African elephants can detect water from natural and artificial sources via olfactory cues

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Abstract

Water is vital for mammals. Yet, as ephemeral sources can be difficult to find, it raises the question, how do mammals locate water? Elephants (*Loxodonta africana*) are water-dependent herbivores that possess exceptional olfactory capabilities, and it has been suggested that they may locate water via smell. However, there is no evidence to support this claim. To explore this, we performed two olfactory choice experiments with semi-tame elephants. In the first, we tested whether elephants could locate water using olfactory cues alone. For this, we used water from two natural dams and a drinking trough utilised by the elephants. Distilled water acted as a control. In the second, we explored whether elephants could detect three key volatile organic compounds (VOCs) commonly associated with water (geosmin, 2-methylisoborneol, and dimethyl sulphide). We found that the elephants could locate water olfactorily, but not the distilled water. Moreover, they were also able to detect the three VOCs associated with water. However, these VOCs were not in the odour profiles of the water sources in our experiments. This suggests that the elephants were either able to detect the unique odour profiles of the different water sources or used other VOCs that they associate with water. Ultimately, our findings indicate that elephants can locate water olfactorily at small spatial scales, but the extent to which they, and other mammals, can detect water over larger scales (e.g. km) remains unclear.

Keywords: 2-methylisoborneol, dimethyl sulphide, geosmin, smell of water, surface water, volatile organic compounds

Introduction

For mammals, food and water are essential resources. Water is important for many vital physiological processes such as digestion (Silanikove 1992), thermoregulation (Cain et al. 2006), lactation (Maltz and Shkolnik 1980), maintenance of blood plasma volume (Al-Toum and Al-Johany 2000), and body-fluid homeostasis (Noda and Sakuta 2013). Moreover, it even influences food intake (e.g. Shrader et al. 2008). Thus, water is vital for life.

Finding water is more difficult in some environments compared to others. For instance, large mammalian herbivores (hereafter, large herbivores) living in arid environments are challenged by the limited distribution of water sources across the landscape. Permanent water bodies are rare, and the many ephemeral water sources such as pans, streams, shallow pools, and puddles dry up as the dry season progresses (Chamaillé-Jammes et al. 2007; Naidoo et al. 2020; Redfern et al. 2005). Many species in these regions have evolved behavioural, physiological, and morphological mechanisms to reduce water loss or obtain more water from food (see Cain et al. 2006; Kihwele et al. 2020 for details). Yet, many large herbivores remain dependent on surface-water and need to drink every day or two (Kihwele et al. 2020; Western 1975). Accordingly, surface water is a key factor that determines these mammals daily and seasonal activities and movement (Gaylard et al. 2003; McKee et al. 2015; Naidoo et al. 2020; Redfern et al. 2003; Thrash et al. 1995; Valls-Fox et al. 2018a). As the availability and distribution of the ephemeral water sources are dependent on rainfall patterns, the predictability of their locations will vary both within and between years (Redfern et al. 2005). The question that arises then is: how do water-dependant mammals locate these ephemeral water sources?

One animal that is heavily dependent on water is the African savanna elephant (*Loxodonta africana*) (Chamaillé-Jammes et al. 2007; Gaylard et al. 2003; Tsalyuk et al. 2019; Tshipa et al. 2017; Valls-Fox et al. 2018b). Elephants generally drink on a daily basis but can go 2-3 days between drinks (Chamaillé-Jammes et al. 2013; Owen-Smith 1996). Bax and Sheldrick (1963) suggested that elephants were able to track rainfall events and the subsequent re-establishment of temporary water sources. While this might be true, Bax and Sheldrick (1963) did not explore the cognitive processes driving the elephants' movements. Thus, it is unclear what cues the elephants may have used to determine when to leave and in which direction to travel.

One possibility is that elephants rely on spatial memory to make both small and large-scale movements between permanent water sources (Polansky et al. 2015; Presotto et al. 2019). Yet, this does not explain how elephants detect when ephemeral water sources contain surface water, or how they might track rainfall events. One possibility is that elephants may use olfactory cues to locate surface water (Presotto et al. 2019). However, to date there are no data to support these claims for elephants or any other mammal. Nevertheless, elephants possess an exceptional olfactory sense (Shoshani and Foley 2000; Shoshani et al. 2006), which can likely discriminate between subtle differences in structurally related odours (Niimura et al. 2014). For example, elephants can differentiate between organic compounds that differ by a single carbon chain (Rizvanovic et al. 2012). In addition, they can olfactorily distinguish between ethnic groups of humans (i.e. Maasai and Kamba) that they frequently encounter (Bates et al. 2007), discriminate between the scents of different humans including related individuals (von Dürckheim et al. 2018), recognise related and unrelated elephants by urine scents (Bates et al. 2008), and the smell of lion dung (Valenta et al. 2021). Moreover, they make foraging decisions across a range of spatial scales using only olfactory cues (Schmitt et al. 2018), can locate marula fruit with the highest sugar content using scent alone (Nevo et al. 2020), and can find preferred plant species through a 'noisy' olfactory background (McArthur et al. 2019). With regards to water, Ramey et al. (2013) suggested that elephants in the Kunene region of Namibia may have avoided drinking from small water holes containing high faecal coliform bacteria levels by keying off of either olfactory cues or the taste of the water.

Three volatile organic compounds (VOCs) generally associated with water are geosmin, 2-methylisoborneol (2-MIB), and dimethyl sulphide (Izaguirre et al. 1982; Jenkins et al. 1967; Jüttner and Watson 2007). Geosmin and 2-MIB are known to be produced by aquatic microorganisms like cyanobacteria as well as other actinomycetes (Izaguirre et al. 1982; Jüttner and Watson 2007). These and other bacteria are common and abundant within natural water reservoirs (Izaguirre et al. 1982). In some cases, however, geosmin and 2-MIB are not inherently part of water sources but rather are present in the surrounding soil (Jüttner and Watson 2007). When dry soil is exposed to rainfall, both geosmin and 2-MIB are released and give off distinctive musty odours akin to damp soil or the smell associated with the first rains of the season (Bear and Thomas 1964; Izaguirre et al. 1982; Jüttner and Watson 2007; Suffet et al. 2004). Dimethyl sulphide (DMS) is another compound frequently associated with water sources, giving off a natural gas, sulphur-like odour (Jenkins et al. 1967). Its biosynthesis is associated with blue-green algae that occur in most natural water sources (Jenkins et al. 1967). Although most commonly found in marine environments, where it is used by seabirds to locate good foraging patches (Nevitt and Bonadonna 2005), it has also been identified and isolated in fresh water sources (Bechard and Rayburn 1979).

Despite the importance of surface water to elephants and their excellent sense of smell, little is known about the degree to which elephants can detect the olfactory cues given off by water. Additionally, despite humans being able to detect geosmin, 2-MIB, and dimethyl sulphide at very low concentrations (Izaguirre et al. 1982; Jenkins et al. 1967; Young et al. 1996), it is unclear whether elephants can detect these VOCs, and use them to locate re-established temporary water sources. To address this knowledge gap, we conducted two olfactory choice experiments on semi-tame elephants to explore the extent to which they could detect water from both natural (dams) and artificial (trough) water sources using only olfactory cues. We did this, by presenting the elephants with two buckets with holes drilled into the lids, one with and one without water, so that the odours given off by the water could escape. The elephants were then allowed to smell each bucket before making a choice. In addition, we determined the olfactory profiles of these different water sources. We then determined whether they were able to detect geosmin, 2-MIB, and dimethyl sulphide by smell, and whether these key VOCs were present in the water sources.

We predicted that if the elephants were able to detect water using olfactory cues, that they would choose the buckets containing the water from the study site. However, as the distillation process likely removes VOCs from the water, we predicted that the elephants would not be able to locate the distilled water (i.e. show a pattern of random selection). Yet, if the distillation process did not remove key compounds that the elephants recognised and associated with water, then they should also be able to locate the distilled water. Due to their excellent sense of smell, we predicted that the elephants would be able to detect the three key compounds associated with water (i.e. geosmin, 2-MIB, and dimethyl sulphide). Moreover, we predicted that these compounds would be found in the water sources at the study site, and thus likely act as cues for the elephants. Yet, if these compounds were not present, it would suggest that the elephants were using some other compound or compounds to locate the water.

Materials and methods

We conducted the study during the dry season from 20-Aug to 8-Sept-2019 at the Adventures with Elephants sanctuary near Bela Bela, South Africa (24°46'53.8"S+27°57'03.3"E). Adventures with Elephants operates as an education centre where people can interact with and learn about elephants. To ensure the safety and comfort of the elephants, the professional elephant handlers carried out the experiments and issued the verbal commands under our direction. To determine the extent to which elephants use olfactory cues to detect and locate water at small spatial scales, we conducted choice experiments on five semi-tame, adult African elephants (see details below). All experiments were conducted in a covered open area where the elephants are fed and where educational interactions take place. Each elephant has its own section within this area, which made it possible for us to interact with each elephant separately.

Elephants

The elephants we used in the study consisted of two males (Chova 23 years of age, Chishuru 21 years), and three females (Shan 18 years, Mussina 17 years, Nuanedi 17 years). These animals are housed in a barn at night but free-range over the 500 ha property during the day. The handlers follow the elephants as they forage, but do not limit what and where they eat or how far they travel. Yet, during the day the elephants are brought to the covered interaction area at 08:30, 12:00 and 15:00. We limited our experiments to the 8:30 and 12:00 time slots.

Experimental design

To obtain the data, we followed a similar experimental design to (Schmitt et al. 2018). Specifically, we conducted scent-based choice experiments using four different sources of water. We collected the water from three locations on the property from which the elephants drink on a daily basis. We did this prior to the elephants arriving at the interaction area, thus they did not see where the water came from. The water sources comprised two dams (upper and lower) that refill via rain and runoff, and a plastic trough (artificial water source) that is refilled with underground water provided via a pump. The purpose of using these water sources was that each would likely have a different odour profile. Visually, there were noticeable differences between the water sources with the upper dam having a white cloudy appearance (due to the clay soil of the dam) compared to brownish water of the lower dam, and the clear underground water found in the trough, which also contained a small amount of green algae. Despite the visual differences, we could not detect any olfactory differences between the different water types. Finally, as a control, we used type II distilled water that was distilled at the University of Pretoria, as the distillation process would have likely greatly reduced the odour profile.

Experiment 1: Detecting water via olfactory cues

For the choice experiments, we used two identical 25-litre plastic buckets (32 cm x 32 cm x 40 cm) placed side-by-side (Fig. 1). The buckets each contained a 5-litre glass beaker (Super-duty, low form, CC Imelmann, Robertsham). We ensured that the beaker was held in the centre of the bucket by wrapping a blanket around the outside of the beaker (Fig. 1a). We wrapped the beaker to prevent it from moving, which could produce noise that the elephants may use to determine which bucket contained the water, and to prevent water from spilling out of the beaker when the bucket was moved between trials. Before inserting the beaker into the blanket, we covered the blanket with a 50-litre clear plastic trash bag to prevent the blankets from getting wet during the trials, and thus potentially providing an additional olfactory clue for the elephants.

In each experiment, we poured 3.5 litres of water into the beaker of one of the two buckets and left the beaker in the second bucket empty. To ensure that the elephants could only use olfactory and not visual cues to find the water, we drilled 28, 10 mm holes (separated by ~3 cm) into the lids of the buckets to allow any odours given off by the water to escape (Fig. 1a). Once the lids were attached, the two buckets (one with and one without water) were placed side by side ~5 m in front of the elephant being tested (Fig. 1b). To ensure that the elephant did not know which bucket contained the water prior to smelling, the handlers instructed the elephant to turn 180˚ and face away from where the buckets would be placed. Once the buckets were in place the elephant was instructed to turn and walk up to the buckets. As with (Schmitt et al. 2018), upon reaching the buckets the elephant was instructed to smell each bucket, remove its trunk, and then choose which bucket it wanted by placing its trunk on the lid of the bucket. To prevent the elephants from knocking the buckets over when they smelt, two trainers held the buckets in place on the ground (Fig. 1b). Once the elephant had made its choice, it was rewarded with the contents of the bucket it chose (i.e. water or no water), while the other bucket was taken away without them seeing its contents. This contingency trained the elephants to learn that whatever they chose, they received.

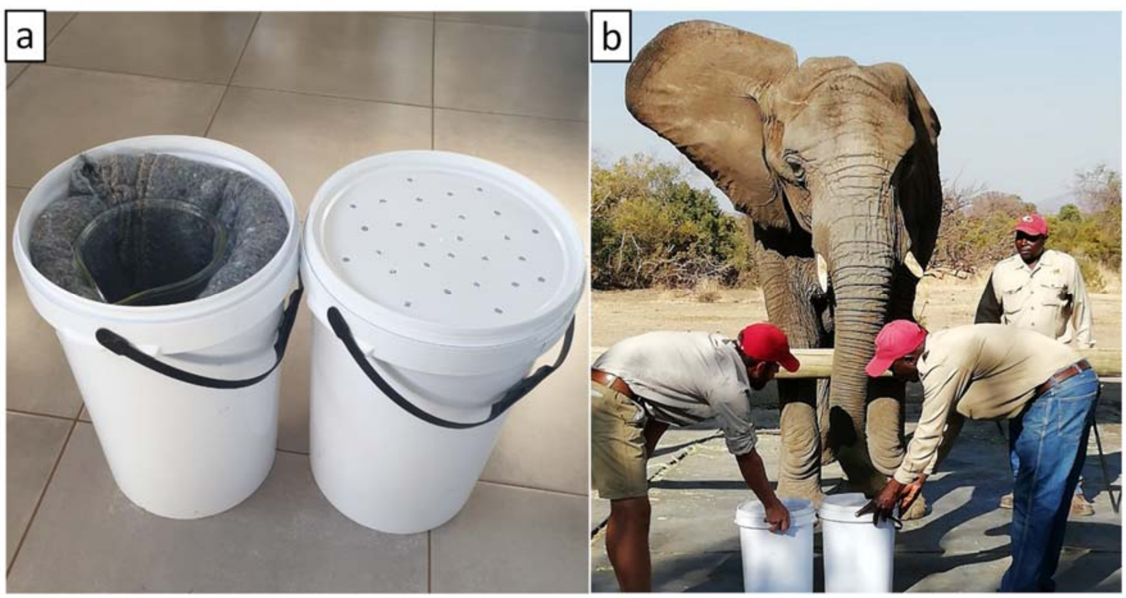

Fig. 1 Experimental setup of experiment one, with a) the blankets used to surround the beaker and holes drilled into the lids, and b) an elephant (*Loxodonta africana*) smelling the buckets that are being held in place by the handlers.

During the experiments, the trainers knew which bucket contained the water but were not aware of where the water came from (i.e. dams, trough, or distilled). As they knew the location of the water, it is possible that the trainers may have inadvertently signalled to the elephants where it was. However, if this was the case, then we would expect that the elephants would consistently locate the water and that there would be no differences in their ability to locate the different water types. As neither of these things happened (see results and supplementary material), we are confident that the trainers did not signal the location of the water to the elephants and that our results thus reflect the elephants' ability to find water using olfactory cues.

Over a three-day period, we tested the ability of all five elephants to locate the four water types using olfactory cues alone. On these days, we ran trials at 08:30 and at 12:00. To ensure that the elephants were thirsty, the handlers prevented them from drinking for a minimum of two hours prior to being tested. This was easy, as during the dry season the only surface water available on the property is found in the two dams and the trough. The water that we used in the different trials was collected ~30 minutes prior to each trial. For logistical reasons, we exposed all five elephants to the same water type during one of the time slots. Thus, we tested two water types each day. In each time slot, the ability of each elephant to locate the water was tested five times. This resulted in 25 separate choice tests in each time slot (i.e. 5 elephants tested 5 times). To control for any side bias or handler effect, the location of the bucket with water, as well as the handlers holding the buckets were randomized for each trial. In addition, we wiped down both lids of the buckets with the same cloth between each trial, and periodically swapped the lids between buckets. For each elephant, new lids were put onto the buckets, and the used ones were cleaned with water, rubbed down with sand, and left to dry in the sun. These were then used again in a later trial.

Training session

Prior to conducting the experiments, we pre-trained the elephants in the procedure. This pre-training involved first presenting them with the two buckets, one with a beaker with water from one of the dams on the property, one with an empty beaker. However, in these initial trials, we removed the lids so that the elephants could smell, touch, and see what was in each bucket. We followed the same experimental procedure as above (i.e. approach, smell, smell, choose). Once all five of the elephants were consistently choosing the bucket containing the water (~2 days), we then put the lids on and continued the training. It only took one day for the elephants to figure out the experimental design with the lids on and demonstrate that they could select the bucket containing the water (i.e. locate the water >75% of the time). As a result, we started data collection the following day.

Odour profiles of the different water sources

To determine the odour profiles of the four water sources (i.e. two dams, a plastic trough, distilled water), we collected 4 x 3 L water samples from each source on the day we exposed the elephants to the water from that source. We then stored these water samples in sealed glass bottles in a refrigerator for two weeks to prevent the VOCs from dissipating prior to analysis.

To determine the odour profiles, we decanted 300 µL from each sample into separate 1.5 mL glass vials with a septum and then incubated at 23 °C for approximately 24 h. The headspace of each vial was sampled for 40 min using a solid-phase-micro extraction fibre coated with 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane solid support (Supelco, Merck). The fibre was injected into the inlet of an Agilent 7890 gas chromatograph coupled to an Agilent 7000D mass spectrometer (GC-MS) equipped with an Agilent J&W DB-Wax column. The inlet of the GC was set at 220 °C and the mobile phase flow rate at 1 mL/min. The initial temperature of 40 °C was held for 2 minutes after which the temperature was ramped up by 5 °C min^{-1} to a final temperature of 180 °C with an additional 5 min hold at 220 °C before re-equilibration to 40 °C. The volatiles eluting from the column were diverted to the ion source of the MS which was kept at 320 °C and ionized at 70 eV. We set the MS to scan mode with a low mass of 40 m z^{-1} and a high mass of 450 m z^{-1}. Unfortunately, when we ran the first three samples (one each of the lower dam, trough, and distilled water), there was an air leak in the GC. As such we discarded the output of these samples, which left us with 13 samples in total. To view the data, we used MassHunter software (Agilent) and then used the NIST v. 98 library (National Institute of Standards and Technology, USA) for tentative identifications of the analytes. We identified potentially important metabolites by comparing the chromatograms and locating unique metabolites not found in the distilled water.

Experiment 2: VOCs associated with water

In addition to determining whether elephants could locate water via olfactory cues alone, we ran a second experiment to determine whether they could detect geosmin, 2-methylisoborneol (2-MIB), and dimethyl sulphide, olfactorily. As we were only interested in whether the elephants were able to detect these compounds, and not their degree of sensitivity to these different compounds, we standardised the compounds by creating a 1% solution of each by diluting them into dipropylene glycol, a relatively odourless solvent. For the choice experiments, 2 ml of the solutions were poured into separate Eppendorf tubes.

As with the first experiment, we ran an odour-based choice experiment. However, in this experiment we used two plastic boxes (29 cm x 21.5 cm x 16.5 cm) with 15, 1 cm diameter holes drilled into the lids. In each box, we taped an open 2 ml Eppendorf tube to the inside wall of the container, halfway up the side (~8 cm from the top and bottom). In one box, the Eppendorf contained 2 ml of the VOC solution, while the Eppendorf in the second box contained only 2 ml of dipropylene glycol. As in the first experiment, we had the elephants smell each box and then choose the one that they wanted. However, in contrast to the first experiment, the elephants were not given what was inside the box. Rather, if they chose the box containing the VOC, we gave them four animal food pellets (Alzu Game Feeds: Grazer, South Africa) as a reward. This acted as a positive reinforcement and thus, if the elephants made a significant number of correct choices, this would indicate that the elephants could detect the VOCs associated with water. Similar to the first experiment, the location of VOCs and handlers holding the boxes were randomised between each trial. Additionally, to ensure that the elephants were not associating the positive reward with another odour cue (e.g. the smell of the handler deposited on the box, or another random odour), we wiped down the boxes and lids with alcohol between each trial, as well as switching the lids randomly during each trial.

Prior to the experiments, we trained the elephants to learn that if they indicated to the box containing the VOC, we would give them the food pellets as a reward. This was done by first having the elephants smell the VOC and then immediately giving them the pellets. This helped them associate the VOC with a reward. We did this 10 times for each elephant for each VOC. We, however, only exposed the elephants to one VOC a day, thus it took 3 days to do the initial training. We then introduced the elephants to the two boxes and ran the experimental procedure 10 times for each VOC for each elephant during one day.

Statistics

As both experiments comprised a series of binary choices (i.e. selection between two buckets or boxes), and we tested the same elephants multiple times, we treated the results from each individual elephant as repeated measures in generalized estimating equations (GEE's; see

Schmitt et al. (2018) for similar procedure). The GEEs incorporated an exchangeable correlation matrix and binomial error distribution with a logit link function. As such, the GEEs allow modelling the proportion of time elephants make a given choice and comparing it to a 50% distribution expected under random selection for that given choice. Data were back transformed from logit-scale for graphical representation. We used the means and 95% confidence intervals (CI) to determine whether the elephants' choice differed from the expected 50% random selection for each of the water types.

To statistically analyse the odour profiles, we converted the GC-MS raw data to .mzXML file format using the msConvert GUI in the Protewizard tool (Kessner et al. 2008). We then uploaded the converted data to XCMS online (Tautenhahn et al. 2012) for peak picking and retention time correction. Once the retention time was corrected, we log-transformed the deconvoluted dataset and analysed it in R using the Metaboanalyst v. 3 (Chong and Xia 2018) platform.

We conducted a standard principal component analysis to determine the similarity of the four water sources. We then ran a random forest analysis to determine the specific analyte differences between the water sources (Biau 2012). Finally, we then ran separate 1-way ANOVAs to determine if the quantities of five key volatiles (see below) varied between the different water samples taken from the four water sources ($N = 13$ samples). For the principal component analysis, the random forest analysis, and the ANOVAs, we log transformed the data for normality.

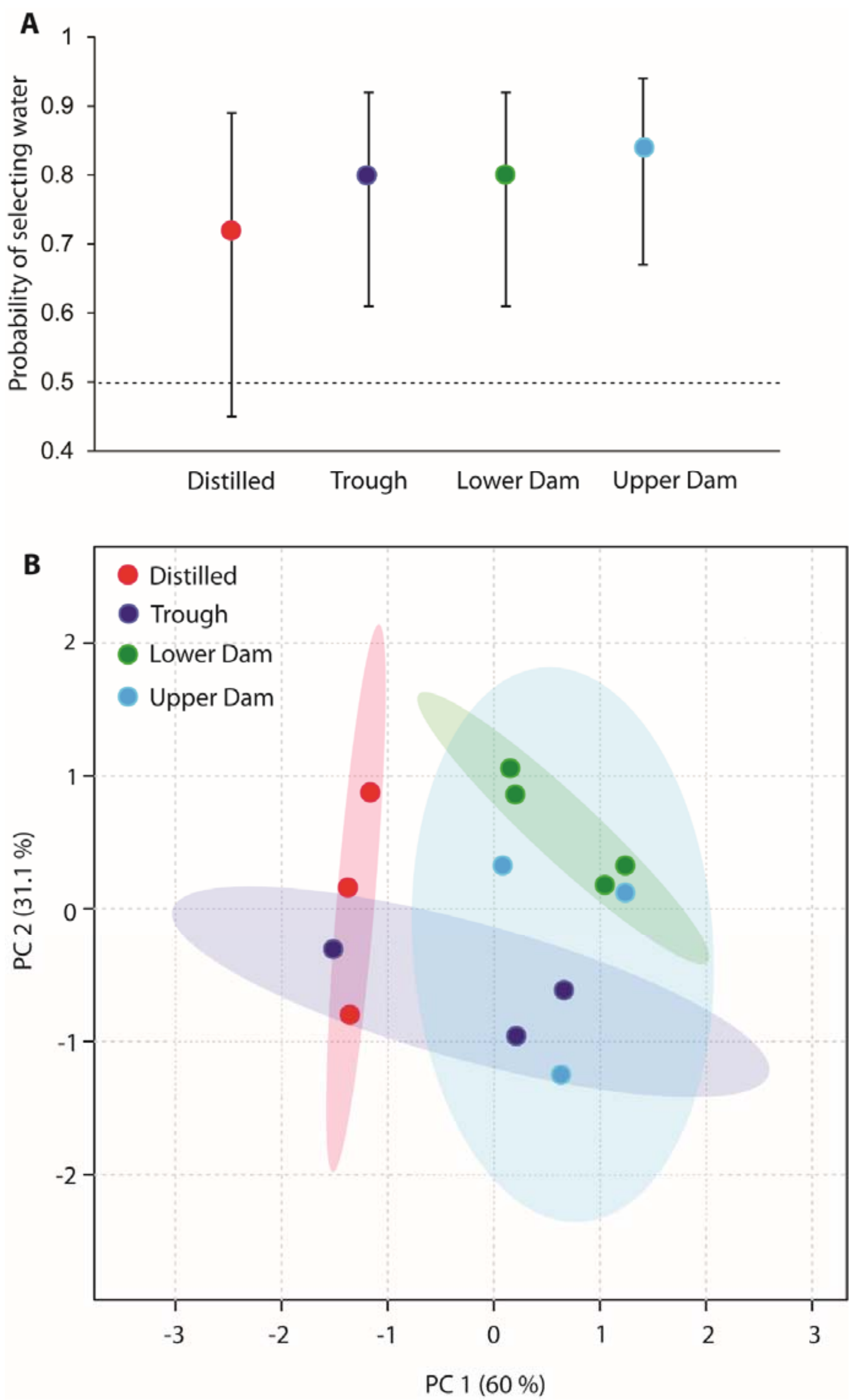

Fig. 2 a) Probability of elephants selecting for water when given the choice between water and the empty control, and b) PCA plot showing the differences between water samples from 4 different sources. Marginal means (±95% CI) are plotted in (a). Dotted line represents the 0.5 expectation (i.e. random selection). Confidence intervals that overlap the 0.5 indicate no difference in selection, while means and confidence intervals above the 0.5 expectation indicate detection and selection for the bucket containing water.

Results

Experiment 1: Detecting water via olfactory cues

Using only olfactory cues, the probability that elephants located water when it came from either one of the two dams or from the trough, was much higher than expected by chance (i.e. the 95% CI of the marginal means were above the 0.5 expectation; Fig. 2a). Yet, this was not the case for the distilled water (Fig. 2a), for which elephants' successes were highly variable (i.e. the 95% CI of the marginal means overlapped the 0.5 expectation), leading to their selection of the water source to be random and not significantly different from chance (GEE: $\chi 2= 2.119$, df= 3, $p= 0.548$, Fig. 2a). Individually, the elephants' ability to locate the different water types varied (Table SM1 supplementary material).

Odour profiles of the different water sources

When we explored the scent profiles of the water sources, the PCA showed a clear distinction between the distilled water and the three water sources from the property (Fig. 2b). These three water sources had very similar odour profiles (Fig. 2b, Fig SM1 supplementary material), but each contained unique VOCs that we identified using the peaks on the chromatograms (Figs. SM2 & SM3 supplementary material). These were a nitrogenous volatile ($F_{3,9}= 348.53$, $p<0.0001$), aliphatic hydrocarbon ($F_{3,9}= 134.64$, $p<0.0001$), putatively oxygenated terpenoid ($F_{3,9}= 202.63$, $p<0.0001$), and two separate unique phenolic volatiles (Phenolic volatile 1: $F_{3,9}= 255.49$, $p<0.0001$; Phenolic volatile 2: $F_{3,9}= 430.76$, $p<0.0001$). We found two of the unique compounds in the water from the two dams, which included the nitrogenous volatile and the putatively oxygenated monoterpene (Fig. SM2). There were two unique phenolic volatiles emanating from the upper dam samples that we also detected to a lesser degree in the trough water samples (phenolic volatile 1 and 2; Fig. SM2). We found the aliphatic hydrocarbon in the water from the two dams and the trough (Fig. SM2).

These volatiles could not be specifically identified, as closely matching spectra were not available in the NIST v. 98 spectral library. Therefore, no candidate compounds were identified for retention index calculations (See mass spectra in Fig. SM3). Thus, we provide a tentative identification based on the mass spectra (aliphatic vs phenolic vs nitrogenous).

For the distilled water, we detected a greater number of unique compounds compared to the other three water sources (Fig. 2b). However, these were mainly plasticisers and other impurities derived from processing of the water at the University of Pretoria.

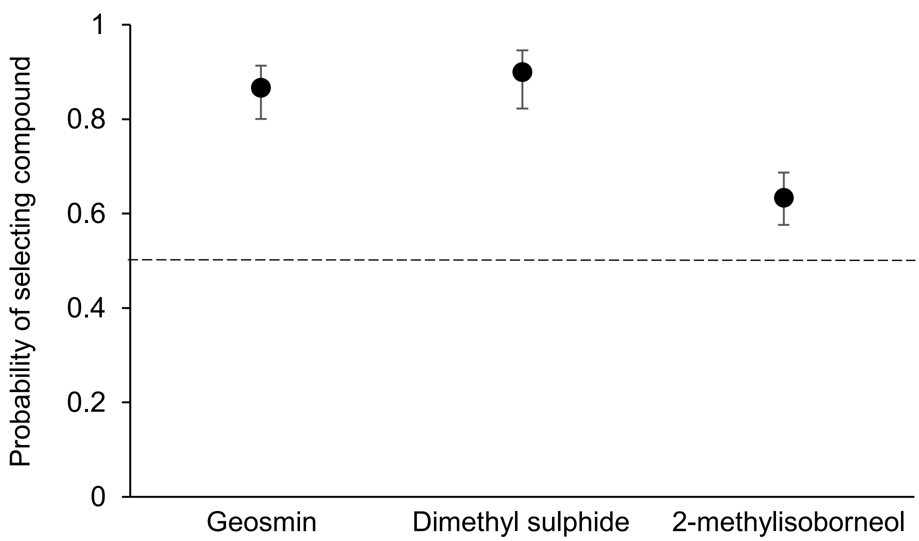

Fig. 3 Probability of elephants selecting the different volatile organic compounds compared to the control. Marginal means (±95% CI) are plotted. Dotted line represents the 0.5 expectation (i.e. random selection). Confidence intervals that overlap the 0.5 indicate no difference in selection, while means and confidence intervals above the 0.5 expectation indicate detection and selection for a specific compound.

Experiment 2: VOCs associated with water

When presented with the VOCs commonly emitted from water (i.e. geosmin, 2-methylisoborneol and dimethyl sulphide), the elephants were able to detect and locate all three of the VOCs using olfactory cues alone (GEE: $\chi 2= 35.138$, df= 2, $p< 0.001$; Fig. 3).

However, the ability to locate the specific VOCs varied between individuals (Table SM2 supplementary material). Interestingly, none of these three VOCs were found in any of the scent profiles of the three water sources (i.e. the two dams and the trough) found on the property.

Discussion

Small ephemeral water sources (e.g. pans, pools, puddles) are key for water-dependant herbivores living in arid environments, yet the temporary nature of these water sources makes predicting their location and availability difficult (Redfern et al. 2005). Using choice experiments, we found that the elephants were able to detect water at a small spatial scale using only olfactory cues. Furthermore, we found that they were able to detect three VOCs commonly associated with natural water sources and rainfall, namely geosmin, 2-MIB, and dimethyl sulphide.

Despite geosmin, 2-methylisoborneol, and dimethyl sulphide being considered common to the odour profiles of water sources (Izaguirre et al. 1982; Jenkins et al. 1967; Jüttner and Watson 2007), and us finding that the elephants could detect these VOCs, we did not find these VOCs in the odour profiles of the water we used in our experiments. As such, we are unable to comment on the extent to which elephants may use these key VOCs as cues when searching for water. Nevertheless, the elephants still located the water from the three water sources found on the property olfactorily.

The results of the PCA indicated that the odour profiles of the three water sources on the property were very similar, and that they all differed to that of the distilled water. As such, it is possible that the elephants used specific VOCs, other than geosmin, 2-methylisoborneol, and dimethyl sulphide, or combination of VOCs (e.g. aliphatic hydrocarbons) found within the odour profiles of the water sources that were eliminated from the distilled water during the distillation process. However, as each water source contained unique VOCs, which would result in different odour profiles, it is possible that the elephants may have learnt and now recognise the specific odour profiles of these water sources. As such, they could have used these odours to locate the water from the sources on the property. Either way, our results suggest that elephants do not solely rely on the presence of geosmin, 2-methylisoborneol, or dimethyl sulphide to locate water, which opens up an interesting and important field of future study.

Ultimately, our study is the first to demonstrate that elephants can detect water using olfactory cues alone. Moreover, despite us only focussing on a few individuals, the fact these elephants were unrelated, semi-tame, and free ranging suggests that our findings go beyond these individuals and likely apply to all African savanna elephants. Yet, as we focused on a small spatial scale, the extent to which elephants can detect water at larger spatial scales (e.g. km) remains unclear. Nevertheless, the fact that the elephants in our study were able to detect water olfactorily opens the possibility that they, especially with their exceptional sense of smell (Niimura et al. 2014; Shoshani and Foley 2000; Shoshani et al. 2006), may be able to detect water sources at distances beyond their visual range. Moreover, the VOC's being given off by the larger surface area of a body of water would likely be greater than those emitted from the small surface area of the beakers we used in our experiment (Aydin et al. 2014; Marneweck et al. 2018).

This same logic may also apply to the suggestion that elephants can track rainfall events (Bax and Sheldrick 1963). For example, geosmin and 2-MIB are released by bacteria in the soil when rain hits dry ground (Asquith et al. 2018; Gerber 1967; Izaguirre et al. 1982). As rain tends to fall over large areas, it is possible that the volume of VOCs released by the newly wet soil may allow elephants to detect key VOCs or combination of VOCs over large spatial scales. This then may allow elephants to track rainfall events and the subsequent re-establishment of temporary water sources (Bax and Sheldrick 1963; Garstang et al. 2014). Yet, the distance that these VOCs can travel and the concentration at which elephants can detect them is unknown. Moreover, it is also possible that elephants may rely on auditory cues generated by thunderstorms to track distant rainfall events (Garstang et al. 2014). As such, further study is required to determine the distances and concentrations at which elephants can detect olfactory cues associated with water, and whether they can use these to track rainfall.

Despite our focus on elephants, other mammalian herbivore species may also rely on olfactory cues to locate surface water. For example, Western (1975) suggested that the large-scale movements of wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*), and zebra (*Equus burchelli*), in Amboseli National Park, Kenya were correlated with rainfall events. In addition, the distribution of water-dependent herbivores like wildebeest, zebra, and sable antelope (*Hippotragus niger*) can change depending on the availability of ephemeral and artificial water points (Redfern et al. 2005; Smit et al. 2007). Yet, the extent to which the movements of these herbivores are driven by olfactory cues is unclear. Nevertheless, our results indicate that elephants can use olfactory cues to locate water. Thus, it is possible that both the small- and large-scale movements of a wide range of herbivore species may be driven by the detection of olfactory cues given off by both large and small water sources.

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Authors' contributions M.W., S. C-J and A.M.S conceived the ideas, designed the methodology, and led the writing of the manuscript. M.W. and A.M.S. collected and analysed the data. A.H. conducted and interpreted the olfactory analysis. All authors contributed critically to the drafts.

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Data Availability Upon publication, all data will be available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.h9w0vt4hc (Wood et al., 2021)

Compliance with ethical standards

Conflict of interests The authors have no conflict of interest to declare.

Ethical Approval All animal experiments were approved by the University of Pretoria's Animal Ethics Committee (reference number NAS075/2019) in compliance with South African laws and regulations.

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