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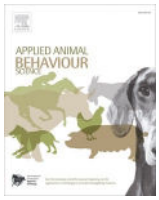

Effect of rapid changes in environmental conditions on canine detection of methyl benzoate

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ABSTRACT

Detection canines are utilized worldwide in some of the most challenging field conditions for the detection of narcotics, explosives, and other targets. Much remains unknown, however, how these challenging conditions impact detection canine performance. Anecdotal evidence suggests that detection dogs require a "start-up" period – a short duration of time working – before performing optimally. These dogs commonly rest in climate-controlled settings (such as in a climate-controlled vehicle) and quickly transition to searches in contrasting conditions, which may exacerbate the issue. Accordingly, this study sought to quantify the magnitude and duration of the start-up period, and to identify any further effects on performance due to rapid transitions into extreme temperature/humidity conditions. The detection threshold of seven dogs was established for methyl benzoate (an odor simulant of illicit cocaine) under standard conditions using an air dilution olfactometer. A series of evaluations were then conducted to determine changes in this threshold when the dogs were rapidly transitioned from standard conditions to one of six temperature/humidity conditions - hot-humid, warm-humid, hot-dry, standard, cold-dry and cold-humid. Temperatures ranged from 0 to 40 °C and relative humidity ranged from <40 % to >85 %. Changes in detection threshold were measured via a series of three "probes" of six trials, with a 2 min inter-probe interval to habituate to environmental conditions. Probes started at the dogs' estimated threshold and decreased in concentration based on correct performance. Overall, dogs showed substantial decrements in the hot-humid condition followed by moderate decrements in hot-dry and warm-humid conditions. Cold conditions did not produce statistically significant decrements. In addition, the data indicate that a start-up period does exist when canines transition from a state of rest directly into a search assignment. The duration of this start-up period was initially measured to be several minutes long; but after the first series of testing (approximately a couple months of training/testing), the dogs only exhibited a decrement in performance on the first trial of a session. Overall, the results suggest that environmental conditions and a brief "start-up" effect should be considered as important variables that can impact detection canine performance.

1. Introduction

Many entities rely on canines for the detection of landmines (Sargisson and Bach, 2012), explosives (Furton and Myers, 2001; Gazit and Terkel, 2003), narcotics (Marks, 2007; Riva et al., 2012), and human scent (Greatbatch et al., 2015a; Settle et al., 1994), among many others. Despite the wide usage of these working canines, many variables that influence performance remain unknown, or only have limited anecdotal or correlational evidence to aid in informed decision making.

Ambient temperature has been observed to affect odor detection in both humans and animals, but not on a consistent basis (see Table 1). Table 1 summarizes the outcome of various studies that have included analyses of temperature and humidity effects on odor detection performance. Nearly all studies, however, are correlational in nature and utilize temperature and humidity as covariates of other analyses. Importantly, though, several studies have found some effects of

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temperature and humidity on detection canine performance.

Mendel et al. (2018) analyzed three dogs' capabilities of detecting Laurel wilt infected avocado wood and Raffaelea lauricola (fungal pathogen which leads to wilt disease). The three dogs had shorter detection time when searching in warmer weather than cooler weather, but found no significant effect of humidity (Mendel et al., 2018). However, Greatbatch et al. (2015a) failed to find a correlation between ambient temperature and dog performance for their 10 handler/air-scenting search and rescue (SAR) pairs, who attempted to locate human targets along pre-determined paths. These results could partially result from the lack of aging the humans' tracks prior to the dogs running the trails. Jinn et al. (2020) evaluated the effects of ambient environmental conditions on SAR dog performance. The dogs were tested three times a day between June and December. The dogs' task was to locate a researcher who laid a track approximately one to three hours before the team arrived using a scent article that was left at the start of the trail. Researchers found that while accuracy did not waiver (93 % success rate finding the human), dogs did appear to change their search patterns in relation to the temperature. In cooler temperatures, dogs remained closer to the original track (Jinn et al., 2020). It further remains unclear to what extent variations in outcomes might be related to the variations of the target odors used across the studies.

While humidity greatly fluctuates depending on the temperature and season, it appears to play little into an animal's detection ability. Cablk and Heaton (2006) conducted a study to observe the search patterns of detection dogs when finding tortoises. Due to the nature of this job, dogs were subjected to humidity ranges from 16 % to 85 %. Despite the fluctuating humidity, there was no effect on the dogs' performance finding the tortoises (Cablk and Heaton, 2006). Likewise, Greatbatch et al. (2015a) found that humidity levels (55–78.4 %) played no role in the lowland SAR dogs' performance. The aforementioned study conducted by Mendel et al. (2018), found that in addition to effects of temperature, high humidity increased dogs' search time by about 10 s, although this result did not reach statistical significance. Lastly, Jinn et al. (2020) saw that in higher humidity, SAR dogs remained closer to the path laid by a researcher.

In humans, Kuehn et al. (2007) tested 75 volunteers for their ability to discriminate and conduct threshold tests with butanol under varying barometric and humidity conditions. They found that thresholds were lower in humid conditions compared to those in dry conditions (Kuehn et. al, 2007). Similarly, Alfonso Collado and Vallés Varela (2008) analyzed 154 volunteers' abilities to differentiate four concentrations of pyridine. Average relative humidity was 50 % +/- 20 %. No statistical differences were found in relation to intensity or discrimination ability (Alfonso Collado and Vallés Varela, 2008).

Together, these results suggest that temperature and humidity can impact detection dog performance, as well as human olfactory capabilities, but the magnitude of effects and implications remain unclear. It is vital to recognize that while temperature and humidity appear to influence human/canine olfactory capabilities, the environment's interaction with the target odor can have its own independent effects. For example, temperature changes can impact the volatility of the target odor and potential odor transport. Importantly, prior research has been almost extensively correlational in which temperature and humidity are one of many other variables (e.g., wind, specific target odor chemistry, odor prevalence of the target, detection threshold for the target, etc.) that could be impacting canine field performance. Thus, there remains a need for experimental evaluation of the effects of environmental conditions on canine detection.

In addition to environmental effects, another largely anecdotal concern for detection canines is that of a potential "warm-up" or "start-up" effect, where canine detection sensitivity may not be optimal at the immediate start of a search. Some detection handlers have been taught to start searches prior to an area of interest or to plant an odor for the dog to find to motivate search before beginning an actual search to ensure the dog is working optimally for the start of the critical search area (Bunker, personal experience). However, within published literature, such an effect has yet to be demonstrated scientifically. Despite "warm-up" or "start-up" effects not frequently being demonstrated scientifically in its own sake, it is commonly noted that performance is poorer at the beginning of behavioral training sessions. As just one example, Klink et al. (2006) analyzed an adaptive threshold and constant-stimuli procedure for tone detection thresholds in mice. In the adaptive threshold tests, the first two reversals were considered a "warm-up" period and not calculated in the overall threshold. The constant-stimuli procedure removed the first 10 trials from overall threshold calculations, too.

Secondly, it is possible that the magnitude of this start-up effect, should it exist, be greater if the dog is rapidly transitioning from a climate-controlled environment (such as a building or vehicle) to a vastly different working environment (outdoor hot or cold conditions). If "warm-up" effects are found to occur in a detection dog scenario, this could have important implications for how dogs should optimally work, especially when transitioning from a climate-controlled environment to

Table 1
Environmental effect on detection canines. Condition refers to the main condition that was analyzed.

Conditions	Study	Species	Effect	Type of Evidence	Sample Size
Temperature 19– 32.5°C	(Mendel et al., 2018)	Dog	No effect on detection performance; increasing temperatures lead to a decrease in search time during training	Correlational	3
Temperature 7– 27°C	(Greatbatch et al., 2015b)	Dog	No effect on detection performance	Correlational	10
Temperature 16.60 –30.96°C	(Jinn et al., 2020)	Dog	No effect on detection performance; in cooler temperatures, dogs remained closer to original track; in hot/dry temperatures, dogs moved more slowly when sampling odors	Correlational	6
Humidity 40 –95 %	(Mendel et al., 2018)	Dog	No effect on detection performance; increased search time in higher humidity, but not statistically significant	Correlational	3
Humidity 15.75–87.87 %	(Cablk and Heaton, 2006)	Dog	No effect on detection performance	Correlational	2
Humidity 55.0–78.4 %	(Greatbatch et al., 2015b)	Dog	No effect on detection performance; trend increase in search time, but not statistically significant	Correlational	10
Humidity 41.79 –74.92 %	(Jinn et al., 2020)	Dog	No effect on detection performance; in higher relative humidity, dogs remained closer to original track; in hot/dry conditions, dogs moved more slowly when sampling odors	Correlational	6
Humidity 42–67 %	(Alfonso Collado and Vallés Varela, 2008)	Humans	No effect on detection performance	Correlational	154
Humidity 20–55 %	(Kuehn et al., 2007)	Humans	Higher humidity lowered odor threshold	Experimental	75

an extreme operational environment. Such information would be useful to establish standard practices to ensure a canine is working optimally for the entire search area. Given the potential of such effects influencing detection performance, this warranted initial scientific investigation.

The objective of this project was to quantify the magnitude and duration of the start-up period, and to identify any further effects on performance due to rapid transitions into extreme temperature/humidity working conditions.

2. Methodology

2.1. Subjects

Six German Shorthaired Pointers and one Labrador Retriever were utilized in this study. Of the seven dogs, their average age was four years old. There were two spayed females and five neutered males. The dogs were previously disqualified from a federal explosives detection dog training program for a variety of reasons and participated in this study prior to moving to new working positions. Dogs were largely previously disqualified due to specific environmental sensitives associated with their specific work environment, except for ambient temperature and humidity. Initial training was conducted by Chiron K9 in Somerset, Texas after which, the dogs were trained and tested at the Canine Olfaction Lab at Texas Tech University. Eligible dogs were pair housed. Each dog received multiple daily walks and play sessions in addition to daily training and an enrichment program. Enrichment typically occurred outdoors in ambient temperature and humidity conditions; otherwise, the dogs were housed indoors in temperature-controlled kennels ranging from 21 to 26 ºC. Each dog had at least one hour of rest in the temperature-controlled kennels prior to participating in the study. All experimental procedures were reviewed and approved by Texas Tech University Institutional Animal Care and Use Committee (Protocol # 20027-04).

2.2. Materials

2.2.1. Odorant

Methyl benzoate was selected as the target odorant due to its relevance in canine narcotics detection; it is considered one of the key volatiles associated with the detection of illicit cocaine (Dejarme et al., 1997; Furton et al., 2002; Waggoner et al., 1997). Methyl benzoate (ACROS Organics; Product #AC126345000), was diluted in mineral oil (Bluewater Chemgroup, Fort Wayne, IN) at a 10^{-4} (volume/volume) dilution for use in initial training on the odor panel and in later training/testing in the air dilution olfactometer. These solutions were made fresh daily in 40 mL amber glass jars made of borosilicate glass with a PTFE septa screw top lid. These jars were cleaned at the end of every day in an ultrasonic cleaner, rinsed with RO water, and baked at 105ºC. Mineral oil dilution is a common practice in olfactory research to provide a stable and predictable odor concentration (Cometto-Muñiz et al., 2003) and was necessary to reduce the concentrations produced by the olfactometer to realistic and threshold-challenging levels.

2.2.2. Environmental chamber

The environmental chamber was a ~ 3 m x 3 m room with dedicated heating, ventilation, and air conditioning (HVAC) and additional heater units to allow temperature adjustments below 0 °C and above 40 °C, +/- 2.5 °C. Supplemental humidifiers and de-humidifiers enabled further control of the relative humidity (RH). Humidity levels were adjusted between 40 % and 85 %, with an allotted error of +/− 10 % caused by entering the chamber. The chamber had a sealed door which was closed during trials and between trials to maintain the designated environmental measurements. In the chamber, a three port olfactometer was placed along the front wall (Fig. 1).

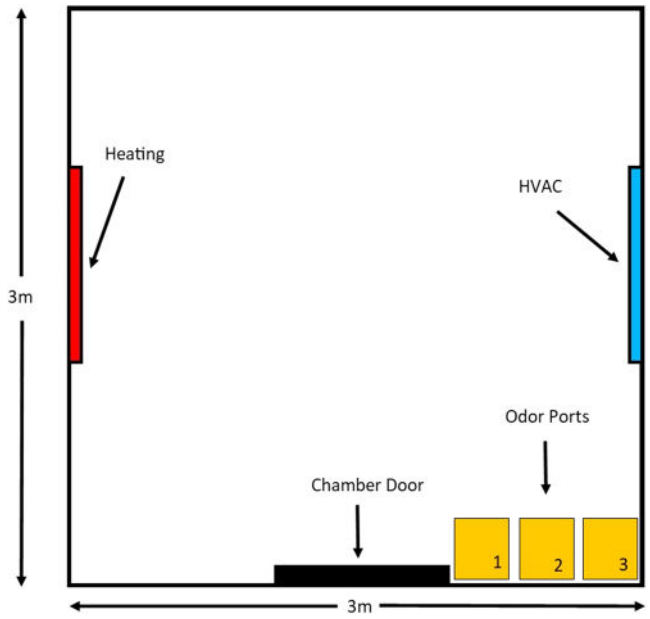

Fig. 1. The environmental chamber is illustrated above. Due to the placement of the heating and cooling elements, the olfactometers were placed along the front wall.

2.2.3. Environmental conditions

Conditions within the environmental chamber were monitored using a SensorPush® Wireless Thermometer/Hygrometer. Five conditions were selected to best represent some of the more extreme conditions working dogs may encounter in the field (see Table 2). These included a standard room temperature, hot-humid, hot-dry, cold-humid, and cold-dry conditions. An additional condition, warm-humid, was added to serve as a less extreme version of the hot-humid condition after the first series of testing.

To account for learning effects, dogs were randomly assigned to one of two groups that worked in either a cold to hot direction or hot to cold direction (see Table 2). Table 2 shows the conditions order for both groups including when the warm-humid condition was tested. Due to time the chamber required to reach conditions, it was not feasible to full randomize condition testing order, but this was selected as the most feasible way to minimize order effects.

2.2.4. Air-dilution olfactometer

Fig. 2 shows the design of the air dilution olfactometer used. Zero air (Praxair UN1002 Air Compressed zero air) was delivered to two ports and a mixture of zero air and methyl benzoate/mineral oil headspace was delivered to the third port. Nine AliCat® (Tucson, AZ, USA) mass air

Table 2
Testing conditions utilized in the present study.

Group 1	Group 2
Series 1	
Cold, dry (0 ºC, < 40 % RH)	Hot, humid (40 ºC, > 85 % RH)
Cold, humid (0 ºC, > 85 % RH)	Hot, dry (40 ºC, < 40 % RH)
Standard (22 ºC, 60 % RH)	Standard (22 ºC, 60 % RH)
Hot, dry (40 ºC, < 40 % RH)	Cold, humid (0 ºC, > 85 % RH)
Hot, humid (40 ºC, > 85 % RH)	Cold, dry (0 ºC, < 40 % RH)
Warm, humid (32 ºC, > 85 % RH)	Warm, humid (32 ºC,>85 % RH)
Series 2	
Hot, humid (40 ºC, > 85 % RH)	Cold, dry (0 ºC, < 40 % RH)
Warm, humid (32 ºC, > 85 % RH)	Cold, humid (0 ºC, > 85 % RH)
Hot, dry (40 ºC, < 40 % RH)	Standard (22 ºC, 60 % RH)
Standard (22 ºC, 60 % RH)	Hot, dry (40 ºC, < 40 % RH)
Cold, humid (0 ºC, > 85 % RH)	Warm, humid (32 ºC, > 85 % RH)
Cold, dry (0 ºC, < 40 % RH)	Hot, humid (40 ºC, > 85 % RH)

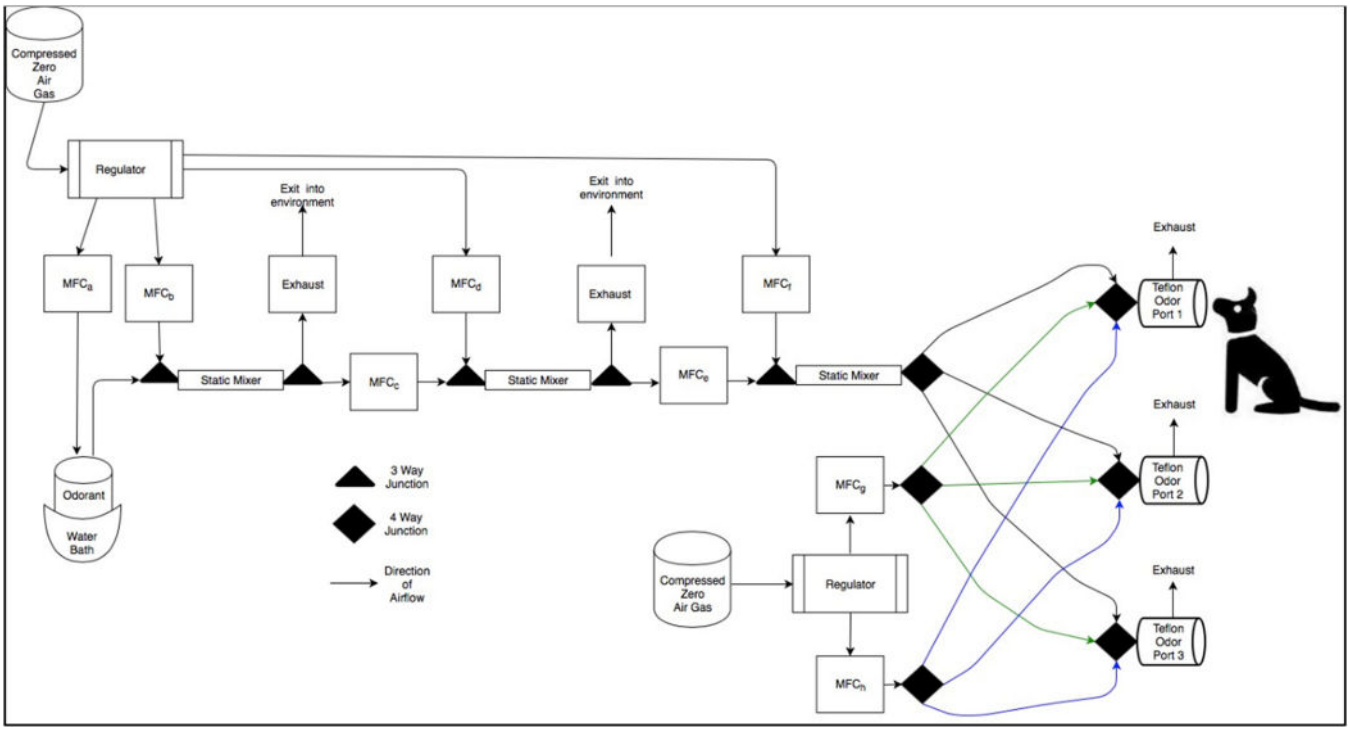

Fig. 2. Design of Air Dilution Olfactometer. Zero air from a compressed air source is used. Odorant sits in a water bath and a regulated flow is introduced to collect the odorant source. A series of mass air flow controllers then perform a series of air dilutions. Comparison clean lines are generated from the same air source. A series of relay valves are then used to direct the odorant to any of three ports at which the dog can sniff. Odor ports are inside the environmental chamber, but all other olfactometer equipment is held outside the chamber at standard conditions.

flow controllers conducted an air serial dilution of the odor from 1×10^{-2} to 1×10^{-9} air dilution. The odorant was kept in a glass vial and held at 36 ºC using an odor bath. The olfactometer interface was a polypropylene plastic sheet that measured 0.85 m wide x 0.28 m tall. Along this interface, three stainless steel ports were mounted flush with the plastic approximately 0.23 m apart. These ports allowed the dogs to put their nose inside to sample the odor or air output from the olfactometer. Target delivery was balanced between the three odor ports, but the order of delivery to port 1, 2 or 3 was randomized. The handler, who was present in the chamber with the dog, did not know which port contained the target odor. Two of the three ports each trial had clean air, the third port contained the odorant-air mixture at varying dilutions, and all ports delivered an identical flow of 10 slpm. Thus, dogs were tested using a 3 alternative forced choice test (3AFC).

An IR beam pair was used to measure the duration of a nose hold from the dog, which was trained as the alert response. The response criterion for an alert was a 4 s continuous hold duration. If a dog's nose was present in the port for four seconds, the trial was terminated and scored as correct or incorrect. The computer then emitted a tone to indicate to the handler as to whether the response was correct or incorrect. Thus, all testing was blinded. Incorrect responses were not reinforced whereas correct responses were rewarded with a toy or treats. The handler delivered the reward as soon as the toned sounded correct. The reward lasted no longer than the 60 s between trials. If a dog failed to respond to a port within 60 s of the start of a trial (indicated by a start tone) a timeout or "no response" was scored and counted as incorrect.

2.3. Procedure

2.3.1. Experimental design

The experimental design was tailored to quantify the magnitude and duration of the start-up period, and to identify any further effects on performance due to rapid transitions into extreme temperature/humidity working conditions. To accomplish this, all dogs were first trained to detect and discriminate methyl benzoate (*Pre-training)* from a variety of distracters using our previously described olfactometer and procedure (Aviles-Rosa et al., 2021). Odor responding was further confirmed in a control test (*Control Test)*. Next, dogs were transferred to an air dilution olfactometer system and completed a baseline threshold test to methyl benzoate (*Baseline Threshold Testing)*. A final control threshold test was conducted to the diluent to ensure threshold represented detection of the target (i.e., methyl benzoate). The measured methyl benzoate threshold was then set as the individual dog's odor concentration used for detection threshold probe assessments.

On test days, dogs were held at standard climate-controlled conditions for one hour and then rapidly introduced to the environmental chamber set to the test conditions (see Fig. 3). Dogs then engaged in a six-trial detection threshold "probe" (*Detection Threshold Probe Assessments*). This brief test started at the individual dog's baseline methyl benzoate threshold and measured the dog's ability to detect the odorant at this concentration or lower. Dogs then rested at the environmental conditions for 2 min and repeated an identical detection threshold probe (probe 2). This was followed by another 2 min of rest and the final detection threshold probe (probe 3). Comparison of performance across all three probes for different environmental conditions yielded information on the effects of environmental conditions. Changes in performance from probe 1 to probes 2 and 3 were indicative of start-up effects *(Statistical Analysis)*.

All dogs completed two series of tests as outlined in Table 2. Testing at an additional environmental condition (warm-humid) was performed at the end of Series 1 to collect data under conditions that reduced the extremity of the hot-humid condition. In between Series 1 and Series 2, dogs were subjected to an unscored session at standard conditions in order to return performance to baseline before starting Series 2.

Throughout the entirety of the study, all data was collected double-blind. The computer controlled all odor presentation and randomization. The handler never knew the correct location of the odor or whether odor was present. The computer simply emitted tones at the end of the

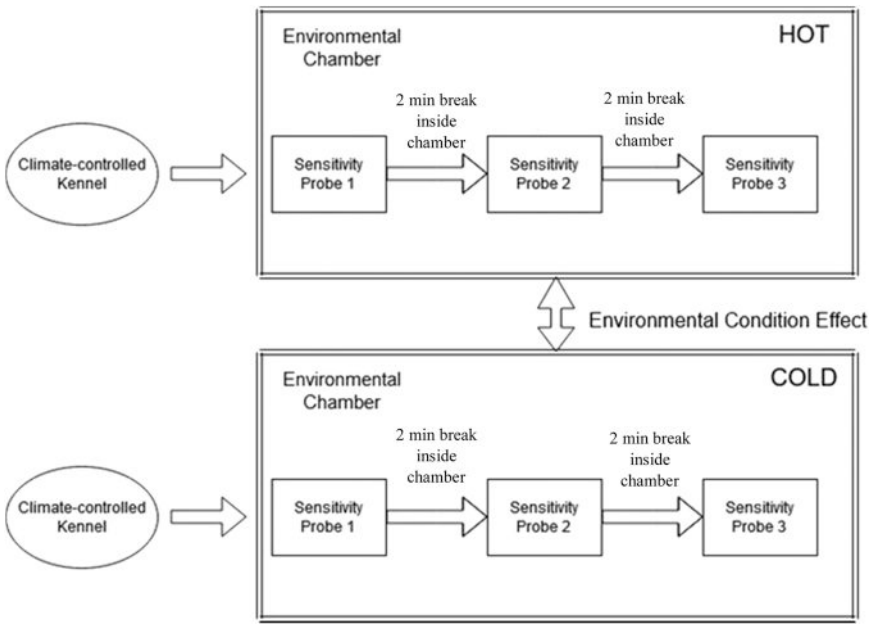

Fig. 3. The experimental design and analysis. Dogs moved from a climate-controlled environment to the environmental chamber at either standard or one of five other environmental conditions. Dogs immediately started a six trial "probe" which was a 3AFC threshold test starting at the concentration previously determined to one step above detection threshold in a previous baseline test. Dogs then rested for 2 min, followed by probe 2, then 3. Comparison in overall performance across all three probes allowed for assessment of climate effects. Comparison between probes 1, 2, and 3 allowed for assessment of start-up effects.

trial to indicate to the handler whether or not to reinforce.

2.3.2. Pre-training

Prior to air dilution training with methyl benzoate, dogs were trained to discriminate methyl benzoate using a multi-odor olfactometer. The olfactometers were based on prior work at TTU (Aviles-Rosa et al., 2021). Carbon filtered air from a commercial air pump was controlled by rotameter flow meters and directed via six channels of automated solenoid valves to push the headspace of an odorant from a 40 mL glass vial. This allowed a session to present any of six different odorants to the dog. Pre-training lasted about one and a half months and consisted of one to two training sessions a day, five days a week. Initially, dogs started at a 0.5 s nose hold and built up their alert duration to 4 s. Once dogs had a 4 s nose hold to methyl benzoate, distracter odors were introduced – the diluent (mineral oil) and four other distracters – clean air, a bird's feather, limonene, and rocks from outside. An alert was scored if the dog made a 4 s continuous nose hold in the odor port. If the first response occurred to the port presenting the target odor a correct response or "hit" was scored. A 4 s response to a distracter odor was scored as a false alert and terminated the trial. If no response was scored within 60 s, the trial timed out and it was marked as "incorrect". On 20 % of the trials, no target odor was presented. On these trials, dogs were required to sample each odor port, then remove their nose from the ports for 4 continuous seconds to indicate an "all clear". Dogs typically returned to the handler during this time. Training continued until the dogs scored 90 % accuracy in two forty trial sessions. The dogs were then required to complete a forty-trial test with 90 % accuracy with a novel set of distracters (not previously used in training) Following meeting criterion, dogs began training with the air dilution olfactometer.

2.3.3. Pre-training control test

A control test was conducted to verify pre-training test results were controlled by odor detection and not due to potential unintentional olfactometer cues. Dogs were subjected to a brief 10-trial session in which all the vials contained mineral oil (no target odorant). These trials measured whether the dogs used *any* cue other than the odor to make correct responses. If audible cues/olfactometer cues, *or any other cues* controlled canine behavior, they would be identified with performances greater than chance. If *only* olfactory cues were controlling canine behavior, performance during the control test would be expected to drop to chance, or canines not to respond, even though the same valves were being activated as normal and a session was otherwise conducted as normal. As expected, all dogs performed at or below chance indicating performance was directly mediated by the odor and not unintentional cues.

2.3.4. Air dilution olfactometer training

After passing the pre-training phase, dogs were familiarized and tested with the air dilution olfactometer under a 3AFC procedure until reaching 90 % accuracy in a blinded session (40-trials). In the 3AFC procedure, odor was present in one of three ports every trial. The dog was required to respond to one of the three odor ports. If a dog did not respond to a port within 1 min, a "no response" was recorded. Unlike the olfactometer in pre-training that could present a variety of distractors, the air dilution olfactometer only presented the target and two comparison clean air lines. Thus, the air dilution was a detection task of the target from comparison clean air.

2.3.5. Baseline threshold testing

Threshold was measured using a 3AFC procedure using a 2-down, 1-up descending staircase adaptive threshold procedure (Leek, 2001) that continued until dogs completed eight reversals in the direction of concentration change. Each of these assessments were conducted under standard conditions (22 °C, 60 % RH). Each dog completed three assessments on separate days. Threshold was calculated as the geometric mean of the last six reversal points of each individual test and the three threshold values were averaged to produce an overall threshold (Table 3). The threshold was then rounded to the next highest half-log dilution that the olfactometer generated and was used as that dog's starting concentration for detection threshold probes. To further ensure that detection thresholds reflected detection of the target odorant (methyl benzoate), dogs were given a threshold assessment where the diluent (mineral oil) was used as the target. If detection thresholds were substantially lower when methyl benzoate was included compared to the diluent only, it was concluded that thresholds reflected methyl benzoate detection rather than diluent detection (see Table 3).

2.3.6. Detection threshold probe assessments

For one hour prior to testing, dogs were housed in standard temperatures. Each dog completed one full assessment per day. The assessments were comprised of three detection threshold probes. Each probe was comprised of six trials and began at the *Baseline Threshold*

Table 3
Overall detection limits to methyl benzoate and the diluent (mineral oil) only. Grey shaded lines show the estimated threshold to the diluent only.

Dog	Odorant	Session	Dilution Factor at Last Six Reversal Numbers						Geometric Mean
			3	4	5	6	7	8	
Bin	Methyl Benzoate	1	1.00E-05	3.16E-05	1.00E-05	3.16E-05	3.16E-06	3.16E-05	8.79E-06
Bin	Methyl Benzoate	2	1.00E-05	1.00E-04	3.16E-06	1.00E-04	1.00E-05	1.00E-04	
Bin	Methyl Benzoate	3	3.16E-06	1.00E-05	3.16E-07	1.00E-06	3.16E-07	1.00E-05	
Bin	Mineral Oil	4	0.001	0.01	0.00316	0.01	0.000316	0.01	3.16E-03
Boki	Methyl Benzoate	1	3.16E-05	1.00E-04	3.16E-05	1.00E-04	3.16E-05	1.00E-04	1.14E-04
Boki	Methyl Benzoate	2	0.001	0.00316	0.001	0.00316	3.16E-06	1.00E-05	
Boki	Methyl Benzoate	3	0.001	0.00316	1.00E-05	3.16E-05	1.00E-05	1.00E-04	
Boki	Mineral Oil	4	0.00316	0.01	0.00316	0.01	0.00316	0.01	5.62E-03
Dokk	Methyl Benzoate	1	3.16E-05	1.00E-04	1.00E-05	1.00E-04	3.16E-05	0.001	3.59E-04
Dokk	Methyl Benzoate	2	0.001	0.01	3.16E-05	1.00E-04	3.16E-05	0.000316	
Dokk	Methyl Benzoate	3	0.001	0.01	0.00316	0.01	0.000316	0.00316	
Dokk	Mineral Oil	4	0.00316	0.01	0.00316	0.01	0.00316	0.01	5.62E-03
Leo	Methyl Benzoate	1	3.16E-05	1.00E-04	3.16E-05	1.00E-04	3.16E-05	1.00E-04	1.21E-04
Leo	Methyl Benzoate	2	0.001	0.00316	0.000316	0.001	1.00E-05	3.16E-05	
Leo	Methyl Benzoate	3	1.00E-04	0.00316	3.16E-05	1.00E-04	3.16E-05	1.00E-04	
Leo	Mineral Oil	4	0.001	0.00316	0.001	0.01	0.00316	0.01	3.16E-03
Luna	Methyl Benzoate	1	3.16E-05	1.00E-04	3.16E-06	1.00E-05	3.16E-06	1.00E-04	2.78E-05
Luna	Methyl Benzoate	2	3.16E-05	1.00E-04	3.16E-05	0.000316	3.16E-05	0.000316	
Luna	Methyl Benzoate	3	3.16E-06	3.16E-05	1.00E-05	1.00E-04	1.00E-06	1.00E-04	
Luna	Mineral Oil	4	0.000316	0.01	0.00316	0.01	0.00316	0.01	3.83E-03
Lunya	Methyl Benzoate	1	0.001	0.00316	0.001	0.01	0.000316	0.00316	2.02E-03
Lunya	Methyl Benzoate	2	0.001	0.00316	0.001	0.00316	0.001	0.00316	
Lunya	Methyl Benzoate	3	0.00316	0.01	0.001	0.00316	0.001	0.00316	
Lunya	Mineral Oil	4	0.00316	0.01	0.00316	0.01	0.001	0.01	4.64E-03
Pena	Methyl Benzoate	1	0.00316	0.01	3.16E-05	1.00E-04	1.00E-05	0.000316	8.25E-05
Pena	Methyl Benzoate	2	3.16E-05	1.00E-04	3.16E-05	1.00E-04	1.00E-05	1.00E-04	
Pena	Methyl Benzoate	3	3.16E-05	1.00E-04	3.16E-05	1.00E-04	1.00E-05	1.00E-04	
Pena	Mineral Oil	4	No reversals (Did not get two correct in a row)						0.01

determined dilution. For the six trials of the probe, which equated to approximately five minutes, the dogs' threshold was evaluated using the same 2-down, 1-up procedure implemented in the *Baseline Threshold Testing*. However, the maximum concentration presented was the starting concentration (i.e., concentration did not increase above the initial dilution, rather dogs continued at that same dilution). After six trials, two minutes of habituation to the environment elapsed between probe one and probe two. During the two minutes, dogs rested in the environmental chamber with no specific programmed activity. The same methodology was utilized between probe 2 and probe 3.

The assessment continued until dogs completed all three trials, unless the dog met a pre-determined welfare criterion for the extreme condition. If a dog timed out on 8 or more trials during the first two probes (i.e., timed out on 8 or more of the 12 trials in probe 1 and 2), the session was terminated. In addition, if after five minutes, a dog had timed out on all but one trial, the session was terminated. Sessions that were terminated early, the remainder trials were scored as a "no response". This occurred for 19 of the 84 sessions. This was comprised of 2 warm humid sessions, 4 hot dry sessions and 13 hot-humid sessions.

2.3.7. Air dilution olfactometer validation

Solid phase micro extraction (SPME) fibers (grey hub selected based on pilot optimization work) were inserted directly into the air-dilution olfactometer output line to 1) verify the linear change in odorant concentration as a factor of dilution stage and 2) verify that no detectable amount of residual methyl benzoate was present in the output line when clean air was delivered (i.e., no methyl benzoate was retained by the odor lines).

Five air dilutions were tested (0.01, 0.008, 0.005, 0.003, and 0.002) with six replicates at each air dilution. The order of sampling was conducted from low to high dilutions. A 10^{-2} methyl benzoate v/v in mineral oil was used in the olfactometer to generate odor. These dilution stages and the solution concentration are higher than those used during canine testing but were necessary to generate results within the detection range of the GC/MS.

A total of six blank samples were collected after an odor presentation at 50 % dilution (1 LPM odorant air and 1 LPM dilution air) to ensure no residual methyl benzoate remained in the olfactometer output line. This was completed by performing a 60 s extraction from the output line of a blank air purge after a 60 s odorant purge and a 30 s clearing purge had been completed. No detectable amount of methyl benzoate was found in these blank samples.

2.3.8. Air-dilution olfactometer environmental sampling

To verify that the air dilution olfactometer maintained consistent concentrations across the various temperature and humidity conditions, SPME fibers were inserted into the end of the output line consistent with procedures outlined above. A total of 10 replicates were performed under each environmental condition. For these tests, the 0.01 air-dilution setting was used with the 10^{-2} methyl benzoate v/v in mineral oil.

2.3.9. Statistical analysis

Detection canine outcome measures included trial accuracy (correct vs. incorrect), log-transformed concentration tested (the concentration determined by the 2-down, 1up algorithm for each trial), response probability (alerted to a port or timeout), log-transformed latency (time from start of the trial to the first nose insertion; set to 60 s if no response was made), and total time in the odor ports (total time spent sampling the odor ports subtracting out 4 s for an alert). The tests at standard conditions conducted between series 1 and series 2 were only used to recover canine performance to baseline levels and were not used in analysis.

The environmental condition effect and probe effect were assessed for each outcome measure using a generalized linear mixed effect model including fixed effects of environmental condition (6 level factor for each tested condition) and probe number (3 level factor). Separate analyses were conducted for the first and second series to reduce model complexity and facilitate analysis for each series independently. A random intercept for each dog was included (assuming compound symmetry). A binomial distribution was used for binomial variables, otherwise a gaussian distribution was assumed or variables were log

transformed. Models were fit using the lme4 package in R. Statistical significance for each fixed effect was determined through nested model comparison, starting with the interaction term, and removing non-significant terms from the model. Levels of a fixed effect were compared using Tukey-adjusted post-hoc tests from the lsmeans package in R. For graphical purposes, 95 % confidence intervals were estimated using a bootstrapping procedure from the hmisc package and ggplot2 in R.

3. Results

3.1. Baseline threshold testing

Table 3 shows the detection threshold results for each individual dog for three methyl benzoate sessions and the diluent only session. A Wilcoxon signed test was performed to test if the median difference between the threshold for mineral oil (diluent) and the threshold for methyl benzoate was greater than zero. Dogs showed a statistically higher (poorer) threshold for mineral oil compared to methyl benzoate ($p = 0.008$), indicating that, in general, the threshold obtained during testing reflected methyl benzoate detection and not that of mineral oil or an unintentional cue of the olfactometer. The geometric mean threshold shown for each dog in Table 3 was used to establish the concentration used for the detection threshold probes.

3.2. Accuracy

Fig. 4A shows the bootstrap estimated 95 % confidence interval for each environmental condition and probe for all conditions. Separate plots were made for the first and second series of data collection (Fig. 4A: left and right respectively). Supplemental Fig. 1 shows that the change in accuracy across conditions and probes was consistent at the individual dog level and shows that each dog showed a similar response pattern.

The small confidence interval for the hot-humid probe 3 condition in Fig. 4A indicates that all dogs met the welfare termination criterion in that condition and therefore showed 0 % accuracy. For the first series, the generalized linear mixed effect model indicated there was no significant interaction between the probe number and environmental condition ($X^2 = 13.63$, df = 10, $p = 0.19$), however there was a main effect of probe ($X^2 = 5.83$, df = 2, $p = 0.05$) and environmental condition ($X^2 = 252.58$, df = 5, $p < 0.001$). Tukey-adjusted post-hoc tests indicate that cold-dry, cold-humid, and standard conditions were similar (all $p > 0.67$), but the hot-dry, warm-humid, and hot-humid conditions led to overall poorer accuracy compared to the cold and standard conditions. Lastly, accuracy during the hot-dry and warm-humid condition was significantly better than during the hot-humid condition ($z = 5.36$, $p < 0.001$; $z = 5.44$, $p < 0.001$).

Tukey-adjusted post-hoc tests for the effect of the probe number indicate that accuracy was slightly lower in the probe 1 compared to probe 2 ($z = 2.30$, $p = 0.05$), but that probe 1 and 3 did not differ ($z = 1.75$, $p = 0.18$) nor probe 2 and 3 ($z = 0.55$, $p = 0.85$).

Fig. 4 A indicates that the second series of test generally showed a similar pattern. Identical to the first series, there was no significant interaction between the environmental condition and probe number ($X^2 = 16.41$, df = 10, $p = 0.08$) and there was a main effect of environment ($X^2 = 231.50$, df = 5, $p < 0.001$). However, unlike the first series, the overall effect of probe number was no longer present ($X^2 = 2.02$, df = 2, $p = 0.36$).

For the effect of environmental condition, a similar pattern of results emerged, with the exception that dogs performed best in the cold-dry condition compared to cold-humid ($z = 2.91$, $p = 0.04$) and standard conditions ($z = 3.67$, $p = 0.003$). Regarding the hot/warm conditions, a similar and significant decrement was observed again for the hot-dry, warm-humid, and hot-humid conditions compared to standard and cold conditions, with the most extreme decrement in the hot-humid

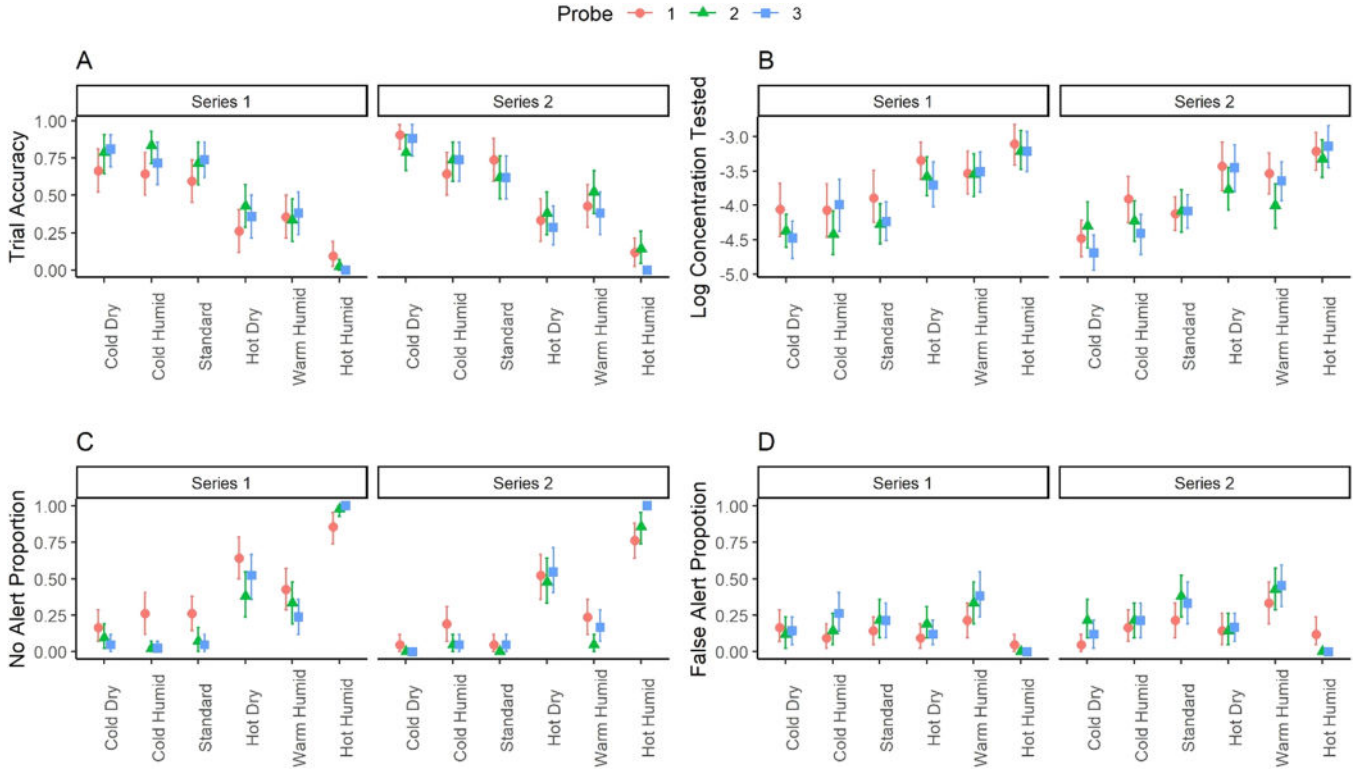

Fig. 4. Shows the bootstrap estimated 95 % confidence intervals for each environmental conditions and probes. Left graphs show the results from the first series of tests and right shows the second series. Each horizontal panel (A, B, C, D) shows a different outcome variable. Panel B shows the log of the concentration in the form of proportion of vapor saturation.

condition (all $p < 0.001$).

To further investigate the lack of an effect of probe number, it was investigated whether the "start-up" effect occurred over a shorter timescale than across the six trials of the probe. Fig. 5 shows the trial accuracy (top) across the first six trials averaged over every session for series 2. A linear-mixed effect model that predicted response accuracy by the environmental condition and trial number indicated a significant main effect of trial number ($X^2 = 6.74$, df = 1, $p < 0.01$). Post-hoc tests indicate that accuracy on the first trial was lower than on the second trial ($z = 2.54$, $p = 0.01$) as indicated in Fig. 5. Thus, the start-up effect in the second series largely only occurred in the first trial of probe 1.

3.3. Concentration tested

Fig. 4B shows the bootstrap estimated 95 % confidence interval for the log concentration tested under each environmental condition and probe for both series of tests. During the first series of tests, the linear mixed effect model for log-transformed concentration indicated there was no significant interaction between the probe number and environmental condition ($\chi^2 = 16.50$, df = 10, $p = 0.09$), however there was a main effect of probe ($\chi^2 = 16.43$, df = 2, $p < 0.001$) and environment ($\chi^2 = 237.1$, df = 5, $p < 0.001$). Tukey-adjusted post-hoc tests indicate that cold-dry, cold-humid, and standard conditions were similar in concentration tested (all $p > 0.30$), but the hot-dry, warm-humid and hot-humid conditions led to higher concentrations (poorer detection) compared to the cold-dry, cold-humid and standard conditions. Lastly, the hot-dry and warm-humid conditions led to similar performance decrements ($t = 0.14$, $p = 1.00$) but the hot-humid condition led to significantly poorer detection in comparison to hot-dry and warm-humid conditions ($t = 4.26$, $p < 0.001$; $t = 4.12$, $p < 0.001$).

Tukey-adjusted post-hoc tests for the effect of the probe number in this first series indicate that the concentration tested was poorer (i.e., higher) in probe 1 compared to probe 2 ($t = 3.82$, $p < 0.001$) and compared to probe 3 ($t = 3.07$, $p = 0.006$), but there was no difference between probe 2 and 3 ($t = 0.75$ $p = 0.73$).

During the second series of tests an overall similar pattern was observed (see Fig. 4B), but in the second series, the effect of the probe depended on the environmental condition (probe by environment interaction: $X^2 = 36.31$, df = 10, $p < 0.001$). Tukey adjusted post-hoc tests indicate that the start-up effect (lower performance in Probe 1 compared to Probe 2 or 3) was observed only in the cold-humid and warm-humid conditions. Under standard conditions and hot-humid conditions, performance was similar across all three probes. An analysis at the individual trial level is not possible for concentration tested because the concentration tested during trial 1 was preset by the testing parameters of the study.

The effect of environment was also similar to the first series of tests, where performance was poorest in the hot/warm (hot-dry, warm-humid, hot-humid) conditions and best in the standard and cold conditions. The hot-humid, again, led to the poorest performance.

3.4. No alert response

Fig. 4C shows the bootstrap estimated 95 % confidence interval for the probability of a no response for each environmental condition and probe for series 1 (left) and series 2 (right).

For the first series of tests, the generalized linear (binomial link) mixed effect model including an interaction term showed model convergence issues, likely due to probe 3 of the hot humid condition being made nearly entirely of timeouts. The interaction term was therefore not included. Excluding the interaction term, there was a main effect of probe ($X^2 = 24.89$, df = 2, $p < 0.001$) and environment ($X^2 = 411.1$, df = 4, $p < 0.001$). Tukey-adjusted post-hoc tests indicate that cold-dry, cold-humid, and standard conditions were similar in the number of responses (all $p > 0.96$), but the hot-dry, warm-humid, and hot-humid conditions led to overall more no responses ("timeouts") compared to the cold-dry, cold-humid, and standard conditions (all $p < 0.05$). Lastly, there were fewer timeouts during the warm-humid than the hot-dry condition and both conditions yielded fewer timeouts than during the hot-humid condition ($t = 7.23$, $p < 0.001$; $t = 9.07$, $p < 0.001$).

Tukey-adjusted post-hoc tests for the effect of the probe number indicate that there were more no responses in probe 1 compared to probe 2 ($t = 4.18$, $p < 0.001$) and compared to probe 3 ($t = 4.18$, $p < 0.001$), but there was no difference between probe 2 and 3 in response probability ($t = 0.10$ $p = 0.99$).

For the second series of tests, results were again similar with a significant main effect of probe number ($X^2 = 8.49$, df = 2, $p = 0.01$) and environmental condition ($X^2 = 443.22$, df = 5, $p < 0.001$) on response probability. Tukey-adjusted post hoc tests indicate that response probability was again similar in the cold and standard conditions, but significantly lower in the hot/warm conditions. Amongst the hot conditions, the fewest responses were made in the hot-humid conditions and the most in the warm-humid condition, with hot-dry yielding an

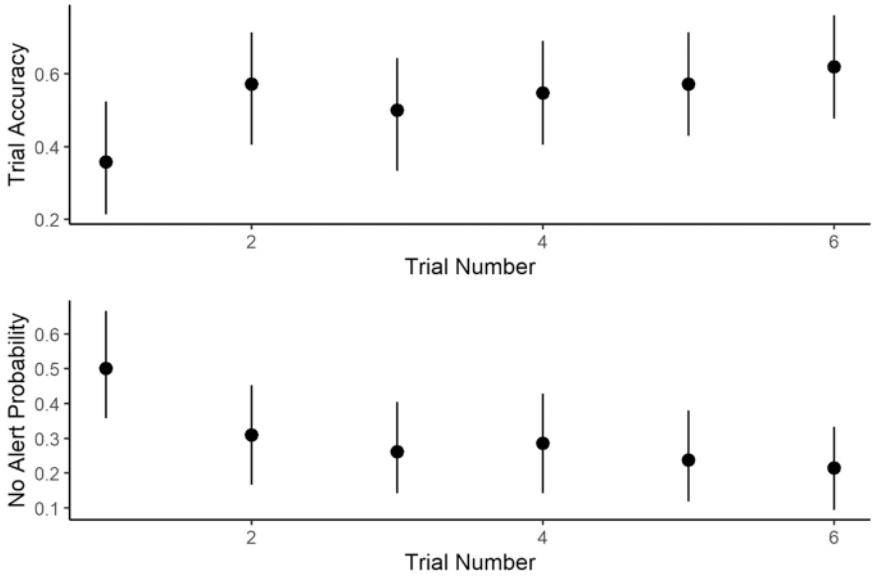

Fig. 5. Effect of trial in Probe 1 on the second series of testing. Error bars show the bootstrap estimated 95 % confidence interval.

intermediate number of no responses. Additionally, more trials without responses occurred in the first probe compared to the second ($z = 2.50$, $p = 0.03$), but there was no difference between the first and third probe or second and third ($p > 0.05$). Fig. 5 further shows that the probability of a trial without an alert was highest specifically in the first trial of Probe 1 compared to the other trials.

3.5. False alerts

Fig. 4D shows the proportion of trials ending in a false alert for each environmental condition and probe. False alerts and no responses are the two error types a dog could make on a trial.

For the first series of tests, the generalized linear (binomial link) mixed effect model including an interaction term showed model convergence issues. The interaction term was therefore not included. There was a main effect of environment ($X^2 = 31.30$, df = 5, $p < 0.001$), but no main effect of probe ($X^2 = 3.77$, df = 2, $p = 0.15$). Tukey-adjusted post-hoc tests were therefore conducted to test the differences in environmental conditions. The warm-humid condition showed the highest number of false alerts compared to hot-humid, hot-dry, and cold-dry (all $p < 0.05$). This indicates that although timeouts were low in warm-humid, false alerts were elevated. In contrast, false alerts were low in hot-humid compared to all other conditions (all $p < 0.05$). This reflects the elevated timeouts/no responses in the hot-humid condition, which implies false alerts could not occur if the dog failed to respond/alert and/or met the welfare criterion for participation.

For the second series of tests, the interaction term was again not included due to model convergence. Excluding the interaction term, there was a main effect of environment ($X^2 = 57.23$, df = 5, $p < 0.001$), but no main effect of probe ($X^2 = 3.30$, df = 2, $p = 0.20$). Tukey-adjusted post-hoc tests showed again that the warm-humid condition showed the highest number of false alerts compared to hot-humid, hot-dry, cold-humid and cold-dry (all $p < 0.05$). Also similar to series 1, false alerts were low in hot-humid compared to warm-humid, standard and cold-humid (all $p < 0.05$).

3.6. Latency

Fig. 6A shows the bootstrap estimated 95 % confidence interval for each environmental condition and probe within condition for log-transformed latency for the first and second series of testing. Latency refers to the time from the start of the trial to when the dog first inserts their nose into one of the ports. This measure captures, in part, the dog's motivation or willingness to rapidly engage in a search. Latency for trials in which the dog was removed due to the welfare exclusion criteria were scored as missing.

For the first series of tests, the linear mixed effect model indicated there was a significant interaction between the probe number and

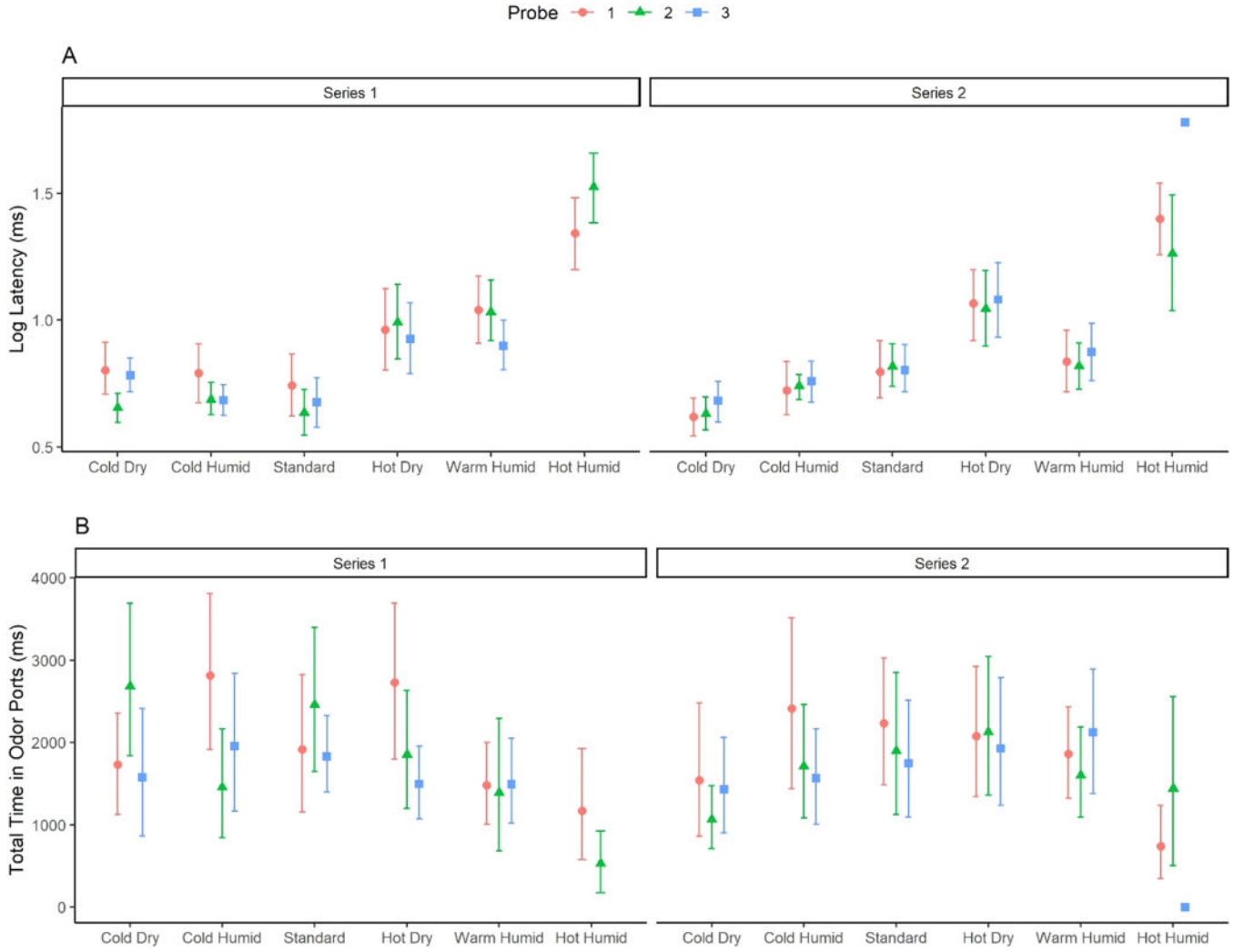

Fig. 6. Shows the bootstrap estimated 95 % confidence intervals for each environmental conditions and probes. Each panel (A and B) shows a different outcome variable. Total time in odor port excludes the 4 s from the alert.

environmental condition (X^2 = 17.142, df = 9, $p = 0.046$). Tukey-adjusted post-hoc tests indicate that cold-dry, cold-humid, and standard conditions were similar in latency during probes 1, 2 and 3 (all $p > 0.05$). The hot-dry, warm-humid, and hot-humid conditions, however, showed longer latency to initiate a search for probes 1, 2, and 3 (with the exception that hot-humid data is missing for probe 3; all $p < 0.05$). However, in probe 1, latency during the hot-dry and cold-humid were similar ($t = 2.34$, $p = 0.13$) as well as hot-dry and cold-dry ($t = 2.29$, $p = 0.19$).

Tukey-adjusted post-hoc tests for the effect of the probe number indicate that latency was similar between all probes for each environmental condition with the exception that latency was longer in probe 2 compared to probe 1 in the hot-humid condition ($t = 2.46$, $p = 0.04$). This highlights that dogs largely initiated trials at a similar speed across all three probes.

For the second series of tests, the linear mixed effect model indicated there was no significant interaction between the probe number and environmental condition ($X^2 = 12.69$, df = 10, $p = 0.24$) or main effect of probe ($X^2 = 4.52$, df = 2, $p = 0.10$). There was, however, a main effect of environment ($X^2 = 261.45$, df = 5, $p < 0.001$). Tukey adjusted post-hoc tests indicate that latency was shortest under the cold-dry condition, and cold-humid, standard, and warm-humid had similar latency. Hot-dry produced longer latencies than the other conditions, and hot-humid produced the longest latencies.

3.7. Total time in odor port

Fig. 6B shows the bootstrap estimated 95 % confidence interval for each environmental condition and probe within condition for the total time spent with their nose in the odor ports for the first and second series (subtracting out the 4 s from an alert/response). Trials for which the dog was removed (due to the welfare exclusion) were scored as missing.

For the first series, the linear mixed effect model showed a significant interaction between the probe number and environmental condition ($X^2 = 17.37$, df = 9, $p = 0.04$). Tukey-adjusted post-hoc tests indicate that the total time with the nose in the ports was similar between the cold-dry, cold-humid, standard, warm-humid, and hot-dry conditions for probes 1, 2 and 3. The hot-humid condition, however, showed reduced sniff time in probe 1 compared to the hot-dry condition and cold-humid conditions, and in probe 2 compared to the standard and cold-dry conditions. Thus, the total time with the nose in the port was overall similar between all conditions except for some comparisons to the hot-humid condition.

For the second series, there was no significant interaction between the probe number and environment ($X^2 = 8.20$, df = 10, $p = 0.61$) nor a main effect of probe number ($X^2 = 1.86$, df = 2, $p = 0.39$). There was a main effect of environmental condition on nose-port entry time ($X^2 = 16.73$, df = 5, $p = 0.005$). Tukey-adjusted post hoc tests indicate that nose port entry time was overall lowest in the hot-humid condition compared to hot-dry, warm-humid, standard, and cold-humid conditions (all $p < 0.05$).

3.8. Air dilution olfactometer validation

Fig. 7 shows the relationship between the olfactometer generated air dilution and the calculated concentration of methyl benzoate based on SPME GC-MS. An R^2 of 0.98 was found suggesting that within the range tested, the air dilution olfactometer did show acceptable linearity in manipulating odor concentration as presented at the odor port. Furthermore, no methyl benzoate was found in clean air samples taken after target air samples, indicating no detectable levels of residual methyl benzoate existed in the odor delivery lines.

Lastly, an ANOVA comparing the effect of environmental condition on the odor concentration measured at the odor port (n = 10 replicates/condition), when the olfactometer was set to deliver the same concentration across all conditions, indicated there was not a statistically significant variation in odor concentration between the conditions ($p = 0.0830$). This indicates that there were not substantial differences in measured concentration at the olfactometer output line across the environmental conditions, indicating that differences in performance across conditions are not likely due to changes in the olfactometer concentration delivery.

4. Discussion

Overall, results showed that hot-dry, warm-humid, and hot-humid conditions each led to poorer overall accuracy, but hot-humid conditions had the lowest performance. The dogs had the lowest thresholds to methyl benzoate in standard/cold conditions. In addition, a start-up effect was present. Dogs performed the worst during the first probe and/or trial as compared to later trials; however, these start-up effects were generalized across all environmental conditions.

These results highlight three potential decrements in performance that should be mitigated: 1) it may take dogs a brief period to reach

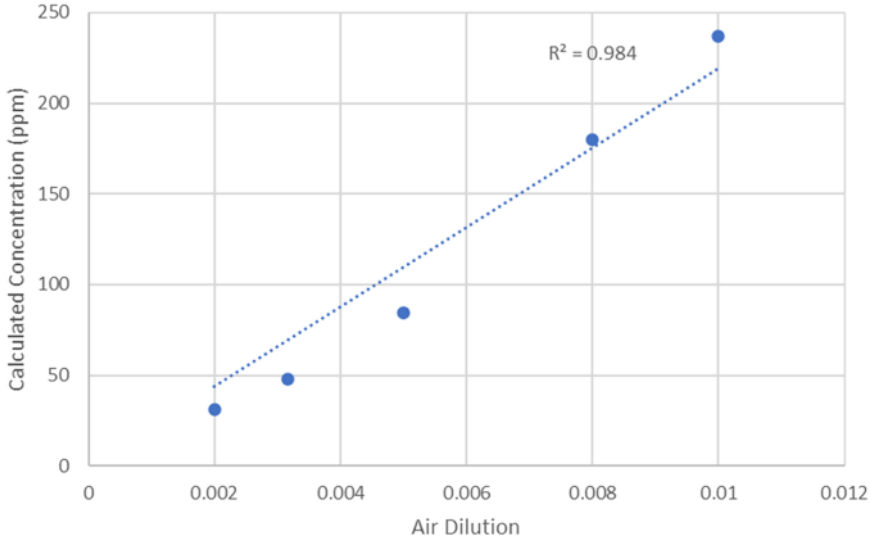

Fig. 7. Air-Dilution Olfactometer Odorant Concentration Analysis. Shows the relationship between olfactometer generated dilution and calculated concentration measured from SPME GC-MS.

optimum performance when transitioning from rest to active searching (exemplified by poorer performance on probe 1 or trial 1); 2) dogs may exhibit adequate search behaviors in warm-humid and hot-dry conditions, but accuracy may be substantially impacted; and 3) dogs transitioning to hot-humid conditions may be impacted to the point of being unable to work. The effect of the hot-humid (40 ºC, > 85 % RH) condition showed the most dramatic impact on canine behavior with all dogs failing to complete the assessment, leading to poor detection outcomes.

The hot-dry and warm-humid condition also led to a significant decrement in performance, partially due to a lack of responding, but also due to incorrect responses. Interestingly, however, in the hot-dry and warm-humid condition, poor performance was not related to the dogs failing to investigate the odor ports (although they were slower to initiate search). Dogs spent similar times searching each port per trial in the hot-dry and warm-humid condition compared the standard, cold-wet and cold-dry. Even though they spent similar times investigating each port, dogs still showed a lower probability to alert to the target port and a longer latency to start search. It is notable that dogs were observed by the trainer engaging in substantial panting during the hot conditions, and even though they approached the ports in the hot-dry and warm-humid condition, heat-associated panting may have led to poorer detection. Heat induced panting has previously been researched and indicated that panting negatively impacts a canine's olfactory capabilities (Gazit et al., 2003; Gazit and Terkel, 2003; Settles et al., 2003). The dogs in this study may have also experienced reduced motivation to alert with a nose-hold. This reduced motivation may in part by due to a dog's focus on returning to homeostasis rather than engaging in the behavioral task (Gazit and Terkel, 2003). Future research leveraging respiration sensors would be an important next step.

Interestingly, there was no effect of the cold (dry or humid) condition on canine performance compared to the standard conditions in this case. In fact, based on some outcome measures, dogs performed best in the cold-dry condition. This effect may indicate dogs perform better under cold-dry conditions or it may reflect a nature of the testing order that was required. The cold-dry condition was either tested first or last and was least susceptible to potential carryover effects from the hot conditions that created suppression in performance. Additional research that directly compares the cold-dry and standard conditions would be necessary to more definitively resolve whether dogs generally perform better in cold-dry conditions.

In comparison to the results of this study, Mendel et al. (2018) found that dogs could detect odorants faster in the warmer climate (30–33 ºC) than they could in the cooler climate (19 ºC). However, one important variation is that the current study controlled for potential differences of odor availability that would normally change if an odor source was placed in warmer conditions. Thus, there remains further need to conduct an experimental manipulation of environmental conditions when odor availability is also allowed to change, to simulate real-world conditions.

In the first series of tests, there was an overall start-up effect in which performance during probe 1 was poorer (accuracy, concentration, and probability of a response) compared to the following probe(s). Similar results were also seen in Gazit and Terkel's (2003) experiment where performance was lower in detecting the first and second explosive than the third during a "calm search" and during a "strenuous search", which occured after exercise. The start-up effect in this present study did not depend on the environmental conditions (i.e., no statistical interaction), indicating that the start-up effect is general across environmental conditions and not exacerbated in extreme conditions. In the second series of tests, this start-up effect was no longer significant for trial accuracy when comparing between probes, but instead was significant when comparing performance across trials within the first probe. Performance was substantially lower on the first trial in Series 2. These results suggest that experience over Series 1 tests reduced the duration of the start-up effect, but nonetheless remains an important effect for handlers to consider. The potential mechanism of the start-up effect, however, is not immediately clear and could result from a physiological origin (e.g., physiological adaptation to the test) or psychological (e.g. arousal/-preparedness for the task). Future work to explore the potential mechanism would be useful to identify the best methods to mitigate this effect.

There were important limitations to this study. Firstly, methyl benzoate was the only odorant utilized in the present study. Therefore, the results of this study may not be generalizable across a range of odorants. In addition, the study is limited by the variables manipulated. Environmental conditions in the field will also include factors such as solar radiance, wind speed, and altitude, which were not included in the present study.

Nonetheless the present results suggest several actionable outcomes. First, dogs should be trained, or at minimum evaluated, under any extreme temperature conditions the dogs may work operationally. Handler protocols should include collecting variables such as temperature, humidity and heat index, and handlers should exert caution (or not deploy) if the heat or heat index exceeds conditions in which the dog has been formally evaluated, as decrements can be expected based on the present results. It is further notable that performance under the hot-dry and warm-humid conditions showed performance decrements even when dogs may exhibit adequate search behaviors. This suggests that in situ tests should be conducted to confirm expected canine sensitivity in all potential operational temperature and humidity conditions.

Second, there is potential benefit of a brief "pre-search" area to help ensure dogs are performing optimally at the start of any critical search area. Results suggested that even with much training and repetition, performance remained poorest on the very first trial of every session. Therefore, there is a potential decrement for handlers to be aware of at the immediate start of a search. More research is needed to determine the validity and effectiveness of a pre-search in an applied setting.

Lastly, the number of canines in this study was limited due to time, space, and budget restrictions. Further research would be necessary to determine whether these results are representative of the larger working dog community.

Overall, the results highlight the need for further studies evaluating anecdotal or correlational evidence of variables impacting canine detection performance. Here, impacts of environmental conditions and a potential start up effect on detection canine performance were addressed. Results indicate that extreme environmental conditions can influence detection canine performance, and that performance tended to be poorest at the very beginning of the detection task, but any detriment was quickly alleviated within 1–6 trials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105924.

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