### Comparing the Differential Effects of Neighborhood and Nature Walks on Behavior and Urinary

Cortisol Levels of Dogs

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#### ABSTRACT

Dog training practitioners suggest that walks in nature provide more welfare enhancing benefits than the neighborhood walks that most dogs experience daily. While the benefits of nature walks are a well-studied phenomenon in humans, to date there has been little investigation of this topic in companion dogs using objective measures. This study compared the effects of thirty-minute walks in nature to walks in the dogs' home neighborhoods. Fifteen dogs took part in a withinsubjects design that measured physiology and behavior to examine the effects of the two types of walks. Dogs had free catch urine samples taken multiple times per day for urinary cortisol analysis, were video recorded during walks, and were outfitted with activity monitoring collars. We found significant variation in cortisol levels in accordance with time of day, but no difference was found between the neighborhood and nature walks. Several stress, movement, and exploratory/foraging behaviors were found to differ between conditions. While we found that the experimental conditions did influence some of the dogs' behavior in this study, they did not impact cortisol levels.

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#### GENERAL PUBLIC ABSTRACT

Dog training practitioners suggest that walks in nature are more beneficial for dogs than the neighborhood walks that most experience daily. While walks in nature have been shown to have significant benefits in humans, this type of activity is not well-studied in dogs. This study compared the effects of thirty-minute walks in nature to walks in the dogs' home neighborhoods. The effects of walk type on behavior, overall activity levels, and cortisol (a hormone often considered to be indicative of stress) was measured in fifteen dogs. Each dog was video recorded during walks, outfitted with an activity monitoring collar, and provided multiple urine samples per day. We found significant variation in cortisol levels in accordance with time of day, but no difference was seen between neighborhood and nature walks. Several stress, movement, and exploratory/foraging behaviors were found to differ between conditions. While experimental conditions did impact some behaviors of the dogs in this study, they did not impact cortisol levels.

## DEDICATION

I dedicate this thesis to the memory of my heart dog and best girl, Portia. She continues to be the reason I travel this path today.

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#### **INTRODUCTION**

#### 1.1 Canine Welfare

Dogs (*Canis lupus familiaris*) are ubiquitous, with an estimated population of 1 billion individuals worldwide (Bryce, 2021). In the United States, nearly half of all households own a dog, resulting in an estimated 83 million dogs throughout the country (Christian et al., 2016). The popularity of pet dogs, alongside the increasingly urban lifestyle of their human caretakers, is thought to expose these animals to particular sources of welfare reduction, such as obesity (Bland & Hill, 2011; Chan et al., 2005; German, 2006; McGreevy & Bennett, 2010), separation anxiety (Amaya et al., 2020; Bradshaw et al., 2002; Butler et al., 2011; Guthrie, 1999; Jones et al., 2014; Karagiannis et al., 2015; Landsberg et al., 2003; Sonntag & Overall, 2014; Wells, 2004), and reduced choice and agency in their daily lives (Cobb et al., 2020; Coppinger & Coppinger, 2016). Notably, control over one's environment is considered to be a contributing factor to positive welfare in animals (Broom, 1993; Clubb & Mason, 2007; Frézard & Le Pape, 2003; Hughes & Duncan, 1988; Janssen et al., 2014; Markowitz, 1982; Mellor et al., 2020; Morgan & Tromborg, 2007; Veasey et al., 1996).

The increasingly indoor lifestyle of contemporary dogs contributes to dogs experiencing more confinement, and therefore, more socially and environmentally isolated lives (Meyer et al., 2022; Stephan et al., 2021). Many dogs are unable to access adequate opportunities to engage in physical activity. Despite being provided with the necessities of survival (i.e., food, water, shelter, veterinary care), companion dogs' behavioral repertoire may otherwise be limited, constituting a reduction in welfare. Free-ranging dogs, in contrast, enjoy considerable control over their social lives, reproduction, and behavior (Cobb et al., 2020; Coppinger & Coppinger, 2016), but at the expense of readily accessible necessities like food and water.

#### **1.2 Welfare Assessment**

Attempts to assess animal welfare have generally fallen within three paradigms. The first among these is production based (Broom, 1993). This view of welfare is centered around outputs and biological functioning, such as animal longevity, growth, and reproduction resulting in tangible outcomes like viable offspring or milk production (Broom, 1993; Fraser, 2003). The second lens through which welfare may be viewed, affective state, is more animal-centric and emphasizes reduction of distress and suffering while promoting "a life worth living" (Berteselli et al., 2019; FAWC, 2009; Fraser, 2003; Mellor et al., 2020). The third paradigm elevates the provision of semi-natural environments and abundant opportunities to engage in natural behaviors, such as those that an animal's wild counterparts display in their natural habitat (Bracke & Hopster, 2006; Clark et al., 2016; Clubb & Mason, 2007; Cobb et al., 2020; Coppinger & Coppinger, 2016; Duranton & Horowitz, 2019; Fraser, 2003; Mellor & Beausoleil, 2015; Morgan & Tromborg, 2007; Veasey et al., 1996). For companion dogs, such behaviors would include those displayed by their free-ranging counterparts or possibly their closest living relative, the grey wolf (*Canis lupus lupus*).

While these three paradigms have been used to alleviate animal suffering through the measurement and reduction of negative welfare states, recently the field has focused more on the promotion of positive welfare states alongside the elimination of negative ones (Fraser, 2008; Mellor & Beausoleil, 2015). One example of such focus can be found in the Five Domains Model, which includes appraisal of the animal's overall mental state as part of the welfare assessment (Mellor & Beausoleil, 2015). Originally developed in 1994, the Five Domains Model was later expanded to include positive welfare states through, for example, the exercise of agency (Mellor & Beausoleil, 2015; Mellor & Reid, 1994). Another example of the incorporation

of positive welfare states is the Five Opportunities to Thrive, used by the San Diego Zoo Global (Janssen et al., 2014). The Five Opportunities to Thrive state that an animal should be given the opportunity to self-maintain, enjoy optimal health, engage in species-typical behavior, and have the ability to exercise choice and control (Janssen et al., 2014). When applying these concepts to companion dogs, for example, such provisions may include being permitted to engage in self-directed behaviors during daily walks, such as foraging, sniffing, or walking at their own pace.

#### **1.3 Specific Welfare Assessment Parameters**

Behavior is considered to be an external indicator of an individual's internal state (Amaya et al., 2020). An animal's welfare can be evaluated through a multimodal assessment in which both behavior and physiology are measured simultaneously and interpreted in conjunction with one another (Beerda et al., 1997; Beerda et al., 1998; Clark et al., 1997; Clubb & Mason, 2007). For example, in one study, Beerda et al. (1998) reported that dogs responding to the sudden appearance of unexpected stimuli with a lowered posture was indicative of stress, as the behavior occurred in tandem with an increase in dogs' salivary cortisol. In another study, Herbel and colleagues (2020) found that during transportation in a car, changes in dog behavior, particularly lip-licking, correlated with an increase in heart rate and neutrophil-to-leukocyte ratio, the latter of which is a stress response produced by the immune system in response to cortisol production (Burgeuez et al., 1983; Herbel et al., 2020; Kent & Ewbank, 1986).

Along with specific instances of body language, such as lip-licking or cowering, overall activity levels are another way in which behavior can be described to evaluate an animal's welfare. For welfare to be considered optimal, rest and activity levels of animals living in confinement should be similar to what would be displayed by the animal's wild counterparts. In both captive wolves and kenneled dogs, higher activity levels are associated with reduced

welfare. Frézard and Le Pape (2003) used the balance between rest and activity to measure the welfare of captive wolves, finding that wolves provided with more space spent more time resting, an indication of positive welfare. An earlier study on laboratory housed dogs concluded that greater degrees of social isolation, thought to be detrimental to this social species, led to increases in movement while kenneled (Hetts et al., 1992). Taken together, these studies point to the notion that the amount of time an animal spends resting versus actively moving can be taken as an indication of welfare. Animals living in greater confinement may display more activity via pacing, while animals provided with adequate space and social access spend more time resting.

Another way in which behavior can be used to evaluate welfare is by measuring its diversity (Brereton & Fernandez, 2022; Frézard & Le Pape, 2003; Miller et al., 2020). Behavioral diversity can be described as the number of behaviors or behavioral categories displayed by an animal during a given period (Brereton & Fernandez, 2022). For canids, examples of such behavioral categories include locomotion, play, feeding, hunting, exploration, and social interactions (Walker et al., 2016; Way et al., 2006; Westgarth et al., 2010). Using this approach, animals living in enriched environments are assumed to be able to engage in a broader array of behaviors than those living in impoverished environments, indicating better welfare (Brereton & Fernandez, 2022). For example, Watters and colleagues (2010) found that placing a foraging apparatus with an unpredictable food output schedule in the enclosure of zoo-housed fennec foxes (*Vulpes zerda*) lead to increased diversity in the foxes' behavioral time budgets. If we were to apply this to domestic dogs, dogs displaying a wider array of behaviors during a period of observation would be considered to have greater behavioral diversity, and therefore better welfare, than dogs displaying fewer behaviors.

The expression of species-specific behavior is also used to evaluate an animal's welfare while in captivity (Miller et al., 2020; Schipper et al., 2008). A species-specific behavior is a behavior that is displayed by a captive animal's non-captive or closely related wild counterpart (Veasey et al., 1996). Bracke and Hopster (2006) defined this category of behavior as what an animal will exhibit in natural conditions due to the provision of automatic reinforcement or improved biological functioning, such as foraging and exploratory behaviors. In a commentary on the topic of animal welfare, Fraser (2003) discussed the idea that the welfare of captive animals may be improved by providing them with more ecologically relevant habitats or enclosures and additional opportunities to engage in natural behaviors. In an example of providing additional opportunities to engage in natural behaviors, Schipper and colleagues (2008) used species-specific behaviors, such as those observed during feeding (e.g., chewing licking, carrying), to evaluate the effects of food enrichment with kenneled dogs. The researchers found that their enrichment intervention reduced barking behavior, suggesting an increase in the dogs' welfare.

Another way of measuring welfare using behavioral observations is through cognitive bias testing, such as those used to measure dogs' spatial cognitive bias (Duranton & Horowitz, 2019; Harding et al., 2004; Karagiannis et al., 2015). With this type of testing, the latency in which dogs approach ambiguous stimuli is used to indicate an animal's underlying affective state (Karagiannis et al., 2015). For example, Duranton and Horowitz (2019) trained dogs to discriminate between two bowls consistently presented in opposing locations, one of which always contained food. Dogs were then tested in how quickly they approached a bowl presented in an ambiguous location between the location that had previously contained food and the other,

which did not. Dogs that approached the ambiguous bowl more quickly post-intervention were assumed to have improved affect, and, thereby, better welfare.

In addition to behavior, physiological measures, such as the measurement of hormonal levels, are often employed to assess the welfare of animals. In particular, cortisol has been used as an indicator of acute and chronic stress in a variety of studies with domestic dogs (Akiyama & Ohta, 2021; Alberghina et al., 2019; Citron et al., 2020; Gunter et al., 2019; Gunter et al., 2021; Hiby et al., 2006; Jones et al., 2014). The production of cortisol is triggered by activity in the hypothalamic pituitary adrenal (HPA) axis of the brain (Alberghina et al., 2019; Beerda et al., 1996; d'Angelo et al., 2021; Ward Thompson et al., 2012) and activated in response to stressful situations or stimuli as perceived by the animal (d'Angelo et al., 2021; Hiby et al., 2006; Terlouw et al., 1997). For example, Citron et al. (2020) found dogs' cortisol levels to be higher during their stay in a veterinary hospital as compared to when the dogs were at home. Likely, these increased levels were related to the stress of transport (d'Angelo et al., 2021; Herbel et al., 2020; Kuhn et al., 1991), in addition to the veterinary clinic environment. Similarly, d'Angelo et al. (2021) reported increased cortisol concentrations for dogs after an hour long transport from their kennel to a prison facility for an animal-assisted intervention program.

Gunter et al. (2019) used urinary cortisol levels to determine the effects of one and two nights in a foster home for shelter dogs and found that these sleepovers resulted in reduced urinary cortisol levels and longer bouts of continuous rest for the dogs, indicating better welfare. In another fostering study, Gunter et al. (2021) utilized urinary cortisol and activity levels to determine the possible impacts of a two-and-a-half-hour field trip for shelter dogs. They found that dogs' cortisol levels were higher the afternoon of the field trip in comparison to the afternoons before and after the outing, even after accounting for dogs increased high-intensity

activity. The authors ultimately concluded that short field trips did not reduce the dogs' physiological stress in the same way sleepovers did.

Urinary cortisol is considered to be a valid approach to assess the stress response in canines while also having the added benefit of using a non-invasive method of biological collection (Beerda et al., 1998). Nevertheless, when urinary cortisol is used as a measurement of stress, diurnal variability (Alberghina et al., 2019; Gunter et al., 2021), individual variability (Alberghina et al., 2019), and activity (Durocher et al., 2007; Jones et al., 2014) need to be considered in interpretation. Furthermore, because cortisol is simply a measure of arousal without differentiation between eustress and distress, elevated cortisol levels alone may not necessarily indicate reduced welfare (Pastore et al., 2011), highlighting the importance of using a multimodal approach to assess an animal's experience.

#### **1.4 Welfare Interventions**

Enrichment, the addition of and interaction with environmental diversity, is a common intervention for improving the welfare of pet dogs. Environmental enrichment strategies include the provision of novel strategies for delivering an animal's food (Hunt et al., 2022; Lopes et al., 2022), exposure to olfactory stimuli (Amaya et al., 2020), and supplying additional socialization opportunities (Figueira et al., 2021; Hoy et al., 2009; Hubrecht, 1993). To provide dogs with food-based enrichment, meals can be hidden in boxes, scattered throughout the animal's enclosure, or provided in objects, such as a rubber KONG<sup>TM</sup> toy (Frézard & Le Pape, 2003; Newberry, 1995; Schipper et al., 2008). Food enrichment strategies have been found to positively impact welfare. For example, Schipper et al. (2008) found that providing laboratory dogs with a KONG<sup>TM</sup> toy stuffed with food resulted in more behavioral transitions and a reduction in barking.

Olfactory, or scent-based, enrichment is of particular relevance for dogs, a species known for their olfactory acuity (Wells, 2004). Duranton and Horowitz (2019) found that dogs that engaged in scent training, in comparison to dogs that underwent training to walk alongside their handler, displayed an increase in positive affect as demonstrated by their performance on a cognitive bias test. Amaya et al. (2020) reported that when dogs were exposed to lavender scent in their kennels, their arousal-related behaviors, such as vocalization, reduced. Similarly, Figueira et al. (2021) found that captive crab-eating foxes (*Cerdocyon thous*), a member of the canid family, were exposed to various types of olfactory stimuli (i.e., meat, cheese, rat urine, and egg), the foxes exhibited an increase in behaviors like sniffing and relaxing, indicating an improvement in their well-being.

A third type of enrichment, social interaction, is considered to be an essential component of canine welfare (Hubrecht, 1993; Polgár et al., 2019). The provision of social contact as a form of enrichment for dogs can occur with conspecifics or other species, such as humans (Wells, 2004). In a study examining different enrichment protocols used with dogs housed in an office environment, Hunt et al. (2022) found that increasing the duration of conspecific play led to the greatest positive behavior change. Conversely, Hubrecht et al. (1993) concluded that providing dogs with human socialization was the most beneficial enrichment intervention in their study (even when compared to conspecific socialization), as it led to decreased chewing of kennel furniture while increasing the dogs' solicitation of attention from the lab technician that provided the socialization. Hunt et al. (2022) also found in their study that spending time with a person reduced dogs' alerting behaviors.

#### **1.5 A Novel Welfare Intervention**

One popular form of enrichment with purported welfare enhancing benefits recommended by dog training practitioners for companion dogs is known as a "decompression walk" (Catan, n.d.; Stremming, 2018b). While conducted either with the dog off-leash or on a long-line in a natural setting, such walks are hypothesized to positively impact dogs' welfare by allowing them free movement and the ability to engage in species-specific behaviors (Catan, n.d.; Efimova, 2019; Stremming, 2018b). Dogs can sniff, roll on the ground, and have more overall choice in their activities as opposed to being more tightly managed on a short leash. Despite their name, "decompression walks" have yet to be experimentally evaluated for their effects on dog welfare; thus, throughout this document, this type of walk with owners will be described as a "nature walk."

When understanding nature walks through an animal welfare perspective, components of both The Five Freedoms and Five Opportunities to Thrive (Janssen et al., 2014) are most relevant for evaluating their potential effects on behavior, and consequently, dog welfare. Within The Five Freedoms model, it is recommended that animals be given the ability to express species-typical behaviors while also being provisioned with sufficient space to engage in such behaviors (FAWC, 2009). Additionally, in their description of the Five Opportunities to Thrive, Janssen et al. (2014) recommend providing animals with opportunities for choice and control in their activities.

As these walks are taken in a natural setting while allowing the dog to engage in freedom of movement and direct their own activity, they have the potential to positively impact welfare. Time in nature could result in automatic exposure to a variety of olfactory stimuli that the dog would not otherwise encounter, such as wildlife. As noted earlier, olfactory enrichment has been demonstrated to have a welfare enhancing effect (Amaya et al., 2020; Duranton & Horowitz,

2019; Figueira et al., 2021). Furthermore, the olfactory stimuli present in nature are more likely to be biologically relevant (e.g., scent of other animals) and may therefore represent an enhanced enrichment opportunity (Rafacz & Santymire, 2013). When relating this concept to the daily experience of companion dogs, it is possible that walks in nature could present them with additional opportunities to engage with more diverse stimuli than a typical neighborhood walk, which is often done on a short leash on concrete or pavement and often surrounded by noise from traffic (Baranyiová et al., 2005; Faraco & Seminotti, 2006).

The scientific literature provides limited information about what occurs on walks between dogs and their owners. In one of the few studies on this topic, Westgarth et al. (2021) found that UK dog owners engage in different types of dog walks that serve different purposes in their lives. Through conducting in-depth interviews with owners, accompanying participants on walks with their dogs, and examining two-years' worth of auto ethnographical diary entries, Westgarth and colleagues identified two dog walk types: functional and recreational. Functional walks are typically shorter and have the primary goal of exercising the dog in a convenient location while the second type is recreational. Recreational dog walks are typically longer, taken in environments that allow for off-leash activities, and are perceived as benefiting both the dog and the owner.

Dog training practitioners espousing the use of nature walks with dogs have suggested that their benefits may be similar to those observed in studies of humans walking in natural settings (Stremming, 2019). Multiple human studies investigating the effects of physical activity performed in natural areas have found that participants, after spending time in nature, report decreased feelings of anxiety and rumination on post-walk questionnaires (Bratman et al., 2015; Legrand et al., 2022; MacKerron & Mourato, 2013; Marselle et al., 2013; Park et al., 2011;

White et al., 2019). Other effects of time spent in or in proximity to nature for humans include improved sleep quality (Morita et al., 2011), as well as a reduction in heart rate (Song et al., 2015) and amygdala activity (Sudimac et al., 2022).

Considering the increased popularity of nature walks for dogs and the ability of dogs to engage in species-specific behavior during these walks, along with human studies that point to the advantages of outdoor recreation, we chose to compare the potential impacts of nature walks to neighborhood walks with owned dogs. We assessed the impact of the walks through dogs' urinary cortisol and activity levels, and their behavior during these walks. We hypothesized that dogs' stress-related behaviors and cortisol levels would be lower during and immediately following nature walks, respectively, when compared to neighborhood walks. Furthermore, we hypothesized that dogs' engagement in species-specific behaviors, such as sniffing and rolling, would be greater during nature walks with more resting by dogs following their nature walk compared to walks in the neighborhood. In total, decreases in dogs' stress-related behaviors and cortisol levels as well as increases in rest and species-specific behaviors would indicate a positive impact on the welfare of dogs living in homes.

#### **METHODS**

#### 2.1 Subjects

To participate in the study, dogs needed to be at least nine months of age. Dogs with metabolic issues that could impact cortisol levels (i.e., kidney disease, Addison's disease, or Cushing's syndrome) were excluded from the study (Jones et al., 1990). Due to the nature walks necessitating a car ride to reach the walking location, subjects that displayed car sickness were excluded from the study. Demographic information of the dogs can be found in Table 1.

Subjects were recruited via social media, email outreach, word of mouth, and paper flyers posted in veterinary offices, dog parks, a dog training facility, and a pet supply store. All participants that completed data collection received a thank you card and were entered into a raffle to win a \$50 PetSmart gift card. Subjects were located in New Jersey, Pennsylvania, and North Carolina.

#### **2.2 Procedure**

Subjects were exposed to two experimental conditions: neighborhood and nature walks, both with their owner. Each condition consisted of three consecutive days in which the experimental walk occurred in the late morning or early afternoon followed by a three-day washout period prior to the beginning of the next condition. Half of the dogs began with the neighborhood walk condition, the other half with walks in nature. Owners video recorded their dogs during these walks for behavioral coding and collected urine samples for cortisol: creatinine (UCCR) analysis. Dogs also wore activity monitoring collars for the duration of the study to measure activity levels and rest patterns. Owners recorded when walks, urine sample collections, and collar placement occurred to maintain experimental integrity.

#### 2.3 Walk Conditions

Walks in both conditions were approximately 30 minutes in duration. During the 12 days of the study, owners were asked to avoid taking their dogs on recreational walks aside from those described in the data collection schedule.

#### 2.3a Neighborhood Walks

Dogs were walked in their neighborhoods on an approximately 1.83m leash (varying brands, provided by owners). Owners clipped the leash to their dog's walking equipment, which consisted of either a collar, harness (either front or back clip), or head halter. For this condition, owners were instructed to walk their dogs as they typically would with no further direction provided by the experimenter.

#### 2.3b Nature Walks

The nature walks took place in a nearby park or natural area (open fields, wooded areas, or beaches) on an approximately 6.1m biothane leash (Viper K9, 2020) clipped to the dog's collar or harness. Head halters were prohibited on nature walks, as they would likely impede the dog's movement and allow for the possibility of injury when attached to the long leash.

For our study participants, nature walks required travel by car to the walking location, which was required to be within a 15-minute drive or less, such that the car ride itself did not exceed the duration of the nature walk. Owners walked their dogs from the parking lot on their dog's typical leash and then replaced it with the provisioned long leash upon reaching the natural area. During this experimental condition, owners were instructed to 1) allow their dogs to move freely, including engaging in behaviors other than walking (e.g. sniffing, pawing, rolling) and 2) keep pace with their dogs as much as they were able. Prompting by the owner, either verbal or physical, to continue walking or cease other behavior was only to occur if the dog needed to be moved away from an animal, person, unsafe object, or natural barrier.

#### 2.4 Video Recording

Owners video recorded their dogs behavior during both experimental conditions using either a GoPro HERO7 Silver or a GoPro HERO8 Black camera. The camera was attached to the owner using a GoPro Chesty performance chest mount. For neighborhood walks, owners began recording on the camera and started a thirty-minute timer upon exiting the doorway of their home or apartment building. During nature walks, owners began the camera recording and walk timer after switching to the long leash. To capture the dogs within the camera's video frame, owners either turned to face their dogs or adjusted the harness to the side. Using the Behavioral Observation Research Interactive Software (BORIS) program (Friard & Gamba, 2016), the experimenter later coded the videos for 22 behaviors (Table 2).

#### **2.5 Urine Sample Collection**

Owners collected urine at 28 time points from their dogs for UCCR analysis in order to measure the dogs' stress before, during, and after walks (Gunter et al., 2019; Hay et al., 2000; Hiby et al., 2006; Schatz & Palme, 2001) (Table 3). Baseline samples were collected in the afternoon and evening prior to the start of the experimental condition, which began the following day, with three samples collected per day during each treatment.

Owners collected urine samples using the free catch method by holding a plastic tray (Olympic Veterinary) underneath the dog as the bladder was naturally voided. After collecting the urine, owners transferred the sample into a plastic vial. Samples were frozen in owners' household freezers until being transported on freezer packs to the Smithsonian Conservation Biology Institute (Front Royal, VA) for cortisol and creatinine analysis.

Urine samples remained frozen at -20C until the time of sample preparation. When thawed, the samples were diluted with an enzyme immunoassay buffer to a dilution of 1:200.

The diluted samples were then run on the Cortisol EIA (R4866), a conventional cortisol enzyme immuno-assay (Pahuja & Narayan, 2023). In cases where the urine sample was too dilute (n = 7), samples were diluted with an enzyme immunoassay buffer to a smaller dilution of 1:50 to get a readable hormone value. Urinary creatinine was determined using the Jaffe method (1886) modified for 96 well microplate with 1:50 diluted samples.

#### 2.6 Activity Monitor Collar

Dogs wore an activity monitoring collar (Fi, 2022) which recorded the number of steps taken, distance traveled, and movement/non-movement of the dog throughout the data collection period. Dogs wore the activity monitors continuously for twelve consecutive days, aside from when the collars were removed for charging purposes or to bathe the dog. Owners placed these collars on their dogs in the morning of Day 1, prior to the start of data collection, and removed them on Day 12 after data collection had ended (Table 3).

#### 2.7 Data Analysis

#### 2.7*a* UCCR

While 28 urine samples were collected per subject, not all were analyzed. Afternoon and evening samples were prioritized to detect a potential effect of the walk itself on urinary cortisol levels (afternoon samples), as well as the potential for the walk to impact urinary cortisol levels later in the day (evening samples). To investigate whether our intervention influenced dogs' cortisol responding, we analyzed dogs' cortisol: creatinine ratios using a linear mixed model. Based on prior research (Gunter et al., 2019; Gunter et al., 2021), the model included the following fixed effects as these variables have been shown to affect cortisol levels and were entered into the model as covariates: dogs' weight (kg) and age (months) in addition to the intervention condition (baseline, neighborhood, or nature walk), time of day (afternoon or

evening), and a condition-by-time-of-day interaction. Dog and intercept were included as random effects and time point was included as a repeated effect. A variance covariance matrix was employed, and a diagonal covariance matrix for the repeated measure of phase. The method of Restricted Maximum Likelihood (REML) was used for estimating parameter values.

To test whether the baseline conditions prior to the neighborhood and nature walks differed in our study, two one-way analyses of variance was used to examine cortisol levels in the afternoon and evening. When *post hoc* comparisons were conducted as part of any of 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 utilized throughout. 2.7b Behavior

To investigate whether our intervention influenced dogs' behavioral responding, we coded each dogs' behavior using the Behavioral Observation Research Interactive Software (BORIS) program (Friard & Gamba, 2016). Utilizing a sample criteria previously employed by Rooney et al. (2007), behaviors displayed by at least one third of dogs were used for statistical analysis in order to capture behaviors that were somewhat common within our sample population whilst excluding those that were relatively rare. Based on this criterion, laying down, freezing, retreat, piloerection, rolling, and digging were excluded, and 14 of the 22 coded behaviors were used for statistical analysis in this study. Point behaviors were converted to rate of behavior per minute:

# # of occurences total observed time (minutes) = rate

Duration behaviors were first converted to ratio and then to percentage, as follows:

$$\frac{duration of behavior (minutes)}{total observed time (minutes)} = ratio (100) = percentage$$

Based on the paired experimental design and not assuming Gaussian distribution,

behavioral data were analyzed with the Wilcoxon-matched pairs rank test and corrected using the two-stage step-up method of Benjamini, Krieger, and Yekutieli (2006) using GraphPad Prism for Mac (GraphPad Software). Behavior results from comparable walks were tested across conditions (e.g., Walk 1 in both the neighborhood and nature conditions). To determine whether habituation or sensitization occurred, behavioral comparisons between walks (e.g., Walk 1 and Walk 2) within conditions were also tested.

#### 2.7c Activity Levels

A request for raw data was sent to the activity monitoring collar manufacturer. Analysis and subsequent results will be presented in a later publication.

#### RESULTS

#### **3.1 Sample Demographic**

Our sample consisted of 15 owned dogs. Three dogs that participated in the study were excluded from statistical analysis. Two dogs (Izzy and Winston) would not reliably urinate in the collection tray. One dog (Hollie) was off-leash in the nature walk condition. The 12 dogs used for statistical analysis had a mean weight of 27.03 kg (SD = 16.07), age of 85.84 months (SD = 47.63), and cortisol: creatinine ratio of 176.44 ng/mg (SD = 97.22). These dogs were more often male (58.3%). Complete demographic information for all dogs can be found in Table 1.

#### **3.2 Behavior**

Of the 12 participant dogs used for statistical analysis, a total of 72 walk videos were recorded and subsequently coded using the BORIS program (Friard & Gamba, 2016). Two videos did not depict the walks in their entirety due to the GoPro battery dying. Nature walks captured in full on the GoPro (n = 36) were an average of 30.97 minutes in duration (SD = 3.67). Neighborhood walks captured in full on the GoPro (n = 34) were an average duration of 30.36 minutes (SD = 2.9).

Median and interquartile range for the 17 analyzed behaviors are displayed in Table 4 (movement), Table 5 (point behaviors), and Table 6 (duration behaviors), having been grouped according to behavior category or measurement type. Since dogs were always displaying some form of movement behavior while being observed on a walk, the mean for all movements totaling 100% of each walk have been graphed and are displayed in Figure 1. Median and interquartile range for non-movement behaviors have been graphed and can be found in Figure 2 (rate per minute behaviors) and Figure 3 (duration behaviors). Lip-licking, as measured by the number of occurrences within one minute, was significantly different between conditions in the first (p = 0.021) and second (p = 0.012) walk of each setting; such that lip-licking occurred less

often during the nature condition (walk 1 - Mdn (IQR) 0.27 (0.12 - 0.54); walk 2 - Mdn (IQR) 0.22 (0.10 - 0.59)) than in the neighborhood condition (walk 1 - Mdn (IQR) 0.73 (0.31 - 1.45); walk 2 - Mdn (IQR) 0.65 (0.30 - 1.78)). Air scenting was significantly different between conditions in the first walk (p = 0.016) of each setting; such that the percentage of the walk spent engaging in air scenting was higher in the neighborhood condition (Mdn (IQR) 0.17 (0.00 -(0.83)) than in the nature condition (*Mdn (IQR)* (0.00 - 0.11)). Standing was significantly different between conditions in the first (p = 0.016) and second (p = 0.034) walks in each setting; such that the percentage of the walk spent standing was higher in the nature condition (walk 1 -Mdn (IOR) 23.47 (16.97 - 31.91); walk 2 - Mdn (IOR) 21.91 (14.81 - 32.46)) than in the neighborhood condition (walk 1 - Mdn (IOR) 13.79 (10.02 - 17.36); walk 2 - Mdn(IOR) 14.66 (10.26 - 18.88)). Walking was significantly different between conditions in walk two in each setting, with percentage of the time spent walking being higher in the neighborhood condition (*Mdn (IQR* 74.24 (62.70 – 84.88)) than in the nature condition (*Mdn (IQR*) 59.37 (45.40 – 70.63)). No behaviors were found to significantly differ between conditions in walk three. When testing within conditions using the Wilcoxon-matched pairs signed rank test and two-stage stepup correction method (Benjamini et al., 2006), body shake was significantly different (p = 0.049) between the second (*Mdn* (*IQR*) 0.07 (0.01 - 0.21)) and third (*Mdn* (*IQR*) 0.04 (0.01 - 0.19)) neighborhood walks.

#### **3.3 Cortisol**

Dogs that participated in the present study contributed 166 urinary cortisol values that were used in our analysis. Eight of the dogs provided both afternoon and evening samples across all eight days of the study containing urine sample collection. A total of four dogs did not have baseline samples, either before neighborhood or nature walks. One dog, Hazel, had four samples removed from analysis (time points three, four, five and seven), as they were over three standard deviation units above the overall sample mean.

We used two one-way analyses of variance to compare baseline (prior to neighborhood and nature walks) urinary cortisol in the afternoon and evening. In the afternoon analysis, we found no difference in the average cortisol levels of dogs in the baseline condition before their neighborhood and nature walks, F(1, 14) = 0.06, p = 0.804. Similarly in the evening analysis, we found that dogs' average cortisol levels during baseline did not differ prior to their nature and neighborhood walks, F(1, 13) = 0.17, p = 0.899.

To explore the effect of the study's intervention, dogs' urinary cortisol values were statistically analyzed to detect an effect of condition, time of day, or a condition-by-time-of-day interaction. Dogs' age and weight were also entered into the model. Estimates of mean and standard error per condition (per time of day and collapsed over time) are displayed in Figure 4 and Figure 5, respectively. We found the variables of time of day (p = .041) and age (p = .046) were statistically significant. As dogs' age increased, so did their cortisol values. With regards to the time of day in which the dogs' urine samples were collected, we found that those that were collected in the afternoon were higher (M = 183.02, SE = 21.42) than those that were collected in the evening (M = 171.43, SE = 21.20), indicating a diurnal rhythm. The variables of condition (p = .159) and weight (p = .191) as well as the interaction of condition and time of day (p = .121) were not statistically significant. As such, we did not find an effect of walk type on urinary cortisol levels overall or by time of day.

#### DISCUSSION

#### 4.1 General Overview of Findings

To explore the potential physiological and behavioral effects of providing dogs with walks that occur in their neighborhoods in comparison to those that occur in nature, we measured dogs' urinary cortisol, behavior, and activity levels during these walks. We hypothesized that during the nature walks, dogs would display fewer stress behaviors, more exploratory behaviors, and lower cortisol levels.

In support of our hypothesis, we found that dogs lip-licked, a stress behavior, at a lower rate in the nature condition. In contrast to our expectations, air scenting, an exploratory/foraging behavior, took place at a higher percentage during neighborhood walks. The hypothesis that dogs would experience less stress, as demonstrated by lower urinary cortisol, during the nature walk condition was not supported. We found no effect of the intervention on dogs' cortisol levels.

We did, however, find a significant difference between the time of day that we collected dogs' urine and the corresponding cortisol levels, such that dogs had higher cortisol levels in the afternoon as compared to the evening. We also found a relationship between dogs' cortisol and their age: as dogs' age increased, so did their cortisol levels. Overall, there was considerable variation in both the urinary cortisol levels and the behavior of dogs in our study.

#### 4.2 Behavior

Movement is considered to be an important component of welfare assessment, as the inability to move freely is considered to be a contributing factor to poor welfare in captive animals (Hediger, 1955, 1964; Morgan & Tromborg, 2007). Within the dog movement behaviors that were coded in our study, standing was found to occur more often during nature walks. One quarter of the time, dogs were simply standing on nature walks compared to less than twenty

percent of the time during neighborhood walks. Moreover, standing was often recorded concurrently with sniffing. Like the increase in the sniffing behavior of captive crab-eating foxes exposed to various olfactory stimuli reported by Figueira and colleagues (2021), greater time spent standing and sniffing by the dogs in our study may indicate the presence of more biologically significant olfactory stimuli within the nature condition.

We found that walking occurred during a significantly greater percentage of the walks in the neighborhood condition in comparison to those in the nature condition. This may be due to being restricted to a shorter length leash on these walks. Other movement behaviors did not differ between conditions or were rare and not analyzed.

Time in nature has been shown to have stress-reducing benefits for humans (Bratman et al., 2015; Legrand et al., 2022; MacKerron & Mourato, 2013; Marselle et al., 2013; Park et al., 2011; White et al., 2019). As such, we hypothesized that nature walks would be less stressful for dogs and expected to find lower rates of stress-related behaviors. Yet, we only found one stress-related behavior, lip-licking, that was significantly lower in nature than in the dogs' neighborhood. Lip-licking is considered to be a stress-related behavior in dogs (Alberghina et al., 2019; Polgár et al., 2019; Shih et al., 2020) and has been shown to increase in response to aversive stimuli, such as harsh training methods (Schwizgebel, 1982), transportation (Herbel et al., 2020), and excessive noise (Beerda et al., 1997).

A significantly lower rate of lip-licking in the nature condition supports our hypothesis, suggesting that perhaps less aversive stimuli were encountered on the dogs' nature walks, such as the presence of loud noises, traffic, or fewer unknown dogs and people. Nevertheless, an alternative explanation is that due to owners feeding their dogs more often during the neighborhood walks than in nature, as lip-licking may be related to the provision of food

(Gerencsér et al., 2013; Tobie et al., 2015) and is thereby not solely an indicator of stress. We attempted to account for this confound in our video coding by excluding lip-licks that occurred within 5 seconds of the dogs being fed. This was not a sufficient delay to avoid recording instances of food-related lip-licking. Future studies may consider restricting feeding if lip-licking is to be behaviorally coded to account for this potential source of bias or include the number of times dogs were fed as a covariate in their statistical analyses. It should also be noted that lip-licking may have been less visually accessible for coding in the nature condition due to the dogs being further away from the camera.

In contrast to lip-licking, other stress behaviors did not differ significantly between conditions or were rare and, therefore, not included in the analyses. Yawning, paw-lifting, and body shaking did not differ significantly between conditions. Shivering (body shaking that occurs over time) was not displayed by any dog in the study. Notably, only two dogs, Hazel and Brandi, displayed behaviors categorized as fear, (i.e., freezing and retreat), but the occurrence of these behaviors did not meet the frequency threshold for statistical analysis.

Neither of our vocalization behaviors, barking and whining, significantly differed between conditions. Both barking and whining may be considered stress-based behaviors (Berteselli et al., 2022; Polgár et al., 2019). Barking is often used as a behavioral measure when evaluating the outcomes of enrichment strategies in kenneled dogs. For example, Schipper et al. (2008) concluded that the provision of food enrichment toys promoted welfare, in part because of the subsequent reduction in barking behavior. In a review of environmental enrichment for domestic dogs living in kennels, Wells et al. (2004) concluded that increased barking behavior occurs in response to social isolation.

Due to the likelihood of the nature condition presenting more novel olfactory stimuli (e.g., wildlife), we hypothesized that exploratory and foraging behaviors, which are natural behaviors for wild canids and domestic dogs (Bracke & Hopster, 2006; Figueira et al., 2021; Mellor & Beausoleil, 2015; Mellor et al., 2020; Miller et al., 2020), would be greater within this condition. When investigating potential scent enrichment protocols for captive African wild dogs, Rafacz et al. (2014) found that the provision of feces from gazelles, a natural prey species, led to increases in wild dogs' positive social behaviors. With domestic dogs, the provision of scent-based enrichment can positively affect their behavior and is considered beneficial for their welfare. For example, Graham et al. (2005) found that dogs in the animal shelter spent more time resting in response to lavender and chamomile scents.

We found that air scenting significantly differed between our two experimental conditions, such that the behavior occurred more often when dogs were walked in their neighborhoods. This was an unexpected result, as we originally hypothesized that all behaviors related to exploration would be higher within the nature walk condition. This may be an indication that olfactory stimuli of interest to dogs in the neighborhood condition were more likely to be carried in the air, rather than found on the ground. The neighborhood walking path was more often on concrete or pavement, with considerably less organic material in comparison to nature walks. It is also possible, though, that this increased observation of air scenting may have been an artifact of the shorter leash length utilized during these walks. Air scenting is a subtle behavior and difficult to observe; as such, the closer proximity to the camera may have allowed for greater observation and coding. Unlike sniffing, air scenting occurs when the dog holds its nose aloft in the air and is followed by rapid inhalations. Sniffing, in contrast, occurs when dogs have their noses on or near the ground, which is easier to perceive on video

recordings. Unlike air scenting, the percentage of time dogs spent sniffing did not significantly differ between conditions.

Except for one dog, Hazel, eating grass did not occur on neighborhood walks. Although eating grass is a common behavior of domestic dogs, the etiology of the behavior is not well understood (McKenzie et al., 2010), though it is often thought to be related to gastrointestinal distress (Sueda et al., 2008). Higher densities of vegetation present in the areas where nature walks occurred, combined with the experimental instructions that discouraged owners from directing their dogs' activities and instead encouraged dogs engaging in self-directed behavior, may have led to the observation of eating grass on nature walks. The ability of dogs to eat grass in the nature condition may also represent a natural behavior in which companion dogs are not often allowed to engage. A study by Sueda et al. (2008) concluded that plant-eating is a typical behavior in domestic dogs. Ingestion of plant material has also been documented in grey wolves (Sueda et al., 2008) spanning North America (Mech, 1966; Stahler et al., 2006), Latvia (Andersone & Ozoliņš, 2004), and Greece (Papageorgiou et al., 1994). As this may be a normal behavior within the canid species, the opportunity to engage in such species-specific behavior may constitute a positive welfare outcome for domestic dogs.

Body shaking did not differ overall between conditions, though it was found to occur significantly less during the third neighborhood walk in comparison to the second neighborhood walk. In previous research, body shaking in dogs has been shown to be a response to stressful environmental stimuli, such as the presentation of heavy metal music (Binks & Montrose, 2022; Kogan et al., 2012), an agility competition environment (Pastore et al., 2011), and the sound of a door slamming (Beerda et al., 2000). As such, the decrease in body shaking across the days of

neighborhood walking may be evidence that dogs' stress response to the presentation of environmental stimuli encountered in the neighborhood condition decreased over time.

#### 4.3 Cortisol

We found no difference in urinary cortisol levels between conditions, indicating that neither walks in nature on a long line or neighborhood walks on a standard-length leash produced significant effects on dogs' welfare in either direction as indicated by this measure. This is in contrast to our original hypothesis, in which we predicted that the combination of increased opportunities to engage in natural behaviors, such as interacting with olfactory stimuli (Amaya et al., 2020; Duranton & Horowitz, 2019; Figueira et al., 2021; Rafacz & Santymire, 2013), as well as the ability to move more freely in a quieter environment (Baranyiová et al., 2005; Lopes et al., 2022), would lead to a reduction in cortisol levels.

The lack of an effect of the intervention may be related to the transportation component inherent to the nature walk condition. By design, this condition included travel to access the natural spaces. This is ecologically relevant, as dogs are often driven by their owners to such locations for on- and off-leash activities in their daily lives. Nevertheless, previous research by d'Angelo and colleagues (2021) found that dogs taking part in a prison-based animal assisted intervention (AAI) program showed significant increases in salivary cortisol when traveling from their kennel to the prison. Similarly, dogs in a study by Kuhn et al. (1991) displayed significant increases in plasma cortisol levels during transport when compared to control dogs that did not leave the kennel facility.

As such, it is possible that the nature walks could impact dogs' cortisol levels, but the stress of transportation to the nature setting may have obscured their effect. In our study, dogs that experienced a car ride (n = 10) to and from their nature walk destination were transported for an

average of approximately 17 minutes. Conversely, none of the dogs needed transportation to their neighborhood walks, and instead walked directly from their homes on familiar routes. Furthermore, the novelty of the nature walk location, in comparison to the familiarity of the neighborhood walk location, may have resulted in higher stress levels. If this, in fact, occurred, three days of exposure could have been insufficient for detecting a reduction in stress due to acclimation to the nature walk location. Similar to our results, Gunter et al. (2021) found that shelter dogs taken on brief outings displayed an increase in cortisol levels, even after accounting for their activity. It may be that the dog leaving their primary place of dwelling alone is sufficient to cause an increase in cortisol.

Exercise has also been shown to increase cortisol levels in dogs (Angle et al., 2009; Clark et al., 1997; Durocher et al., 2007; Fergestad et al., 2016). As such, the increase in cortisol may be due to dogs experiencing more physical stress during nature walks, as displayed by greater amounts of running. Due to the number of such potentially confounding factors, cortisol is an imperfect measure of stress.

When considering the lack of difference in urinary cortisol by walk type, in addition to the drawbacks of using cortisol as a measure of stress, the behavior displayed during walks takes on greater significance. Despite the potential stress of transport, dogs still displayed significantly lesser rates of lip-licking in the nature condition. This may point to nature walks being less stressful for dogs despite the transportation component.

Interestingly, we found a time-of-day effect on dogs' cortisol with dogs having higher levels in the afternoon as compared to the evening. In people, it has been established that cortisol typically peaks during the morning upon waking and decreases as the day progresses (Posener et al., 1996) which would be consistent with our findings. A reduction in cortisol across the day has

been found in some studies carried out with dogs (Alberghina et al., 2019; Beerda et al., 1996; Xue et al., 2017) but not others (Colussi et al., 2018; Jones et al., 1990). Beerda et al. (1996) found that urine samples taken from dogs during the morning had cortisol values that were nearly 50% higher than samples collected in the afternoon. Xue et al. (2017) also observed a rise in urinary cortisol levels from the morning to the afternoon for neutered dogs in their study, but a decline across the day for intact dogs. In research by Alberghina et al. (2019), the presence of diurnal fluctuation in shelter dogs' urinary cortisol depended upon whether the dogs had interacted with another dog during the day. On days when the dog did not have a socialization session, their cortisol levels were lower in the morning and higher in the evening. When dogs were socialized with another dog, a decrease in cortisol was observed across the day.

However, other studies have not found evidence of a diurnal rhythm with dogs' cortisol levels in either direction. Jones et al. (1990) failed to find a consistent fluctuation in urinary cortisol in domestic dogs based on the time of day. Additionally, Colussi et al. (2018) did not find a difference between concentrations of salivary cortisol taken at three different time points each day across three non-consecutive days.

Our findings suggest that there is indeed a time-of-day effect on urinary cortisol levels and that difference can be detected even with a relatively small sample size. Considering that the studies also employed relatively small sample sizes (from 8 to 27 dogs), as well as a mixture of ages, breeds, and sexes, it is possible that the method of collection impacted their results or lack thereof. Importantly, studies that reported cortisol fluctuations according to the time of day (Alberghina et al., 2019; Beerda et al., 1996; Xue et al., 2017) utilized urinary cortisol, while those that did not report a difference (Colussi et al., 2018; Kemppaienen & Sartin, 1989) utilized saliva and plasma samples. This is likely due to the reflection period provided by these different

measures. Plasma and salivary cortisol represent more acute measures, while urinary cortisol manifests over a greater amount of time (Polgár et al., 2019). While Jones et al. (1990) did employ urine samples to measure dogs' cortisol levels and their subsequent report indicated no diurnal variability, examination of their data visualizations suggests that dogs' cortisol levels were higher in the morning and decreased as the day progressed for three out of the four experimental days. As such, this may suggest that future researchers should consider that the biological collection method could impact findings regarding diurnal variability of cortisol.

The lack of difference in cortisol levels between conditions in this study does not preclude the overall benefits of walks, regardless of setting. The simple exercise component of walks may constitute an improvement to welfare, as exercise is a substantial part of the current recommended treatment for weight reduction in pet dogs (German, 2006). This is of particular importance due to obesity being considered the number one nutritional disorder affecting companion dogs (Bland & Hill, 2011; Chan et al., 2005; German, 2006; McGreevy & Bennett, 2010). Obesity is correlated with a wide variety of health issues such as hormonal diseases, orthopedic diseases, respiratory system dysfunction, kidney problems, and skin disorders, many of which reduce longevity (German, 2006). As such, if owners find walks in nature with their dog more enjoyable, this has the potential to increase the amount of regular exercise the dog is receiving and positively impact health outcomes. This also has the potential to positively influence the human-animal relationship, for if owners are positively impacted by walks in nature, as has been demonstrated in the human literature (Bratman et al., 2015; Legrand et al., 2022; MacKerron & Mourato, 2013; Marselle et al., 2013; Morita et al., 2011; Park et al., 2011; Sudimac et al., 2022; White et al., 2019), interactions with their dogs may subsequently benefit, indicating that nature walks may indirectly effect dog welfare in a positive manner.

#### **4.4 Limitations and Future Directions**

When considering the limitations of the current study, a larger sample size would have better accounted for individual variations in cortisol levels and behavior between dogs. Furthermore, many of our owner participants were dog training professionals whose dogs may not have been representative of companion dogs as a population.

Another specific limitation of this study is the way in which walk videos were coded. While the dogs being completely off camera was coded as "unobserved" and subtracted from the total walk time when calculating rate and ratio of behaviors, many of the behaviors listed in the ethogram were likely missed due to reduced visibility. For example, behaviors such as lip-licking and air scenting, would only be visible if the dog was facing the camera, which was often not the case. Future studies should account for this by including an additional video coding parameter, in which the direction the dog is facing or distance from the camera are also accounted for.

As discussed earlier, the transportation component, which was present in the nature walk condition but absent in neighborhood walks, may have increased dogs' stress, reducing our ability to detect a possible stress-reducing effect of the nature walks if present. Future studies could account for this potential confound by including a car ride of the same duration prior to both walking conditions. Alternatively, solely owners and dogs living with natural settings within walking distances of their homes could participate in the study, as was the case for two of our subject dogs (Gatsby and Tenzin). Notably however, Tenzin and Gatsby's urinary cortisol levels did not vary considerably when compared to the remainder of our subjects, suggesting that the transportation component may not have had a huge effect.

Additionally, nature walk conditions in our study varied widely across participants, from beaches and urban parks to more isolated, forested areas. More uniform natural conditions may

have provided more consistent results, as different walk settings, despite being within the broader category of nature, may have included differing novel or stressful stimuli. Nevertheless, the variation in natural settings was the result of taking a more naturalistic approach, representing the variety of environments owners walk with their dogs in nature. It would be worthwhile to compare different types of nature walk settings to determine what type, if any, is most beneficial to dog welfare.

In addition to the type of nature walk condition the dogs experienced, the type of walks that the dogs typically experienced was not accounted for. This may have considerably impacted our results. For example, if dogs were accustomed to being allowed off-leash in nature, being restrained to a long line might constitute a stressful event. Furthermore, the data collection schedule itself may have been a source of stress, particularly if dogs were accustomed to being walked multiple times per day and were subsequently reduced to once daily walks, or no walks at all as in the baseline condition.

This study also represents an exploration of the minimum requirements, in terms of walk duration and leash length, for nature walks to purportedly impact welfare (Stremming, personal communication). Sarah Stremming, originator of the term "decompression walk," suggests that dogs be allowed off-leash during nature walks (2018a). Stremming also suggests that nature walks last at least forty-five minutes (2018a), as opposed to the thirty minutes used in this study. It may be that the nature walk parameters in this study were not robust enough to elucidate an effect, and as such, study of longer walks, as well as walks in which dogs are allowed off-leash, are warranted.

Furthermore, while we intended for our experimental conditions to vary in both the environment in which the dog was being walked as well as the way in which the owner managed

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their dog (i.e. short or long leash), it would be important for future studies to disambiguate their effects to identify whether the environment or owner behavior is most impactful on dogs' welfare. Additionally, the measures we utilized in this study to investigate these walking conditions, cortisol, behavior, and activity levels, are not the only means by which to determine the potential impacts of walk setting. Future research might, for example, want to employ preference assessment testing to determine if dogs prefer walks in their neighborhoods to those in nature or perhaps if their preferences vary from day to day. By pairing individual leashes or equipment with walk types, dogs' preferences could be learned, their preferred walks provided, and their impact on dogs' welfare measured.

#### CONCLUSION

There is currently a dearth of information on the impacts of environmental walk conditions on dog welfare, despite claims that nature walks represent a relatively simple way to enhance welfare (Catan, n.d.; Efimova, 2019; Stremming, 2018c). To our knowledge this study represents the first investigation that measures dogs' behavior and cortisol levels when experiencing walks in their neighborhoods and those in nature. We found that cortisol levels varied significantly according to time of day but did not differ between experimental conditions as hypothesized.

Notably, despite a number of studies in humans demonstrating the psychological benefits of spending time in nature, Veitch et al. (2022) did not find a statistically significant difference in the salivary cortisol levels of human participants when they experienced urban and nature walks. As such, it is possible that cortisol is not the best physiological measure for comparing these two types of walking conditions, irrespective of the species being studied. Given the limitations of cortisol as a measure of stress, alternative welfare measures such as cognitive bias testing or observation of post-walk in-home behavior should be explored. These measurements may be stronger indicators of the ways in which the two types of walks impact dogs' welfare.

Only one stress behavior, lip-licking, varied significantly between conditions, such that it occurred more often during dogs' neighborhood walks as opposed to those in nature. While the directionality of this behavior supports our hypothesis, it is also possible that the increased lip-licking was an artefact of higher food consumption during neighborhood walks. While these results do not point to nature walks appearing to be less stressful for dogs, further research is needed to explore this topic more thoroughly.

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As this study represents the first attempt to understand the effects of walk type on canine welfare, there is much work to be done before strong conclusions can be made. The study designs of future research should address the many components that differ between these two types of walks beyond setting, such as transportation needed to access natural settings, the length of the leash, and the behavior of the owner.

Our findings suggest that nature walks do not constitute a less stressful walk experience. However, when we consider the importance of providing physical activity to the overall health of dogs (Bland & Hill, 2011; German, 2006), any potential benefits of nature walks as a whole, especially in regard to owner preferences, warrant further investigation. If dog owners find nature walks more enjoyable, dogs may be more likely to receive walks of this type with longer durations, leading to the provision of more exercise. Conversely, if access to nature is limited for owners, the ease of neighborhood walks may lead to greater frequency and better health for their dogs. To better understand the effects of walking environment on owner behavior, post-walk questionnaires could be deployed, or activity monitors paired with walking diaries to track frequency, intensity, and duration of dog walking activity. Given the relationship between humans and their companion dogs, it is imperative that the sustainability of providing nature walks (or walks in general) on a regular and long-term basis is also taken into consideration. It may be that the walk condition itself is less important, and that the priority should instead lie in making sure that dogs are walked on a regular basis.

# TABLES

### Table 1

Subject Dog Population Information

Name	Age	Gender/neuter status	Breed	Weight (in kg)	First Condition	
Arlo	9y 5m Male (neutered)		Vizsla	24.94	Nature	
Asher	5y 9m	Male (neutered)	Great Dane	69.40	Nature	
Bart	2y 10m	Male (neutered)	English Bulldog Mix	29.48	Neighborhood	
Brandi	10y	Female (spayed)	Golden Doodle	27.03	Neighborhood	
Derby	2y 8m	Male (neutered)	Border Collie	18.14	Neighborhood	
Ember	1y 9m	Female (spayed)	Welsh Springer Spaniel	17.69	Nature	
Franny	9y 11m	Female (spayed)	Miniature Poodle Mix	6.35	Neighborhood	
Gatsby	7у	Male (neutered)	Shepherd Mix	29.48	Nature	
Hazel	11y 11m	Female (spayed)	Pitbull Mix	20.41	Neighborhood	
Hobie	13y 5m	Male (neutered)	Shih Tzu	6.35	Nature	
Hollie	3y 7m	Female (spayed)	English Setter	32.66	Neighborhood	
lzzy	1y 6m	Female (spayed)	Miniature Golden Doodle	11.79	Neighborhood	
Maggie	8y 3m	Female (spayed)	Pitbull Mix	37.65	Nature	
Tenzin	1y 5m	Male (intact)	Labrador Retriever	30.39	Nature	
Winston	2y 3m	Male (neutered)	German Wirehaired Pointer Mix	29.94	Nature	

Behavioral Ethogram

Category	Behavior	Definition					
	0		Туре				
	Standing	Dog is immobile while erect on all four legs	Duration				
	Walking	Dog moves slowly with lateral legs moving together, two legs are always on the ground at one time	Duration				
	Running	Fast paced movement with all four paws often in the air – includes loping and cantering	Duration				
Movement	Trotting	Moving at a pace faster than a walk and slower than a run, similar to jogging in humans	Duration				
	Sitting	Back legs are tucked up under the body while front legs remain in a standing-like position	Duration				
	Laying down	Body flat against the ground; head may be held up	Duration				
Fear	Freezing	Dog ceases all movement and vocalization while the body is held still, posture may appear stiff/rigid	Duration				
	Retreat	Dog quickly moves away from a stimulus	Duration				
	Yawn	Dog opens mouth wide with no vocalization	Point				
	Paw-lift	Dog raises and holds front paw off the ground for at least 2 seconds	Point				
	Shivering	Entire body is shaking/quivering despite ambient temperature	Duration				
Stress	Lip-lick	Dog flicks tongue quickly outside/around mouth; exclude if dog has been given treat in the previous 5 seconds					
01000	Piloerection	Hair on the back of the dog's neck stands up					
		Dog moves body rapidly from head to tail, as though just emerging from	Duration				
	Body shake	water. Exclude if the dog is in a body of water or has emerged from water					
		within the previous 30 seconds.					
	Bark	Short, fast, and often repetitive vocalization emitted with an open mouth	Point				
Vocalization	Whining	High pitched vocalization, mouth may or may not be open; generally, spans several seconds					
	Sniffing	Nose is down near the ground or held close to an object with mouth closed	Duration				
	Air scenting	Dog holds nose up and appears to inhale rapidly	Duration				
Exploratory/Foraging	Digging	Dog uses front paws to scratch ground and create an indentation	Duration				
	Eating grass	Dog ingests plant matter	Duration				
Other	Rolling	Dog moves back and forth on ground while in lateral or dorsal recumbency	Duration				
	Unobserved	Dog is out of sight/completely off camera	Duration				
Uncategorized	Walk Duration	For neighborhood walks, time between dog exiting/returning home; for nature walks, time between dog leaving/returning to parking lot or time between long line being clipped on/removed	Duration				

Note. Adapted from (Essler; Pritchett et al., 2021; Walker et al., 2016; Way et al., 2006)

Example Data Collection Timeline	9
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Day	Morning Urine Collection (7:00a.m.–9:00a.m.)	Walk Type (11:00a.m1:00p.m.)	Afternoon Urine Collection (3:00–5:00p.m.)	Evening Urine Collection (7:00-9:00p.m.)	Notes
1		No walk	Sample #1	Sample #2	Activity monitor attached (note time)
2	Sample #3	Treatment #1 30m	Sample #4	Sample #5	
3	Sample #6	Treatment #1 30m	Sample #7	Sample #8	
4	Sample #9	Treatment #1 30m	Sample #10	Sample #11	
5	Sample #12	No walk	Sample #13	Sample #14	
6		Dogs do usual dai	ly activities – no urine s	ample collection.	
7		No walk	Sample #15	Sample #16	
8	Sample #17	Treatment #2 30m	Sample #18	Sample #19	
9	Sample #20	Treatment #2 30m	Sample #21	Sample #22	
10	Sample #23	Treatment #2 30m	Sample #24	Sample #25	
11	Sample #26	No walk	Sample #27	Sample #28	
					Activity monitor removed (note time);
12					Animal released from study & remains in owner's care.

Note. Adapted from Gunter et al., (2021). Treatment #'s 1 & 2 alternate between neighborhood and nature walk conditions.

Types of Movement Displayed by Dogs and Percentage of Time Spent in Those Movements During 30-Minute Neighborhood and Nature Walks

		r	Neighborh	nood Walk	s		Nature Walks							
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3			
	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR		
Movement														
Standing	13.79	10.02 _ 17.36	14.65	10.26 _ 18.88	14.61	12.96  23.25	23.47	16.97  31.91	21.91	14.81 	25.09	11.32 		
Walking	73.79	57.29 _ 85.89	74.24	62.70 _ 84.88	76.89	52.16 _ 83.26	54.35	47.41 _ 72.14	59.37	45.40  70.63	54.73	47.47 _ 67.63		
Running	0	0  0.26	0	0  0.37	0	0  0.08	0.15	0  0.60	0.05	0  0.57	0.20	0  0.79		
Trotting	4.09	1.12 _ 17.04	6.96	0.53 _ 15.47	3.68	1.31 _ 7.83	11.69	7.60 _ 18.42	12.01	5.56  21.51	16.39	4.04 _ 26.93		
Sitting	0	0  0.43	0	0 _ 1.01	0	0	0	0	0	0	0	0		

Types of Point Behaviors Displayed by Dogs and Rate per Minute of Those Behaviors During 30-Minute Neighborhood and Nature Walks

			Neighborh	ood Walks	6	Nature Walks							
	Day 1		Day 2		Da	Day 3		Day 1		Day 2		Day 3	
	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR	
Behavior													
Yawn	0	0	0	0  0.02	0	0  0.02	0	0	0	0  0.02	0	0  0.05	
Paw-lift	0	0  0.04	0	0	0	0  0.02	0	0  0.06	0	0  0.02	0	0  0.04	
Lip-lick	0.73	0.31 _ 1.45	0.65	0.30 _ 1.78	0.45	0.15 _ 1.43	0.27	0.12 _ 0.54	0.11	0.10 _ 0.59	0.14	0.08 _ 0.60	
Body shake	0.09	0.03 _ 0.18	0.07	0.01 _ 0.21	0.04	0.01 _ 0.19	0.09	0.04 _ 0.18	0.11	0.04 _ 0.29	0.06	0.03 _ 0.16	
Bark	0	0	0	0  0.03	0	0  0.07	0	0	0	0	0	0	

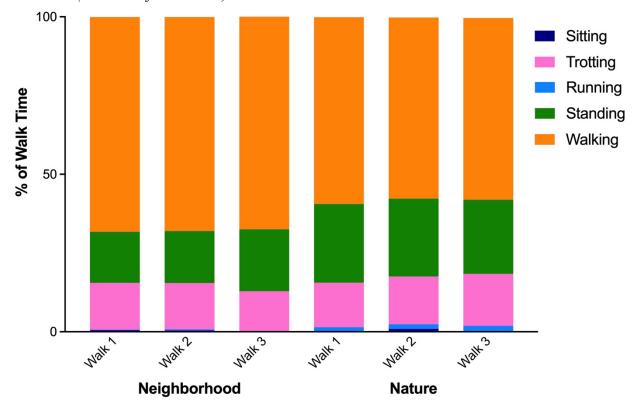
Types of Duration Behaviors Displayed by Dogs and Percentage of Time Spent Engaging in Those Behaviors During 30-Minute Neighborhood and Nature Walks

	Neighborhood Walks							Nature Walks						
	Da	y 1	Day 2		Day 3		Day 1		Day 2		Day 3			
	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR	Mdn	IQR		
Behavior														
Sniffing	16.99	13.18 _ 24.23	12.20	7.72 _ 27.46	15.63	12.28 _ 21.24	25.24	20.75 	26.40	19.64 _ 34.54	27.32	15.77 		
Air scenting	0.17	0 _ 0.83	0.09	0  0.67	0	0  0.18	0	0  0.11	0	0	0	0  0.06		
Eating grass	0	0	0	0	0	0	0	0  0.72	0	0 _ 1.33	0	0  0.08		
Whining	0	0  0.22	0	0	0	0	0	0  0.01	0	0	0	0  0.02		

# **FIGURES**

Figure 1Error! Reference source not found.

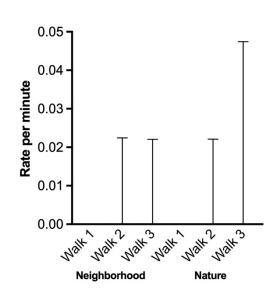
Movement (Mean % of Walk Time)

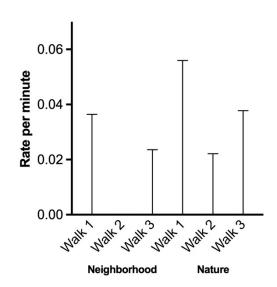


Behavior (Median Rate per Minute and Interquartile Range)



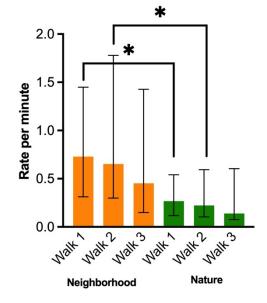


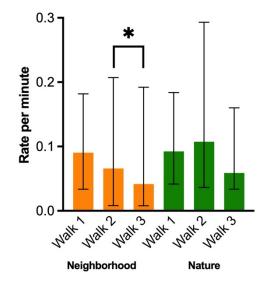




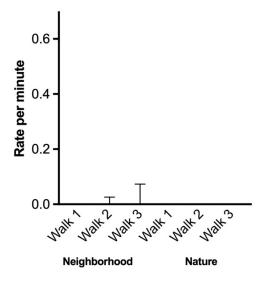
c. Lip-lick

d. Body shake





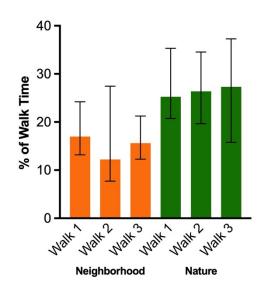


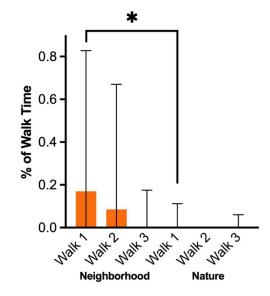


Behavior (Median % of Walk Time and Interquartile Range)

# a. Sniffing

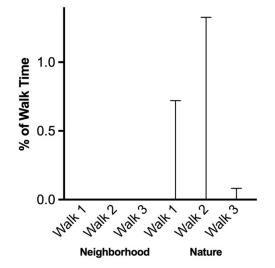
# b. Air scenting

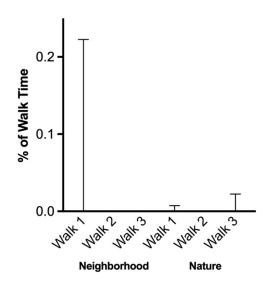




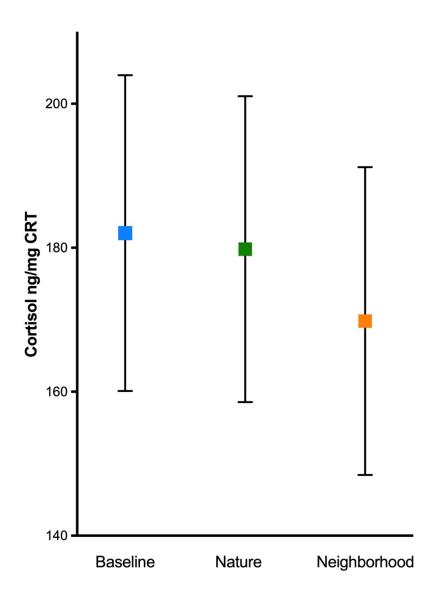
c. Eating grass

d. Whining

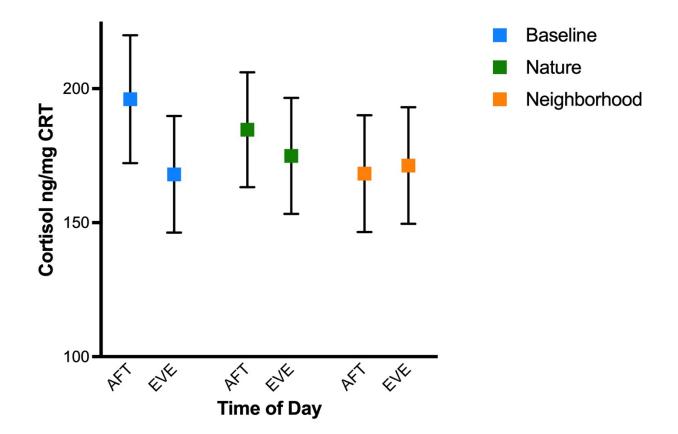




Urinary Cortisol per Condition (Mean and Standard Error)



Cortisol per Time of Day (Mean and Standard Error)



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