Effect of Multidog Play Groups on Cortisol Levels and Behavior of Dogs (Canis lupus familiaris) Housed in a Humane Society

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Abstract

Dogs waiting for adoption in humane societies face several stress-inducing stimuli. The aim of this research was to study the effect of multidog play groups on cortisol levels and stress-related behaviors in kenneled dogs in a humane society. Forty dogs were randomly assigned to an experimental group and a control group. The experimental dogs went to a 30-minute play group each day. Salivary cortisol specimens were obtained at baseline, 30 minutes after play group, and 3 hours after play group. Video recordings were conducted at the same time points for each of the groups. We found no significant difference in the cortisol levels between groups and across days. We did obtain significance with the video analysis for specific behaviors between groups and across days. Overall, there were fewer stress behaviors in dogs who had the opportunity to participate in play groups than in those who did not. Offering humane society dogs the opportunity to play can be a great outlet to release stress and improve conditions for animal welfare.

Keywords: animal welfare, canine play, kennel stress, salivary cortisol, social behavior, stress

Introduction

Canine stress is a state in which a dog reacts to an endogenous or exogenous threat and focuses its energies on coping with a dangerous situation (Scholz & Von Reinhardt, 2007). This stress is a response of an organism to a demand placed upon it to change or adapt (O’Heare, 2005). The stress response is divided into three general stages: the recognition of a stressor, the biological defense against the stressor, and the consequences of the stress response (Moberg, 2000). There are numerous emotional states that could elicit a stress response, including arousal, fear, nervousness, agitation, and frustration. Kennel stress is visible in dogs that are confined for a period of time and exhibit specific stress markers (Beerra, Schilder, Van Hooff, & DeVries, 1997; Beerra, Schilder, & Van Hooff, 1999; Blecha, 2000; Hiby, Roosey, & Bradshaw, 2006; Stephen & Ledger, 2006).

Although humane societies and rescue groups prepare dogs for adoption, not all dogs are adopted immediately, and when housed for long periods of time in kennels these dogs may manifest various stress-related symptoms (Hewson, Hiby, & Bradshaw, 1997; Hiby et al., 2006). The associated physiological events related to stress can impact immune response and overall health (Beerra et al., 1997, 1999; Blecha, 2000; Hewson et al., 1997; Hiby et al., 2006; Moberg, 1985). This so-called “kennel stress” can be severely deleterious (Taylor & Mills, 2007). It is identifiable by a number of overt behavioral symptoms, including backing away on approach, barking, figure-eight spinning, growling, vertical jumping, exaggerated autogrooming (air licking, flank or facial licking), repetitive pacing, panting, destructive ripping, distress whining, yawning, facial muscular rigidity, intense staring, rounded topline of the dog’s body with a lowering of the head, and/or hiding (Abrantes, 1997; Aloff,
A number of known factors elicit stress in shelter dogs. Among them are high noise levels (including other dogs’ barking) (Sales, Hubrecht, Peyvandi, Milligan, & Shield, 1987; Wells et al., 2002), arousal associated with potential adopter interactions (Hewson et al., 1997), arrival of shelter employees, feeding and kennel cleaning rituals, and ancillary noises associated with vehicle traffic, trains or sirens (Sales et al., 1987). Shelter dogs are described as “quieter” after kennel lights are off (Wells, 2004), and dogs are reported to rest while lying or sitting (versus standing) when they listened to classical (versus “heavy metal”) music (Wells et al., 2002). Kennel stress affects the dog’s endocrine system (altering hormone levels), and sensory systems (auditory and visual systems, in particular) (Blecha, 2000; Moberg, 2000).

One reported physiological marker of such stress is elevated cortisol levels (Beerda et al., 1997; Dreschel & Granger, 2009; Haverbeke et al., 2009; Horvath, Doka, & Miklosi, 2008; Russell et al., 2007). Corticoids appear to be produced in association with stress, and have behavioral effects associated with the maintenance of bodily functions. The sympathetic nervous system is activated and will increase glucose, blood pressure and heart rate (Chrousos & Gold, 1992). Cortisol release is affected by numerous conditions, including stress, exercise, fever, trauma, pain, and extreme temperature.

Dreschel and Granger (2005) evaluated stress in dogs in a study on exposure to a noise stimulus that was similar to a thunderstorm. Findings included significant increases in canine salivary cortisol levels related to fear of a thunderstorm noise stimulus. The dogs were also observed to exhibit such behavioral signs as pacing, panting, hiding, positioning near the owner, and whining. Another study on canine stress due to noise sensitivity showed increased vital signs in canines exposed to 1-minute increments of aversive stimuli, such as sound blasts, electric shock, an open umbrella, and restraint (Beerda, Schilder, Van Hooff, DeVries, & Mol, 1998). A number of studies report that cortisol levels in dogs increase with exercise and activity. Sprint-racing sled-dog teams were researched, and serum cortisol levels were significantly elevated after a few days of racing (Wakshlag, Snedden, & Reynolds, 2004). Alternating exercise and cage rest in sled dogs over a 12-week period showed elevated plasma cortisol levels. The external demands of racing placed enough stress on the dogs to cause the increase. Hinchcliff and colleagues (1993) conducted a study whose findings were contrary to other studies on cortisol being elevated from exercise. They found no changes in cortisol levels in long-distance sled-racing dogs. The findings suggested the dogs were not severely stressed by the demands of competing in the race. Cortisol levels can change in opposite directions depending on human interaction with the dog, which can be affiliatory or authoritarian (Horvath et al., 2008). In addition, Hewson et al. (1997) found that kennel-housed female dogs exhibited more stress symptoms than males. In essence, cortisol levels can vary and increase with numerous stimuli, including noise and exercise.

Play is motor activity performed postnatally in which motor patterns from other contexts may often be used in modified forms (Bekoff, 1972, 1998). The styles of play of domestic dogs can follow patterns of breed-specific jobs such as hunting, guarding, tracking, or other behavior patterns necessary for later survival (Beaver, 1994). While play may appear purposeless to the human observer, it is assumed to be a form of “practice” for living. When dogs play, they use the same forms of body language as those used for status battles, reproduction, and hunting (Aloff, 2005; Horowitz, 2006).

Play can ameliorate the effects of kennel stress (Bekoff, 1972, 1998; Horvath et al., 2008). Play has a calming effect, especially if it has an exercise component. So-called “object-
related” play with a person, such as tug-of-war or ball retrieving, can calm a stressed dog (Toth, Gácsi, Topal, & Miklósi, 2008). Hence, play interventions by caregivers may decrease kennel stress and the associated behavioral problems in dogs. Incidentally, this can lead to more timely and successful adoptions (American Kennel Club, 2009).

The purpose of this study was to test whether intraspecific play intervention lowers (1) stress levels in kennel-housed dogs (measured by the amount of stress-related behaviors manifested), and (2) canine salivary cortisol levels.

Materials and Methods

The experimental protocol was approved by Northeastern Illinois University Animal Care and Use Committee as well as the Longmont Humane Society Executive Board. Procedures were in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, as well as standards for humane care and treatment of animals through the United States Department of Agriculture (USDA).

Expanded Range HS Salivary Cortisol EIA Kits, sorbettes, and collection supplies were obtained from Salimetrics Laboratory in Pennsylvania. All other chemicals and supplies were obtained from Fisher Scientific Company in Massachusetts.

Forty dogs were selected from the Longmont Humane Society (LHS) and were randomly assigned to experimental and control groups. These dogs had been in the shelter through the initial screening phase and had acclimated to their surroundings for a minimum of 48 hours prior to being in the study. The dogs received a veterinary exam and were noted as being in good health. Each of the dogs was temperament tested, found to be stable, and kenneled on the adoption floor. The subjects ranged in age from 6 months to 13 years. There were a variety of breed types (purebreed and mixed) in the study, including border collie, Australian cattle dog, American pit bull terrier, miniature poodle, German shepherd, labrador retriever, shiba inu, beauceron, Lakeland terrier, harrier hound, kelpie, wirehaired terrier, boxer, golden retriever, otterhound, American Staffordshire terrier, Rottweiler, and Lhasa apso. There were 18 females and 22 males in the study. Each dog was identified by their specimen number, which was attached to the dog’s collar and kennel, and handwritten on duct tape attached to their collar for the purposes of play-group sessions. The medication records of the study dogs were reviewed with the veterinary staff and evaluated for appropriateness for selection in the study. None of the dogs were on any corticosteroids.

The study was conducted over a 4-day period. The 20 experimental dogs took part in a daily 30-minute play-group session. The 20 control dogs remained in their kennels and did not take part in the daily play-group session. Specimens were collected from all dogs at baseline (each morning), 30 minutes after a play-group session, and 3 hours after a play-group session. Before each specimen collection, the dog was videotaped in their kennel for 1 minute for behavior analysis. The daily play-group sessions were also videotaped.

Hand sanitizer was used by handlers between specimen collections from different dogs, to prevent any possible spread of infection. The dogs were observed to see if there was any reaction to the smell of sanitizer on the handlers’ hands. The dogs did not seem to be affected by the sanitizer. Before the study, the team of five professional dog trainers/handlers (subinvestigators—SIs) attended a class and were informed of their role in the research. The USDA standards for humane care and treatment of animals were reviewed. The SIs were taught how to collect saliva specimens and properly store them, and completed a return demonstration of the collection protocol. The SIs worked in teams of two to collect specimens. The team kept detailed records of saliva collection times and play-group start and finish times. One of the professional trainers facilitated the multidog play-group session each day and used a timer to maintain the schedule. Handlers reviewed the behavioral signs of stress in the dogs. The dogs were desensitized to the
swabbing procedure, to prevent resistance or a stress response to specimen collection.

Many LHS volunteers helped each day with transporting dogs to maintain the rigorous schedule of the study. All dogs were allowed to eliminate outside. Control dogs were immediately brought back to their kennels without the benefit of play group. Experimental dogs were immediately brought to the play yard after elimination. Once the video recordings and specimens were collected on the dogs, all the dogs returned to their normal routines for the rest of the day.

The dogs’ morning meal was withheld until after the second swabbing for each of the 4 days until the study was completed. We were concerned that the dogs could become stressed if they were conditioned to expect a meal at a certain time and none was offered. To control for this, before the study, the dogs’ morning meal was withheld so that they would not notice any change in routine once the study began. Water was withheld 30 minutes before swabbing since drinking water could dilute the saliva and give false readings of cortisol levels (Salimetrics, 2009). Food was withheld 1 hour before swabbing since the presence of food could alter salivary pH and ultimately alter specimen results (Salimetrics, 2009). Canine saliva was collected using two sorbettes obtained from Salimetrics Lab (Figure 2). Once the sorbettes swelled from saliva saturation, they were placed in a prelabeled conical storage tube. The tube was immediately placed in a tube-organizer storage box in the freezer. The label read #1-1-1 for Dog 1, Day 1, Sample 1 and followed this pattern throughout the 4 days.

Enzyme-linked immunosorbent assay of saliva samples was done at the Northeastern Illinois University Biology Department Laboratory, according to the manufacturer’s protocol. The procedure used 96-well plate Expanded Range HS Salivary Cortisol EIA Kits. Cortisol samples were thawed on ice and centrifuged at 3000 rpm at room temperature for 20 minutes. The saliva was transferred to small prelabeled microfuge tubes. Samples that did not have enough saliva were diluted with the reagent in the kit to make up 25 μl of fluid. Mean cortisol levels (μg/dL) and standard deviations were compared individually and between groups. Cortisol level percentage change was assessed across samples.

The SI who completed the video recordings took precautions to be nonintrusive during videotaping. Behavior counts from the video analysis were conducted (blind) at the end of the study by two people who were not dog professionals. They logged the number of times specific behaviors were viewed on the videos. These behaviors included figure-eight spinning, vertical jumping, pacing, facial licking, panting, yawning, distress whining, and barking.

LHS has a unique outdoor play-yard area. The fenced area has three large attached yards, with three small fenced entrance areas. While it would be beneficial for the fenced yards to be larger, allowing the dogs even more room to run and maneuver freely, the current setup is conducive to exercise and play between all three areas, with the opportunity to close off sections to divide dogs for size and/or play-style appropriateness. During the play groups (Figure 6, Figure 7), the handlers continuously open and close the areas to support healthy interaction with groups of dogs that are best suited for one another. The small entrance areas provide a safe place for volunteers to enter with a dog, separate from the playing group. As the new dog enters, there is no risk of an escapee. In addition, if an altercation breaks out where dogs need to be separated, the entrance pens can be used for this purpose.

Operational definitions of behaviors monitored included the following:

**Vertical jumping** was scored as one point per episode of jumping at kennel gate or wall. After the dog had stopped the behavior and reached baseline, as evidenced by no jumping, the dog would receive another point when they started the behavior again.

**Barking** was scored as one point for each episode of vocalization. When the dog returned
to a nonbarking state, the measurement would begin again.

*Facial licking* (Figure 3) was scored as one point per episode. (This behavior is not associated with food or water, since there was none available prior to specimen collection times.)

*Panting behavior* was timed and received one point for every 5 seconds of panting.

*Pacing behavior* was measured as one point for each time the dog walked the length of the kennel and back.

*Figure-eight spinning* episodes (Figure 4) were exhibited when the dog jumped up and turned in a figure-eight pattern, usually bouncing off one of the two side walls of the kennel, spinning and then bouncing up against the opposite kennel wall. This behavior was counted as one point for each full figure-eight pattern observed.

*Distress whining* is a nonbarking vocalization that tends to be repeated during stress. This behavior was given one point per episode of whining.

*Yawning and growling* were given one point per episode of behavior.

The video analysis was initially conducted by two people who were not dog professionals. When dog professionals viewed the video recordings, it was noted the dogs were exhibiting other signs of stress in which they were not relaxed. These behaviors included air licking, flank licking, scratching, destructive ripping behaviors, backing away from stimuli, and a patterned behavior in which the dog showed facial muscular rigidity, a rounded topline, a hard eye stare, and an attempt to hide (Aloff, 2005; Handelman, 2008). The operational definitions for these behaviors included the following:

The dog received one point when they showed a four-step patterned behavior that was noted a number of times upon video analysis. We defined it as *facial muscular rigidity, rounded topline of the dog’s body and lowered head, hard-eyed stare, followed by an attempt to hide.*

*Destructive ripping* (Figure 5) behavior episodes were timed and scored one point for every 5 seconds.

*Backing away* to hide was given one point per episode. *Growling* was given one point per episode.

*Air licking, flank licking* and *scratching* were given one point for every 5 seconds the dog presented this behavior. The scratching behavior was observed to be a very fast, continuous behavior on an inanimate object.

Three dogs were dropped from the study due to fearful behavior, tremoring, and anorexia. The final sample was n = 37.

Data analysis was completed with standard statistical analysis through testing with DataDesk (DataDesk®, Data Description, Inc., Ithaca, NY 14850, USA, www.datadesk.com). A chi-square analysis was conducted on the video recorded data.

**Results**

Refer to Figure 1 for the mean cortisol levels (µg/dL) of the dogs in the experimental and control groups over three samples (baseline, 30 minutes post–play group, and 3 hours post–play group) and across 4 days. Normality testing was done, and the data were not normally distributed. Although there were increases and decreases in the cortisol levels throughout the experiment, the differences in cortisol levels were not statistically significant over the three samples and across the 4 days.
Behavior frequencies are presented in Table 1. Overall, the behaviors can be divided into three categories according to their frequencies: high (counts = 86–170), medium (counts = 14–66), and low (counts = 6–7) frequency behaviors. We obtained significant differences in specific behaviors between groups over the 4 days. Of the 781 stress behaviors noted on video analysis, the majority occurred in the control group dogs who did not have access to the play group (control: 427 stress behaviors; experimental: 354 stress behaviors; $\chi^2 = 133.4$, $df = 14$, $p < .0001$). Analysis of the behaviors in each category gave the following results:

- **High frequency behaviors** $(\chi^2 = 66.1$, $df = 3$, $p < .0001)$
- **Medium frequency behaviors** $(\chi^2 = 38.22$, $df = 5$, $p < .0001)$
- **Low frequency behaviors** $(\chi^2 = 17.94$, $df = 4$, $p < .003)$

![Figure 1](image-url)
Table 1. Summation of stress-related behaviors ranked by total frequency of occurrence (orange = high frequency, blue = medium frequency, lavender = low frequency)

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Control</th>
<th>Experimental</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical jumping</td>
<td>87</td>
<td>83</td>
<td>170</td>
</tr>
<tr>
<td>Barking</td>
<td>81</td>
<td>71</td>
<td>152</td>
</tr>
<tr>
<td>Facial licking</td>
<td>56</td>
<td>76</td>
<td>132</td>
</tr>
<tr>
<td>Muscle rigid/stare/hide</td>
<td>82</td>
<td>4</td>
<td>86</td>
</tr>
<tr>
<td>Panting</td>
<td>31</td>
<td>35</td>
<td>66</td>
</tr>
<tr>
<td>Pacing</td>
<td>22</td>
<td>31</td>
<td>53</td>
</tr>
<tr>
<td>Stress whining</td>
<td>17</td>
<td>15</td>
<td>32</td>
</tr>
<tr>
<td>Figure-eight spin</td>
<td>1</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Yawning</td>
<td>5</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Ripping</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Growling</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Back away</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Air licking</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Flank licking</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Scratching</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>427</td>
<td>354</td>
<td>781</td>
</tr>
</tbody>
</table>
**Figure 2.** Handlers obtaining canine salivary cortisol specimens with sorbette swabs

**Figure 3.** Licking behavior

**Figure 4.** Spinning behavior

**Figure 5.** Destructive behavior (ripping blanket)

**Figure 6.** Play group
Figure 7. Play group

Discussion

Long periods of confinement are known to cause stress in dogs (Beerda et al., 1997, 1999). Stress is manifested in both behavior and physiology. Much of the early work on canine stress centered on behavioral symptoms; however, recent reports suggest a link between canine stress levels and cortisol (Haverbeke et al., 2009). Kennelled dogs are exposed daily to several stress factors, such as confinement, loud noises, and visitors. Kenneling can lead to stressed behaviors. Although cortisol is a marker for stress, in this study cortisol levels were not significantly different between the control and experimental groups. On Days 2, 3 and 4, both groups showed a nonsignificant increase in cortisol levels at the 30-minute post-play sample collection.

Cortisol level is known to be affected by various factors, such as exercise, excitement, and arousal (Rooney et al., 2003; Rooney, Gaines, & Bradshaw, 2007; Stephen & Ledger, 2006; Taylor & Mills, 2007). It is possible that the heavy exercise during play may have resulted in higher levels of cortisol in the play group, whereas the anticipation of their morning meals could have increased the cortisol levels in the control group.

In addition, there were extraneous variables that may have further affected cortisol levels. Short of closing the shelter doors for the length of the study, it was challenging to control these variables. The shelter opened its doors to potential adopters late morning. At that time, families would come in to find a pet. Some of the dogs became very aroused as people walked past their kennels, talking and pointing to them. One early morning, the county had an administrative meeting that was held at the shelter without the knowledge of the researchers. Before our research staff began the study, a number of meeting participants stopped to see the shelter dogs after their meeting. All the dogs were quite aroused by the time we got there to conduct our research. On Day 3, unbeknownst to the research team, summer camp children had arrived at the shelter and washed kennel windows to perform an act of kindness for the dogs. This excited the dogs, even though we were promptly able to clear the children from the area.

Cortisol levels showed an increase on Days 3 and 4 in the experimental dogs, and a decrease on Days 3 and 4 in the control dogs, but the changes were not statistically significant. It is possible that the dogs became habituated to the kennel environment and routine of the study (Hinchcliff et al., 1993). As their stress level dropped, they may have shown lower cortisol levels.
Play has long been known to be relaxing, for humans as well as animals. LHS uses play groups as enrichment, as an assessment tool, and to modify behavior. The role of the handler is to observe behavior and interfere only when necessary. The purpose of play groups is to allow the dogs the opportunity to respond and relate to one another in a social setting. For some dogs who have not been afforded this opportunity in their lives, a learning process is involved. LHS’s position is that dogs are the best teachers for one another when it comes to dog–dog interactions. The handlers provide support by interrupting posturing and displays that are disproportionate to the feedback being given by another dog. If inappropriate posturing or displays are successfully diffused or diverted by another dog, the handler does not need to interfere. Various aversives are used if needed to interrupt inappropriate behavior. Examples of the aversives used include a water squirt bottle, shake can of pennies, or an air horn. The dogs may respond differently to each aversive. The act of playing and socializing with one another is considered a self-rewarding behavior and therefore qualifies as positive reinforceer for social interaction. When the dogs communicate with one another to change or diffuse an interaction, they use a combination of positive punishment and negative reinforcement. The handlers use these techniques as well, to assist in changing or diffusing inappropriate interactions when necessary.

During the play time over the 4 days, some dogs initiated play with a play bow, some ignored each other, and some played chase. Some dogs mounted another dog. On Day 2, a fight broke out between five dogs and had to be interrupted by the lead person via a horn. There was loud vocalization and arguing for a short time. Two of the dogs had to be separated initially by a gate, but all the other dogs resumed playing as though nothing had happened. When the fight was over, there was a 1-inch pink scratch on the inside of the ear of the dog who had been mounting the other dogs. The rest of play group was uneventful. The cortisol levels in the dogs involved in the fight were elevated, as expected, 30 minutes post–play group. Their cortisol levels decreased dramatically 3 hours post–play group. On the other days, the play group was uneventful with the same mix of dogs.

Although there were no differences in cortisol levels between control and experimental groups, the experimental dogs were observed to be calmer after the play-group session at the 3-hour time point. Some of the experimental dogs were lying belly up watching quietly as people walked by looking for an adoptable pet. Although all of the dogs had access to bedding, it was observed that a number of dogs in the control group ripped apart bedding at the 3-hour time point. These dogs did not present relaxed behaviors, and the researchers regarded this behavior as stress.

The researchers did not note any correlation between either breed or sex of the dogs and either cortisol levels or stress behaviors. One dog was coprophagic in the kennel. This particular dog had a history of coprophagia. We did not consider this to be a stress behavior on video analysis. Although there were a few dogs who smeared feces in their kennels, most of their stools were solid. There were no unusual elimination patterns, such as diarrhea, which can sometimes be noted in times of stress. However, a number of dogs (toy breeds) had dry mouths. We attempted to stimulate saliva production through the use of food treats, by keeping them available in a pouch the handler wore, or allowing the dogs to smell food. Some of the dogs increased saliva production with this process, others did not. A small number of sorbettes had blood on them with specimen collection. In reviewing the analysis, the sorbettes tinged with blood did not seem to have an effect on cortisol level results.

Conclusion

In this study, we found that cortisol levels varied within groups and across days, but the differences between the control and experimental groups were not significant. Our studies suggest that cortisol may not be the best chemical to monitor for stress in canines, due to the various factors that can affect its level. Literature does show variability in other cortisol studies. The video analysis was helpful in this study in showing stress behaviors in dogs, even though cortisol levels were not significant. A larger sample size, an increased length of play time, and controlled shelter activity may give
more consistent results. One might say that an open shelter is a better test of what would really affect dogs, but it can be challenging to control for extraneous variables.

Monitoring for stress behaviors in kennel situations can be a first step in promoting behavioral health in shelter dogs. Awareness and education for shelter staff regarding kennel stress in dogs can bring about new levels of wellbeing for canines housed in humane societies. Improving behavioral health in shelter dogs can provide more adoptable pets, which, overall, improves the quality of pet behavior in the community.

**Acknowledgments**

We want to thank the Longmont Humane Society in Longmont, Colorado, their staff and volunteers, and all of the dogs who graciously gave to this study. We also want to thank CampBowWow, Denver, Colorado for funding the supplies for this research and for their dedication and support of scientific research. A heartfelt thanks to Nancy Dreschel DVM, Penn State University, for her expertise in canine salivary cortisol experimentation, her excellence in professionalism, and the many gifts of wisdom and kindness she gives to the animal behavior profession. Thanks to Sharon, Nancy and Mary at Salimetrics Lab for the laboratory support offered to us. Thank you Don Whittemore, for stopping your life, on a dime, to assist us with video and computer work, which took so much longer than we thought. A warm thanks to Richard and Dylan King for assisting in the many hours of video analysis and mathematical formulations. And lastly, thanks to Dr. Frederick Prete for statistical analysis assistance and supervision.

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