Olfactory Generalisation in Dogs (*Canis familiaris)* Trained to Detect Koi Carp (*Cyprinus rubrofuscus)*

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Abstract

Koi carp (*Cyprinus rubrofuscus*) are an invasive fish species that threaten the biodiversity and ecological health of New Zealand's freshwater systems. To prevent the spread of koi carp, populations must be identified early so management methods can be implemented. However, the methods currently used to detect koi carp populations are either not suited to particular waterbodies, are too expensive, or are not sensitive enough to detect koi carp populations at low biomasses. Domestic dogs (*Canis familiaris*) are an emerging technology for the detection of koi carp. Previous studies have shown that dogs can detect koi carp at a biomass of 9.3 kg/ha in aquaria water samples, well below the proposed 50 kg/ha threshold for ecological decline. The present study used an automated scent detection apparatus to present dogs with naturally sourced carp-present and carp-absent lakes. The use of lake samples presented a unique challenge to the dogs because of the large variety of distractor odours in a natural environment, potentially complicating the detection of koi carp. In Phase A, dogs evaluated two carp-absent lake samples spiked with koi carp scent at an ecologically relevant biomass of 310 kg/ha. All dogs met the criteria of a hit rate and correct rejection rate ≥ 80% on these lakes, demonstrating that dogs can detect koi carp amongst lake water distractor odours. Phase B presented dogs with 12 positive (carp-present) and 12 negative (carp-absent) probe samples interspersed among samples from the lakes used in Phase A. The dogs' first responses to positive probes were examined to evaluate generalisation to koi carp scent in novel contexts, and negative probes tested the dogs' ability to reject lake samples not containing koi carp. Accuracy was stable across Phase A (range = 76% - 84%) and Phase B first responses (range = 71% - 81%). This stability across phases was interpreted as evidence for generalisation. Overall, generalisation was observed, but these generalisation data are interpreted cautiously due to the complexity of lake samples. This

complexity may have resulted in the dogs using cues irrelevant to the presence or absence of carp to make responses, such as the differing scent profiles of carp-present and carp-absent lakes. Despite these potential issues, this study acts as a proof of concept for the operational viability of dogs as a koi carp detection technology.

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Introduction

The Impacts of Invasive Fish Species on Freshwater Ecosystems

Freshwater ecosystems have significant biodiversity, housing 6% of the world's species while comprising only 0.8% of the world's total landmass (Dudgeon et al., 2006). This biodiversity is declining, affecting numerous economic and cultural human activities (Pascual et al., 2017; Pimentel et al., 2001). Declines in biodiversity are partly due to human-mediated introductions of invasive species (Strayer & Dudgeon., 2010; Thomaz et al., 2015). Invasive species reduce biodiversity by competing with native species for resources, directly preying on native species, hybridisation, and habitat alteration (Collier & Grainger., 2015). For example, in invaded African lakes, Nile tilapia (*Oreochromis niloticu*) out-compete native tilapia species for resources and threaten to replace native tilapia through hybridisation (Firmat et al., 2013; Stauffer et al., 2022). In New Zealand, brown trout (*Salmo trutta*) contribute to a decline in native galaxiid species, such as the banded kōkopu (*Galaxias fasciatus*) and inanga (*Galaxias maculatus*), through predation and competition for macroinvertebrate prey (McIntosh et al., 2010; Woodford & McIntosh, 2013). Invasive benthic species such as tench (*Tinca tinca*), brown bullhead catfish (*Ameiurus nebulosus*), and koi carp (*Carpio rubrofuscus*) are associated with a decline in water quality through bioturbation in shallow New Zealand lakes (Rowe, 2007; Schallenberg & Sorrell, 2008). Of these benthic species, koi carp are one of New Zealand's most prolific invaders, often dominating the total biomass of the freshwater systems they inhabit (Collier & Granger, 2015; Hicks & Ling, 2015).

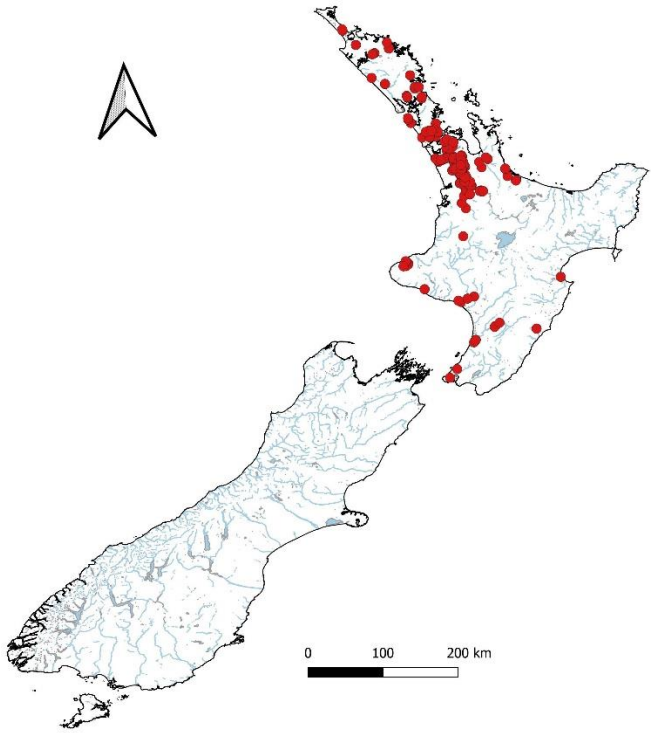
Carp as an Invasive Species

The common carp (*Cyprinus carpio*) is one of the most introduced species globally, with self-sustained populations in 91 of the 120 countries in which they have been introduced (Casal,

2006). New Zealand contains koi carp (hereafter referred to as carp), an ornamental variant of the common carp with origins in East Asia (De Kock & Gomelsky, 2015; Xu et al., 2014). Carp arrived in New Zealand in the 1960s and became established in the Waikato River in the 1980s (Collier & Granger, 2015). The arrival of carp led to the creation of a containment zone surrounding the Waikato and Auckland regions (Grainger, 2015). The spread of carp has been primarily contained within this zone, with large populations in the Waikato, Northland, and Auckland regions, and smaller populations scattered around the North Island (Collier & Granger, 2015; Hicks & Ling, 2015). In the Waikato region, the foraging behaviour of carp while feeding has contributed to a reduction in macrophyte biomass and reduced water quality, leading to their classification as a noxious and unwanted fish species (Collier & Granger, 2015; Hicks & Ling, 2015; New Zealand Government, 1983; New Zealand Government, 1993).

Figure 1

Distribution of Carp in New Zealand



Note. *Credit: Grant Tempero. Information retrieved from New Zealand Freshwater Fish Database, December 2022*

Carp are benthic omnivores, feeding on invertebrates and plant material by foraging through sediment (Collier & Granger, 2015). This foraging behaviour uproots macrophytes and resuspends sediment, increasing suspended particles and nutrients in the water column and reducing water clarity (Adámek & Maršálek, 2013; Bjaer et al., 2009; Qiu et al., 2019). Reduced water clarity reduces light availability to submerged macrophytes, ultimately leading to the loss of submerged aquatic vegetation and further sediment destabilisation by wind resuspension (Adámek & Maršálek, 2013; Hicks & Ling., 2015). The aquatic system then changes from a clear water macrophyte system to a turbid algal dominated system (Akhurst, 2012; Barton et al., 2000; Crivelli, 1983; Driver et al., 2005; Jackson et al., 2010). Increased nutrient cycling from carp feeding and excretion may also result in increased eutrophication, furthering phytoplankton dominance (Driver et al., 2005).

The effects of carp are widespread in the Waikato region due to their abundance (Hicks et al., 2015a). A boat electrofishing survey of the lower Waikato River in 2005 reported estimated biomasses ranging between 39.6 and 307.8 kg/ha, comprising 69% of total fish caught (Hicks et al., 2005). Similarly, the Whangamarino River, a tributary of the Waikato River, was estimated to have a mean biomass of 326 kg/ha in 2008 (Hicks et al., 2008). These biomasses are much higher than the 100 kg/ha threshold that Bjaer et al. (2009) found to induce macrophyte loss and reduce water quality. Lakes in the Waikato region also exceed this threshold, with many lakes estimated to surpass 200 kg/ha in a 2006 boat electrofishing survey (Hicks et al., 2006). At biomasses under 50 kg/ha, carp have minimal effects on macrophyte biomass and water quality (Bjaer et al., 2009). However, biomass is rarely under 50 kg/ha in the lower Waikato region, with

carp often completely dominating environments where they are present (Hicks & Ling, 2015). In order to prevent the further spread of carp, populations need to be detected early so that management methods can be put in place to mitigate or prevent ecological damage (Jerde et al., 2013).

Carp Detection Methods

Early detection of a carp population provides the greatest chance of eradication (David et al., 2021). For example, New Zealand's only successful carp eradication was in 2001, when the spread of carp to the Marlborough region was detected before carp populations became established (Grainger, 2015). In that case, the Department of Conservation applied the piscicide rotenone to several small ponds with limited biodiversity, eradicating these isolated carp populations (Grainger, 2015). There have been successful carp eradications overseas in larger water bodies, such as Lake Crescent, Tasmania (Yick et al., 2021). In the example of Lake Crescent, eradication was possible by early detection through visual observation and 12 years of eradication efforts; since 2007, no carp have been captured and the spread of carp to other waterbodies was prevented (Yick et al., 2021). The example of Yick et al. (2021) highlights the need for early detection methods to inform proactive population management while carp are at low biomasses. Current detection methods cannot achieve this goal, as they lack the sensitivity to detect carp at low biomasses and their sensitivity is not easily quantified (David et al., 2021).

A surveillance method often used to survey carp is boat electrofishing (Hicks et al., 2015). Electrofishing can be effective for generating population estimates in non-wadeable waters, and captured fish can also be assessed for information about sex, age, and condition (Hicks et al., 2015). However, electrofishing is ineffective in deeper lakes, and there is only one boat fit for purpose in New Zealand, limiting the number of waterbodies that can be surveyed

(Hicks et al., 2015). Depending on fish density, overnight baited traps and netting can be cost- and time-effective methods (Hicks et al., 2015; Yick et al., 2021). However, both may result in unintended bycatch of native or sports fish and lack the sensitivity to detect carp at low biomasses (Hicks et al., 2015; Lake, 2013). Early detection methods are needed as electrofishing, netting, and traps are highly intrusive and largely reactive measures for surveillance when biomass is already at high levels (Banks et al., 2021).

Environmental DNA (eDNA) is a relatively recent technology allowing carp and other species detection at low biomasses (Banks et al., 2021; Wilcox et al., 2013). eDNA detects carp through cells released into the environment, meaning carp do not need to be visually observed and can be detected at low biomasses (Banks et al., 2021; David et al., 2021). eDNA is also a non-intrusive and relatively low-effort method compared to netting and boat electrofishing (Hinlo et al., 2017; Jerde et al., 2011). For example, Itakura et al. (2019) utilised eDNA to detect the endangered Japanese eel (*Anguilla japonica*) and compared its efficacy to electrofishing. Electrofishing caught Japanese eels in 61 different sites, while eDNA detected Japanese eels in 56 of these sites, in addition to 35 sites where electrofishing failed to detect eels. These findings suggest that eDNA may have greater sensitivity in detecting Japanese eels. However, there are several considerations to be researched before the use of eDNA can become more standardised. While the collection of eDNA is relatively straightforward, errors can occur during sample collection and storage (Furlan & Gleeson, 2017). It is also unknown how long DNA persists in an environment, but it is estimated to be under a month (Dejean et al., 2011). However, this can vary depending on factors such as water temperature (Kasai et al., 2020), UV exposure (Strickler et al., 2015), and the behaviour of the target species (Wilcox et al., 2016; David et al., 2021). Detection limits are also relatively unknown, along with rates of false positives and false

negatives (David et al., 2021). Overall, there needs to be more investigation of the environmental factors likely to impact the efficacy of eDNA as a detection tool (Bohmann et al., 2014). eDNA shows promise and could be an effective tool in conjunction with other detection and management measures (Hinlo et al., 2017). Other non-intrusive methods for detecting carp at low biomasses should also be investigated.

Dogs in the Detection of Carp

Dogs (*Canis familiaris*) are another emerging technology for the detection of carp at low biomasses. Collins et al. (2022) found that dogs trained to detect carp in laboratory water samples have similar levels of sensitivity to eDNA, detecting carp at 9.3 kg/ha. This evidence suggests there is value in exploring the use of dogs as a non-invasive technology for detecting carp. The use of dogs as a detection technology is grounded in their olfactory ability, trainability, and cost-effectiveness compared to traditional methods (Beebe et al., 2016). Dogs have been extensively used in non-biological scent detection, including narcotics (Francis et al., 2019; Lancaster et al., 2017) and explosives detection (Furton & Myers, 2001; Lazarowski & Dorman, 2014). Dogs have also been used to detect a wide array of biological scents in medical detection (Pickel et al., 2004; Pirrone & Albertini, 2017), the detection of missing persons (Osterkamp, 2011), and the detection of endangered and pest species (Beebe et al., 2016; Browne et al., 2006).

Scent Detection Dogs in Conservation

Dogs have a long history in conservation, with the first application in New Zealand in the 1890s to detect kiwi (*Apteryx* spp.) and kākāpō (*Strigops habroptilus*) (Browne et al., 2006). The use of dogs for conservation work includes pest detection and monitoring of endangered species (Beebe et al., 2016; Browne et al., 2006). This can be achieved through training dogs to detect

the target scent of a live organism or traces of the target organism (e.g., hair, scat). Scats are often an ideal target scent due to their abundance and because using traces of an organism keeps detector dogs as non-invasive as possible (Wasser et al., 2009). Across various detection tasks on different species, dogs have proven to be more time efficient and accurate than alternative survey methods. For example, in a review of 422 scientific papers, Grimm-Seyfarth et al. (2021) found that dogs detected targets (e.g. live animals, scats, plants) better than alternative methods (e.g. camera traps, hair snares, and experienced human searchers) 88% of the time. However, training a dog to a high standard of detection is time-consuming and expensive (Beebee et al., 2016). Therefore, the right dog must be chosen for each detection task. There are several considerations for potential training candidates, such as olfactory abilities and traits such as food motivation (DeMatteo et al., 2019).

Canine Olfaction

Dogs have exceptional olfactory abilities, facilitating the location of partners for reproduction, the location of food, and threat detection (Kokocinska-Kusiak, 2021). Volatile organic compounds (VOCs) are taken in through the nasal cavity and dissolved in the mucus lining when a dog sniffs, and are then bound to odorant-binding proteins (Craven et al., 2010). VOCs are chemical molecules or combinations of molecules emitted by targets of interest, such as people, animals, narcotics and explosives (Angle et al., 2016). Organisms release VOCs into the environment through processes such as skin secretion, breathing, saliva, and blood, each producing distinct scent profiles which dogs can be trained to detect (Angle et al., 2016). Walker et al. (2006) found that dogs could detect the VOCs of n-amyl acetate at as low as 1-2 parts per trillion. This sensitivity is between 10,000 and 100,000 times greater than humans (Walker et al., 2006). A dogs nasal cavity, which houses the olfactory epithelium, is much larger compared to a

humans, containing approximately 200 million olfactory receptor cells (ORCs) compared to 5 million in humans (Quignon et al., 2003; Jenkins et al., 2018). Each ORC has hundreds of cilia, compared to 25 in humans, allowing for the detection of an estimated half a million trace odours (Buszewski et al., 2012; Jenkins et al., 2018). Canine nasal architecture also produces unique airflow patterns across the respiratory epithelium while sniffing, allowing for efficient transportation of VOCs to olfactory receptor cells (Craven et al., 2010). These factors all contribute to dogs being a highly sensitive detection technology, but the presence of traits such as food motivation is equally important (Beebee et al., 2016; Grimm-Seyfarth et al., 2021).

Selecting the Appropriate Scent Detection Dog

Traditionally, dolichocephalic breeds such as Labrador retrievers, pointing dogs, border collies, and German shepherds have been chosen for scent detection work based on behavioural traits such as athleticism, allowing dogs to work in, at times, harsh conditions for extended periods (Ferrando & Dahl, 2022; Grimm-Seyfarth et al., 2021). Dolichocephalic breeds also generally have a larger olfactory epithelium and thus more ORCs, indicating superior olfactory ability (Bebee et al., 2016). However, breed is not always a significant predictor of performance in laboratory detection tasks (Grimm-Seyfarth et al., 2021; Hall et al., 2015). For example, Hall et al. (2015) found that pugs, a brachycephalic breed, outperformed German shepherds in a laboratory odour discrimination task and maintained this performance as the concentration of the target odour was decreased. These findings are unlikely to be based on differing motivation levels between German shepherds and pugs, as both breeds performed similarly in a prior visual discrimination task (Hall et al., 2015). Other studies have found conflicting results, with dolichocephalic breeds outperforming brachycephalic breeds in other detection tasks (Ferrando & Dahl, 2022; Jezierski et al., 2014). It is difficult to compare these studies due to differing

methodologies. However, at the very least, Hall et al. (2015) show that all breeds should be considered for laboratory detection tasks, regardless of preconceived ideas about the breed's suitability. Any detection dog should be highly motivated to obtain the chosen reinforcer (i.e. food, play), as this establishes the reinforcer as valuable, making dogs more receptive to operant conditioning (Beebe et al., 2016; Grimm-Seyfarth et al., 2021). Other traits to consider are aggression toward humans and other dogs, fearfulness, and the ability to cooperate with a trainer (Bebee et al., 2016; DeMatteo et al., 2019). Overall, detection performance is not purely linked to olfactory abilities; it is heavily influenced by the individual dog's characteristics which can make training through operant conditioning more effective (DeMatteo et al., 2019).

Training Scent Detection Dogs

The method used for training detection dogs will differ between each detection task depending on what the target scent is and whether the task is based in the field or a laboratory (Beebe et al., 2016; Bennett et al., 2019; Martin et al., 2020). Regardless of the task, the underlying mechanism of learning in detection tasks is operant conditioning, with the consequences of behaviour selecting the probability of similar behaviour occurring in the future (Skinner, 1982). Positive reinforcement is commonly used in scent detection training, aiming to increase the probability of an indication response (such as sitting) occurring in the presence of a target odour. The target odour is a discriminative stimulus, as it signals the availability of reinforcement. When the indication response is emitted in the presence of this discriminative stimulus, the response is reinforced, increasing the probability of that response being selected in future presentations (Edwards et al., 2017; Moser & McCulloch, 2010; Porritt et al., 2015). Training often utilises respondent conditioning by pairing a neutral stimulus (such as a clicker) with the delivery of unconditioned reinforcement, establishing the previously neutral stimulus as

a conditioned reinforcer (Feng et al., 2016; Pfaller-Sadovsky et al., 2020). Successful training establishes the target odour as a discriminative stimulus and gives the target odour stimulus control over the indication response, making it more likely that, in future, the indication response will be selected in the presence of the target odour (Hall & Wynne, 2016).

Scent Discrimination

Scent discrimination is an essential mechanism for the specificity of dogs as a detection tool (DeGreef et al., 2021). Discrimination is seen through a dog not indicating the presence of the target odour when it is absent (Lazarowski et al., 2020). Dogs learn to discriminate through differential reinforcement, only being provided reinforcement when the response is performed in the presence of the target odour and not being provided reinforcement for emitting the response to non-targets (Hall et al., 2013; Hall, 2017). For example, Fukuzawa and Sashara (2019) trained dogs through differential reinforcement to detect scats of the invasive Carolina anole (*Anolis carolinensis*) and discriminate these scats from those of native reptiles from the same habitat. Dogs have also been trained to detect the scats of endangered species, such as the Marlborough gecko (*Naultinus manukanus*), and discriminate these scats from those of the closely related forest gecko (*Hoplodactylus granulatus*) (Browne et al., 2015). Dogs must also generalise to untrained variations of the target odour while retaining this specificity against non-targets (DeGreef et al., 2022).

Scent Generalisation

In a detection task, generalisation is seen through dogs emitting the indication response to novel variations of the target odour (Ghirlanda & Enquist, 2000; Moser et al., 2019; Oldenburg et al., 2016). For example, a dog trained to respond to an odour of a particular concentration may still exhibit the indication response when presented with the same odour at a different

concentration (DeChant et al., 2021). However, odour concentration as a dimension for generalisation is challenging to measure and control. Generalisation research on other stimuli such as light frequency often produces generalisation gradients in the shape of a bell curve with peak responding at the trained stimulus and gradually lowering levels of responding to either side (Blough, 1972). Contrarily, olfactory gradients often show a response bias towards stimuli that are higher in concentration rather than lower, resulting in a peak shift towards higher concentrations (Moser et al., 2019; DeChant et al., 2021). The target odour can also be variable, so to encourage generalisation to novel variations of the target odour a sufficient number of variations should be presented in training (Edwards et al., 2017). This training of multiple variations forms a concept formation of the target odour, increasing the probability of generalisation to novel variations (Oldenburg et al., 2016). Another way that the target odour can vary is when it is mixed with other odours, which can lead to several perceptual issues (DeGreef et al., 2022; Hall & Wynne, 2016; Prichard et al., 2020).

Odour Perception

Scent discrimination is aided by ORC coding in the OE. Each presented odourant activates a unique pattern of ORCs, with some activating many and others activating a small amount (Kokocinska-Kusiak et al., 2021; Kurian et al., 2021). As an odourant concentration varies, different groups of ORCs are activated, and the number of ORCs activated changes based on odour intensity (Kurian et al., 2021; Sirotin et al., 2015). This differing activation produces a mechanism through which odourants are perceived as distinct (Kokocinska-Kusiak et al., 2021; Kurian et al., 2021). Impulses from ORCs move through olfactory axons to the olfactory bulb where olfactory glomeruli initially interpret stimuli (Kokocinska-Kusiak et al., 2021). At this level of processing, discriminations between odours are made and target odours are filtered from

background odours (Barrios et al., 2014; Doucette et al., 2007; Jia et al., 2014; Kokocinska-Kusiak et al., 2021). Olfactory glomeruli assist in a dogs ability to generalise to novel variations of a target odour (Hall et al., 2016; Moser et al., 2019). Odours of the same and similar carbon chain length are mapped closely together, so structurally similar compounds activate overlapping olfactory glomeruli, eliciting a generalised behavioural response (DeGreef et al., 2020; Hall et al., 2016; Moser et al., 2019). Notable olfactory processing also occurs in the amygdala and piriform cortex, where stimulus-reward associations are formed (Prichard et al., 2020). Additionally, the hippocampal formation is involved in the recognition of odours (Kokocinska-Kusiak et al., 2021).

This relatively simple view of ORC coding cannot account for patterns of activation seen in more complex blends of odours, particularly when multiple odours in a mixture activate overlapping ORCs (Xu et al., 2020). Controlled laboratory experiments investigating a single odour along a chemical dimension have little applicability in natural environments where the olfactory system is exposed to complex mixtures of odourants (DeGreef et al., 2022; Kurian et al., 2021). Understanding of canine olfactory coding is limited, but in studies of other mammalian species ORC activation is modulated depending on the components of a mixture (Kurian et al., 2021; Xu et al., 2020; Pfister et al., 2020). More complex mixtures of odourants produce agonistic and antagonistic effects where ORC responses are either suppressed or enhanced depending on the components of a mixture (Xu et al., 2020). This assists in discriminating odourants in mixtures and makes each mixture more distinct (Xu et al., 2020). The activation observed in response to odourants is also modulated when odourants in a mixture are presented at differing concentrations (Grabe & Sachse, 2018; Kurian et al., 2021).

Mixtures of odours are believed to be perceived either elementally, where each component of a mixture is processed individually, or configurally, where each mixture is perceived as a new stimulus (DeGreef et al., 2022; Hall & Wynne, 2016; Prichard et al., 2020). These processes occur in olfactory regions such as the amygdala and piriform cortex and appear to be based on stimulus-reward associations (Prichard et al., 2020). Whether a mixture is perceived configurally or elementally is a contextually dependent process influenced by the characteristics of the target odour and the non-target odours present (DeGreef et al., 2022; Hall & Wyne, 2016). The salience of the target odour matters, and if the concentration of the target odour is too low compared to non-target odours its binding can be affected through overshadowing or masking (DeGreef et al., 2022; Hall & Wyne, 2016). This highlights issues that can impact generalisation to new scents, as a target odour may not be associated with reward while in a mixture due to it being perceived as a new stimulus (Prichard et al., 2020). Therefore, scent detection training should include target odours among distractors; this encourages elemental processing and has been shown to improve a dog's ability to detect target odours amidst distractors (Fischer-Tenhagen et al., 2017; Hall & Wynne, 2018).

The Methodology can Alter Outcomes

Beyond choosing an appropriate dog and target odour to be trained, the training methodology should be considered (DeMatteo et al., 2019; Johen et al., 2013; Lazarowski et al., 2020). Many scent detection tasks heavily involve human trainers, making them manual tasks (Edwards, 2019). A manual scent detection task can confound results through unintentional cueing from the trainer (Szetei et al., 2003; Edwards et al., 2019; Johen et al., 2017; Lit et al., 2011). For example, dogs have been shown to make indications based on human cues even when this contradicts perceptual information (Szetei et al., 2003; Larazowski et al., 2019; Lit et al.,

2011). Manual tasks can also lead to subjectivity in how responses are coded, especially if the topography of the target behaviour is not well-defined (Concha et al., 2014; Edwards, 2019; Larazowski et al., 2020). Human presence can confound results even in double-blinded studies, with handler beliefs of a sample's location making dogs more likely to indicate on a sample when the target scent is not present (Lit et al., 2011). In a manual task, trainers are also unlikely to deliver reinforcement with appropriate timing (Ferry et al., 2019). This is an issue because delaying reinforcement, even by a second, decreases the likelihood of the dog learning a task (Browne, 2015).

In scent detection research, the sample presentation method is often suboptimal, with many studies using forced-choice line-ups (Edwards et al., 2017; Edwards, 2019). In these tasks, dogs must indicate a positive sample from a set of samples in a line (Ferry et al., 2019; Lazarowski et al., 2020). The trainer may misread the dog's response, or as discussed the dog may rely on human cues (Edwards et al., 2017). Additionally, forced-choice line-ups have no applicability to settings where the positive or negative status of samples may be unknown. In such conditions there may be multiple positive samples, while only one of these is able to be indicated (Edwards et al., 2017). Specificity calculations also cannot be made as only one sample can be indicated in a trial, while every other sample is considered rejected. Therefore, if there are one positive and five negative samples in the line-up, and the target is missed, no data can be gathered regarding the other negative samples (Edwards et al., 2017). Another more general issue is that many studies do not provide adequate details concerning training methodology, making it impossible to confirm that contrasting results between studies are not a result of methodological differences (Johnen et al., 2017; Edwards et al., 2017). Instead of relying on a manual procedure, Edwards (2019) suggests a fully automated approach.

An Automated Methodology

Edwards (2019) has developed an automated apparatus allowing stimulus presentation, response recording, and reinforcement without a human present during sessions. This mostly removes the chance for cueing and observation bias as the investigator is in a separate room (Edwards et al., 2017). Timing of reinforcement is kept consistent through software delivering it at an optimal interval following a response. In each trial, there are four possible outcomes based on whether or not the dog indicates the presence of the target and whether that target is positive or negative (Edwards et a., 2017). If a sample is positive and it is indicated, this is a hit; if it is not indicated, this is a miss. If the sample is negative and it is indicated, this is a false alarm; if it is not indicated, this is a correct rejection. Only hits are reinforced, while every other outcome has no programmed consequence (Edwards et al., 2017). This allows for the easy coding of common errors and calculations of sensitivity and specificity. Additionally, as reinforcement is not given on every trial, this approach could also translate to operational settings (Edwards et al., 2017; Edwards, 2019). This apparatus has been used to train dogs to detect an array of target scents, including invasive aquatic species such as carp (Collins et al., 2022; Quaife, 2018).

Dogs as a Carp Detection Technology

Utilising the scent-detection apparatus described by Edwards (2019), water samples from lakes and ponds containing carp can be collected in the field and presented to dogs in a controlled laboratory setting. Quaife (2018) found that dogs could detect carp at an equivalent biomass of 37.4 kg/ha, a biomass lower than carp are often found in the Waikato Region (Hicks et al., 2005; Hicks et al., 2006). It was also found that dogs could discriminate between carp and the closely related goldfish (Quaife, 2018). Collins et al. (2022) compared the detection thresholds of dogs and eDNA; a dog could reliably detect carp in laboratory conditions at an

equivalent biomass concentration of 9.3 kg/ha, which was comparable to eDNA detection. These studies demonstrate that dogs can detect carp at biomasses equivalent to 3-4 adult carp per hectare. However, these studies used water samples from a municipal supply, and carp were housed in aquaria. Further research into the ability of dogs to detect carp in naturally sourced lake water is required. This would also allow the evaluation of dogs' ability to generalise their detection abilities to samples with more 'noise'.

The Present Investigation

Due to the negative environmental impacts carp have at high biomasses, novel early detection methods must be developed. To demonstrate operational viability, a study was undertaken to assess dogs' ability to detect carp in water samples from natural sources. The present study specifically investigated dogs' ability to generalise their detection abilities to novel lakes containing carp while rejecting novel lakes without carp. Each lake presented a unique challenge for the dogs due to differing background VOCs from non-target species and plant materials. It was expected that dogs would successfully detect carp in novel lakes that contained carp and reject those which did not contain carp.

The first phase of the study aimed to investigate whether dogs were able to detect carp in lake water samples spiked with laboratory water containing carp at a constant biomass of 310 kg/ha. The second phase aimed to investigate dogs' ability to generalise to novel lakes containing carp of unknown biomass, while rejecting novel lakes without carp.

Methodology

Subjects

Four domestic dogs participated in this experiment. Sabi, Aspen, and Harlee were recruited and trained to use the apparatus by past students. Cairo was recruited and started training from the initial phases; no other candidates were screened. The recruitment process for potential dogs consisted of social media, word of mouth, and advertisements at the University of Waikato campus. Potential candidates were screened for suitability through a questionnaire completed by dog owners. Candidates viewed as suitable attended an initial interview without the owner present that assessed food motivation, interactions with the trainer, and behaviours associated with separation anxiety.

Table 1

Details of Dog Participants

Dog	Breed	Sex	Age
Sabi	Labrador retriever	Female*	10
Cairo	Labrador retriever	Female*	6
Aspen	Labrador retriever	Male	4
Harlee	Labrador retriever	Female*	4

Figure 2

Dog Participants, from left to right: Sabi, Cairo, Aspen, and Harlee

Carp

Carp were collected via boat electrofishing from Lake Ohinewai, east of Huntly. The University of Waikato's Aquatic Research Centre kept experimental carp in a 195 L high-density polyethylene tank. In the same laboratory, reserve carp were housed amongst other fish species in a 5000 L fibreglass tank. Both tanks had a continuous supply of dechlorinated tap water (0.5 L/minute), and oxygen levels were maintained through a submerged aerator. Carp were fed commercial fish pellets once a week, with a slightly increased feed in the summer months.

Animal Ethics Statement

The University of Waikato Animal Ethics Committee (protocol AEC#1141) approved the use of domestic dogs and carp for this experiment. Following a standard operating procedure (SOP) approved by the Animal Ethics Committee, dogs were housed in individual crates with water available at all times. Dogs were taken for a 10-minute walk every two hours and an additional 30-minute walk at midday if they were in the laboratory for the whole day.

Apparatus and Experimental Setting

Experiments took place at the University of Waikato's Scent Detection Research Facility. Dogs were housed in crates within a central room. The facility had a room for sample preparation and an experimental room containing the scent detection apparatus (SDA). The SDA, as shown in Figure 2, was designed to present odour samples to dogs without human input during sessions. The SDA had a carousel that could hold 17 odour samples. Each of these 17 samples sat inside a stainless-steel segment, and a stainless-steel lid was placed on top of the segments to trap volatile organic compounds (VOCs) evaporating from the samples. The segments were positioned behind an acrylic panel with a 10cm port in the centre, giving access to a flap that dogs could push open to assess samples. Infrared beams ran across the port and an audible tone would sound when these were broken during an observation. An omnidirectional switch (lever) was located on the right side of the apparatus. Dogs could press the lever to reject a sample, advancing the apparatus to the next sample. The SDA was connected to a feeder (Treat & Train Remote Dog Trainer, manufactured by PetSafe) that automatically dispensed kibble from the other side of the room. When dispensing kibble, a 'click' would sound. Two cameras within the experimental room were connected to a computer in the central room, allowing the investigator to view sessions and for these sessions to be recorded. A second computer ran custom software that controlled the SDA and collected response data. Each dog had a configuration file loaded into the custom software where the status of each sample was input, along with the dog's minimum observation time and indication threshold. The minimum observation time was the minimum amount of time dogs had to hold their nose in the port for the custom software to recognise a sample observation. After this minimum observation time was reached, the dogs were able to press the lever to advance to the next sample. The indication

threshold was the length of time dogs had to hold their nose in the apparatus port to indicate that a sample contained carp. A full rotation of the apparatus was referred to as a session, and each of the 17 sample presentations in a session was referred to as a trial. A full explanation of the SDA can be found in Edwards (2019).

Figure 3

The Scent Detection Apparatus: Front and Side Views

Sample Collection

Aquaria water samples were collected from the University of Waikato's Aquatic Research Facility. The experimental tank containing carp was drained to a standby level 24 hours before sample collection. This standby level was calculated based on the weight of the fish and corresponded to 15.5 g/L. The control tank was not drained, and a steady flow of dechlorinated water was maintained. After 24 hours, samples from both tanks were collected in carp- or control-specific 1 L plastic containers and placed into separate zip-lock bags. To prevent contamination, control samples were collected before carp samples and gloves were changed when handling different sample types. Tanks were scrubbed with their corresponding sponge,

and the experimental tank was refilled. If sample collection was happening the next day, the experimental tank was drained back to its standby level. The tank was left with a steady flow of dechlorinated water if the experimental week was over.

Lake water samples were collected a few metres from the shoreline or off a jetty near the shoreline. Water was collected in 2 L plastic containers. These containers were rinsed three times with lake water before being filled. They were then placed into a zip-lock bag and a label was placed inside the bag with the name of the lake and the date of collection. Samples were put into a cooler alongside ice packs to keep them chilled. These samples were frozen for at least 24 hours before being defrosted overnight before experimental days.

Sample Preparation

Before preparing samples, a boiled kettle was poured over the metal bench in the preparation room and wiped with paper towels. The bench was then sprayed with 70% isopropyl alcohol (IPA) and wiped with paper towels. Paper towels were placed on either side of the sink, with the left side reserved for negative samples and the right for positive samples. Gloves were worn at all times and were changed when preparing different sample types. A pattern was made to identify each sample type using a sticker on the bottom of the plastic container.

In phase A, samples were prepared in 200 mL plastic containers and always contained 100 mL of lake water. Positive training samples were spiked with 100 µL of carp aquaria water, corresponding to a standard areal biomass of 310 kg/ha (assuming a two-metre deep water body). This biomass was considered ecologically relevant, as this is a biomass at which carp populations are commonly found in the Waikato Region, and carp have been shown to produce adverse effects on the environment at this biomass (Hicks et al., 2005; Hicks et al., 2006; Bjaer et al., 2009). Negative training samples were spiked with 100 µL of control water. In phase B,

positive and negative probe samples were added. These probe samples contained 100 mL of unspiked lake water.

Samples were placed onto a three-tiered transportation trolley and moved into the experimental room. Negative samples were on the top, and positive samples were on the bottom. Samples were placed in their randomised position on the apparatus, and segments were placed over each of them. To allow VOCs to evaporate into the segments, samples were left with the lid on for a minimum of 15 minutes before beginning sessions.

Training Procedure

Sabi, Harlee, and Aspen had been trained to operate the SDA by previous students. Due to COVID-19 lockdowns there was a break of multiple months prior to the project start. Therefore, these dogs took some time to reach their previous baseline performance. Cairo began training from the shaping phases.

Dogs were trained through shaping. This is the differential reinforcement of successive approximations toward a target behaviour (Edwards, 2019). Each target behaviour had to be exhibited three times before progressing to successive target behaviours. Initially, prompts were required for some target behaviours. This prompt was kept consistent, such as pointing a finger at the sample port or lever. Prompts were faded before moving on to subsequent target behaviours.

The first step in training was to establish the sound of the feeder dispensing food as a conditioned reinforcer. This involved the investigator using a handheld remote to release food from the feeder and allowing the dog to approach the feeder to eat the kibble. Once the dog

approached the feeder within three seconds of food delivery three times in a row, the sound of the feeder was considered to be established as a conditioned reinforcer.

The dog was then trained to place their nose into the sample port. Reinforcement was initially provided when the dog approached the apparatus and then when the dog placed its nose in the port. A segment was then placed behind the port, and reinforcement was provided when the dog touched its nose to the segment flap and, finally, for opening the segment flap with its nose. Two samples containing 100 mL of carp aquaria water were then placed on the carousel. The dogs' corresponding configuration file was set to a minimum observation time of 500 ms, after which the dog could press the apparatus lever and advance to the following sample. The indication threshold was set to 1000 ms. A tone would sound for the duration of the beam break, giving feedback to the dogs. After 1000 ms, kibble was dispensed from the feeder. After a session at an indication threshold of 1000 ms, the dog was removed from the room, and the indication time was gradually increased in subsequent sessions by 100 ms until a session had been completed at 1500 ms.

The apparatus was then turned off, and the next step of training was shaping towards 10 lever presses in a row without prompting from the investigator. The topography of behaviour exhibited while activating the lever differed between dogs, with the majority pushing the lever with their head or pulling it with their teeth.

Following this, one positive sample with 100 mL of carp water and one negative sample with 100 mL of control water were placed next to each other on the apparatus. The dogs' configuration file was set to have these two samples alternate until a session was completed. Prompting was required for some dogs and would be provided after 20 seconds of a dog not interacting with the apparatus or lever. After a session with no prompts, the apparatus was loaded

in a randomised order with approximately half of the samples being positive and half negative. The sample order was reversed after three sessions, and the indication threshold was gradually increased to 4501 ms following two out of three sessions where the dogs hit rate and correct rejections were above 80%. A full description of the shaping procedure can be found in Appendix A.

Once the dog was working at an indication threshold of 4501 ms, positive samples were diluted gradually to 100 µL (310.7 kg/ha). Decreases in dilution occurred following two out of three consecutive sessions with hit rate and correct rejections above 80%. The dilution was then reduced by 50% until achieving detection at a biomass of 310 kg/ha. Dogs remained at 310 kg/ha until achieving the criteria of two out of three consecutive sessions with a hit rate and correct rejection rate.

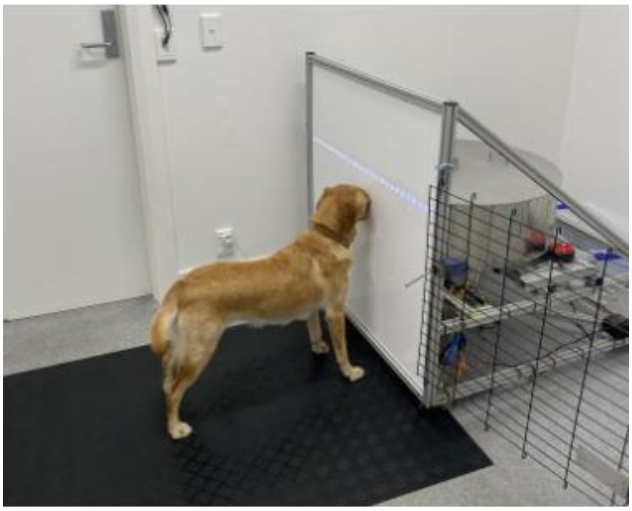
Experimental Procedure

Experiments were conducted between 8am and 12pm on Wednesdays and Thursdays each week. To ensure dogs were motivated, owners were instructed to feed their dogs a reduced amount two hours before arriving at the laboratory. Each dog completed six sessions on experimental days. A session was one full rotation of 17 samples, and this order was reversed following the third session to avoid dogs remembering the position of positive samples. Before beginning a session, each dog's configuration file was updated to that day's randomised sample order. Sessions involved each dog placing its nose into the segment to analyse the sample within. Dogs indicated the presence of carp by holding their nose in the segment for a predetermined indication threshold. This threshold was 5001ms for Cairo, Aspen, and Harlee. Sabi's indication threshold was decreased from 5501ms to 4501ms prior to the beginning of Phase A to increase

the latency of her correct rejections. Dogs rejected samples by pushing the lever after the minimum observation time was reached and before the indication threshold was reached.

Figure 4

Cairo Assessing a Sample



Four responses could be recorded in each trial. If carp was present in a sample and the dog indicated, this was recorded as a hit. If carp was present in the sample and the dog did not indicate, this was recorded as a miss. If the sample was carp-absent and the dog indicated, this was recorded as a false alarm. If the sample was carp-absent and the dog did not indicate, this was recorded as a correct rejection. Reinforcement was provided after hits, and every other response had no consequence. After any response that was not a hit, dogs had to push the lever to initiate the next trial. Whether a dog indicated on a sample or not was recorded, and then these were used to calculate each dog's hit rate and correct rejection rate. The start and end time of each session was recorded, along with the temperature and humidity in the experimental room.

Cleaning

Segments were cleaned in warm water with a dissolved dishwashing tablet. The segments were then rinsed, and all sides were submerged in 50% IPA before being left to air dry. The SDA carousel, front panel, lid, and lever were wiped with paper towels using 70% IPA.

The 1L plastic containers, 2L plastic containers used for lake water, and glass measuring cylinders were all soaked in 10% hydrochloric acid and reverse osmosis (RO) water. All sample types had corresponding acid baths and were left for at least 12 hours. They were then rinsed three times using RO water and placed into a drying oven set at 45°C. A full description of the acid-washing procedure can be found in Appendix B.

The experimental and control tanks were cleaned once a month. Carp were removed and placed in the reserve tanks. Tanks were then drained and wiped with their corresponding sponge. The tanks were sprayed with 10% hydrogen peroxide and left for 15 minutes. Tanks were rinsed with dechlorinated water, refilled, and supplied with a steady flow of dechlorinated water. The experimental tank was left with a flow of water for at least 24 hours before a new carp was added.

Experimental Design

Phase A of the experiment was criterion based, with dogs needing to achieve a hit rate and correct rejection rate of 80% on two out of three consecutive sessions before progressing to the second lake and, ultimately, phase B of the experiment. In phase A, 17 apparatus segments were assigned to five positive and 12 negative samples. Positive samples contained 100 mL of lake water spiked with 100 µL of carp aquaria. Negative samples contained 100 mL of lake water spiked with 100 µL of control aquaria water. The location of these samples on the apparatus was randomised each day. Dogs completed six sessions each experimental day,

meaning 12 sessions were conducted each week. The first lake presented to the dogs was Lake Rotomā, Bay of Plenty and this was presented for 24 sessions (two weeks). Each dog met the criteria before moving onto Lake Rotoroa, Waikato. This lake was presented for 24 sessions, and after meeting the criteria, dogs could advance to phase B.

Phase B of the experiment was a multiple probe design. The probes were either positive or negative. Positive probes were positive lakes believed to contain carp, and negative probes were lakes believed to be carp absent. Twelve positive and 12 negative probes were presented to the dogs over 12 weeks, with one positive and one negative probe being used each experimental day.

Table 2

Probe Samples Presented on Each Experimental Day of Phase B

Day	Positive probe	Negative probe
1	Whangape (Waikato)	Tarawera (BoP)
2	Waahi (Waikato)	Ōkāreka (BoP)
3	Puketirini (Waikato)	Tikitapu (BoP)
4	Mountfield Rd Dam (Northland)	Okaro (BoP)
5	Hakanoa (Waikato)	Ōkataina (BoP)
6	Waipu GC pond (Northland)	Rotoiti (BoP)
7	Ngaroto (Waikato)	Rotoehu (BoP)
8	Oranga Lake (Waikato)	Ngāhewa (BoP)
9	Waikere (Waikato)	Ngāpouri (BoP)
10	Kimihia (Waikato)	Tutaenga (BoP)
11	Rotongaro (Waikato)	Areare (Waikato)
12	Ohinewai (Waikato)	Chapel lake (Waikato)

Note. BoP stands for Bay of Plenty

The dogs' first response to positive probes tested the dogs' ability to generalise to novel variations of carp scent. The dogs' first response to negative probes tested the dogs' ability to reject novel lakes that did not contain carp. Out of the 17 segments, positive probes were presented four times, and negative probes were presented four times. The remaining samples were from one of the two lakes encountered in phase A, two being carp-spiked positive samples and seven being control-spiked negative samples. These training lakes rotated between Lake Rotoroa and Lake Rotomā every two weeks. This made the ratio of positive to negative samples 6:11. Indications on both positive probes and positive training samples were reinforced, and indications on negative probes and negative training samples had no programmed consequences.

Statistical Analysis

As shown in Table 3, each trial had four possible outcomes, and these were used to calculate each dogs hit rate (HR), correct rejection rate (CRR), and accuracy.

Table 3

The Four Possible Outcomes in a Trial

	Carp-present	Carp-absent
Indicated	Hit True Positive (TP)	False alarm False Positive (FP)
Not indicated	Miss False Negative (FN)	Correct rejection True Negative (TN)

In Phase A, each dogs HR for positive samples was calculated using the equation HR = TP / TP+FN. Each dogs CRR was calculated using the equation CRR = TN / TN+FP. Accuracy was also calculated using the equation TP+TN / TP+TN+FP+FN.

In Phase B, HR was calculated for positive training samples and positive probe samples. CRR was calculated for negative training samples and positive probe samples. The dogs' HR and CRR for each session were presented on tables, allowing for the visual analysis of data. The dogs' mean HR and CRR on each positive and negative probe were calculated. The dogs' HR and CRR on the first response to positive and negative probes were calculated. A chi-square analysis of the relationship between probe status (positive or negative) and whether the probe was indicated (yes or no) on the first response was also conducted.

In both Phase A and B, tables and figures were made using excel. In Phase B, SPSS was used for chi-square analysis.

Results

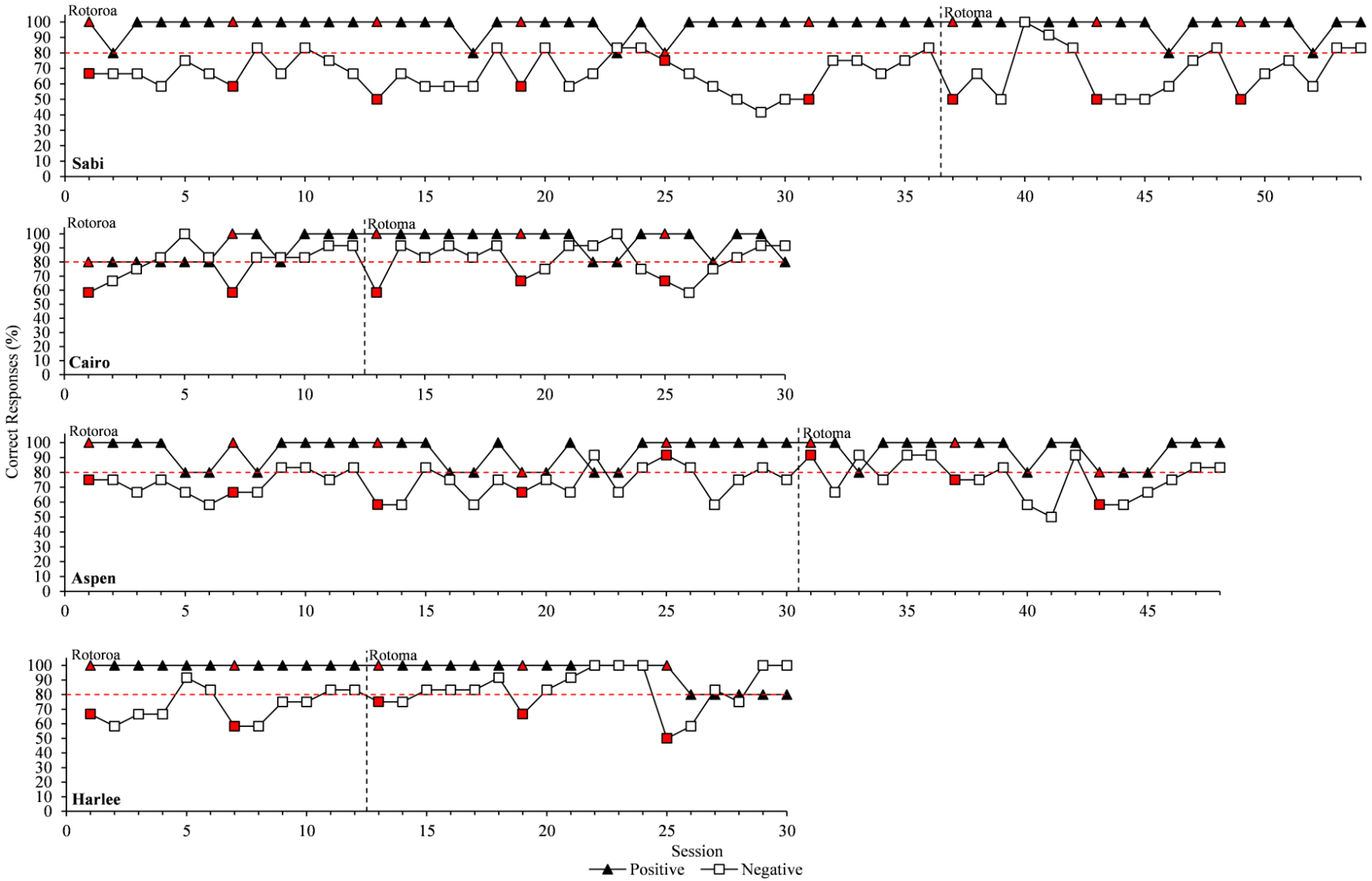
Phase A Hit Rate and Correct Rejection Rate

Phase A tested the dogs' ability to detect target odour in spiked lake water samples. It was assumed that lake water had more background odour than the aquaria samples on which the dogs were trained. During this phase, all four dogs met the criteria of a hit rate (HR) and correct rejection rate (CRR) above 80% on two out of three consecutive sessions on both Lake Rotoroa and Lake Rotomā. Sabi and Aspen spent considerably more time on Lake Rotoroa while waiting for Cairo and Harlee to return from unforeseen absences. Once Cairo and Harlee returned, all dogs moved onto Lake Rotomā simultaneously.

The HR and CRR of Sabi, Cairo, Aspen, and Harlee in each session of Phase A are displayed in Figure 5. Sabi had a near-perfect HR across Phase A (*M* = 98%, *Mdn* = 100%); comparatively, her CRR was low (*M* = 67, *Mdn* = 67). Cairo's mean HR was 91%, with a median of 100%. Cairo's CRR of 80% for negative samples was the highest amongst the dogs in this phase (*Mdn* = 83%). Aspen had a mean HR of 93% (*Mdn* = 100%) and a CRR of 75% (*Mdn* = 75%). Harlee's HR was high (*M* = 97%, *Mdn* = 100%), as was her CRR (*M* = 79%, *Mdn* = 83.3%). Therefore, Sabi, Cairo, Aspen, and Harlee had an accuracy of 76%, 84%, 80%, and 84%, respectively (*M* = 81%, *Mdn* = 82%). The dogs' combined mean HR was 95%, with a median of 100%. The combined CRR was lower, at 74%, with a median of 75%. The dogs showed a bias to positive responses, and more variability was seen in CRRs compared to HRs.

Figure 5

Sabi, Cairo, Aspen, and Harlee's Phase A HR and CRR for Lake Rotoroa and Rotomā



Note. A red marker indicates the first session of an experimental day, and the horizontal red dashed line indicates the HR and CRR to meet criteria.

Phase B Overall Performance

Phase B introduced novel, unspiked positive and negative probe samples alongside the positive and negative training samples from Phase A. This allowed for the recording of responses to novel probe samples from natural settings alongside the spiked lakes that the dogs' had already encountered. Hit rate and CRR for both training and probe samples are provided in separate graphs for Sabi (Figure 6), Cairo (Figure 7), Aspen (Figure 8), and Harlee (Figure 9). A summary of each dog's HR and CRR for each positive and negative probe is displayed in Table 4. Sabi was absent for the day Lake Waikere and Ngapouri were presented; therefore, those data are not present in Figure 6 or Table 4.

Figure 6 shows Sabi's performance across all sample types. During Phase B, Sabi consistently achieved HRs of 100% for positive probe samples, never falling below 75%, except in the first session of Phase B. Her mean HR across all positive probes was 95%, with a median of 96% (range = 88-100%). Sabi's CRR on negative probes was more variable, ranging from 38% to 100%. Still, her mean CRR across negative probes was 87%, with a median of 92%. Sabi's HR was typically high on positive training samples, with a mean of 87%. Sabi's mean CRR for negative training samples was 63%, and her median CRR was 71.4%. On these negative training samples, Sabi showed the most variability across sessions, rarely reaching 80% and dropping to 28.5% for three consecutive sessions between sessions 26 to 28. In general, Sabi demonstrated a bias toward positive responses and was least accurate with negative training samples.

Figure 7 presents Cairo's performance across all sample types. Cairo often had a HR at or near 100% for positive probes, and her mean HR across all positive probes was 93%, ranging from 67% on Lake Waikere to 100% on multiple other lakes, giving a median HR of 100%.

Across all negative probes, Cairo's CRR was 87% (range = 46-100%), and the median CRR was 92%. For positive training samples, Cairo had a mean HR of 36% and a median HR of 50%, her lowest rate of correct responses out of all sample types. Cairo consistently rejected negative training samples, giving a mean CRR of 91% and a median CRR of 100%. Overall, Cairo was biased toward negative responses, primarily due to her low HR with positive training samples.

Figure 8 shows Aspen's performance across all sample types. Aspen's HR for positive probes rarely fell below 100%, giving him a median HR of 100% and a mean HR of 97% across all probes (range = 83-100%). Aspen's CRR ranged from 4% to 100% on negative probes. Despite this wide range, Aspen's CRR across all negative probes was 78%, with a median CRR of 83%. Aspen had a mean HR of 79% for positive training samples, with some full experimental days at 100% and others dropping to 50%. Aspen's mean CRR was 76% on negative training samples, with a median HR of 71.4; these CRRs varied between experimental days. Overall, Aspen showed a slight bias to negative responses and had some periods of variability.

Figure 9 presents Harlee's performance on all sample types. Harlee's HR for positive probes was consistently high, ranging from 88% to 100% (M = 93%, Mdn = 100%). Harlee's CRR for negative probes varied, ranging from 25% for Chapel Lake to 100% for Lake Tikitapu. Across all negative probes, Harlee's mean CRR was 78%, with a median of 88%. Harlee had consistent misses on positive training samples at the beginning of Phase B, giving her a mean HR of 69% and a median HR of 100% after her performance on these samples recovered. For negative training samples, Harlee had periods with low CRRs, giving a mean CRR of 81% and a median CRR of 86%.

Overall, the dogs had high HRs to positive probes, with a mean HR of 96% across all dogs and probes. Out of all positive probes, the lowest HR was recorded by Cairo on Lake

Waikere, at 67%. CRRs for negative probes showed more variability, ranging from 4% to 100% (*M* = 82%, *Mdn* = 88%). The negative probe with the lowest CRR was Chapel Lake, where CRR ranged from 4% to 46%. The dogs also had varied HRs to positive training samples, ranging from 36% to 87%, with a mean HR of 67%. On negative training samples, CRRs ranged from 63% to 90%, with an overall mean of 78% (*Mdn* = 85.7). In general, the dogs' showed significant variability on all sample types, apart from positive probes.

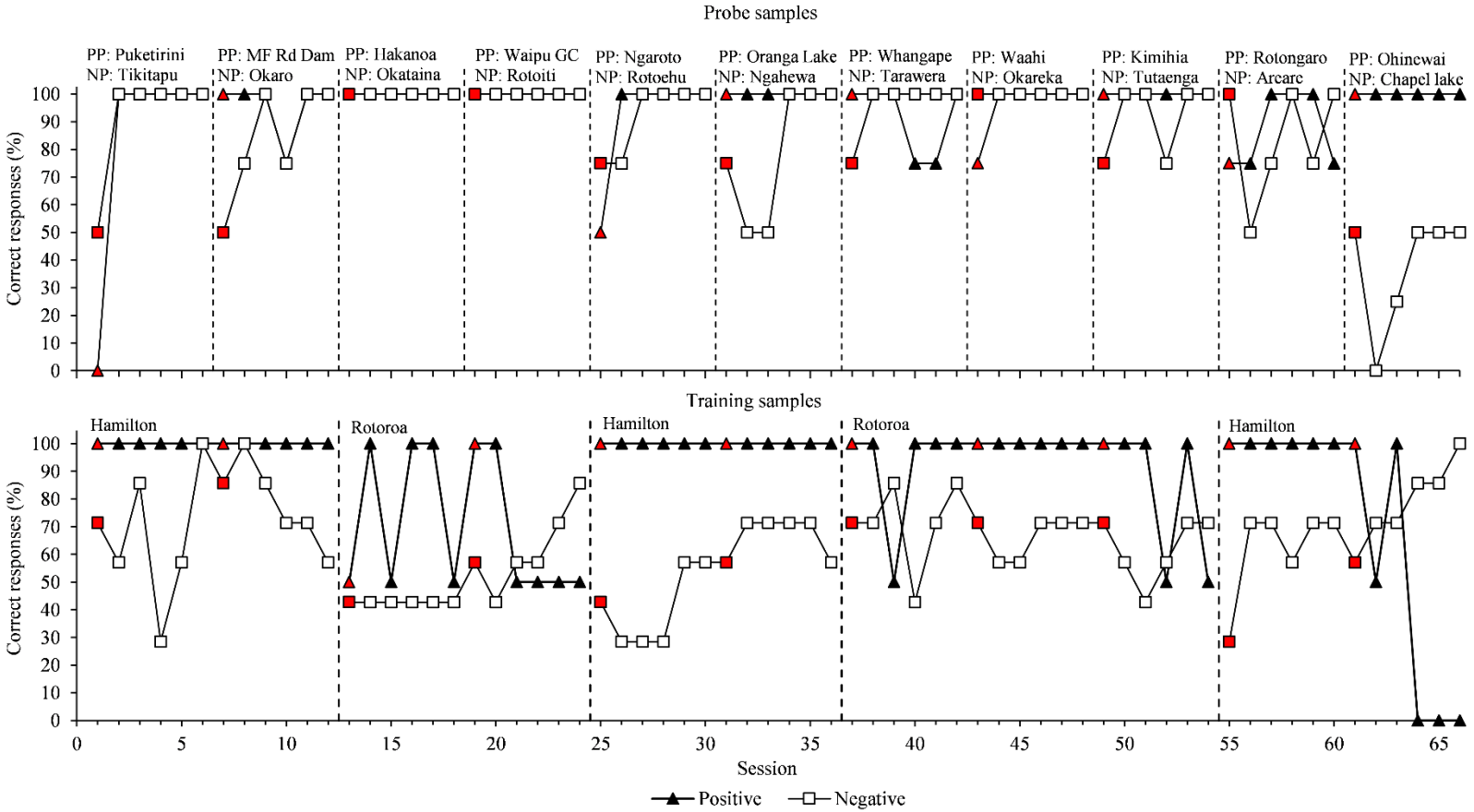
Table 4

Summary of Each Dog's Mean HR for Positive Probes and CRR for Negative Probes

Positive Probe	Sabi	Cairo	Aspen	Harlee	Mean ± SE	Negative Probe	Sabi	Cairo	Aspen	Harlee	Mean ± SE
Whangape	92%	96%	100%	96%	96% ± 1.90	Tarawera	96%	71%	96%	71%	83% ± 6.01
Waahi	96%	100%	100%	100%	98% ± 1.02	Ōkāreka	100%	88%	96%	96%	95% ± 2.07
Puketirini	83%	100%	83%	100%	91% ± 4.34	Tikitapu	92%	92%	96%	100%	95% ± 2.94
Mountfield Rd Dam	96%	100%	100%	100%	99% ± 1.02	Okaro	83%	92%	100%	92%	92% ± 3.51
Hakanoa	100%	100%	100%	100%	100% ± 0.00	Ōkataina	100%	92%	83%	88%	91% ± 3.85
Waipu GC pond	100%	100%	92%	100%	98% ± 2.04	Rotoiti	100%	100%	96%	92%	96% ± 1.90
Ngaroto	92%	88%	100%	88%	92% ± 3.18	Rotoehu	92%	96%	83%	88%	90% ± 2.92
Oranga Lake	100%	92%	100%	100%	98% ± 1.41	Ngāhewa	79%	100%	83%	79%	85% ± 3.87
Waikere	-	67%	96%	96%	86% ± 4.04	Ngāpouri	-	75%	83%	63%	73% ± 4.15
Kimihia	100%	100%	100%	100%	100% ± 0.00	Tutaenga	92%	88%	58%	96%	83% ± 5.03
Rotongaro	88%	100%	100%	100%	97% ± 1.69	Areare	83%	100%	63%	54%	75% ± 5.51
Ohinewai	100%	79%	92%	88%	90% ± 2.92	Chapel lake	38%	46%	4%	25%	28% ± 4.73
Overall HR	95%	93%	97%	97%	96% ± 1.40	Overall CRR	87%	87%	78%	78%	82% ± 1.59

Figure 6

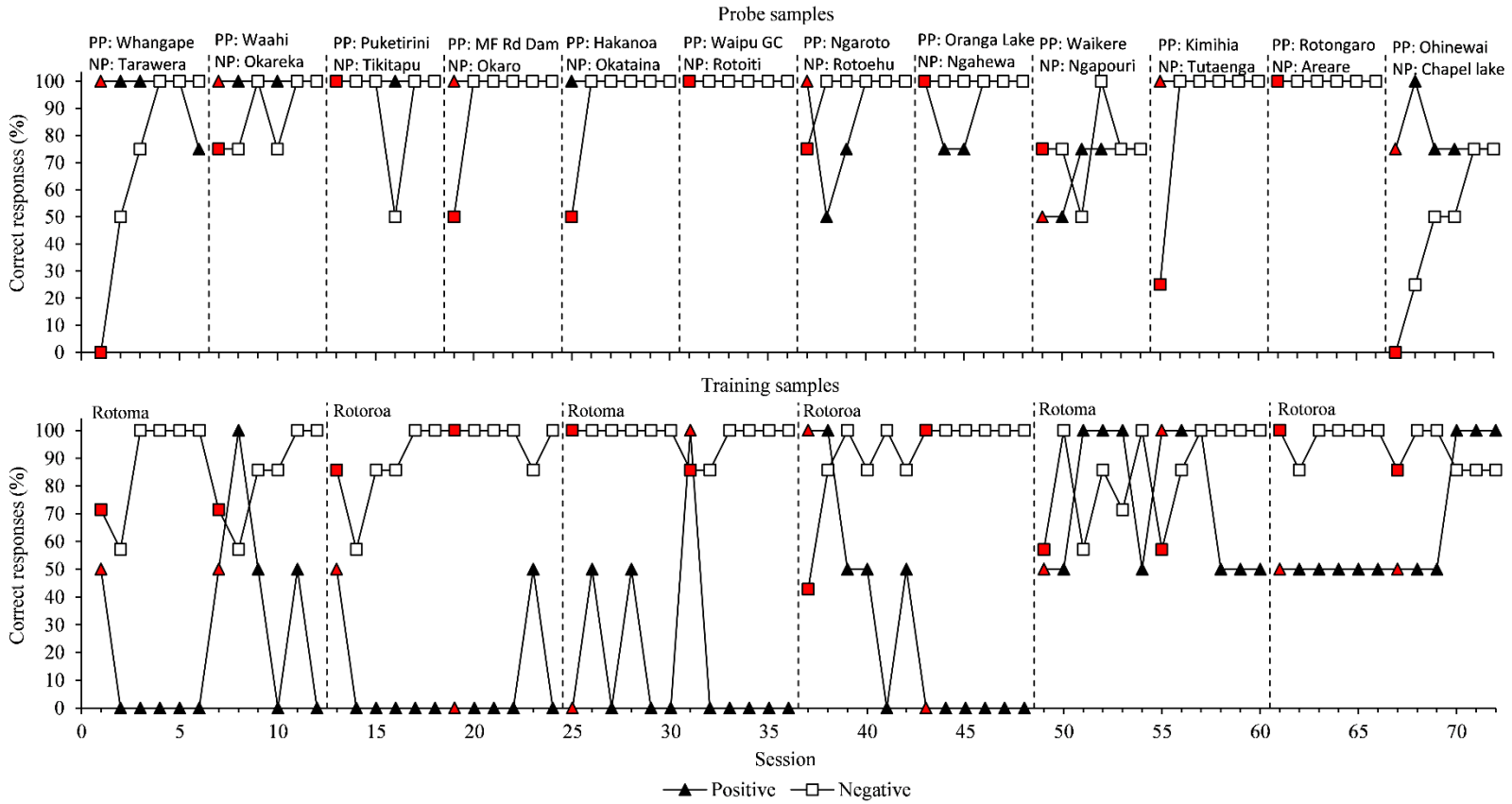
Sabi's Phase B HR and CRR on Probe Samples and Training Samples



Note. Each Marker Represents a Session, and a Red Marker Indicates the First Session of an Experimental Day. PP Stands for Positive Probe, and NP stands for Negative Probe

Figure 7

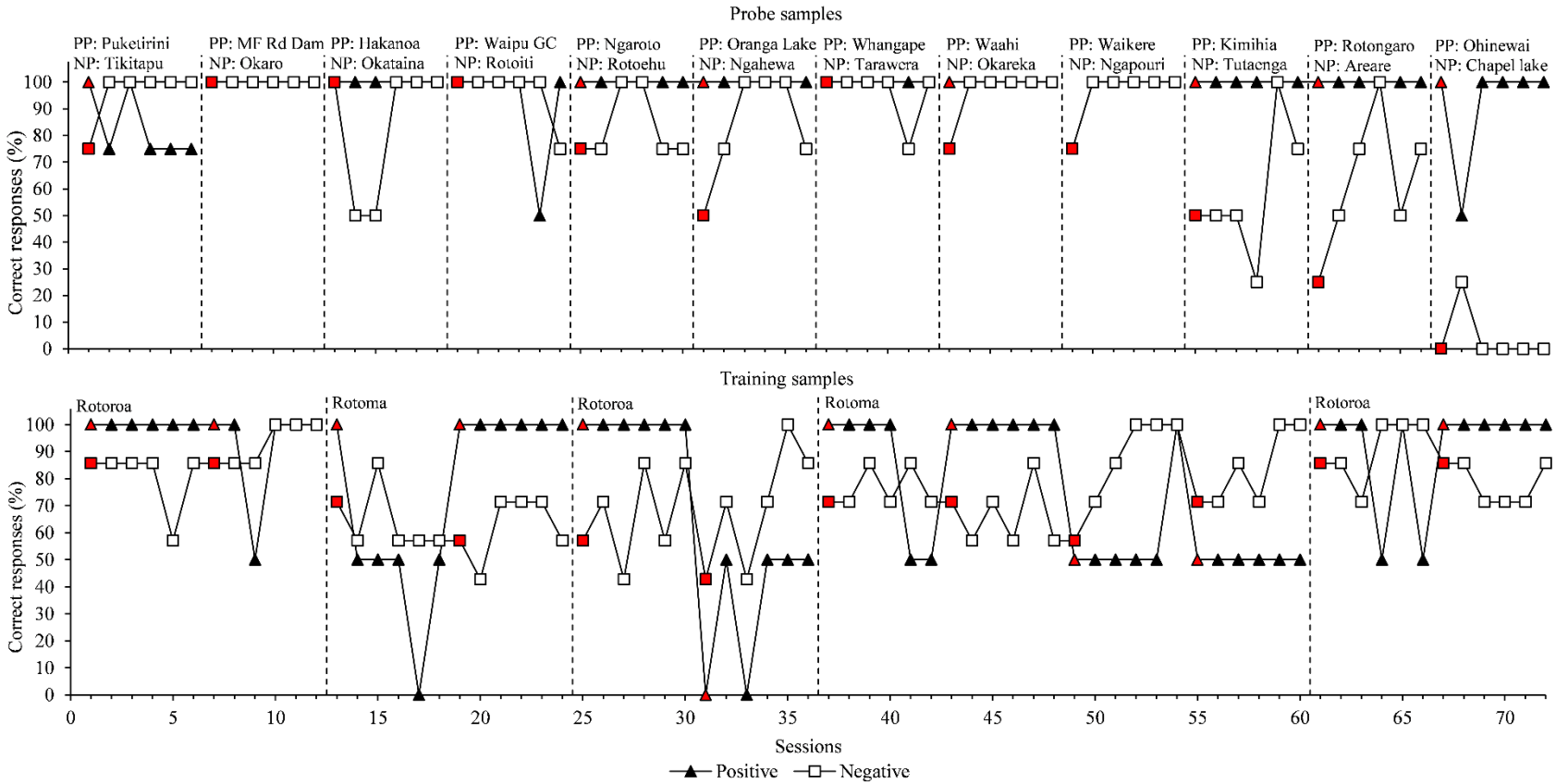
Cairo's Phase B HR and CRR on Probe Samples and Training Samples



Note. Each Marker Represents a Session, and a Red Marker Indicates the First Session of an Experimental Day. PP Stands for Positive Probe, and NP stands for Negative Probe

Figure 8

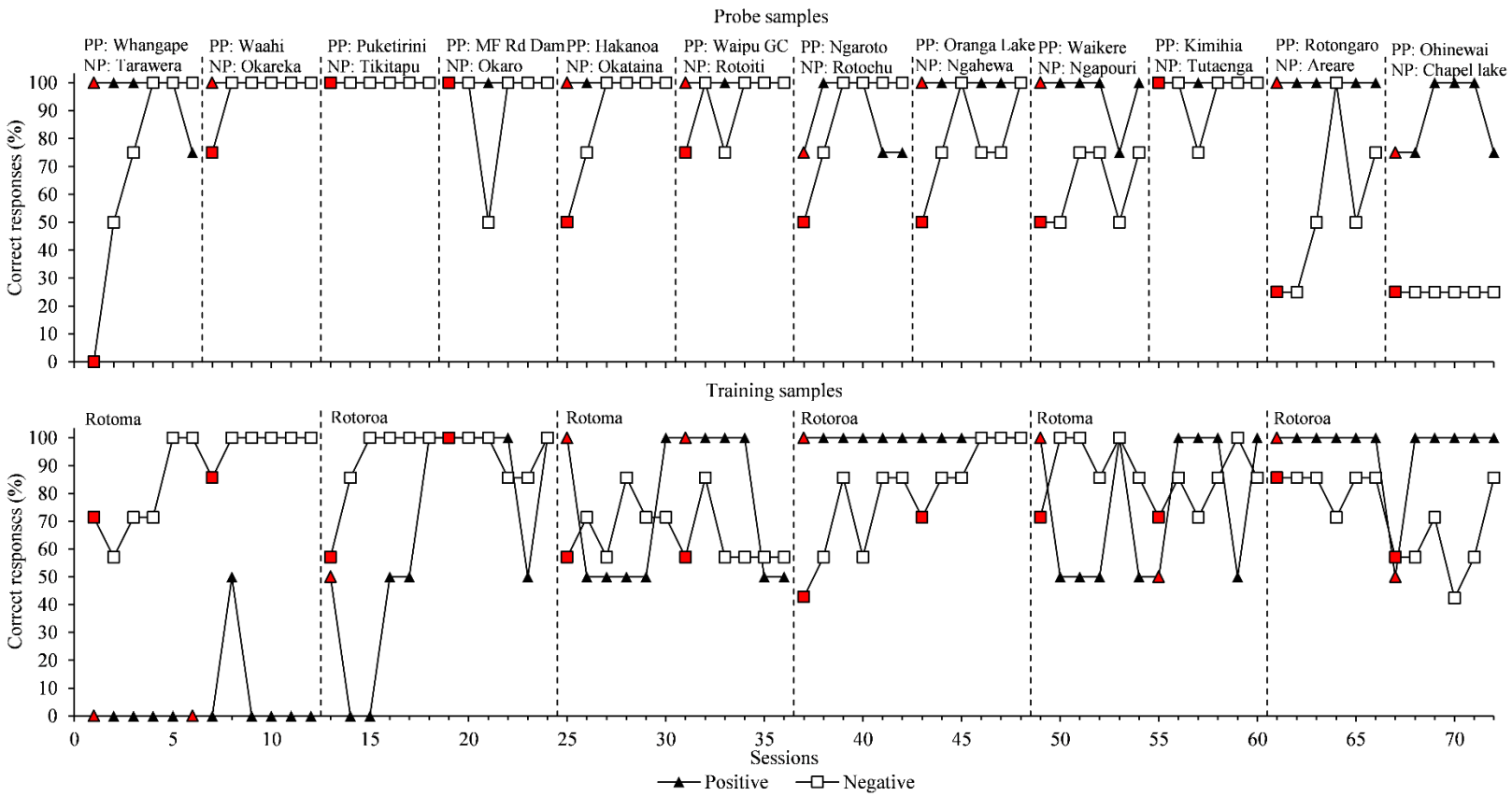
Aspen's Phase B HR and CRR on Probe Samples and Training Samples



Note. Each Marker Represents a Session, and a Red Marker Indicates the First Session of an Experimental Day. PP Stands for Positive Probe, and NP stands for Negative Probe

Figure 9

Harlee's Phase B HR and CRR on Probe Samples and Training Samples



Note. Each Marker Represents a Session, and a Red Marker Indicates the First Session of an Experimental Day. PP Stands for Positive Probe, and NP stands for Negative Probe

Phase B First Response Data

Each dog's first responses to positive and negative probes are presented in Table 5. The dogs' first responses to positive probes were used to evaluate generalisation to novel targets. The dogs' first responses to negative probes tested whether the dogs would reject novel non-targets.

Table 5

All Dogs' First Responses to Positive and Negative Probes

Positive probe	Sabi	Cairo	Aspen	Harlee	Negative probe	Sabi	Cairo	Aspen	Harlee
Whangape	1	1	1	1	Tarawera	1	0	1	0
Waahi	1	1	1	1	Ōkāreka	1	0	1	1
Puketirini	0	1	1	1	Tikitapu	1	1	0	1
Mountfield Rd Dam	1	1	1	1	Okaro	1	1	1	1
Hakanoa	1	1	1	1	Ōkataina	1	1	1	0
Waipu GC pond	1	1	1	1	Rotoiti	1	1	1	1
Ngaroto	0	1	1	0	Rotoehu	1	1	0	1
Oranga Lake	1	1	1	1	Ngāhewa	1	1	0	0
Waikere	-	0	1	1	Ngāpouri	-	0	0	1
Kimihia	1	1	1	1	Tutaenga	0	0	0	1
Rotongaro	0	1	1	1	Areare	1	1	0	0
Ohinewai	1	0	1	0	Chapel lake	1	0	0	1
Overall HR	73%	83%	100%	83%	Overall CRR	91%	58%	42%	67%

Note. 1 indicates a correct response, 0 an incorrect response

Sabi had the lowest HR for the first response to positive probes at 73% but had the highest CRR for negative probes at 91%. Aspen had the highest HR for positive probes at 100% but the lowest CRR for negative probes at 42%. Cairo and Harlee both had a HR of 83% for positive probes and a CRR of 58% and 67%, respectively. Therefore, all dogs besides Sabi had HRs for positive probes, which were, on average, 33% higher than correct rejections on negative probes (range = 16 - 58%). Overall, dogs recorded misses for five positive probes and ten negative probes. This gave Sabi, Cairo, Aspen, and Harlee an accuracy of 81%, 70%, 70%, and 75%, respectively, on the first response to probe samples.

As shown in Table 4, for positive probes with incorrect first responses, the dogs' hit rate nearly always recovered to 80% or higher, except Cairo for Lake Waikere and Lake Ohinewai, with an overall hit rate of 67% and 79%, respectively. This was similar to the recovery for negative probes; Harlee had a false alarm for Lake Areare, CRR only recovered to 54%, and Cairo had a false alarm for Chapel Lake, which recovered to 46%. Aspen was the exception, having three lakes that were incorrectly indicated where CRR did not recover to at least 70%.

Chi-Square Analysis of First Responses

A chi-square test was conducted to examine the association between the dogs' first responses to probe samples and the carp status of probes. The data presented in Table 5 were used as input, with two variables: the carp status of probes (positive or negative) and whether a sample was indicated on the first response (yes or no). The test revealed that indications were not randomly distributed between probes of differing carp status, as evidenced by significant chi-square values ($p < 0.05$) for all dogs, as shown in Table 6. These results suggest that the presence or absence of carp scent in probe samples affected indication rates, demonstrating that the dogs discriminated between positive and negative probes on the first response.

Table 6

All Dogs' Chi-Square Output for the Association between First Response and Lake Status

Dog	X^2	df	N	p-value
Sabi	9.21	1	22	0.002
Cairo	4.44	1	24	0.035
Aspen	6.17	1	24	0.013
Harlee	6.32	1	24	0.012

Discussion

The present study first investigated dogs' ability to detect carp in spiked lake water samples at an ecologically relevant biomass of 310 kg/ha. In Phase A, the dogs could detect carp at this biomass, demonstrating that dogs can detect carp in samples containing more background odour while rejecting samples that did not contain carp. In Phase B, novel probe samples were introduced to investigate whether trained dogs can generalise to novel variations of carp scent and reject samples from novel lakes that did not contain carp. On the first response to novel positive probes, the dogs maintained a high hit rate (HR), with some variance between dogs. Compared to first response HRs, correct rejection rates (CRRs) for novel negative probes were consistently lower, with Sabi being the exception. Overall, the dogs' accuracy was comparable between Phase A and B; this was interpreted as evidence for the generalisation of detection ability. However, these generalisation data should be treated with caution, and the reasons are discussed below. In subsequent Phase B learning data, the dogs had near-perfect HRs to positive probes, and CRRs to negative probes were generally high excluding some outliers. Overall, generalisation was observed, and these learning data demonstrate that dogs can detect carp in naturally sourced water samples. This study acts as a proof of concept for the future operational viability of carp detection dogs.

Phase A

In Phase A, all dogs met the criteria on samples from Lake Rotoroa in a maximum of 10 sessions, and on Lake Rotomā samples in a maximum of five sessions. This supported the hypothesis that the dogs would be able to detect carp in spiked samples from carp-absent lakes. The fact the dogs met the criteria on both lakes is not unexpected, as Sabi, Aspen, and Harlee had encountered spiked lake water samples with a biomass of 310 kg/ha in a prior experiment

(Collins, unpublished data). In the prior experiment, Sabi and Harlee met the criteria on the three lakes presented, while Aspen failed to meet the criteria on these lakes (Collins, unpublished data). Cairo had never encountered spiked lake water samples before but still met the criteria on both lakes in the lowest total number of sessions. Across Phase A, the dogs had HRs ranging from 91% to 98% and CRRs ranging from 67% to 84%.

The main finding from this phase was the dogs used in the present study could detect carp in samples with additional background odour, known as 'noise' (Helton, 2009). The additional noise in this phase came from the array of distractor odours in lake water, such as the VOCs emitted from aquatic vegetation, non-target fish species, the by-products of some microorganisms, and human-made products (Pozzer et al., 2022). Increased noise changes the ratio of signal-to-noise, with carp being the signal, potentially making carp less detectable (Hoffman et al., 2009). The dogs' sensitivity to carp and specificity against non-targets is likely because of the dogs' history of reinforcement for indicating the presence of carp and not having reinforcement provided for indications to non-targets (Lazarowski et al., 2020). This history of reinforcement contributes to the dogs' concept of carp scent, so even when additional noise is added, the dogs can still indicate the presence of carp and reject samples that do not contain carp (Oldenburg, 2016).

Phase B First Responses

In Phase B, it was hypothesised that due to the dogs meeting criteria on spiked lake water samples with more noise, their detection ability would generalise to novel lakes. The dogs' first responses to novel probe samples were key to observing generalisation. This is because the dogs' first responses to positive probes are a measure of how the dogs respond to novel variations of carp scent (Crawford et al., 2022; Edwards et al., 2017). Subsequent data do not represent

generalisation, as the dogs may have been responding to an odour or combination of odours specific to the lake and irrelevant to carp scent (Edwards et al., 2017). In observing generalisation, recording the first response to negative probes is also essential, as negative probes tested whether the dogs were simply indicating samples because they had novel scents instead of responses being based on the presence or absence of carp. For generalisation to be observed, CRR and HR must be similar across Phases A and B. This comparison is necessary because the dogs' prior learning of carp scent was developed in Phase A, which is what the dogs generalise from. To provide this comparison, an accuracy measure was used, combining CRR and HR into a single measure.

In Phase A, Sabi, Cairo, Aspen, and Harlee had an accuracy of 76%, 84%, 80%, and 84%, respectively. For Phase B first responses, Sabi, Cairo, Aspen, and Harlee had an accuracy of 81%, 70%, 70%, and 75%, respectively. Therefore, accuracy was lower for all dogs in Phase B, besides Sabi. However, these differences are not too large to rule out generalisation, with Sabi, Cairo, Aspen, and Harlee having differences of 5%, 14%, 10%, and 9%, respectively. Alongside stable accuracy across phases, significant chi-square values for each dog give support for generalisation, with positive and negative probes being differentially indicated on the first response. It is important to note that the dogs had a bias toward positive responses. In Phase B, Sabi, Aspen, Cairo, and Harlee had HRs to novel positive probes of 73%, 83%, 100%, and 83%, compared to CRRs to novel negative probes of 91%, 58%, 42%, and 67%. Therefore, while accuracy was relatively stable across phases, supporting the idea that the dogs were generalising, the dogs' CRR was generally low to negative probes on the first response.

A factor in these low CRRs on negative probes could be the complexity of probe samples. As with the lakes presented in Phase A, the use of lake water samples introduced scents

irrelevant to carp, and this could have an impact on the detection of carp (Hoffman et al., 2009). Additionally, in a carp-present lake, the level of noise may be considerably higher than in the carp-absent lakes presented in Phase A. This noise could come from many factors associated with carp-present lakes, such as resuspended particles, dislodged plant material, nutrient cycling, phytoplankton dominance, and algae (Adámek & Maršálek, 2013; Bjaer et al., 2009; Qiu et al., 2019). Also, if negative probes do not share this same noise, they may become discriminable from positive probes through factors irrelevant to the presence or absence of carp. Dogs are efficient problem solvers, so if the presence or absence of carp is less detectable, they may use cues such as common distractor odours or the differences between samples (Hall et al., 2013).

Aspen's results are of particular interest when discussing noise-related differences between probe samples. This is because from the presentation of Lake Rotoehu onwards, the decision was made to include negative probes sharing more common features with positive probes, such as eutrophication and turbidity. Before this change, the negative probes consisted of non-turbid lakes from the Rotorua Region, potentially making them discriminable from positive probes through features irrelevant to the presence or absence of carp. In these conditions, Aspen correctly rejected all but one negative probe. However, when negative probes more closely resembled positive probes, Aspen falsely indicated every subsequent negative probe. Therefore, Aspen may have been rejecting the clearer negative probes due to their noise-related differences rather than the absence of carp. Aspen's responses to positive probes are now also brought into question. His false indications to eutrophic negative probes could signal that Aspen was not indicating the presence of carp in positive samples but a more salient distractor odour or a combination of distractors. Therefore, when negative probes were added that shared more commonalities with positive probes, Aspen may have been indicating this distractor odour that

was also present in the eutrophic negative probes. This transfer of stimulus control could be caused by carp-related VOCs in positive probe samples being masked or overshadowed by a more salient odour and responses to both positive and negative probes being under the stimulus control of this odour (Hall & Wynne, 2016).

It would be unexpected that carp odour could be masked or overshadowed, as carp often dominate the total fish biomass of invaded lakes (Collier & Grainger, 2015; Hicks & Ling, 2015). However, in scent detection work, a common issue is a lack of knowledge regarding what a dog is detecting or even if the dog is detecting what the researcher assumes they are (Horowitz & Franks, 2020). For example, Furton et al. (2002) found that of six VOCs related to cocaine, only methyl benzoate elicited a response from dogs trained to detect cocaine. The reason methyl benzoate elicited a response and not the other VOCs in the odour profile of cocaine may be that it has a lower odour detection threshold (ODT) (Kelley & Cadwallader, 2017). VOCs with lower ODTs may be less abundant in the odour profile and a mixture of odours yet still have a greater odour activity value (OAV) (Kelley & Cadwallader, 2017). VOCs with a greater OAV are often perceived as more intense, even when less abundant in the headspace of a sample (Du et al., 2010). Now, when reconsidering eutrophic lake water, the hundreds of VOCs that become volatile in the headspace of a sample can all have differing OAVs. If VOCs with a greater OAV bind to the same glomeruli as VOCs with lesser OAV, there is competition for olfactory receptor sites. As a result, VOCs with a greater OAV can block the reception and therefore perception of VOCs with a lower OAV (Kokocińska-Kusiak et al., 2021). This makes it possible that competing odourants could overshadow or mask the VOCs of the carp profile (DeGreef & Maughan, 2022). Therefore, the complexity of probe samples could have reduced the

detectability of carp through masking or overshadowing, contributing to a drop in CRRs for negative probes and subsequently bringing Aspen's perfect HR to positive probes into question.

Phase B Learning

On each experimental day, each dog completed a total of 102 trials across both probe and training samples. Of these 102 trials, 48 were probe samples, 24 being positive and 24 being negative. All of these trials were included when reporting the dogs' learning, while the generalisation data consisted of only the dogs' first response to probe samples. The dogs' learning was not directly relevant to testing the hypothesis that dogs would generalise their detection abilities. This is because, in subsequent responses no new information regarding the dogs' ability to detect carp is provided (Crawford et al., 2022). Instead, responses may be under the control of features specific to the probe in question rather than features of the carp scent profile (Edwards et al., 2017). However, subsequent learning data were still recorded, though it should be noted that these responses may not be indicative of the dogs' ability to detect carp. As expected, the dogs' HR and CRR improved when considering subsequent responses. HRs for positive probes ranged from 93% to 97%, and CRR ranged from 75% to 87% for negative probes.

Learning data such as these subsequent responses are often taken as evidence of target acquisition, and the ability of detection dogs to learn to detect targets in complex mixtures of VOCs while simultaneously rejecting equally complex mixtures is well-reported (Aviles-Rosa et al., 2022). The dogs' first responses are considered less often, which is a major issue in the reliability of many scent detection findings. Without testing first responses to novel sources, no new information about the dog's ability to detect a target is provided (Edwards et al., 2017). For example, dogs have demonstrated the ability to detect lung cancer in breath samples and

discriminate against cancer-absent samples (Crawford et al., 2022; Crawford et al., 2023). Crawford et al. (2022) also recorded the dogs' first responses to novel samples over a considerably larger set of novel samples. They found that the first response HR and CRR were considerably more variable than the overall learning data while acknowledging that these learning data are not indicative of target acquisition.

One outlier in these learning data is the dogs' responses to samples from Chapel Lake, a lake on the University of Waikato campus. Before samples were collected from Chapel Lake, it was believed to be carp-absent, with the last survey conducted in 2015 failing to detect carp presence (G. Tempero, personal communication, January 17, 2023). However, the dogs' responses may be an indication that carp was, in fact, present as of sample collection in October of 2022. Chapel Lake was the only negative probe to which every dog had low CRRs. For example, the dogs' CRR never fell below 50% on any other negative probe, but on Chapel Lake, no dog had a CRR above 50%. Aspen only correctly rejected this lake once, giving him a CRR of 4%, potentially indicating the status of this sample was incorrect. While this is highly speculative, in the case Chapel Lake was carp present, this may provide an insight into how the dogs' could perform under schedules of intermittent reinforcement.

If carp detector dogs are to become operationally viable, a requirement is that dogs evaluate samples of unknown status (Edwards et al., 2017). In these double-blinded conditions, reinforcement would be on a variable, intermittent schedule. Hits to samples of an unknown status could not be reinforced, but reinforcement would be interspersed with hits on positive samples of known status. However, thinning the schedule of reinforcement could have a negative effect on learning. For example, Sargisson and McLean (2010) trained dogs to detect remote explosives and found superior performance with higher reinforcement rates compared to a

thinner schedule. An explanation may be that a higher rate of reinforcement strengthens behaviour, making it more resistant to disruption (Podlesnik & DeLeon, 2015). In an experiment that used the same automatic apparatus as the current study to present lung cancer breath samples to dogs, Crawford (2022) found that HR and CRR declined following a transition to intermittent reinforcement. Therefore, in future, the dogs' ability to detect samples under a thinned reinforcement schedule should be evaluated. These data from Chapel Lake may demonstrate how the dogs could perform, but this can not be confirmed.

The final sample type to discuss is the positive and negative training samples. These were lakes encountered in Phase A. Of the 17 samples on the apparatus, nine training samples were loaded on the apparatus: two positive and seven negatives. There were significantly fewer positive training samples to allow more apparatus space for probe samples. It was assumed the dogs would be most accurate on these samples as this type of sample had been previously encountered, and drops in HR and CRR on these samples could indicate an issue with learning. Sabi, Cairo, Aspen, and Harlee had HRs of 87%, 36%, 79%, and 69%, respectively. Harlee and Cairo's lower HRs are largely a result of the beginning of Phase B, where the dogs often missed every positive training sample in a session. Harlee began consistently rejecting positive training samples after 15 sessions, while Cairo took 49 sessions. CRRs on negative training samples were variable between the dogs, with Sabi, Cairo, Aspen, and Harlee having CRRs of 63%, 91%, 76%, and 81%, respectively.

Cairo and Harlee's poor HR at the beginning of the experiment is concerning, as in Phase A, the dogs had near-perfect hit rates on these same lakes. Additionally, Cairo and Harlee had HRs close to perfect on positive probes during these sessions. There are several potential explanations for these poor HRs, the first being that positive training samples were of a lower

carp biomass than positive probes. Biomass estimates of carp-present lakes are infrequent, and as positive probes had unknown biomass, the 310 kg/ha biomass of positive training samples could have been less than positive probes. Also, these samples may not share the same noise as positive probes, causing them to be rejected on that basis. Finally, positive training samples were the least prevalent sample, so ignoring these samples would still provide dogs with close to the most available reinforcement. A combination of these factors could have resulted in the observed inhibition of responses to positive training samples.

Limitations

The number of lakes that could be sampled was limited, and 12 positive and negative probes may not have been enough to accurately examine the dogs' ability to generalise. The dogs were presented with water samples from the majority of carp-present lakes in the Waikato Region, and samples from lakes from Northland were also presented. More positive probes could be sourced in future with the continued support of end-users. However, a challenge would be the sourcing of suitable negative probes. An equal number of positive and negative probes is required, so a wider contact web of end-users and an improved system to transport these samples to the laboratory would be required. Time constraints also limited the number of lakes that could be presented to the dogs.

The project was under time constraints due to a lengthy re-training period before Phase A began. In 2021, COVID-19 lockdowns closed the laboratory and data collection was halted from September 2021 to January 2022. It took from January 2022 until August 2022 to complete this re-training. Part of this extended period of poor performance may be explained by the changing of trainers, as this has been shown to decrease a dog's accuracy and increase distractibility (Jamieson et al., 2018). For example, during re-training, there were times when dogs would not

complete their last session of the day. Aspen even had a short period where he would stop working after only his second session. However, issues of motivation and distractibility slowly decreased over the re-training phase, and there were no similar issues throughout Phases A and B.

The Phase A criteria may not have been a viable comparison to first responses in Phase B. There is no guarantee that the dogs' responses were related to the presence or absence of carp in Phase A. This lack of knowledge regarding what compounds of a lake sample are controlling responses was addressed in Phase B by only considering first responses as providing new information about the dogs' ability to detect carp. However, in testing for generalisation, there does have to be prior criteria to compare performance against. Multiple lakes could have been presented simultaneously instead of a single lake for two weeks at a time. This would not eliminate the possibility of responses being under the control of factors irrelevant to carp, but that design would more closely resemble Phase B, where three lakes were always presented simultaneously. Beyond presenting multiple lakes simultaneously, more carp-absent lakes could have been presented over the course of Phase A. This would give the dogs more exposure to variations of carp scent and could improve generalisation to novel variations.

Future research

All samples presented to the dogs in this experiment were of known status; in future, operational conditions would require the dogs to be presented with samples of unknown status. In operational conditions, agencies could send frozen samples to the laboratory for the dogs to evaluate. The samples sent in would have unknown status, and no reinforcement would be provided for indications. Therefore, the dogs' performance under schedules of intermittent reinforcement needs to be evaluated. This could involve a transition back to the conditions of

Phase A, using spiked lake water, and choosing a certain rate of reinforcement to be gradually thinned. For example, the schedule could be thinned after a dog achieves the Phase A criteria. If the dogs meet a predetermined criterion, such as meeting criteria while having 50% of hits reinforced, then novel probe samples could be re-introduced. With the reintroduction of probe samples, these would now be reinforced intermittently, while correct indications on positive training samples would always be reinforced. The goal of this phase would be to thin the schedule of reinforcement enough that it matches the schedule of reinforcement under operational conditions (Edwards et al., 2017).

Another line of research would be to evaluate carp present samples through VOC analysis technologies, such as gas chromatography-mass spectrometry (GC-MS) (Cairou et al., 2016). Using GC-MS could shed light on what VOCs of the carp scent profile dogs detect. For example, VOCs found to be prevalent in the headspace of a carp sample could be presented to the dog individually to determine if those VOCs elicit an indication. If a specific VOC or multiple VOCs are found to elicit an indication, then this could provide some evidence that those markers of the carp profile function as discriminative stimuli (Furton et al., 2002). However, it may be more likely that the dogs' indications are under much more complicated stimulus control, where VOCs of the carp profile could be important for indications but only if other VOCs are present or absent (Hall & Wynne, 2016). Despite this, knowing whether stimulus control is basic or more complex and conditional would provide useful information for future trainers. Also, if specific VOCs are found to reliably elicit indications in dogs, and these markers are consistently detected by VOC analysis technology, VOC analysis could eventually become a viable method of carp detection.

Conclusion

Carp are an invasive species that contribute to a decline in the biodiversity of New Zealand's waterways (Collier & Grainger, 2015; Hicks & Ling, 2015). In order to control the further spread of carp, there is value in developing novel detection methods to inform future management (Collins et al., 2022). The present study aimed to investigate how domestic dogs would perform when presented with complex, naturally sourced samples. Generalisation was observed based on the dogs' first responses to probe samples, as accuracy was stable across both experimental phases. However, the complexity of probes appears to have impacted the dogs' ability to reject negative probes, and these generalisation data should be treated cautiously because of this. In viewing data following the dogs' first response, HRs to positive probes and CRRs to negative probes were high. However, these subsequent data may not be indicative of the dogs' ability to detect carp, as responses could be under the control of odours specific to that lake. In Phase B, issues were also observed regarding low HRs to positive training samples, though HR on these samples did recover. Despite these issues, this study acts as a proof of concept for the use of domestic dogs in operational settings. Actionable methodological changes have been suggested, such as improving Phase A as a point of comparison for generalisation and presenting dogs with more probe samples. Applying these changes will increase the reliability of future findings. Overall, this study demonstrates that dogs can detect the presence or absence of carp across a variety of complex natural environments. In future, these dogs could be invaluable for conservation work, providing a cost-effective and accurate detection technology.

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Appendix A: Standard Operating Procedure: Training Dogs for Scent Detection Work

Note: This procedure does not include dog selection, habituation, handling and care have been omitted, as requirements are likely to vary among laboratories. The complete standard operating procedures specific to the author's laboratory are available on request.

Apparatus Setup

Position the apparatus in a room without other objects that might distract the dog. Only the front panel should be accessible to the dog, a ramp may be required so the dog can access the sample port hole. Movable partitions may be used to block access to the other sides of the apparatus. The room must have a door that closes/latches and should be equipped with one or two cameras to monitor the dog. The computer(s) used to control the apparatus and monitor the dog should be positioned in an adjacent room.

Basic Training

1. **Introduction**

   Once the dog has been habituated to the environment and the researcher(s), training sessions can be started. During the shaping and early training process, at the first sign of fatigue to disinterest, the session should be terminated, ideally immediately following a correct response and reinforcement. Early shaping/training sessions should not exceed 10 minutes. Dogs should be given a short break between sessions.

2. **Conditioned Reinforcer Establishment**

   The researcher should enter the experimental room with the dog and stand to the side of the apparatus (the side closest to the door is preferred if possible). The researchers should stand with their hands crossed either in front of their body or behind their back

(whichever is more comfortable), holding the feeder remote/hand-switch out of view of the dog. The dog should be allowed to freely explore the experimental room. Dispense food from the automatic feeder using the remote/hand-switch until the dog immediately approaches the feeder upon hearing the sound made when the feeder is activated. Take care not to trigger the feeder if the dog is only sitting and staring at the feeder. The dog should approach the automatic feeder and consume the food within 3 seconds of activation 3 times in a row to continue to the next stage of training.

3. Shaping- Nose to Port

Once the sound of the feeder is established as a conditioned reinforcer, the remote/hand switch is used to train the dog to put its nose into the sample port of the apparatus. Use the method of differential reinforcement of successive approximations to target this behaviour. For initial sessions the apparatus should be turned off, and not loaded with samples but one segment may be placed on the apparatus for the dog to open. The closing of a segment does make a sharp tap noise which can sometimes initially startle the dog. Prompting (e.g. pointing) may be used, but the prompt must be faded and removed before processing to the next step (lever activation).

As soon as the dog is comfortably placing its nose into the port far enough to open the segment and make the closing noise, the dog should be removed from the room. The apparatus should then be loaded with positive samples only and turned on. The subject's configuration file on the computer should then be edited to set the status of the samples in relation to their placement on the carousel, and the response times as 1000ms for the minimum indication time and 500ms for observations. The apparatus will now make a beep sound when the dog places its nose in the port. Continue shaping as required until

the dog begins to trigger the feeder automatically. Once a run (17 samples) at the 1000ms threshold is complete, increase the threshold in 100-500ms intervals to 1500ms. Once a run is complete at 1500ms, continue to the next step.

4. Shaping – Lever Activation

With the apparatus unloaded and turned off, use the method of differential reinforcement of successive approximations to shape lever pressing (see Appendix). Depending on the size and behavioural tendencies of the specific dog, an appropriate topography should be selected for shaping (e.g., use of a paw or nose to activate the lever/omnidirectional switch). Prompting (e.g., pointing) may be used, but the prompt must be faded and removed before processing to the next step. Once the lever/omnidirectional switch has been activated 10 times without prompts (and reinforced via manual activation of the feeder), proceed to the next step.

5. Discrimination Training

Load the apparatus with approximately half positive and half negative samples (e.g., 8 negatives and 9 positives respectively), alternating positive and negative sample placement on the carousel starting with a positive sample in the first position. This pattern status should then be updated in the subject's computer configuration file. Ideally, samples should contain a high concentration of the target/control substance.

Bring the dog into the experiment room and stand in place beside the apparatus. If there is no response given to the apparatus within 20 seconds, prompt as required. When the dog encounters the first negative sample, allow 20 seconds before prompting to see if lever pressing occurs without prompt. Continue prompting when necessary, but fade out

prompts as soon as possible (e.g., wait for increasing amounts of time before prompting). Be sure to prompt with a consistent cue.

Once one run has been completed without prompting, randomise the sample arrangement in subsequent sessions and update this in the subject's configuration file. The same randomisation pattern may be used for up to a maximum 3 sessions in a row before it is needed to be randomised again. Continue until hit rate (correct positive indication) and rejection rate (correct lever pressing) is above 80% without prompt. At this point the experimenter should gradually remove themselves from the room and, once the dog is successfully working on its own, systematically increase the indication threshold in 100-500ms increments until they reach target threshold.

With a standard sample (e.g., amyl acetate) at a high concentration, hit rate and correct rejection rate should reach and stay at approximately 100%. At this point additional runs may be added (i.e., the samples can be presented more than once during a session), or the sample concentration, type or distribution (e.g., positive sample prevalence) can be systematically changed as required (see advanced training).

Advanced Training

1. Increasing Run Number

To introduce dogs to a sample more than once during a session change the run number in the subject configuration file from 1 to 2. If the dog is still performing correct hit and rejection rates above 80% you can add an additional run if necessary or change the sample concentration, type or distribution. If dog is struggling with the extended number of samples you can try increasing the number of food rewards given per correct positive indication.

2. Introducing New Samples

To introduce a new sample that is to be treated like a negative, first you must systematically increase the number of negative control samples and decrease the number of positive samples (e.g. If you are going to be introducing 5 new samples then you need to increase the number of negative samples to 10 and decrease the number of positive samples to 7). If the dogs are still performing well, swap the corresponding number of negative control samples with the number of new negative non-target samples.

It may be necessary to re-enter the room and provide prompt, but then the experimenter must be sure to phase out prompting as soon as possible and gradually remove themselves from the room again.

3. Decreasing Sample Concentration

Once the dog is reliably performing above 80% correct hit and rejection rates after the introduction of new samples and the run number has been increased, it is possible to start to decrease the sample concentration. Dilutions should be done incrementally and for both target (positive) and non-target (negative) samples, the criteria for going down a dilution is a correct hit and rejection rate above 80% for 4 out of five sessions.

Dilution	Volume Control (ml)	Volume Target (ml)
1st Dilution	50	50
2nd Dilution	75	25
3rd Dilution	87.5	12.5
4th Dilution	93.75	6.25

5th Dilution	96.875	3.125
6th Dilution	98.4375	1.5625
7th Dilution	99.21875	0.78125

Appendix: Trouble shooting tips:

If the dog is performing poorly in training:

- Make sure the dog is healthy, deal with any health related issues first.
- Confirm the dog is not being fed by the owner at least 2 hours prior to training.
- Confirm that there has been no significant changes in the dogs home routine (e.g. owner has been away for an extended period, new dog introduced at home, change in diet, fireworks have been let off recently etc.)
- Confirm that food is an effective reinforcer by evaluating approach and consumption and/or by attempting to shape a simple response. If confirmed try selecting a different food (using paired-choice preference assessment procedure).
- Check factors related to sample quality (make sure that samples have been prepared and arranged as specified in the specific sample preparation SOP)
- Return to earlier stages of training as required (e.g. if the lever press is not occurring reliably in discrimination training conduct another lever press shaping session in isolation).
- If dog continues to perform poorly consult with supervisor. The dog may need to cease participation in the study.

If the dog is putting its nose in the port too early (while the apparatus is still moving):

- Turn on the "noise mode" in the subject configuration file. The apparatus will now produce a "buzz" while the carousel is still moving.
- Use a board to create an obstacle the dog must navigate around in order to reach the lever/omnidirectional switch and return to the port.

Appendix: Guide for Shaping

1. Introduction

This document outlines the basic training hierarchy for shaping by successive approximations. As a general rule, each step must be completed 3 times in a row before progressing to the next stage of training. Some dogs, however, may require additional learning trials before progressing. Keep sessions short (Under 5 minutes) and finish on a positive note when possible to ensure that the process is enjoyable for the dog.

2. Procedure

Researcher is to position themselves near the apparatus, ideally near the door, avoiding the dog's gaze to reduce unintentional cueing. This will facilitate fading of the researcher's presence during later trials when the dog is required to be in the experimental room alone. Gestural prompts may be used to facilitate training, but these should be used only as needed as they must be faded out before training is complete.

3. Shaping of sample port entry

1. For initial sessions the apparatus should be turned off, and not loaded with samples but one segment may be placed on the apparatus for the dog to open (The closing of a segment does make a sharp tap noise which can sometimes initially startle the dog).

2. Reinforce moving further and further away from the feeder, until the dog is reliably approaching the side of the room near the apparatus.
3. Reinforce attending to the apparatus (putting nose near or on any part of the front panel).
4. Reinforce nose near port.
5. Reinforce nose in port.
6. Reinforce nose touching and opening the flap (indicated by a tap noise as it closes).
7. Reinforce pushing flap inwards.
8. Turn the apparatus on – when the sample port beam is broken it will now produce a "beep" sound.
9. Continue to reinforce for dog breaking the beam and pushing the flap inward, until the dog is fully opening the flap (nose is fully inside the port).

4. Shaping of lever press

1. Turn apparatus off. Do not have apparatus loaded with samples.
2. Reinforce any movement towards the lever/omnidirectional switch.
3. Reinforce movement of nose or paw toward the lever/omnidirectional switch (as appropriate).
4. Reinforce any contact with the lever/omnidirectional switch (nose or paw, as appropriate).
5. Reinforce any movement of the lever/omnidirectional switch.

6. Reinforce movement of the lever/omnidirectional switch that produces a "click" (microswitch closure).

Appendix B: Standard Operating Procedure: Acid Washing

1. Purpose

This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted while acid washing sample bottles used during scent detection (fish) projects in the R2 laboratory on the University of Waikato Hamilton campus. Only those with prior induction training are authorised to do this.

Hydrochloric acid washing of sample bottles

1. A lab coat, safety glasses and disposable gloves should be worn.
2. There are acid buckets of 10% HCl designated to each sample type, this is labelled on each bucket. There are also labelled long green gloves (need appropriate names for these) designated to each sample type/bucket.
3. Bottles should be put in their designated buckets in order from negative to positive sample type.
4. Sample bottles should have all been emptied down at the dog lab (TT.H.01).
5. Wear the designated long green gloves.
6. Remove the lid from the bucket.
7. Remove the lid from the sample bottles to be placed in the bucket.
8. Place the bottle into the acid solution, do so at a slight angle so the bottle can fill with acid but does not bubble violently. Ensure there are no air bubbles in the bottle. Bottles should be fully submerged.
9. Place the bottle lids into the acid solution, be sure to submerge them.
10. Replace the bucket lid.

11. Rinse the green gloves under the tap and return them to the correct space beside their designated bucket.
12. Repeat steps 1-12 for each sample bottle type.

Taking sample bottles out of hydrochloric acid

13. A lab coat, safety glasses and disposable gloves should be worn.
14. There are acid buckets of 10% HCl designated to each sample type, this is labelled on each bucket. There are also labelled long green gloves designated to each sample type/bucket.
15. Bottles should be handled in order from negative to positive sample type.
16. Wear the designated long green gloves.
17. Remove the lid from the bucket.
18. Remove the sample bottles from the bucket, tip the acid out carefully and slowly to avoid splashing.
19. Place the bottle into the labelled designated rinsing bucket.
20. Remove the lids from the acid solution and place these into the labelled designated rinsing bucket.
21. Replace the acid bucket lid.
22. Rinse the bottles and lids in RO water, and place them on the rinsing bucket lid for transport to the drying incubator.
23. Rinse the long green gloves under the tap and return them to the correct space beside their designated bucket.

24. Take the rinsed bottled to the incubator for drying. Control bottles should be placed on the top shelf, negatives on the middle shelf and positives on the bottom shelf.
25. Dispose of and change gloves (if applicable).
26. Repeat steps 16 to 25 for each sample bottle type.
27. Leave glassware in incubator to dry overnight.
28. Wearing gloves put clean dry glassware in designated storage containers.