced urinary posture in male dogs. Both male and female dogs were more likely to defecate when walked by unfamiliar women than when walked by unfamiliar men. Based on our findings, and those of others, we suggest that the sex of all observers and handlers be reported in behavioral studies of dogs and considered in behavioral evaluations at animal shelters, where results can impact whether or not a dog is made available for adoption.

Abstract: Interactions with humans influence the behavior and physiology of other animals, and the response can vary with sex and familiarity. Dogs in animal shelters face challenging conditions and although contact with humans typically reduces stress and behaviors associated with stress, evidence indicates that shelter dogs react differently to unfamiliar men and women. Given that some aspects of canine scent-marking behavior change under fearful conditions, we examined whether sex of an unfamiliar walker would influence scent-marking behavior of 100 shelter dogs during leash walks. Male dogs urinated at higher rates when walked by unfamiliar women than when walked by unfamiliar men; female dogs urinated at similar rates when walked by unfamiliar women and unfamiliar men. Sex of walker influenced urinary posture in male dogs, but not in female dogs. Both male and female dogs were more likely to defecate when walked by unfamiliar women than by unfamiliar men. Based on our findings that shelter dogs behave differently in the presence of unfamiliar men and women, we suggest that researchers conducting behavioral studies of dogs record, consider in analyses, and report the sex of observers and handlers as standard practice. We also recommend recording the sex of shelter staff present at behavioral evaluations because the results of these evaluations can impact dog welfare.

Keywords: dog; scent marking; urination; urinary posture; defecation; ground scratching; animal shelter; human-animal interactions

1. Introduction

Human presence and handling can affect the behavior and physiology of other animals, including species living in the wild [1–4] and in captive settings, such as zoos [5,6], farms [7–9], and research laboratories [10]. Such effects often depend on the number of people present as well as their distance, behavior, and familiarity [11–15]. Additionally, human physical characteristics, including sex and age, can influence the behavior and physiology of other animals [10,16]. Laboratory rats and mice discriminate human sex using olfactory stimuli [10] and free-ranging elephants discriminate human sex and age using acoustic cues in voices [16]. In many interactions between humans and other animals, humans are perceived as either predators or at least as something to be feared [16,17], thus, human presence often causes increased vigilance, avoidance, and stress.

Domestic dogs present a somewhat special case in which contact with humans typically reduces both stress and the performance of behaviors associated with stress [18–26]. This has been shown in animal shelters where dogs experience challenging conditions, such as isolation, lack of control, and exposure to unfamiliar people, dogs, and surroundings. For shelter dogs, various forms of physical contact with humans (e.g., petting, massaging, and grooming) and different types of interactions with humans (e.g., walks, play sessions, training sessions, and simply having a person sit passively in the same enclosure), have been shown to reduce physiological measures of stress [18–20], produce favorable changes in behavior [21], or both [22–26].

Despite the general pattern that human contact has positive effects on shelter dogs, there is evidence that dogs respond differently to men and women. Shelter dogs enrolled in a human interaction program improved in their sociability toward unfamiliar women but not toward unfamiliar men [26]. When an unfamiliar man or woman stood in front of cages for a few minutes, shelter dogs decreased to a greater extent the time they spent barking and looking at the person when the unfamiliar person was female [27]. An initial report that shelter dogs petted by females had lower cortisol levels than those petted by males [28] was later found to reflect subtle differences in petting techniques of males and females: when men and women received specific training to standardize petting techniques, male and female petters reduced cortisol levels to similar degrees [20,29]. Differential responses to men and women also have been documented for dogs in settings other than animal shelters. For example, dogs in a guide dog training program made more frequent contact with unfamiliar women than with unfamiliar men [30] and during agility competitions, dogs with male handlers experienced greater increases in cortisol than did dogs with female handlers [31]. Finally, from a study in a commercial kennel, male dogs spent less time near an unfamiliar man than an unfamiliar woman, whereas female dogs spent equal amounts of time near an unfamiliar man and an unfamiliar woman; a similar pattern occurred for the frequency of direct body contact [32]. Although the responses of dogs to male and female humans have been studied in diverse settings and ways, we could find no information on how sex of an unfamiliar walker might influence the behavior of dogs during leash walks.

In the present study, we examined whether sex of an unfamiliar walker influenced scent-marking behavior of mature male and female shelter dogs during walks on a leash. Leash walking is commonly used by shelters to provide dogs with opportunities to exercise, socialize with humans, and perform species-typical behaviors, such as sniffing and urine-marking. At least one aspect of canine scent-marking behavior is sensitive to fearful or stressful conditions: adult male dogs that used the raised-leg urinary posture typical of mature males temporarily reverted in fearful situations to using the juvenile lean-forward posture in which all four feet remain on the ground [33,34]. Consistent with this finding, we reported that the percent of urinations in which adult male dogs used the raised-leg posture was lower in our study shelter (73%; [35]) than reported for mature male dogs living under other conditions (94%–97%; [36–39]). We found a similar effect for female dogs: 6% of urinations by adult females involved raising a hindlimb at our study shelter [35] compared with 19%–37% for adult female dogs living under other conditions [36–39]. These observations suggest that monitoring scent-marking behavior of dogs during walks might be a useful way to assess how shelter dogs respond to the sex of an unfamiliar walker. Given that dogs generally respond less favorably to unfamiliar men than unfamiliar women [26,27,30] and that this response can be stronger in male dogs [32], we predicted that the frequency of scent marking behaviors would be lower when dogs were walked by an unfamiliar male than by an unfamiliar female, and that such reductions would be more dramatic in male dogs than in female dogs. We predicted reductions in scent marking behaviors of mature dogs walked by unfamiliar men because mature male dogs reverted to using the juvenile urinary posture in fearful situations [33,34] and less frequent urination, defecation, and ground scratching represent the pattern of scent marking shown by juvenile shelter dogs during leash walks [40]. Finally, we predicted that dogs walked by an unfamiliar male would be more likely to use postures in which all feet remain on the ground (i.e., the lean-forward posture in males and the squat posture in females).

2. Materials and Methods

The data presented here were collected at the Tompkins County SPCA in Ithaca NY, USA, between September 2017 and December 2019, as part of a long-term research program on scent-marking behavior of shelter dogs. Tompkins County SPCA is a no-kill shelter with open-admission and scheduled intake. The shelter has very active volunteer programs for both cats and dogs. Dog volunteers must be at least 18 years old and can be either canine companions or dog walkers. Canine companions help socialize and train dogs in their cubicles, sit with them, and pet and groom them. Dog walkers take the dogs out for walks or to a large outdoor enclosure (Section 2.1). As time permits, volunteer dog walkers sometimes engage in canine companion activities as well. A one-time snapshot of dog volunteers at the end of our study showed that 71% (58/82) were women and 29% (24/82) were men. Although the numbers of male and female staff members are complicated by the variation in the extent of direct interaction with the dogs and the needs of individual dogs (e.g., some dogs may have extensive interactions with Medical Staff, whereas others have much less), staff was also female-biased in most positions over the course of our study (e.g., Animal Care Technicians, 10 females and one or two males; Medical Staff, all females except for one male intern in the past 6 months; Adoptions, Intake, and Behavior Program, approximately equal number of females and males).

2.1. Dogs and Housing

We observed 100 dogs (57 males and 43 females) that were at least one year old (Mean ± *SD*, 4.4 ± 3.4 years; range, 1–17 years). Housing and care of dogs have been described elsewhere [41], thus, we provide a brief description here. Most dogs in our study were mixed breeds. We did not have access to DNA analyses or pedigrees; thus, the number of purebred dogs is unknown. Dogs were either surrendered by owners (n = 44), transferred from other shelters (n = 24), picked up as strays (n = 18), or returned by adopters (n = 12); two dogs were seized by animal control officers. All dogs received veterinary care at intake (e.g., vaccinations, flea control, fecal exam and deworming, heartworm test, and any additional diagnostic tests deemed necessary). Dogs without a microchip received one. Screening blood work, including complete blood count/chemistry profile, was routinely run for older dogs. If owners provided information about urinary issues at the time of surrendering their dog to the shelter or if symptoms of disease were observed in the shelter (e.g., frequent urination), then urinalysis was performed for dogs of any age. We excluded from our study dogs with known medical issues. About 3 days after intake, dogs underwent behavioral evaluation by Behavior Program staff [42,43]. All dogs had received veterinary care, undergone behavioral evaluation, and were on the adoption floor by the time we walked them. Dogs on the adoption floor wore buckle or martingale collars and were individually housed in one of 13 cubicles (from 5.2 m^2 to 7.3 m^2). Each dog had a water bowl, raised bed with blanket, and toys. Staff fed dogs between 08:00 and 09:00 h and between 15:00 and 16:00 h; additionally, a pre-measured bag of small treats was available for each dog each day. Shelter staff or volunteers either walked dogs or brought them to a large outdoor enclosure several times a day. Each day, the start time and end time of each walk or time in the outdoor enclosure were recorded on a dry erase board in the dog wing.

At the shelter, most dogs are spayed or neutered before placement on the adoption floor; all are spayed or neutered before adoption. In research previously conducted at this shelter, one of us (B.M.) found that rates of urination during walks decreased after castration in males but did not change after spaying in females (within-dog study; [44]). Similarly, intact males urinated at higher rates than castrated males, but intact and spayed females urinated at similar rates (between-dog study; [44]). Gonadectomy did not influence likelihood of defecation or ground scratching during walks in either males or females (between-dog study; [44]). Given the effect of reproductive condition on the rate of urination in male dogs, it was essential that we control for reproductive condition within each male dog when walked by male versus female walkers. Of the 57 male dogs that we observed, 56 were neutered for all of their walks and one was intact for all of his walks. Of the 43 female dogs that we observed, 36 were spayed for all of their observations, four were intact for all of their observations, and three were intact for some observations and spayed for others.

2.2. Behavioral Observations

Behavioral observations occurred during walks, which began on shelter grounds and continued into a large field across the street (16.6 ha; 42°28′20″N, 76°26′22″W). The field was bordered by a creek, forest, and other fields of very tall grass; the substrate where we walked was mostly grass, which was occasionally mowed in spring and summer. All procedures were carried out under protocol 2012-0150, which was approved by Cornell University's Institutional Animal Care and Use Committee.

Over the course of the study, five different walkers (two females, B.M. and D.O., and three males, K.F., L.U., and J.C.) conducted behavioral observations during individual first walks of dogs (i.e., only the walker was present with the dog and this was the first time that person had walked the dog). All walkers had extensive experience handling and observing dogs on walks, gained via research activities at the shelter, long-term dog-walking as a volunteer at the shelter, or independent employment as a dog walker. Individual walkers conducted observations between 12:00 and 17:00 h, typically once or twice a week, on days that were convenient for them. All dogs included in the data set were individually walked by at least one male and one female walker (one male walker and one female walker: 50 dogs; one male walker and two female walkers: 30 dogs; two male walkers and one female walker: 10 dogs; two male walkers and two female walkers: 10 dogs). Dogs were adopted throughout our study, which is why the number of times a given dog was walked varied from two (one male walker and one female walker) to four (two male walkers and two female walkers). Records of specific staff or volunteers who had walked each dog prior to our walks were not available.

On each walking day, a walker checked the dry erase board in the dog wing and selected dogs he or she had never walked before and that had not been outside for at least 2 h. Scheduled dog walking shifts at the shelter occur at 12:00, 14:30, and 17:00 h, thus, dogs included in our study were walked approximately 2–3 h after their previous walk. Once a team member had walked a specific dog for the first time, B.M. alerted other team members to prioritize that dog for walking. We used leashes and harnesses provided by the shelter; staff had previously fitted each dog with an appropriate harness (either a PetSafe Easy Walk Harness, Radio Systems Corporation, Knoxville, TN, USA or a Zack and Zoey Nylon Pet Harness, Pet Any Way LLC, model US2395 14 99) and placed the harness and a cloth lead (at least 1.8 m long) on a hook outside the dog's cubicle. Upon entering a dog's cubicle, each walker harnessed the dog, attached the lead and led the dog out of the shelter. Behavioral observations began once the dog was outside and lasted for 20 min, during which time we let dogs determine the pace of the walk (dogs were not kept in a heel position). Per shelter policy, dogs were not allowed to interact with other dogs during walks. We verbally recorded behavioral observations using our cell phones (e.g., the voice memo app on an iPhone 7, model MN9G2LL/A, Apple Inc., Cupertino, CA, USA). We recorded each urination, defecation, and occurrence of ground scratching (backward scraping of the ground with the front feet, hind feet, or both performed by some dogs after urination or defecation). For each urination, we also recorded the posture used (female postures: squat, used by juvenile females and most adult females, and squat raise, used by some adult females; male postures:

lean forward, used by adult males under fearful conditions and juvenile males, and raised leg, typical posture for adult males; [39,45]). At the end of walks, we returned dogs to their cubicles and retrieved relevant information from shelter records (e.g., dog identification number, intake date, source, and age). We used the intake date to calculate the number of days each dog had been at the shelter at the time of each of its walks with us (= time at shelter); for dogs that were adopted and returned to shelter, time at shelter was left blank. (Note that this meant that the 12 returned dogs were dropped from analyses, which included time at shelter as a main effect and the interaction between time at shelter and walker sex; see Section 2.3). Each dog was photographed. We transferred data from verbal recordings to paper check sheets within hours of walks and scanned each check sheet as a .pdf file.

2.3. Statistical Analyses

A linear mixed model was used to model the rate of urination (total number of urinations/20 min) and a generalized linear mixed model with a binominal distribution and logit link was used to model defecation (yes/no) and ground scratching (yes/no) during walks. We used a generalized estimating equation (GEE) to model the predominant urinary posture. We defined the predominant posture as the posture used most frequently during a walk; ties were recorded as such. We coded males whose predominant posture was either the raised leg or a tie between the raised leg and the lean forward as one; those whose predominant posture was the lean forward as zero (i.e., not involving a raised hindlimb). Similarly, we coded females whose predominant posture was the squat raise or a tie between the squat raise and squat as one; females whose predominant posture was the squat were coded as zero (again, not involving a raised hindlimb). All models were initially fit with the fixed effects of the dog's sex and the walker's sex, and the interaction between the dog's sex and the walker's sex. Time at shelter was included as a main effect and interacted with walker's sex. In all of the mixed models, we included the dog's ID as a random effect; in the GEE model, we treated the dog's ID as a cluster effect with an unstructured covariance matrix. Reduced models were obtained by removing interactions that were not significant (except for the interaction between dog sex and walker sex, which was retained in models due to research interest), and then removing the main effects that were not significant. For the rate of urination, we used Cohen's d to calculate the effect size. Data were analyzed using either JMP Pro 12 (2015. SAS Institute, Cary, NC, USA) or R, version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Descriptive statistics for the three scent-marking behaviors and time at shelter are shown in Table 1. The statistics in Table 1 are meant to provide a general overview of the raw behavioral data collected and the length of time dogs had been at the shelter at the time of their walks.

Table 1. Descriptive statistics (Mean ± *SD*) for rate of urination by male and female dogs during a 20-min walk by either male or female walkers, along with time at shelter. Additionally, percentage of walks in which dogs defecated or ground scratched are shown.

Dog's Sex	Walker's Sex	Urination Rate [1]	% Walks with Defecation	% Walks with Ground Scratching	Time at Shelter (Days)
Male	Male	0.20 ± 0.15	43.5	37.7	14.8 ± 8.0
	Female	0.40 ± 0.28	61.5	34.6	13.6 ± 9.0
Female	Male	0.10 ± 0.07	49.0	27.5	13.5 ± 6.4
	Female	0.14 ± 0.10	72.6	24.2	13.1 ± 7.1

[1] Total number of urinations/20 min.

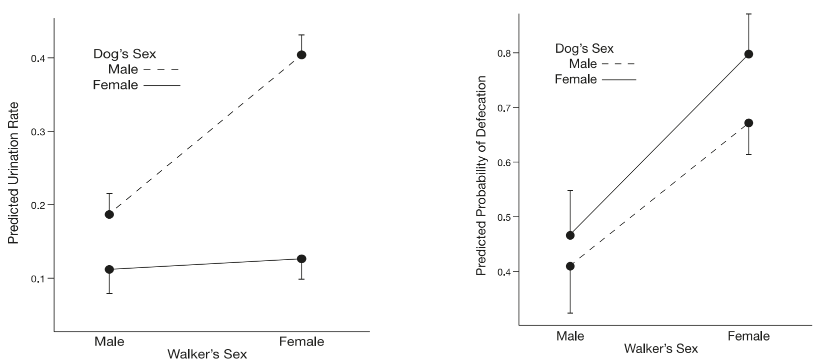
The results that follow are from the reduced models. The results from the full models are provided as Supplementary Material.

3.1. Urination Rate

Of the six male dogs that did not urinate during their walks, five did not urinate when walked by a male walker but did urinate when walked by a female walker, and the remaining dog did not urinate when walked by either male or female walkers. One female dog did not urinate when walked by a male walker but did urinate when walked by a female walker. Dogs that did not urinate during walks were included in analyses. We found a significant interaction between dog sex and walker sex for rate of urination (total number of urinations/20 min; Table 2). Male dogs urinated at higher rates when walked by female walkers than when walked by male walkers (d = 0.87); in contrast, female dogs urinated at similar rates when walked by male walkers and female walkers (d = 0.36; Figure 1a). Additionally, sex differences in rates of urination (urination rates of male dogs > urination rates of female dogs) were apparent with female walkers but not with male walkers (Figure 1a). The main effect for time at shelter also was significant (Table 2).

Table 2. Effects of sex of dog, sex of walker, and time at shelter on rate of urination per min by dogs during a 20-min walk.

Parameter	Estimate	*SE*	*df*	*t* Value	*p*
Intercept	0.243	0.037	173.464	6.638	<0.001
Dog's sex					
Female	−0.078	0.041	136.412	−1.888	0.06
Male					
Walker's sex					
Female	0.218	0.022	139.614	9.644	<0.001
Male					
Time at shelter	−0.004	0.002	206.940	−2.286	0.02
Dog's sex × Walker's sex					
Female × Female	−0.203	0.037	137.708	−5.548	<0.001
Female × Male					
Male × Female					
Male × Male					



(**a**) (**b**)

Figure 1. Scent-marking behaviors of shelter dogs in relation to sex of dog and sex of walker. (**a**) Predicted rates of urination by male and female dogs when walked by male or female walkers. (**b**) Predicted probabilities of defecation by male and female dogs when walked by male or female walkers. Walks were 20 min in duration.

3.2. Likelihood of Defecation

We found a significant main effect of walker sex on likelihood that a dog would defecate during a walk (Table 3). A dog had a 0.441 probability of defecating with a male walker and a 0.740 probability of defecating with a female walker. We did not find a significant interaction between dog sex and walker sex for the likelihood that a dog would defecate during a walk (Table 3; Figure 1b). The odds that a male dog will defecate with a female walker are 2.9 times larger than with a male walker ($p = 0.013$). The odds that a female dog will defecate with a female walker are 4.5 times larger than with a male walker ($p = 0.004$).

Table 3. Effects of sex of dog and sex of walker on likelihood of defecation by dogs during a 20-min walk.

Parameter	Estimate	*SE*	*z* Value	*p*
Intercept	−0.360	0.364	−0.988	0.32
Dog's sex				
Female	0.241	0.555	0.435	0.66
Male				
Walker's sex				
Female	1.073	0.431	2.491	0.013
Male				
Dog's sex × Walker's sex				
Female × Female	0.426	0.646	0.659	0.51
Female × Male				
Male × Female				
Male × Male				

3.3. *Likelihood of Ground Scratching*

There were no significant predictors of ground scratching during a walk (Table 4).

Table 4. Effects of sex of dog and sex of walker on likelihood of ground scratching by dogs during a 20-min walk.

Parameter	Estimate	*SE*	*z* Value	*p*
Intercept	−1.279	0.699	−1.83	0.07
Dog's sex				
Female	−1.317	0.989	−1.33	0.18
Male				
Walker's sex				
Female	−0.214	0.532	−0.40	0.69
Male				
Dog's sex × Walker's sex				
Female × Female	0.081	0.821	0.10	0.92
Female × Male				
Male × Female				
Male × Male				

3.4. *Urinary Postures*

For male dogs, the raw data revealed the following percentages of walks in which the lean forward was the predominant urinary posture (i.e., all limbs remain on the ground when urinating): when walked by male walkers, 20.6%; when walked by female walkers, 13.2%. For female dogs, the raw data revealed the following percentages of walks in which the squat was the predominant urinary posture (again, all limbs remain on the ground when urinating): when walked by male walkers, 92.0%; when walked by female walkers, 95.1%. We found a significant interaction between dog sex and walker sex (Table 5). Male dogs were more likely to use the lean-forward posture when walked by a male walker than when walked by a female walker; in contrast, the likelihood of female dogs using the squat posture did not differ when walked by male walkers or female walkers. The odds of a male dog using the lean-forward posture as its predominant posture were 1.9 times greater when walked by a male walker (predicted probability of 0.222) than when walked by a female walker (predicted probability of 0.127; $p = 0.052$). Finally, the predicted probability of a female dog using the squat posture as its predominant posture was 0.922 when walked by a male walker, which did not differ from the predicted probability of a female dog using the squat posture as its predominant posture when walked by a female walker (0.941; $p = 0.33$).

Table 5. Effects of sex of dog and sex of walker on the likelihood of dogs having a predominant urinary posture in which all limbs remain on the ground (i.e., lean-forward posture in males and squat posture in females).

Parameter	Estimate	*SE*	*z* Value	*p*
Intercept	−1.268	0.310	−4.09	<0.001
Dog's sex				
Female	3.737	0.576	6.48	<0.001
Male				
Walker's sex				
Female	−0.658	0.338	−1.95	0.052
Male				
Dog's sex × Walker's sex				
Female × Female	0.962	0.460	2.09	0.036
Female × Male				
Male × Female				
Male × Male				

4. Discussion

We found that two scent-marking behaviors of shelter dogs—urination (as measured by urination rate) and defecation (as measured by occurrence during a walk)—were influenced by the sex of an unfamiliar walker. Ground scratching, also measured by occurrence during a walk, was not affected by walker sex. The predominant urinary posture during a walk also was affected by walker sex. Only urination rate was affected by time spent at the shelter: rate of urination slightly declined with increasing time spent at the shelter.

In the case of urination rate, the effects of walker sex varied with sex of dog. Male dogs urinated at higher rates when walked by unfamiliar women than when walked by unfamiliar men, whereas female dogs urinated at similar rates when walked by unfamiliar women and unfamiliar men. In fact, the well-established pattern of higher rates of urination by mature male dogs than mature female dogs [37,39,40,46] was present with female walkers but disappeared with male walkers in our study (Figure 1a). For the predominant urinary posture, male dogs were more likely to use the lean-forward posture when walked by unfamiliar men than when walked by unfamiliar women. The urinary posture of female dogs did not differ when walked by unfamiliar men and unfamiliar women. These findings for urination rate and predominant urinary posture support our predictions and are similar to results reported for dogs responding to the presence of either an unfamiliar man or an unfamiliar woman in a commercial kennel setting. Lore and Eisenberg [32] found that male dogs spent less time near an unfamiliar man than an unfamiliar woman, whereas female dogs did not differ in this regard; the authors found a similar pattern for direct body contact with an unfamiliar man and an unfamiliar woman. For the likelihood that a dog would defecate during a walk, we found a main effect of sex of walker but no interaction between sex of walker and sex of dog: both male dogs and female dogs were more likely to defecate when walked by an unfamiliar woman than when walked by an unfamiliar man (Figure 1b). Wells and Hepper [27] found a similar pattern when either an unfamiliar man or an unfamiliar woman stood at the front of cages for a few minutes: both male and female shelter dogs decreased to a greater extent time spent barking and looking at the person when the unfamiliar person was female. In summary, depending on the particular category of behavior used to assess response of dogs to unfamiliar people, the presence of an unfamiliar man can either uniquely affect male dogs (time spent in proximity, time spent in direct contact, rate of urine marking, and predominant urinary posture), affect male and female dogs in a similar manner (time spent barking, time spent looking, and likelihood of defecation), or have no significant effect on either male or female dogs (ground scratching). Ground scratching is performed by a minority of shelter dogs (present study; [40]). Cafazzo et al. [37] studied members of a feral dog pack and found that high ranking individuals ground scratched more frequently than low ranking ones. Perhaps dogs that ground scratch are more confident, which might explain our failure to find an effect of sex of walker for this particular scent-marking behavior.

Our finding that rate of urination declined with increasing time spent at the shelter was unexpected; although the size of this effect was very small, it nonetheless was significant. In a previous study of scent-marking behavior during first walks of dogs at the same shelter, we found that time spent at the shelter did not influence urinary behavior (frequency of urination or percent of urinations directed at targets in the environment) or likelihood of defecation or ground scratching [40]. In a subsequent study in which many dogs were walked multiple times [47], we found that time spent at the shelter positively influenced rate of urination, percent of directed urinations, and likelihood of defecation (ground scratching was not studied). In light of our findings for first walks [40], we interpreted the positive influence of time at shelter in our second study [47] as having resulted from our inclusion of multiple walks on individual dogs, and suggested that the positive influence of time spent at the shelter on marking behavior could reflect dogs becoming more familiar with their surroundings and routine, as well as with us [47]. For these two earlier studies [40,47], there were no single male walkers or single female walkers; we always had two people on each walk, one student (male or female) and B.M. (one person walked the dog and the other recorded behavioral observations). These methodological differences make our current finding regarding urination rate and time at shelter challenging to interpret. One possible interpretation is that more timid dogs are characterized by longer stays at the shelter as well as lower rates of urination.

We did not determine the precise stimuli by which shelter dogs discriminated sex of an unfamiliar walker. Potential stimuli include olfactory, visual, auditory, and tactile/handling differences between male and female walkers. With respect to the latter, subtle differences in petting techniques of males and females appeared responsible for an initial report that shelter dogs petted by females had lower cortisol levels than those petted by males [28]. In two subsequent studies in which men and women received specific training to standardize their petting techniques, male and female petters reduced cortisol levels to the same degree [20,29]. In contrast, androgen-based olfactory cues are used by laboratory mice and rats to discriminate experimenter sex: olfactory cues from men caused a physiological stress response that induced an inability to feel pain in both rodent species [10]. Acoustic cues in voices are used by elephants to discriminate human sex and age: in response to playbacks of either adult male or adult female Maasai voices, members of elephant families were more likely to retreat and exhibit defensive bunching when hearing male voices [16]. It is important to determine which features of unfamiliar men shelter dogs attend to, so that the effectiveness of human interaction during walks, and perhaps other enrichment activities, can be maximized. Additionally, determining whether the observed reactions to unfamiliar male walkers disappear with familiarity would be useful.

Our study focused on scent-marking behaviors of dogs during walks by unfamiliar males and unfamiliar females; we did not measure the physiological responses of dogs to unfamiliar male and female walkers. However, Alberghina et al. [48,49] conducted two studies with shelter dogs to investigate the relationships between scent marking, cortisol, and supervised social exposures with another dog. Social exposures occurred in a fenced area, with both dogs initially on leashes and then eventually off leashes. In the first study, Alberghina et al. [48] found a significant positive relationship between frequency of urine-marking by dogs during social exposures and urinary cortisol-creatinine ratio (C/Cr) measured several hours later and a significant negative relationship between the frequency of defecation during social exposures and C/Cr. In the subsequent study, which differed from the first in some aspects of methodology (e.g., dogs were habituated to muzzles before social exposures in the second study but not in the first), Alberghina et al. [49] found the same patterns with respect to urination and C/Cr and defecation and C/Cr but the results did not reach statistical significance. As suggested by Alberghina et al. [48,49] and Protopopova [50], elevated levels of cortisol could indicate increased arousal and activity, rather than stress in dogs. Results from mammals studied under laboratory conditions suggest a complicated relationship between stress, scent marking, and cortisol. For example, when housed without access to a preferred outdoor cage, common marmosets exhibited elevations in cortisol and increases in scent marking behavior (rubbing scent glands on the substrate) [51]. In contrast, male Mongolian gerbils exhibited elevated cortisol levels but reduced scent marking (rubbing the ventral gland on the substrate) when subjected to social defeat, a stress paradigm in which a male is repeatedly paired with a dominant male conspecific [52]. Thus, elevated cortisol has been associated with both increases and decreases in scent marking behavior in mammals, suggesting the relationship between scent marking, stress, and cortisol requires further study across species and stress paradigms.

A limitation of our study is that age also varied among walkers (female walkers: D.O., 21, B.M., 61; male walkers: J.C., 20, K.F., 22, L.U., 31). Few studies have examined the influence of human age on dog behavior, except in regard to dog bites (e.g., [53]). Although Koda and Shimoju [30] found that dogs enrolled in a guide dog program made more frequent contact with unfamiliar women than unfamiliar men, they found no difference in the frequency with which dogs contacted unfamiliar females who were either between 20 and 40 years old or between 8 and 13 years old. These findings suggest that, at least in the case of unfamiliar females, age might not matter to dogs; Koda and Shimoju [30] did not examine response of dogs to unfamiliar males from different age groups. Dog volunteers at the Tompkins County SPCA ranged from 18 years old to over 70 years old; thus, the dogs in our study likely had some experience interacting with humans of diverse ages before we walked them.

5. Conclusions

Given that sex of an unfamiliar human has been shown to affect both the in-kennel behavior of shelter dogs [27] and their behavior outside the kennel during leash walks (present study), we suggest that researchers conducting behavioral observations of shelter dogs (and perhaps dogs generally) record, consider in their analyses, and report the sex of observers/handlers as standard practice. Based on their findings that experimenter sex influenced the behavior and physiology of laboratory mice and rats, Sorge et al. [10] made a similar recommendation for researchers studying any phenomenon in laboratory rodents that could be affected by stress. Our findings might also have implications for canine behavioral evaluations at animal shelters. Such evaluations are usually conducted a few days after intake, when dogs are likely unfamiliar with at least some staff present at these tests. Additionally, behavioral evaluations often include a subtest in which an unfamiliar person knocks on the door and enters the room where testing is taking place (e.g., Stranger Test in the Modified Assess-A-Pet; [42,43]). Shelter dogs have been shown to differentiate sex of an unfamiliar person during behavioral evaluations: Bergamasco et al. [26] found that shelter dogs enrolled in a human interaction enrichment program and behaviorally evaluated several times over a period of weeks improved in their responses to unfamiliar females but not to unfamiliar males. Thus, sex of the unfamiliar person and perhaps sex of the evaluator/handler and scribe, could potentially influence results of canine behavioral evaluations, which might then affect dog welfare by influencing whether or not a dog is made available for adoption.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2615/10/4/632/s1, Table S1: Effects of sex of dog, sex of walker, and time at shelter on the rate of urination per min by dogs during a 20-min walk. Results are from the full model, Table S2: Effects of sex of dog and sex of walker on the likelihood of defecation by dogs during a 20-min walk. Results are from the full model. Table S3: Effects of sex of dog and sex of walker on the likelihood of ground scratching by dogs during a 20-min walk. Results are from the full model. Table S4: Effects of sex of dog and sex of walker on the likelihood of dogs having a predominant urinary posture in which all limbs remain on the ground (i.e., lean-forward posture in males and squat posture in females). Results are from the full model.

Author Contributions: Author contributions were as follows: conceptualization, B.M.; supervision, B.M.; methodology, B.M. and S.P.; data collection, B.M., K.F., D.O., and L.U.; coordination of statistical analyses, B.M.; statistical analyses, S.P.; writing—original draft preparation, B.M. and K.F.; writing—review and editing, B.M., K.F., D.O., L.U., and S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We thank Jim Bouderau, Executive Director of the Tompkins County SPCA, for permission to walk dogs at the shelter. Emme Hones, Behavior Program Manager, Kat Pannill, Volunteer Coordinator, and Heather Marsella, Shelter Operations Manager, provided information on numbers of male and female staff and volunteers. Jordan Chan joined the walking team toward the end of this study and Emma Rosenbaum entered the data on urinary posture. Kate Bemis and William Bemis reviewed a draft of this manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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ISBN 978-3-0365-0873-3