Canine (*Canis familiaris*) Scent Detection of Invasive Brown Bullhead Catfish (*Ameriurus nebulosus*) in Lake Water Samples

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Abstract

Brown bullhead catfish (*Ameiurus nebulosus*) were deliberately introduced to New Zealand from North America in the 1880s and populations have since increased significantly in the Auckland, Waikato, and Bay of Plenty regions. Catfish pose a major threat to New Zealand's freshwater ecosystems, preying on and competing with native species for food, and decreasing water clarity through bioturbation and nutrient release.

Early detection of the spread of catfish is crucial as it allows for containment and eradication measures to be set up, preventing the establishment of new catfish populations. However, traditional methods such as visual surveying, netting, and electrofishing are time-consuming and resource intensive, resulting in high costs and restricting the number of locations that can be surveyed. In addition, they can be relatively insensitive when the target biomasses are low, such as in newly establishing populations. False negatives can result in a lack of action from governmental agencies, allowing populations to establish and spread undetected.

Domestic dogs (*Canis familiaris*) have previously been used for conservation management programmes, detecting reptiles, birds, insects, and larger wildlife animals. Although the use of dogs to detect targets in aquatic environments is relatively new, research has indicated domestic dogs have the ability to discriminate between similar aquatic species and previous projects related to catfish scent detection have shown domestic dogs can detect the presence of catfish in dechlorinated water samples at biologically relevant concentrations.

The aim of this research was to determine whether domestic dogs could detect the presence of catfish in lake water samples at a biomass concentration equivalent to 43.5 kg/ha

(based on the assumption of a 2 m deep waterbody), a concentration consistent with the estimated populations of catfish in New Zealand lakes. The first stage of the experiment determined whether dogs could detect the presence of catfish in dechlorinated water samples at a biomass concentration of 43.5 kg/ha. Water samples were collected from tanks containing a standard catfish biomass concentration of 15.5 g/L (equivalent to 38,700 kg/ha) and diluted to 311 kg/ha. As the dogs achieved the discrimination criteria, the sample concentration was progressively decreased. All three dogs were able to detect the presence of catfish at a biomass concentration of 43.5 kg/ha. The next stage of the experiment determined whether domestic dogs could detect the presence of catfish in lake water samples. Lake water samples were collected from catfish-absent Lake Rotomā and spiked with catfish aquaria. As the dogs achieved the discrimination criteria, the catfish concentration was progressively diluted. The majority of the dogs in this study successfully detected the presence of catfish at a biomass concentration of 43.5 kg/ha in lake water samples, with one dog successfully detecting catfish at a biomass concentration of 1.55 kg/ha. In New Zealand, it is highly unlikely significant environmental impacts would occur from a catfish biomass of 1.55 kg/ha. However, targeted eradication of a population density this size would be highly advisable and may be feasibly managed depending on available resources, size of the target water body and its connections to other waterways. The findings in this study indicate the potential utility of dogs in the early detection and management of invasive freshwater species. According to the theory of generalisation, the performance of these dogs may be generalised to different lakes and to different biomass equivalent concentrations without specific training on those lakes or concentrations. However, it would be

important to test for this generalisation in future research as differences in stimuli can affect generalisation, with less generalisation occurring as stimuli become increasingly dissimilar from the original training stimulus. Therefore, the differences between the conditions in this study and those in an operational scenario will be important in determining whether the obtained performance will generalise to the operational scenario.

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Introduction

New Zealand Freshwater Ecosystems and the Introduction of Invasive Fish

New Zealand has many freshwater ecosystems which provide habitats for a diverse array of indigenous aquatic species (Dean, 2001). However, many exotic fish have been introduced to these ecosystems either legally or illegally since the 1860s (Dean, 2001), with non-indigenous fish now comprising more than one-quarter of freshwater fish species in New Zealand (Leprieur et al., 2008).

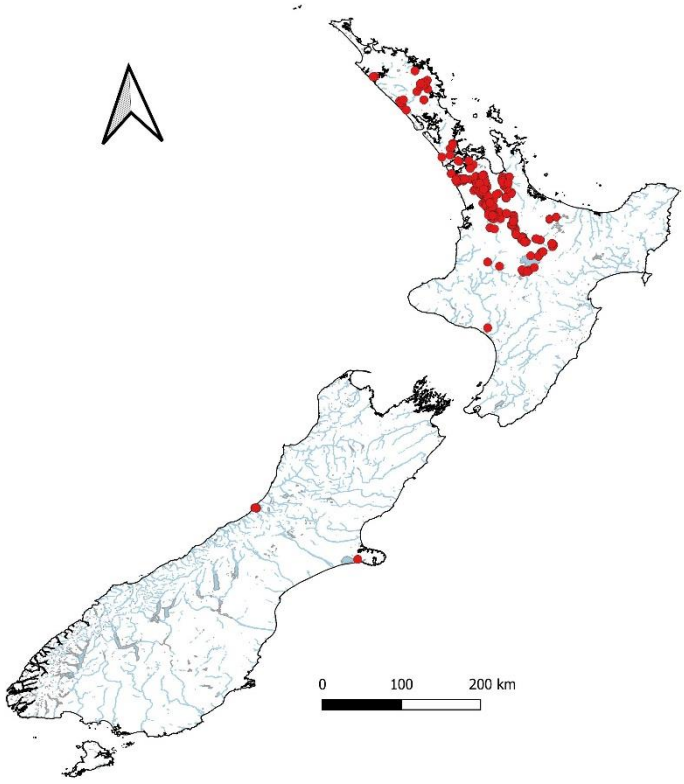
Some of these introduced species have since been labelled as 'invasive,' which can be defined as having significant adverse effects on either the survival or genetic variation of native species, or the integrity and sustainability of natural communities (Chadderton, 2001). The proliferation of invasive species has been noted as one of five significant threats to global freshwater biodiversity (Dudgeon et al., 2006; Rooney et al., 2007) and they often prey on, or compete with native species which can negatively impact on food webs (Córdova-Tapia et al., 2015; Matsuzaki et al., 2011; Smith & Lester, 2007). In addition, it has been proposed that impacts from invasive species will be exacerbated with the warming climate as the spread of invasive species will increase as potential habitats for more equatorial species increase (Rahel & Olden, 2008). Preventing or slowing the spread of invasive species is crucial in maintaining the health and biodiversity of New Zealand's freshwater ecosystems (Vander Zanden & Olden, 2008) and is often the main strategy employed by environmental management agencies.

The Brown Bullhead Catfish

The brown bullhead catfish (*Ameiurus nebulosus*), referred to as catfish hereafter, is the only species of catfish in New Zealand. They originate from the fresh waters of North America and were first introduced to New Zealand in 1877 when 140 individuals were released into St Johns Lake, Auckland (McDowall, 1990). Since then, catfish have been released intentionally and accidentally into lakes and rivers across the North Island (Figure 1), and have recently invaded Lake Rotoiti and Rotorua in the Bay of Plenty over the last decade (Hicks & Allan, 2019).

Figure 1

The distribution of the Brown Bullhead Catfish across New Zealand. Data points were attained from the New Zealand Freshwater Fish Database, December 2022.



Catfish normally grow to a length of 350 mm long and can weigh between 200-300 g (Hicks & Allan, 2019; Scott & Crossman, 1973). They have dark brown or olive scaleless skin, with lighter colouring on their sides and abdomen (McDowall, 1990). They can be most easily identified by their eight whisker-like barbels around their mouth and the rigid spines in their dorsal and pectoral fins (McDowall, 1990). Catfish can occupy a range of different bodies of water such as lakes, ponds, rivers, streams, and wetlands, although slow-moving waters with muddy or weedy beds are preferred (Collier & Grainger, 2015; Scott & Crossman, 1973). Catfish can survive long periods out of water if they are kept cool and their skin wet, they are also highly tolerant of poor water quality including suspended sediment and low dissolved oxygen (Collier & Grainger, 2015). As such, they are more inclined to accidental spread and are difficult to eradicate once established.

One of the most significant adverse impacts of catfish are their predation on and competition with New Zealand native species. They have been implicated in the decline in kōura (freshwater crayfish; *Paranephrops* spp.) populations. Francis (2019) determined that catfish not only had a dietary overlap with kōura, but also predated on kōura. When examining the gut contents of catfish and kōura, both contained chironomid (midge) larvae, Odonata (i.e., damselflies and dragonfly larvae), and common bully (*Gobiomorphus cotidianus*). As a result, catfish may indirectly impact kōura through competition for food. In addition, Barnes and Hicks (2003) discovered large numbers of kōura in the stomachs of catfish residing in Lake Taupō, and according to Clearwater et al. (2014), when populations of catfish were high in the Waikato River, kōura numbers were generally lower. If catfish continue to spread and proliferate, it will likely

cause further harm to kōura populations as there will be an increase in kōura predation rates and competition for food resources.

Catfish can also have adverse impacts through sediment bioturbation and nutrient release. The reduced water clarity in New Zealand lakes has often been attributed to anthropogenic impacts that have caused an increase in both nitrogen and phosphorus in the water and changes to the lake's trophic status (i.e., the amount of productivity it can sustain) (Rowe, 2007). While increases in lake trophic state are primarily due to anthropogenic nutrient inputs, the introduction of exotic fish species such as catfish have exacerbated the decline in water clarity in many New Zealand freshwater systems (Rowe, 2007). For example, a netting survey was completed at Lake Wainamu in 1979 which indicated no exotic fish were present (Thompson, 1979, as cited in Rowe & Smith, 2001). However, by 2004, catfish, European perch (*Perca fluviatilis*), goldfish (*Carassius auratus*), rudd (*Scardinius erythrophthalmus*), and trench (*Tinca tinca*) were found in the lake, and the introduction of these species – apart from goldfish, which may have already been present – likely occurred between 1991 and 1995 (Rowe, 2007). Although there are no records of the water clarity of Lake Wainamu prior to 1955, similar local lakes historically had Secchi disc (a measure of water clarity) values of over 3 m (Cunningham et al., 1953). However, by 1995, the Secchi disc in Lake Wainamu had declined to 1.2 m, and averaged 0.96 m between 1995 and 2004 (Rowe, 2007). Rowe (2007) also found a correlation between water clarity in 49 small North Island lakes and the presence of exotic fish. Where there was an increase in the abundance of exotic fish, there was also a decline in the water clarity, and vice versa. These examples provide strong evidence that catfish contribute to the decline in water

clarity in New Zealand lakes through processes such as increased turbidity and nutrient cycling (Hicks & Allan, 2019; Rowe, 2007).

Current Detection Methods

There are several methods currently used for detecting freshwater fish, and while some involve using off-site technologies, the majority of the techniques rely on employing visual observation or capture. The most common traditional methods are visual surveying, netting, electrofishing, and eDNA, each with their own advantages and disadvantages.

Visual Surveys

Visual surveys involve searching for target species in a body of water such as shallow lakes or rivers. It is a low cost and non-destructive method (Thanopoulou et al., 2018), making it ideal when assessing endangered species or vulnerable habitats. However, it is limited by water clarity and depth and is less effective in waters with high turbidity (Joy et al., 2013). As a result, it is not an ideal method for detecting catfish, who typically occupy low clarity waters. The success of the survey may also be dependent on the observer's ability to identify closely related fish species which may be highly similar in appearance such as the New Zealand longfin (*Anguilla dieffenbachii*) and shortfin (*Anguilla australis*) eels (Thanopoulou et al., 2018).

Netting

There are multiple methods which employ the use of nets to capture fish such as fyke, set, or seine netting. The size of the netting mesh will determine which fish will be trapped and which will pass through the net unaffected (Joy et al., 2013). Netting can be advantageous for surveying large areas and the capture of physical specimens for identification. It also provides

information regarding sex, age, and health of the target species, but can result in the capture and death of unwanted species if performed incorrectly. The predation of smaller fish by larger fish within the net has also been observed (Joy et al., 2013). Furthermore, netting often requires specialist equipment such as boats and experienced personnel to set up and monitor the nets. This can be time-consuming and costly, meaning use of this method may be hindered by budget or limited to areas that can be easily accessed by vessels.

Electrofishing

Electrofishing is the use of pulsed electrical current to capture fish (Hicks et al., 2015). Each pulse of electrical current causes the fish's body to flex and then relax between pulses. The resulting flexing and straightening causes involuntary movement towards the anode (Hicks et al., 2015). Finally, the fish becomes stunned and can be retrieved from the water with dip nets before the effects subside. Correctly conducted, fish will recover within a few minutes and be able to be returned to the water at the end of the survey with no lasting effects. Electrofishing is generally limited to hard-bottomed, shallow (<1 m) waters using either battery powered backpack units or bank-mounted generators. Boat electrofishing can be conducted in deeper, soft-bottomed waters, but the electrical field is limited to about 2 m depth which restricts the area of operation to shallow lakes (Hicks et al., 2015). This limits the effectiveness of boat electrofishing for capturing bottom dwelling species such as catfish. Electrofishing is also hindered by low water clarity and strong water currents (Joy et al., 2013).

Electrofishing is also more expensive than other traditional methods such as visual surveying due to the cost of equipment and the number of personnel required to safely operate

the electrofishing units (Joy et al., 2013). Furthermore, it can impact non-target species who are also exposed to the electrical field and has been reported to cause fish injury and mortality in some cases (Snyder, 2003). Injury and death can also occur from careless capture, handling, and transport (Snyder, 2003).

Environmental DNA (eDNA)

Environmental DNA consists of DNA in tissue, mucus, or waste lost to the environment, which can be used to identify the presence of target species (Jerde et al., 2011). The sensitivity of eDNA detection means it can be used to track rare species or establishing populations, and it has been suggested that the strength of the eDNA signal corresponds to the population abundance at the sample site, allowing a biomass to be inferred (Jerde et al., 2011). However, in aquatic systems, flow rates and turbulence can affect the dilution of the eDNA signal and therefore, conclusions based only on signal strength are not advised (Jerde et al., 2011).

eDNA is non-invasive and has no impact on non-target species compared to traditional methods such as netting and electrofishing. However, the sensitivity of eDNA can lead to an increased likelihood of false positives through cross-contamination. For example, DNA may travel from its point of origin and into the sample area through predation (Symondson, 2002). This means that any DNA confirmed present at a sample site may originate from sources other than the organisms occupying the area, resulting in incorrect assumptions of the presence and abundance of species. Lastly, specialist laboratories are needed to process the eDNA and to prevent DNA contamination (Taberlet et al., 2018). Therefore, lack of access to a laboratory may

hinder the use of eDNA, while the expense associated with this method may also limit the number of areas that can be tested.

Summary

In summary, the traditional survey methods currently employed for the detection and monitoring of invasive fish can be time-consuming and costly. Visual surveying, netting, and electrofishing can be ineffective in detecting establishing populations, leading to false negatives. False negatives can be detrimental in stopping the spread of invasive species to new habitats as they can result in no action being taken by authorities, leading to the potential establishment of the unwanted species. Therefore, a method is needed for the early detection of fish species that is cost-efficient, easy to implement, non-invasive to species and the surrounding habitats, and sufficiently sensitive and accurate.

Theory of Using Scent Detection Dogs and Mechanisms of Smell

While scent detection dogs are widely known for their work in the detection of drugs and explosives, their scenting abilities are utilised by humans across many different fields. For example, they have been used to identify the shifts in blood glucose levels of diabetics (Los et al., 2017) and in locating human scents including cadavers (Oesterhelweg et al., 2008).

The volume of a dog's olfactory bulb, stria, and nasal tract is large, making up 1.95% of a dog's total brain volume compared to just 0.03% in humans (Kavoi & Jameela, 2011). Given that a larger olfactory bulb volume is indicative of greater olfactory function (Haehner et al., 2008), it is not surprising the olfactory acuity of a dog is roughly 10,000–100,000 times that of a human

(Walker et al., 2006; Walker et al., 2003). The gross anatomical structure of an organism's nasal cavity also impacts on the organism's olfactory acuity, with more keen-scented (macrosmatic) animals, such as dogs, possessing a different nasal architecture than feeble-scented (microsmatic) animals (Negus, 1958, as cited in Craven et al., 2010). In macrosmatic organisms, their olfactory mucosa is relegated to an 'olfactory recess' located in the rear of their nasal cavity and excluded from the main respiratory airflow path by a bony plate (Craven et al., 2010). This structuring is critical during sniffing, as a unique nasal airflow pattern is utilised that optimises odourant transport to the olfactory recess. The transport of odourants to the olfactory recess, off the main respiratory passage forces a unidirectional airflow during inspiration (Craven et al., 2010). This airflow pattern allows for the odourants to be deposited and selectively bind to the hundreds of millions of sensory neurons along the olfactory epithelium, enhancing the dog's olfactory discrimination (Craven, 2008; Craven et al., 2010). While humans have ~330 functional olfactory receptor genes, dogs have ~670, allowing them to detect a greater variety of odourants at much lower concentrations (Quignon et al., 2003).

However, while dogs possess a high olfactory acuity, Szetei et al. (2003) found that the presence of human cueing for some dogs is enough to override both visual and olfactory cues. Human cueing occurs when a handler or observer unintentionally prompts the dog through body language to give a certain response according to their own expectations. In a study by Lit et al. (2011), 18 handlers' beliefs about the presence of a target scent were influenced across different experimental conditions and the subsequent handler/dog teams' alert performances were evaluated. The handlers' beliefs were influenced by either being verbally told that a specific

marker indicated a scent location (i.e., human influence; marked condition), by encouraging the dogs to display interest in a specific location with a decoy food scent (i.e., dog influence; unmarked decoy condition), or by the specific marker being placed at the location of the decoy food scent (combined human and dog influence; marked decoy condition). However, no target scent was actually present in the study, so that any alert identified by the handlers was a false alert. The findings show an overwhelming number of false alerts across the conditions, confirming that handler beliefs affect performance. The pattern of alerts in conditions containing a marker compared to the pattern of alerts in the condition containing the unmarked decoy scent suggest that human influence on handler beliefs affected alerts to a greater degree than dog influence on handler beliefs (Lit et al., 2011). Therefore, when testing a dog's true ability during scent detection tasks, it is important that measures are taken to prevent cueing, such as allowing dogs to work in the absence of a handler.

Previous Scent Detection Research in Conservation

Dogs have been used for conservation purposes in New Zealand since the 1890s when they were first used to locate and conserve kiwi (*Apteryx* spp.) and kakapo (*Strigops habroptilus*; Browne, 2005). Since then, dogs have been used to locate pest species, or those classified as 'at risk' or 'threatened' for conservation efforts. For example, Browne et al. (2015) examined dogs' ability to detect tuatara (*Sphenodon punctatus*), Marlborough green gecko (*Naultinus manukanus*), and forest gecko (*Hoplodactylus granulatus*) scents to determine whether they could assist in the conservation of these reptiles. The dogs were highly successful in detecting a range of tuatara and gecko scents, with the dogs achieving success rates as high as 97.8%.

Furthermore, the use of dogs to survey larger areas or areas with dense vegetation can be advantageous over visual surveys by humans (Long et al., 2007; Reed et al., 2011). Not only can dogs locate physical specimens, but they can detect the scats (faeces), urine, hair, and blood of the target species (Kerley, 2010). Locating scats is a non-invasive method that can provide information on the target species such as its sex, reproductive status, diet, and parasitology (Kohn & Wayne, 1997).

While dogs' success in detecting and locating terrestrial species is well studied, research examining their efficacy in locating aquatic species is limited. The process by which scent and scent-bearing materials are transported through water and into the air is not fully understood, although Osterkamp (2011) proposes this may be inferred from how volatile organic compounds (VOCs) behave in terrestrial environments. Scent detection is based on the binding of VOCs – gaseous carbon-based chemicals – to receptors in the dog's nasal epithelium (Craven et al., 2010). All organisms produce VOCs, essentially creating their own individual odour fingerprint or 'smellprint'. This scent profile can change over time with alterations in the individual's metabolism caused by infection, inflammation, disease, and external factors such as medication and diet (de Boer er al., 2014). While dogs cannot smell an organism through water, VOCs from the submerged organism may rise through the water to its surface where they evaporate into the air for the dog to detect (Osterkamp, 2011). Dogs were first used in the USA to detect submerged corpses in the late 1960s and 1970s. Subsequently, the US Navy and Air Force's waterdog program began in 1968 to determine whether dogs could protect assets (boats, bridges, docks) from incoming enemy attacks via surface, snorkel, and open-circuit SCUBA

swimmers (Osterkamp, 2011). Since then, dogs have demonstrated an ability to locate other targets in aquatic environments, such as wastewater in creeks and storm drains (Van De Werfhorst et al., 2014) and faecal samples at sea from North Atlantic right whales (*Eubalaena glacialis*; Rolland et al., 2007).

Previous Research in Scent Detection of Aquatic Species

Recent research has also observed domestic dogs' success in detecting invasive aquatic species. In a study by Deshon et al. (2016), dogs were trained to detect quagga mussel (*Dreissena rostriformis bugensis*) larvae (veligers), an invasive species in the USA. Mussels are not visible until they reach the juvenile stage, and therefore training dogs to detect the veligers would allow for early detection of the species and prevent population establishment in lakes. At ≥31 veligers per 360 mL jar (≥0.086 veligers/ mL), the dogs were able to consistently identify the presence of veliger larvae, indicating that the dogs were successful in detecting this aquatic species and could be trained to identify and alert on varying veliger concentrations (Deshon et al., 2016).

Collins et al. (2022) is the first published study to investigate the potential utility of domestic dogs as a detection tool for invasive fish. The dogs were presented with two non-target sample types (control and goldfish samples) and positive samples containing the target koi carp (*Cyprinus rubrofuscus*) odour. The dogs could detect the presence of koi carp at a biomass equivalent concentration of 9.3 kg/ha, or approximately 2-4 adult carp/ha (based on a water depth of 2 m). The authors suggested a population density of this size could feasibly be managed

before adverse environmental effects occurred, indicating the potential for domestic dogs to be used as an early detection method for invasive freshwater fish.

Little (2020) aimed to determine whether domestic dogs could discriminate between catfish and goldfish species in dechlorinated water samples and at what equivalent biomass concentrations the dogs could detect their presence. The dogs successfully discriminated between the control, catfish, and goldfish samples, with one dog achieving a biomass equivalent dilution of 4,600 kg/ha. Denby (2021) followed up this study, investigating whether domestic dogs could detect the presence of catfish in dechlorinated water samples at 38.8 kg/ha, a concentration consistent with biomass estimations of catfish in New Zealand lakes. The dogs successfully discriminated between control and catfish-positive samples at biomass equivalent concentrations below 38.8 kg/ha, with one dog achieving a concentration as low as 4.6 kg/ha. These studies provide evidence of dogs' ability to detect and discriminate between fish species in dechlorinated water samples at relevant biomass concentrations, and their potential for use in invasive species management programmes.

Relevant Theoretical Issues

Generalisation is a phenomenon where organisms categorize stimuli that are perceptually similar and are therefore likely to share associated outcomes (Moser et al., 2019). Generalisation is said to have occurred when a behaviour established in response to an original training stimulus (S+) is also elicited when presented with similar but novel stimuli (Ghirlanda & Enquist, 2003). For example, in a scent detection task, a dog trained to respond to a specific catfish odour concentration will likely respond when presented with the same catfish odour at varying

concentrations (DeChant & Hall, 2021). Likewise, if a dog is trained to respond to catfish odour in clean lake water, the response may also be elicited when presented with the same catfish odour in dirty lake water. A generalisation gradient in the shape of a Gaussian curve usually occurs, with peak responding at the S+, which then systematically decreases as the stimuli become increasingly dissimilar in either direction from the S+ (DeChant et al., 2021). According to DeChant and Hall (2011), the performance achieved by a dog when trained with a particular odour concentration can generalise to other concentrations within a 10-fold range. This has important implications for studies investigating the lower limits of a dog's detection ability such as the current study, as training at a low odour concentration will allow for generalisation to even lower concentrations than if trained with a high odour concentration (DeChant & Hall, 2021).

Generalisation of an odour can also be impacted when mixtures are made up of two or more odour compounds. Organisms can process odour mixtures either configurationally or elementally (Moser et al., 2019). In configural processing, stimulus parts are integrated into a perceptual whole that conveys a meaning, for example, the sight of a stalking lion (Howard & Gottfried, 2014). If configurationally processing an odour, the mixture would be perceived as a single, unique odour (Moser et al., 2019) and in the case of the lion, this odour might be the mixture of all odours that are being released by the lion. In elemental processing, the stimulus parts themselves hold salience (Howard & Gottfried, 2014). If an odour is processed elementally, each component in the mixture is perceived as separate and identifiable (Moser et al., 2019). In the case of the lion, this might be the specific, individual odour components contained in the lion's scent (Howard & Gottfried, 2014). Although these two types of processing are not mutually

exclusive (Keep et al., 2021), elemental processing can facilitate generalisation between components, whereas configural processing can prevent it (Moser et al., 2019). Furthermore, it has been suggested that a certain degree of overshadowing occurs when mixtures are made up of two or more odour compounds (Keep et al., 2021). Overshadowing is when one odour is more salient than the other and therefore the information from one odour is dominant while the information from the other odour(s) is reduced (Keep et al., 2021). This can lead to a lack of adequate learning of some odour compounds within a mixture, translating into reduced performance in the field. As a result, combining two separate odours such as lake water and catfish aquaria water may result in the dog processing the mixture configurationally (i.e., as a single, unique odour). This may inhibit generalisation of the target catfish odour when presented with other lake water samples. Furthermore, there is the potential for the odours present within the lake water to overshadow or mask the catfish odour, resulting in false negatives.

Project Aims

This research aimed to investigate whether domestic dogs could successfully identify and differentiate the target catfish odour in lake water at operationally useful dilutions. Operationally useful dilutions refer to equivalent biomass concentrations at which catfish are known to occur naturally in lakes in New Zealand. In order for dogs to complement or replace current detection methods in biosecurity monitoring programmes, they must be able to detect the presence of catfish in water at these dilutions or lower. Four dogs were presented with lake water samples collected from Lakes Tikitapu and Rotomā and spiked with decreasing concentrations of either control or catfish aquaria water. The aim of the study was to determine the dogs' detection limits

and a success criteria of ≥80% hit and correct rejection rates was employed for each concentration.

Methods

Subjects and Animal Ethics Statement

Dog Recruitment

Domestic dogs were recruited through University of Waikato advertisements (flyers, social media posts) and word of mouth. Interested dog owners were asked to fill in initial assessment forms which evaluated the dog's suitability for the project (see Appendix 1). It screened their food motivation levels and whether the dog exhibited any anxious or aggressive behaviours. If the dog was found to be suitable, they were brought into the Scent Detection Research Group (SDRG) facility for an assessment. This involved checking whether the dog was comfortable in a crate, around the apparatus, and when separated from their owner. If no issues arose, the owners completed a consent form (see Appendix 2), and the dog began basic training according to a Standard Operating Procedure (SOP) (see Appendix 3).

Four dogs, Cobie, Mika, Tommy, and Saydee had already been recruited for previous projects related to this research. During the course of this project, a standard poodle named Lola was evaluated via the initial assessment form and brought into the SDRG facility for an assessment. However, Lola demonstrated signs of stress when separated from her owner and so did not progress to basic training. In total, four dogs, Cobie, Mika, Tommy, and Saydee, participated in this study (Table 1, Figure 1).

Table 1

Breed, Sex, and Age of the Dogs that participated in the project.

Dog	Breed	Sex	Age (at the start of the project)
Cobie	Labrador retriever x	Female*	10 years
Mika	Border collie x springer spaniel	Female*	3 years
Tommy	Border collie x springer spaniel	Male*	3 years
Saydee	Labrador retriever	Female*	3 years

Note. x Crossed

* Desexed

Figure 2

The Dogs who participated in the project. From left to right: Cobie, Mika, Tommy, and Saydee.

Fish Collection, Housing, and Maintenance

Catfish were collected via boat electrofishing from Lake Rotoroa, Hamilton, and were kept in the University of Waikato Aquatic Research Facility. During mild seasonal months, the fish were kept in the main room of the facility and housed in fiberglass flow-through holding tanks. To reduce osmotic stress on the fish, salt was added to the tanks twice per week to maintain a concentration of 3 parts per thousand (ppt). During peak winter, the fish were moved to glass tanks in a temperature-controlled room that was kept at 15°C. Every fortnight, 10 L of water was removed from these glass tanks and replaced with 10 L of dechlorinated water mixed with salt for a concentration of 4 ppt. Holding tanks always had a continuous flow of dechlorinated water and oxygenation was maintained by a continuous supply of compressed air through bubblers. The fish were fed twice per week with commercial pellets. Any fish showing signs of poor health were removed from the project and euthanised. Due to the Ministry of Primary Industries regulations, all remaining catfish were euthanised at the end of the project.

Animal Ethics Statement

The involvement of domestic dogs and invasive fish in this research was approved by the University of Waikato Animal Ethics Committee (protocol ACE#1141). The participation of the dogs in the project was entirely voluntary and they could be removed at any time at the owner's request. When the dogs were in the SDRG facility and not in a session, they were housed in individual crates. They were walked by either the researcher or a trained volunteer for 10 minutes every 2 hours and were given an extra 30-minute walk at midday if they were in the laboratory for a full day.

Apparatus and Feeder

The apparatus was designed and built at the University of Waikato (Edwards, 2019). It was housed in a room at the SDRG facility, referred to as the experimental room here onwards. This room was temperature controlled during sessions; the temperature kept between 18°C and 20°C. Seventeen stainless steel segments were placed on a carousel which could be rotated clockwise or anti-clockwise. On the front face of each segment was a flap that could be pushed inwards, allowing the dogs access to a sample container placed inside the segment. A stainless-steel lid was placed on top of the segments, trapping the odours from each sample inside their individual segment (Figure 3). In front of the segments was a large, clear acrylic panel with a circular hole cut in the centre (referred to as the port) through which the dogs had access to one segment at a time. Black tape was placed around the hole in the acrylic panel to make it easier for the dogs to locate (Figure 3). Three infrared emitters and receivers were located on the inside of the circular hole, generating three infrared beams which spanned across the port. When any of these beams were broken, it indicated the dog had placed its nose through the port and into the segment. On either side of the apparatus was an omni-directional lever which was only active once the infrared beams had been broken for a set amount of time. When the levers were activated and then pressed, the carousel would rotate to the next sample.

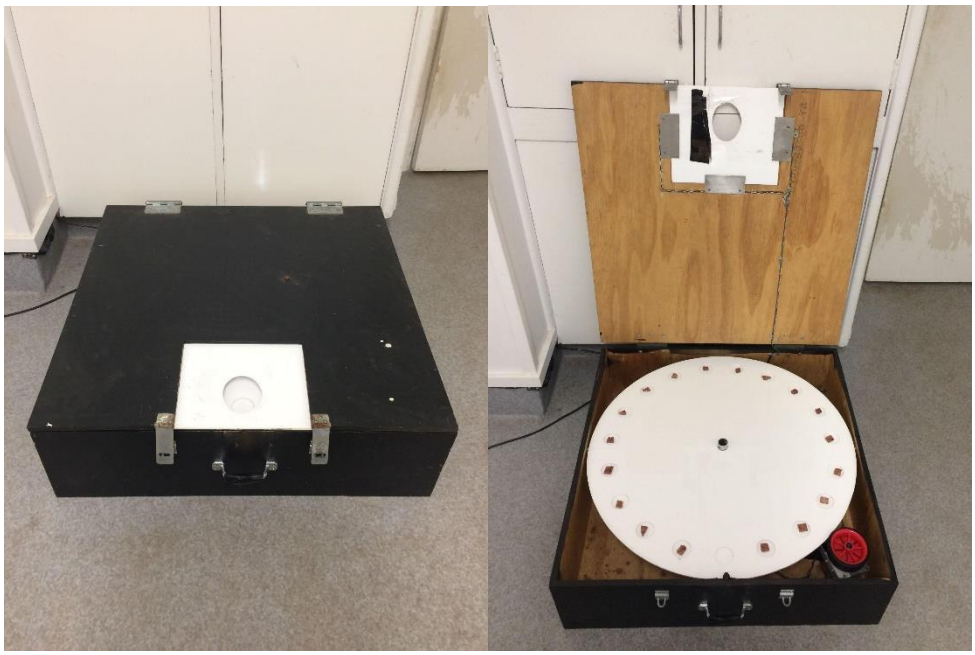
Figure 3

Apparatus, a) Front view of the Apparatus with Omni-Directional Levers either side, b) Birds eye-view of the Apparatus with Segments and Samples loaded on carousel, without lid.

A carousel wooden feeder was also designed and built at the University of Waikato and sat on the opposite side of the room from the apparatus (Figure 4). This distance prevented the odours from the treats interfering with the sample odours. The lid of the feeder had a hole cut in the bottom centre which allowed the dogs access to one treat at a time. Underneath the lid was a large plastic wheel with 18 circular indentations in which the treats (dog roll, brand Possyum) were placed. Possyum was manually loaded into the feeder every two to three sessions.

Figure 4

Wooden Feeder, a) Closed, b) Open and loaded with treats (Dog roll, brand Possyum).



The feeder and apparatus were connected through a wire to a computer located in a separate room where the researcher was stationed during sessions. On the computer were configuration files for each of the dogs. These files could be edited to increase or decrease the dog's observation time (infrared beam break required for the computer to treat the sample as "observed" and to activate the lever), indication threshold (infrared beam break required for the computer to treat the sample as a "positive indication"), and to note which samples on the apparatus were positive or negative. When a dog was in a session, their individual configuration file was entered into a custom programme. Information such as how long the dog's nose was placed in the port, or whether they pressed the lever, was sent from the apparatus to the custom

programme to be compared against the dog's observation time, indication threshold, and which samples were negative or positive.

The dogs could either 'indicate on' or 'reject' a sample, which allowed four possible outcomes (Table 2). To indicate a sample as positive, the dog had to leave their nose in the port until they reached or exceeded their set indication threshold (Figure 5). When the dog correctly indicated on a positive sample (referred to as a hit), the computer triggered the feeder to beep and the wheel inside the feeder to rotate and present a treat. The apparatus also beeped and flashed green on a correct indication. To correctly reject a control sample (referred to as a correct rejection), the dog needed to have met or exceeded their required observation time to activate the omni-directional levers but must not have reached or exceeded their set indication threshold. Either of the levers then had to be pressed to rotate the carousel to the next sample (Figure 6). No treats were given when a dog correctly rejected a control sample. If the dog rejected a positive sample (termed a miss) or indicated on a control sample (termed a false alarm), these responses were incorrect, and no treats were given. A second computer was also connected to two cameras placed in the experimental room. This allowed real-time footage of the dogs during their sessions and for sessions to be recorded for future reference.

Table 2

The four possible outcomes when Indicating on or Rejecting a Positive or Control Sample.

	Indication	Reject
Positive sample	Hit	Miss
Control sample	False alarm	Correct rejection

Figure 5

Saydee Indicating on a Positive Sample.

Figure 6

Tommy Activating an Omni-Directional Lever.

To clean the segments, an unscented dishwashing tablet containing enzymes was dissolved in a sink with warm tap water. The segments were washed in this water and rinsed to remove any soap. They were then placed in a large plastic container containing 50% isopropyl alcohol (IPA) and 50% tap water and lightly scrubbed. After, they were placed on paper towels to air dry. The carousel, underside of the apparatus lid, front of the acrylic panel, levers, and feeder were sprayed with a mix of 70% IPA and 30% tap water and wiped down with paper towels.

Dog Training

Cobie, Mika, and Tommy had already undergone training from a previous project related to this research and were therefore already able to use the apparatus. Saydee had begun training

with the apparatus prior to this study but had regressed in her training due to the break between the projects and so needed to begin training again. Dogs were trained under the SOP provided in Appendix 3, but a brief description is provided here.

Before training started, the dog was first habituated to the researcher and the environment. Training sessions with the apparatus were kept under 10 minutes with breaks in-between and were ended at the first signs of disinterest or fatigue, ideally after a correct response and reinforcement.

Conditioned Reinforcer Establishment

As the feeder beeped when a treat was dispensed, the first step in training was to establish this beeping sound as a conditioned reinforcer. The feeder was manually activated using a remote control until the dog immediately approached the feeder upon hearing the beeping noise. The dog moved to the next stage of training when they consumed the food within 3 seconds of the feeder being activated three times in a row.

Shaping Nose to Port

The apparatus was turned off and one segment was placed at the port for the dog to open. Differential reinforcement of successive approximations was used to shape the behaviour of the dog putting their nose in the port. When the dog was placing its nose far enough into the port to open the segment, the apparatus was loaded with undiluted positive samples. On the dog's configuration file on the computer, the indication threshold was set to 1,000 milliseconds (ms) and the observation time was set to 500 ms. The behaviour of holding their nose in the port and segment flap was manually reinforced until the duration of this behaviour was sufficient to

automatically trigger the feeder. The indication threshold was then increased by 500 ms every session until they reached 4,501 ms.

Shaping Lever Activation

With the apparatus unloaded and turned off, differential reinforcement of successive approximations was used to shape the behaviour of pressing the lever until it clicked. The dog proceeded to the next stage of training when the lever was successfully pressed 10 times without prompting.

Discrimination Training

To teach the dog to discriminate between the positive and control samples, the behaviours of nose to port and lever pressing were combined. The apparatus was loaded with nine positive and eight control samples in an alternating order, with a positive sample placed in the first position. If the dog did not respond to the apparatus for 20 seconds, the researcher prompted them to the apparatus. When the dog encountered the first negative sample, the researcher waited 20 seconds to see if lever pressing occurred before prompting the dog to the lever. After one session with no prompting, the sample arrangement was randomised and these randomised sample patterns were used for a maximum of four sessions in a row. Once the dog was achieving a hit rate (percentage of actual correct indications against all possible correct indications) and correct rejection rate (percentage of actual correct rejections against all possible correct rejections) ≥80%, the researcher gradually removed themselves from the room. Once the dog was working on its own, the dog progressed to the next stage of training.

Decreasing Sample Number and Sample Concentration

The number of positive samples were decreased from nine to seven, and control samples were increased from eight to 10 over time. The dilution of catfish aquaria water in the positive samples was progressively decreased from undiluted catfish aquaria water to the chosen experimental dilution of 100 microlitres (µL) catfish aquaria water in 100 mL control aquaria water. The dogs needed to attain a criteria of ≥80% hit rate and correction rejection rate on two out of three consecutive sessions to move to the next dilution.

When this project started, Cobie, Mika and Tommy were already trained and working at 100 µL catfish aquaria water in 100 mL control aquaria water. Due to needing re-training, Saydee was working at 100 mL of undiluted catfish aquaria water in her positive samples. As she met the criteria, the catfish aquaria water in her positive samples was progressively decreased to 50 mL, 25 mL, 10 mL, and then 100 µL. The control aquaria water in these positive samples was also increased to ensure the samples always contained a total volume of 100 mL. When Saydee reached this latter dilution, the other dogs on the project had moved to lake water samples for the second time. Due to Saydee struggling with the dilution and being near the end of the project, the catfish aquaria water in Saydee's positive samples was increased back up to 50 mL catfish aquaria water in 50 mL control aquaria water and once she met the criteria, Saydee was moved to lake water samples alongside the other dogs.

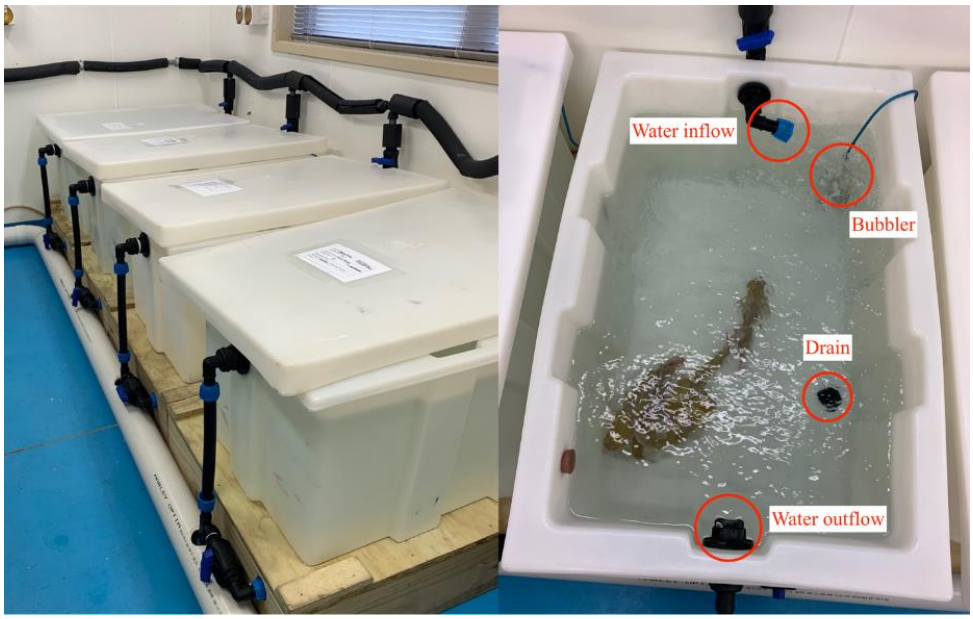
Sample Collection

Aquatic Research Facility: Experimental Tanks and Catfish and Control Aquaria Water Collection

Two experimental tanks were used: one for catfish and the other as a control tank (no catfish). These experimental tanks were placed in a separate room from the holding tanks at the University of Waikato Aquatic Research Facility and the room was set to remain at 17°C. The tanks were made from high-density polyethylene (HDPE) and were covered with HDPE lids to prevent cross contamination (Figure 7). They had a maximum volume of 200 L and were filled with dechlorinated water. Oxygenation was maintained by a continuous supply of compressed air through bubblers.

Figure 7

Left: Experimental Catfish and Control Tanks at the University of Waikato Aquatic Centre. Right: Catfish Experimental Tank under flow-through conditions. Photo Credit: Renee Denby.



Each week, four to six catfish were moved from their holding tanks to the allocated catfish experimental tank 24 hours before the first session was due to take place. Using different catfish each week had two benefits: firstly, it prevented the dogs from learning the odour of a particular fish, and secondly it reduced the stress placed on the fish, helping them to remain healthy. For the catfish biomass concentration to remain at 15.5 g/L in the water samples, the water level of the experimental tank was adjusted according to the total weight of the fish in the tank (referred to from now on as biomass volume). The fish remained at this water level for 24 hours prior to sample collection with no flow-through. The control experimental tank had a continuous flow of dechlorinated water, and the volume of water was not altered. After 24 hours, the control and catfish tank water were collected in sample-specific 1 L glass Schott bottles using plastic cups. The bottles were then stored in clean, separate zip-lock bags. Gloves were worn when handling these bottles and changed between the different sample types. The tanks were then scrubbed with tank-specific sponges. If the experiment was to run the following day, the catfish tank was filled with dechlorinated water and then drained back to the biomass volume for another 24 hours. If the experiment was not due to run the following day, the catfish were returned to their holding tanks and their experimental tank was drained.

The experimental tanks were scrubbed twice weekly with the tank-specific sponges. Once per month, both tanks were drained, sprayed with 10% hydrogen peroxide and scrubbed with the aforementioned sponges to remove accumulated algae and other organic compounds, and then rinsed with dechlorinated water.

Lake Water Collection

Lakes Tikitapu and Rotomā were selected for this project due to being catfish-free, cleaner than surrounding lakes, easily accessible, and within driving range for water collection. Lake water was collected using 2 L plastic bottles. At the selected lakes, the bottles were rinsed with lake water three times and then filled. The bottles were placed in individual zip-lock bags and kept chilled in a cooler bin with ice packs while transported back to the SDRG facility. The bottles were then frozen at -18°C and kept for a maximum of 4 weeks. If the samples were not used within 4 weeks, they were discarded. Twenty-four hours prior to the first session, a 2 L bottle containing the lake water was removed from the freezer and placed in a room at 18°C to thaw.

Sample Preparation

Catfish and Control Aquaria Water

Gloves were worn at all times when preparing and handling the samples and were changed in between preparing the different sample types. To sterilise the stainless-steel bench, it was covered with boiling water twice, wiped with paper towels, sprayed with a mix of 70% IPA and 30% tap water, and then wiped and dried with paper towels. To minimise the risk of cross contamination, the left-hand side of the bench was allocated to control samples and the right-hand side was allocated to positive samples. Ten sample containers were placed on the control designated side and seven containers were placed on the positive designated side of the bench. Label stickers were stuck to the bottom of each sample container to indicate whether they were a control or positive sample. The same type and size stickers were used to ensure the odours

were the same for each sample. Using a glass measuring cylinder and funnel, 100 mL of control aquaria water was measured and poured into the 10 control sample containers and then a predetermined amount was measured and poured into the seven positive samples (Table 3). On the right-hand side of the bench, a separate glass measuring cylinder and funnel was used to measure out a predetermined amount of catfish aquaria water and poured into the seven positive sample containers (Table 3). If less than 10 mL of catfish aquaria water was needed, some was poured into a separate sample container and a pipette was used. The remaining sample water in the Schott glass bottles was stored on sample-specific shelves in the fridge at 4°C until required. If the dogs were working at different dilutions, a new set of samples were prepared after the dog/s working at the lowest dilution had completed their sessions.

Table 3

The Positive Sample Dilutions and their Equivalent Catfish Biomass Concentrations presented in Order of Decreasing Catfish Aquaria Water (not in the order they were presented to the dogs).

Control aquaria water (mL)	Catfish aquaria water	Equivalent biomass concentration (mg/L)	Equivalent environmental biomass (kg/ha)*
0	100 mL	15,548	310,968
50	50 mL	7,774	155,484
100	100 µL	15.5	311
100	25 µL	3.89	77.7
100	14 µL	2.18	43.5

Note. * Based on a water volume of 20,000 m^3 per hectare (i.e., 100 m x 100 m x 2 m water depth).

Lake Water, Catfish and Control Aquaria Water

This sample preparation process was similar to the above, except instead of using control aquaria water, a predetermined amount of lake water was measured and poured into the 17 sample containers (Table 4). After four laboratory days, this methodology was adjusted to include shaking the bottle containing lake water three times each time three samples had been poured in order to distribute the sediment in the lake water among the samples. Using a separate glass measuring cylinder and funnel, a predetermined amount of control aquaria water was measured and added to the 10 designated control sample containers. With a different measuring cylinder and funnel, the same amount of catfish aquaria water was measured and added to the seven designated positive sample containers (Table 4). If less than 10 mL of control and catfish aquaria

water was needed, they were poured into separate sample containers and a pipette was used, with the tip on the pipette changed between the different sample types.

Table 4

The Lake Water and Catfish Aquaria Water Sample Dilutions and their Equivalent Catfish Biomass Concentrations presented in Order of Decreasing Catfish Aquaria Water (not in the order they were presented to the dogs).

Lake water (mL)	Catfish aquaria water	Equivalent biomass concentration (mg/L)	Equivalent environmental biomass (kg/ha) *
50	50 mL	7,774	155,484
90	10 mL	1,555	31,097
100	100 µL	15.5	311
100	50 µL	7.77	155
100	14 µL	2.18	43.5
100	6 µL	0.93	18.7
100	3 µL	0.47	9.33
100	1.5 µL	0.23	4.66
100	0.5 µL	0.08	1.55

Note. * Based on a water volume of 20,000 m^3 per hectare (i.e., 100 m x 100 m x 2 m water depth).

Cleaning

The empty 1 L glass Schott bottles and 2 L plastic bottles were placed in three separate, sample-specific buckets containing 10% hydrochloric acid and 90% reverse osmosis (RO) water. The bottles were left in these buckets for at least 12 hours before being removed, rinsed three times with RO water, and placed in a drying oven on sample-specific shelves at 45°C. (See Appendix 4 for the full acid cleaning procedure).

Experimental Design

Part A - Catfish Aquaria Water at Operationally Useful Dilutions

This part of the design aimed to reduce the catfish aquaria water in the positive samples presented to Cobie, Mika and Tommy to a dilution that was operationally useful. Operationally useful was defined as a dilution of catfish aquaria water representative of the biomass of catfish in New Zealand lakes. The researcher estimated the biomass of catfish in lakes to be around 43.5 kg/ha which is equivalent to 14 µL catfish aquaria water in 100 mL control aquaria water. Therefore, the dilution of catfish aquaria water in the dogs' positive samples was progressively reduced from 100 µL, to 25 µL, to 14 µL, while the control aquaria water in these samples remained at 100 mL. Control samples also contained 100 mL control aquaria water. The dogs were required to attain hit rates and accuracy scores of ≥80% on two out of three consecutive laboratory days in order to move down a dilution. The hit rate was calculated by dividing the dog's total number of hits (TH) achieved that laboratory day by the combined sum of their TH and total number of misses that day (TM). This result was then multiplied by 100 to receive a percentage. Accuracy was calculated by adding the dog's TH with the total number of correct

rejections they achieved that day (TCR). This number was divided by the combined sum of the dog's TH, TM, TCR, and the total number of false alarms that day (TFA). This result was then multiplied by 100 to receive a percentage. Once all three dogs had achieved the criteria when working at 14 μL catfish aquaria water, they moved onto lake water samples.

Part B - Catfish Aquaria Water in Lake Water Samples (Part 1)

The lake water in the samples presented to Cobie, Mika, and Tommy was collected from Lake Tikitapu, Rotorua. This part of the experiment used a probe design that involved the standard catfish dilution of 14 μL in 100 mL lake water for positive samples and a dilution of 7 μL for probe samples. All control samples contained 14 μL control aquaria water in 100 mL lake water.

The 17 sample spots were divided into 10 control samples, four standard catfish samples, and three probe samples. Due to the dogs indicating most samples as positive, part-way through the study the number of positive samples was changed to vary between five or six in the hope that with a low target prevalence, the dogs would be more likely to indicate the target was absent on non-target samples. Consequently, the number of control samples were increased to either 12 or 11, respectively, to ensure 17 samples were presented. Making a rejection response on the control samples may also work as a reinforcer for the dogs as it reduced the delay until their next reinforcer. The five or six positive sample spots were further divided into two probe samples and three or four standard catfish samples, respectively.

As Tommy's correct rejection rate was much lower than the other dogs' and did not improve with the change in number of positive samples, the dilution of catfish aquaria water in his positive samples was increased from 7 µL and 14 µL to 100 µL.

To move onto the next lake, the dogs had to achieve hit rates and accuracy scores ≥80% in two out of three consecutive laboratory days. If this criteria was not met, the dogs were returned to training samples for re-training.

Re-Training

Due to low correct rejection rates when working with lake water samples, Cobie, Mika and Tommy were moved back to samples containing no lake water. Their criteria were also revised so they instead needed to achieve ≥80% hit rate and correct rejection rate in two out of three sessions. To calculate the hit rate achieved in a session, the dog's TH from that session was divided by the combined sum of their TH and TM from the session. This result was then multiplied by 100 to receive a percentage. Correct rejection rate was calculated by dividing the dog's TCR from the session by the combined sum of their TCR and TFA from the session. This result was then multiplied by 100 to receive a percentage. The dogs only needed to achieve the criteria once at any dilution of catfish aquaria water in control aquaria water to move back to lake water samples.

All control samples presented to the dogs contained only control aquaria water. The dogs were first presented with positive samples containing the same dilution of catfish aquaria water they were working at in Part B (Part 1) but in 100 mL control aquaria water instead of lake water (Table 5). If there were no improvements in the dog's correct rejection rates, the dilution of

catfish aquaria water was increased to the standard training dilution of 100 μL in 100 mL control aquaria water (Table 5). If the dog was still unable to meet criteria, they were presented with positive samples containing 100 mL of undiluted catfish aquaria water (Table 5). If the dog met the criteria, the dilution of catfish aquaria water was decreased to 50 mL in 50 mL control aquaria water (Table 5) and if they achieved criteria again, the dogs were moved back to lake water samples with this dilution of catfish aquaria.

This last dilution step was added as in the following phase, the dogs would work at the same dilution of catfish aquaria water they achieved in this phase, except in lake water rather than control aquaria water. If they were moved to the following phase with undiluted catfish aquaria, there would be no difference in the samples; transitioning from 100 mL of undiluted catfish aquaria water in 0 mL control aquaria water to 100 mL undiluted catfish aquaria in 0 mL lake water.

Table 5

The Positive Sample Concentrations presented to Cobie, Mika, and Tommy listed in the order they were presented to the dogs.

Sample dilution number	Control aquaria water (mL)	Catfish aquaria water	Cobie	Mika	Tommy
1	100	14 µL	-	+*	-
2	100	100 µL	+	+*	+*
3	0	100 mL	-	+	+
4	50	50 mL	-	+	+

Note. - Was not presented with the sample dilution.
+ Was presented with the sample dilution.
* Did not meet criteria at this sample dilution.

Part B – Catfish Aquaria Water in Lake Water Samples (Part 2)

Cobie, Mika, and Tommy were returned to Part B of the project for a second time, and Saydee was moved to Part B of the project for the first time having finished her training. In this phase, the dogs were presented with samples containing lake water and either control or catfish aquaria water depending on the sample type. Previously, Cobie, Mika, and Tommy were exposed to samples from Lake Tikitapu (Part B (Part 1)). To avoid any previous learning interfering with their performance, the dogs were presented with lake water samples collected from Lake Rotomā, Rotorua. The criteria remained the same from the retraining phase; the dogs needed to achieve ≥80% hit rate and correct rejection rate in two out of three sessions to move to a lower dilution. The dogs were presented with positive samples containing the same dilution of catfish

aquaria water that they were working at in the previous training or in the retraining phase but in lake water (Table 6). The dilution of control aquaria water in control samples was always equivalent to the dilution of catfish aquaria water in positive samples. As the dogs met the criteria, the dilution of catfish aquaria water was progressively decreased. As the dogs moved down the dilution steps, the lake water in these samples was adjusted accordingly to ensure there was always a total volume of 100 mL (Table 4).

Table 6

The Dilutions of Catfish or Control Aquaria Water in Lake Water presented to Cobie, Mika, Tommy, and Saydee listed in the order they were presented to the dogs.

Sample dilution number	Lake water (mL)	Aquaria water	Cobie	Mika	Tommy	Saydee
1	50	50 mL	-	+	+	+
2	90	10 mL	-	+	+	+
3	100	100 µL	+	+	+	+*
4	100	50 µL	+	+	+	-
5	100	14 µL	+	+	+	-
6	100	6 µL	+	-	-	-
7	100	3 µL	+	-	-	-
8	100	1.5 µL	+	-	-	-
9	100	0.5 µL	+	-	-	-

Note. - Was not presented with the sample dilution.

+ Was presented with the sample dilution.

* Did not meet criteria at this sample dilution.

Experimental Procedure

Sessions ran each week on a Wednesday and Thursday. One session was defined as one rotation of all 17 samples. Cobie completed eight sessions per day while Mika, Tommy, and Saydee completed six due to lower motivation levels. The dog owners were asked to either not feed their dogs or to reduce their feed on these days to ensure the dogs were sufficiently motivated to work for food.

To begin, the 17 available sample locations on the apparatus were divided into 10 control samples and seven positive samples. As mentioned previously, this was later changed to 11 or 12 control samples and six or five positive samples, respectively. At the beginning of each laboratory day, a random number generator was used to determine whether five or six positive samples would be presented, and then to randomise the placement of samples on the apparatus. Once the samples were prepared, they were moved to the experimental room on a three-tier trolley and placed on the apparatus in their randomised order. To avoid cross-contamination, the control samples were always placed on the top tier of the trolley and the positive samples on the second tier. The 17 segments were placed over each of the samples and then covered by the lid of the apparatus. The samples were then left for a minimum of 20 minutes to allow the odours to equalize in their individual segments.

The randomised sample order was entered into each of the dog's individual configuration files on the computer. Cobie and Mika worked at an indication threshold of 4,501 ms; however, throughout the project, Tommy's indication threshold was progressively increased from 4,501

ms to 6,501 ms and then decreased to 5,001 ms, while Saydee's increased from 4,501 ms to 5,001 ms.

Each of the dogs' responses (indication or rejection) were manually recorded by the researcher on digital subject-specific response sheets next to the corresponding sample number. Other factors were also recorded on the response sheet such as the temperature and the humidity of the experimental room during the session, and the start and end time of the session. At the end of each session, the dog's hit and correct rejection rates were calculated and noted on their response sheet. On a separate document, notes about the dogs' performances or any issues with a dog or the apparatus were made.

Statistical Analysis

The hit rate and correct rejection rate each dog achieved during their sessions was recorded and plotted. No statistical comparisons were made as each dog's performance was assessed independently based on predefined criteria.

Results

Part A - Catfish Aquaria Water at Operationally Useful Dilutions

The goal in this phase of the experiment was to reduce the concentration in the positive samples to a dilution that was operationally useful. The samples were prepared with control aquaria water to reduce the potential for distractor scents. For each catfish biomass concentration (i.e., 311 kg/ha, 77.7 kg/ha, 43.5 kg/ha), the dogs were required to attain hit rates and accuracy scores of ≥80% on two out of three consecutive laboratory days.

At the 311 kg/ha sample concentration, Cobie and Mika met the criteria within two and three laboratory days, respectively (Table 7). Tommy maintained a perfect hit rate (Table 8), while his accuracy scores progressively increased across four laboratory days (Table 8), and he achieved the criteria on his fourth day (Table 7). During this phase, Mika and Tommy exhibited low motivation during their sessions, often lying down between samples. Subsequently, the number of sessions they completed in a day was decreased from eight to six, and their motivation improved. At the 77.7 kg/ha sample concentration, both Cobie and Mika met the criteria within two days (Table 7). Tommy achieved hit rates and accuracy scores above 80% on his first and third day (Table 8), but because he had a 3-week break between his first and second laboratory day, he was required to meet 80% on an additional day, which he did (Table 8). At the 43.5 kg/ha sample concentration, Tommy met the criteria within two days (Table 7). Mika met the criteria within three days (Table 7), with scores above 80% on her first and third day (Table 8), and Cobie took four days (Table 7).

Table 7

The Number of Laboratory Days and Sessions (in brackets) completed by Cobie, Mika, and Tommy until they Achieved Criteria at each Dilution.

Equivalent environmental biomass (kg/ha) *	Cobie	Mika	Tommy
311	2 (16)	3 (22)	4 (25)
77.7	2 (16)	2 (12)	4 (24)
43.5	4 (32)	3 (18)	2 (12)

Note. * Based on a water volume of 20,000 m^3 per hectare (i.e., 100 m x 100 m x 2 m water depth).

Table 8

The Hit Rates and Accuracy Scores of Cobie, Mika, and Tommy until they Achieved Criteria while working at Sample Concentrations Equivalent to Catfish Biomasses of 311, 77.7, and 43.5 kg/ha.

Laboratory Day	Equivalent environmental biomass (kg/ha) *	Cobie		Mika		Tommy	
		Hit Rate (%) **	Accuracy (%) **	Hit Rate (%) **	Accuracy (%) **	Hit Rate (%) **	Accuracy (%) **
1	311	91	85	75	82	100	58
2	311	95	86	95	86	100	79
3	311	-	-	95	87	100	81
4	311	-	-	-	-	100	84
5	77.7	88	84	88	88	100	84
6	77.7	96	88	93	83	100	72
7	77.7	-	-	-	-	100	84
8	77.7	-	-	-	-	100	88
9	43.5	82	76	88	88	100	87
10	43.5	91	76	90	79	100	89
11	43.5	100	90	95	85	-	-
12	43.5	89	84	-	-	-	-

Note. * Based on a water volume of 20,000 m^3 per hectare (i.e., 100 m x 100 m x 2 m water depth).

** Calculated from the total number of sessions performed on the day.

Part B - Catfish Aquaria Water in Lake Water Samples (Part 1)

This phase of the experiment investigated the dogs' ability to detect the presence of catfish in lake water samples, which naturally contain potential distractor or masking scents. The dogs were presented with samples containing water from catfish-free Lake Tikitapu spiked with

either control or catfish aquaria water for a biomass equivalent concentration of 43.5 kg/ha. The dogs were required to attain hit rates and accuracy scores ≥80% on two out of three consecutive laboratory days to transition onto the next lake.

Mika achieved the criteria within two laboratory days. Cobie and Tommy were close to meeting criteria, with hit rates above 80% and accuracy scores just below 80% (Table 9). However, on the fifth day, it was determined that sediment settled from the lake water was not being distributed equally across the samples. As the control samples were poured first, followed by positive samples, the settled sediment was disproportionately added to the positive samples. As a result, it was unclear whether the dogs were indicating on the presence of catfish aquaria water in the positive samples or if they were potentially learning to indicate on sediment odours. A change in methodology was implemented on the fifth day by shaking the bottle containing lake water three times every three samples to spread the sediment through the samples. This change was followed by a large decrease in the correct rejection rates for all three dogs (Figures 8, 9, 10). On the first laboratory day following the method change, Cobie achieved an average correct rejection rate of 20% (Figure 8), Mika 6.67% (Figure 9), and Tommy 3.33% (Figure 10). This resulted in a decrease in the dogs' accuracy scores, with a drop of more than 50% in Mika and Tommy's case (Table 9). However, the dogs' hit rates remained relatively high, with Cobie achieving an average rate of 96.4% (Figure 8), Mika 90.5% (Figure 9), and Tommy 73.8% (Figure 10) that first day. This suggested that the dogs were no longer indicating on the target odour. Therefore, it was decided the data collected before this change in methodology was invalid and Mika was no longer eligible to move to the next lake.

Table 9

The Hit Rates and Accuracy Scores of Cobie, Mika, and Tommy 4 days before the Methodology Change and 4 days after when working at a Biomass Equivalent Concentration of 43.5 kg/ha in water from Lake Tikitapu.

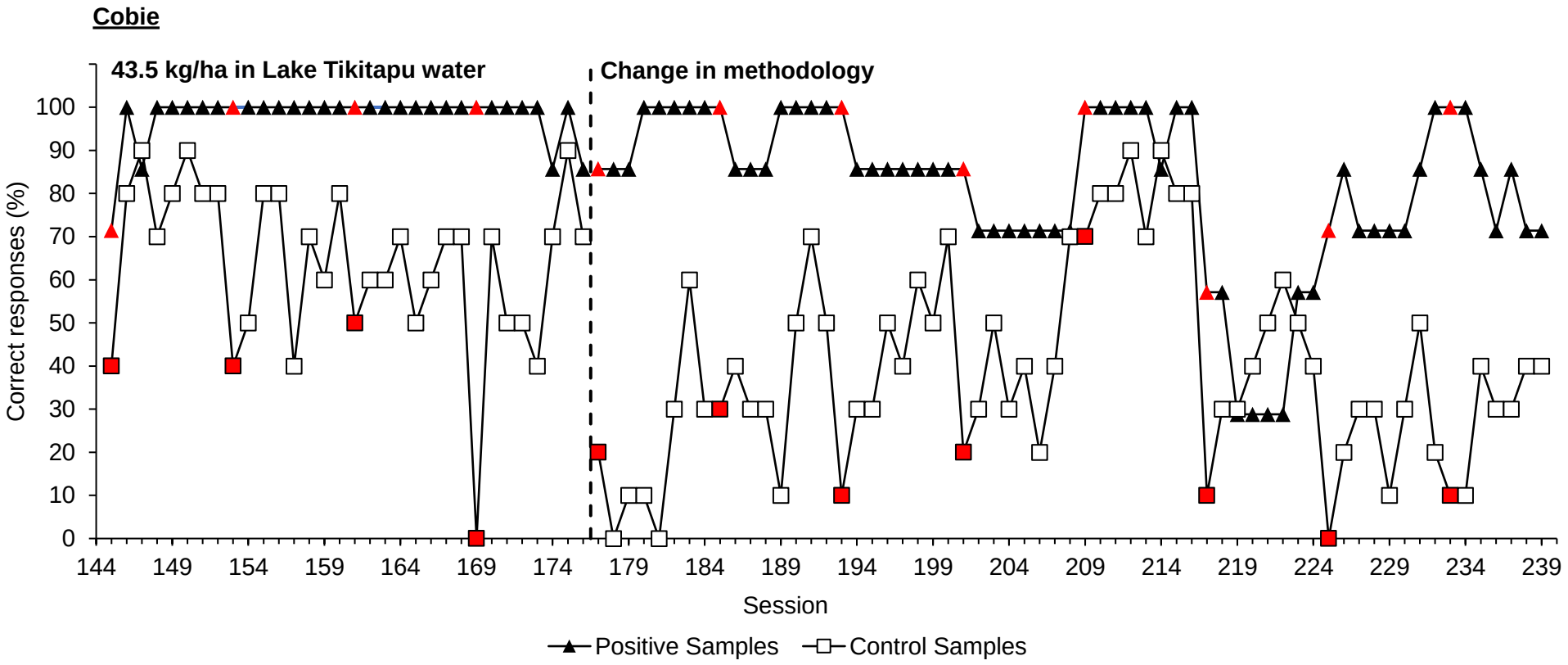
Laboratory Day Number	Cobie		Mika		Tommy	
	Hit Rate (%) **	Accuracy (%) **	Hit Rate (%) **	Accuracy (%) **	Hit Rate (%) **	Accuracy (%) **
1	95	83	81	86	95	79
2	100	78	86	88	98	86
3	100	77	93	75	95	73
4	96	72	93	82	95	76
5*	95	51	90	41	71	31
6	95	63	100	61	100	60
7	88	61	86	56	88	46
8	73	52	62	57	95	52

Note. * Change in methodology

** Calculated from the total number of sessions performed on the day.

Figure 8

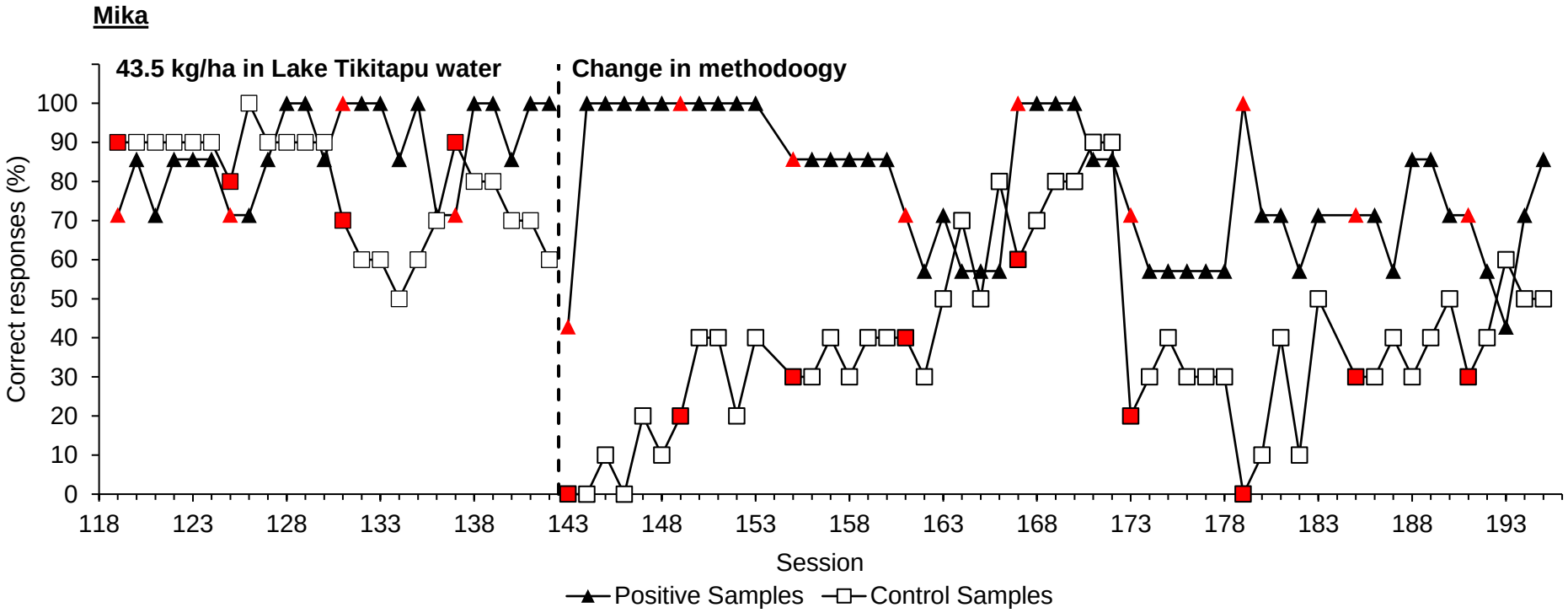
Cobie's percentage of Correct Responses to Positive and Control Samples session by session before the Change in Methodology and afterwards.



Note. Red data point represents first session of the day.

Figure 9

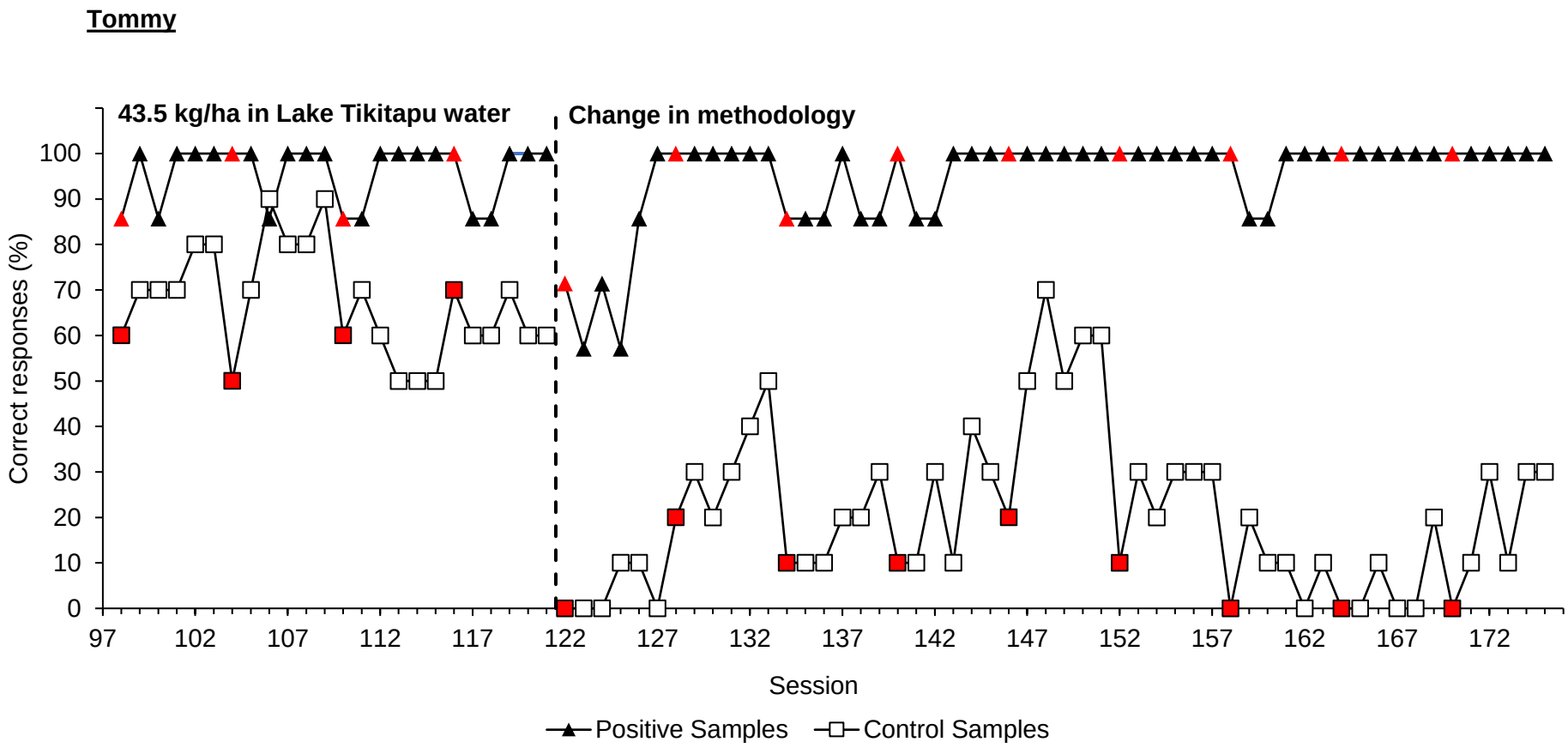
Mika's percentage of Correct Responses to Positive and Control Samples session by session before the Change in Methodology and afterwards.



Note. Red data point represents first session of the day.

Figure 10

Tommy's percentage of Correct Responses to Positive and Control Samples session by session before the Change in Methodology and afterwards.



Note. Red data point represents first session of the day.

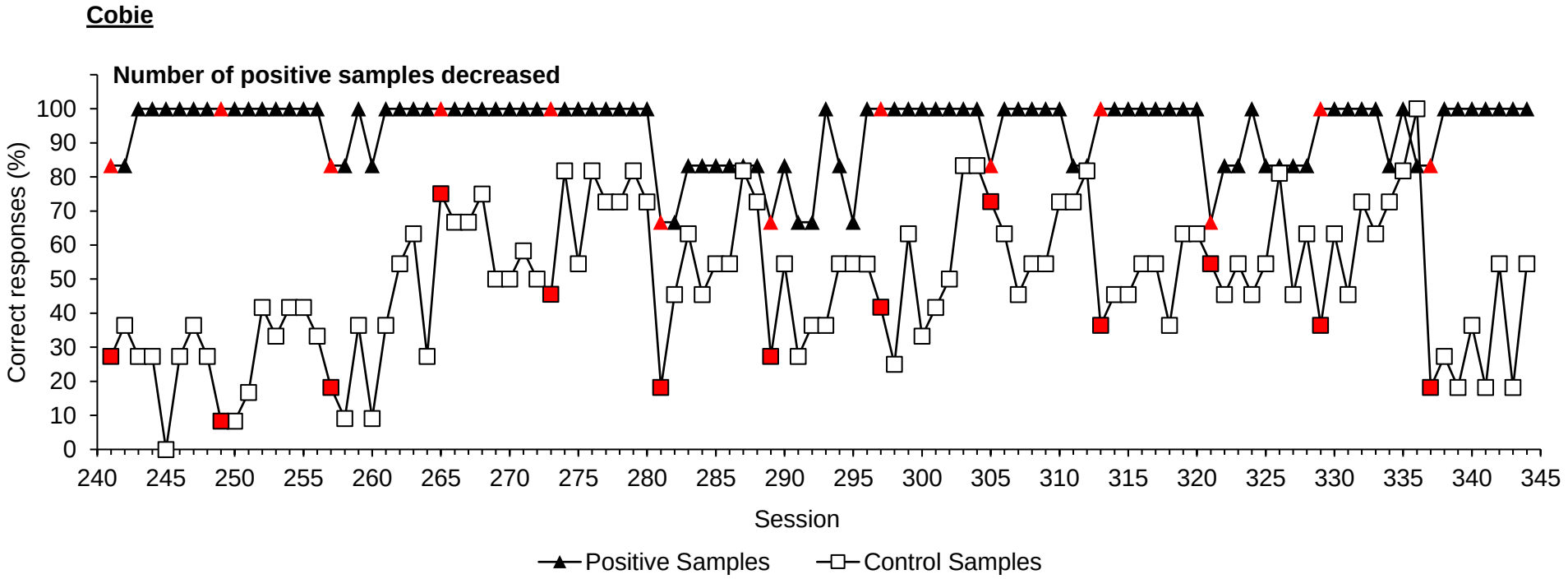
Testing was continued for another six laboratory days under the same sample composition, with Cobie completing 64 sessions, Mika 54, and Tommy 54. However, there were only small or transitory improvements in correct rejection rates while hit rates remained high. It was hypothesized that the dogs were exhibiting a bias toward indicating that the target catfish odour was present due to a high target prevalence. The number of positive samples were decreased from seven to either five or six in the hope that with a low target prevalence, the dogs would be more likely to indicate that the target was absent on non-target samples. However, after a further 104 sessions for Cobie and 72 for Mika, there were still little to no improvements in correct rejection rates (Figure 11, Figure 12).

In the six sessions following the decrease in number of positive samples, there was no improvement in Tommy's correct rejection rate. He achieved an average hit rate of 100%, but an average correct rate of 3.03% (Figure 13) which meant he was indicating on nearly all of the samples. Several methodological changes were tried, including increasing his indication threshold from 4501 ms to 5001 ms and then to 6001 ms later, and increasing his sample concentration to 311 kg/ha. However, he continued to have poor performance on the control samples (Figure 13), meaning he was not effectively discriminating between the sample types.

Excluding the sessions completed before the methodology change, Cobie completed a total of 168 sessions during this part of the experiment, Mika 126, and Tommy 109. At this point, the presentation of lake water samples ceased, and they were all moved to a re-training phase.

Figure 11

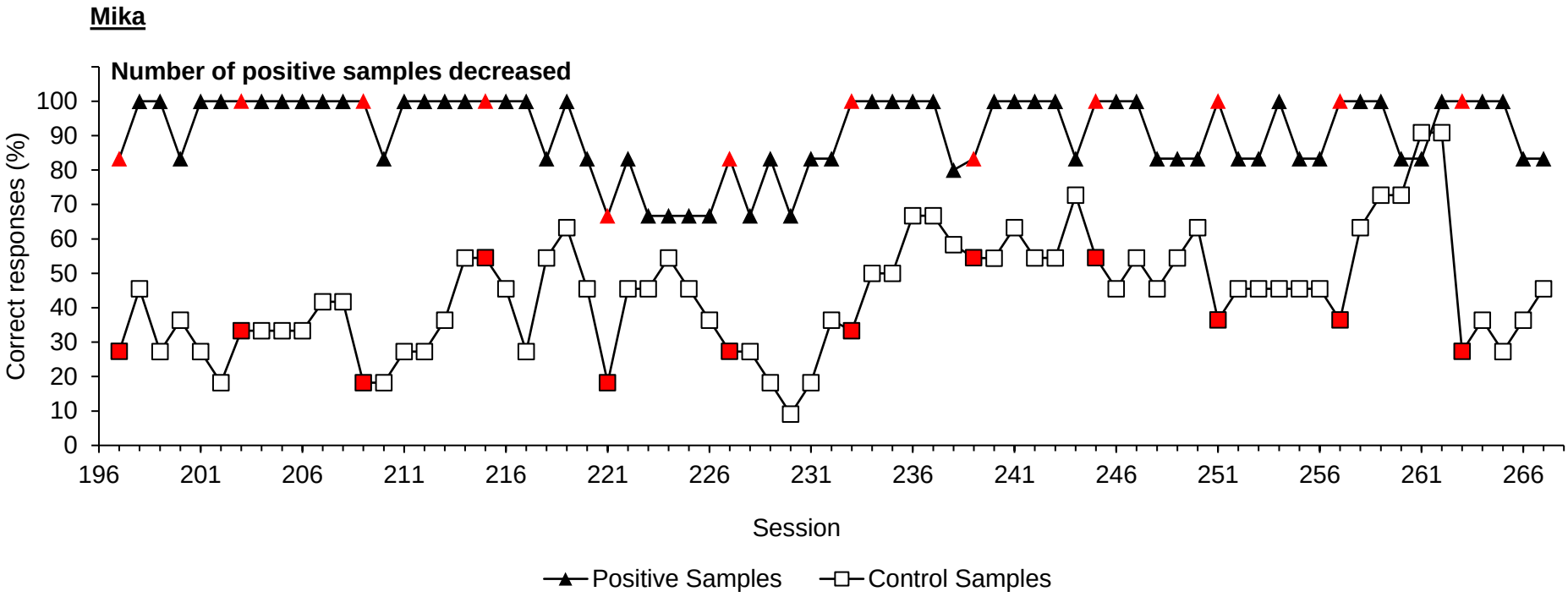
Cobie's percentage of Correct Responses to Positive and Control Samples session by session after the Number of Positive Samples Decreased.



Note. Red data point represents first session of the day.

Figure 12

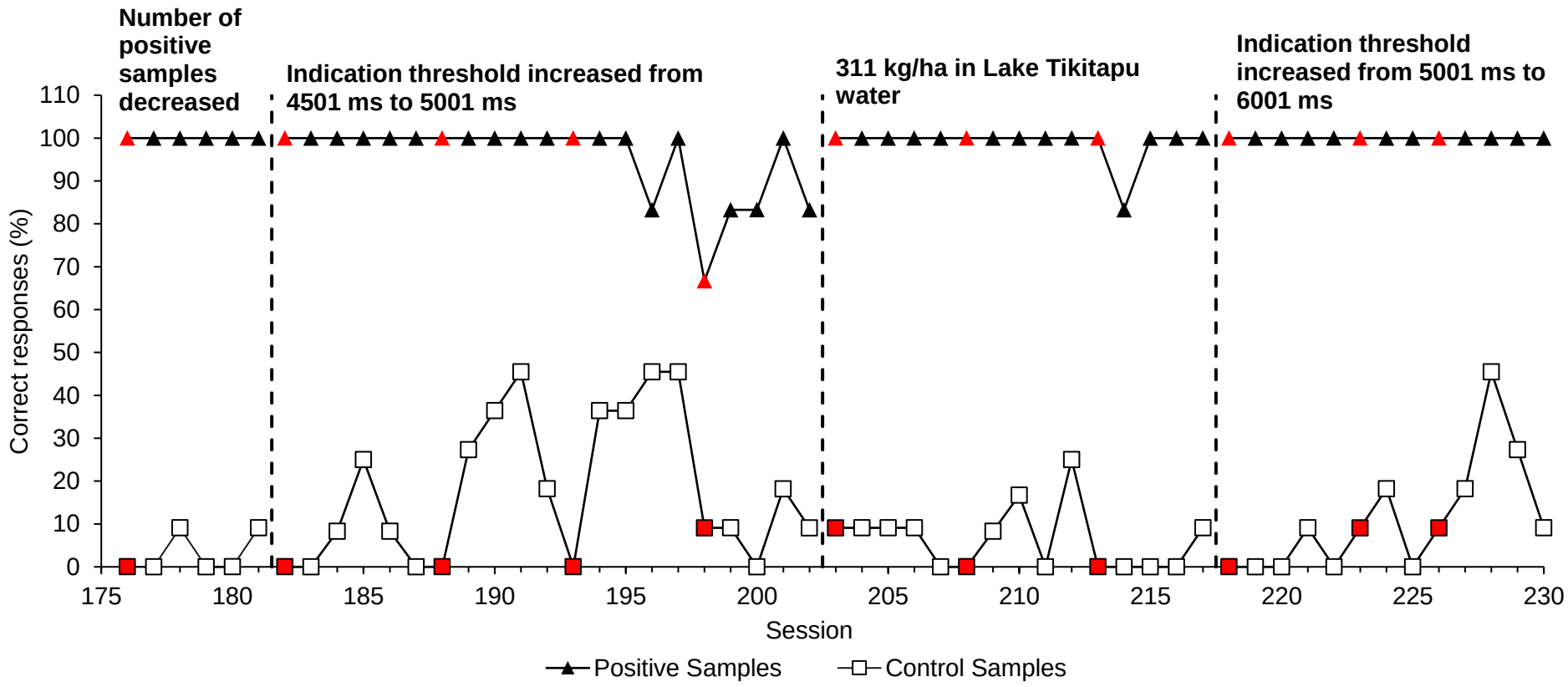
Mika's percentage of Correct Responses to Positive and Control Samples session by session after the Number of Positive Samples Decreased.



Note. Red data point represents first session of the day.

Figure 13

Tommy's percentage of Correct Responses to Positive and Control Samples session by session after the Number of Positive Samples Decreased, and the Increase in his Indication Threshold and Positive Sample Concentration.



Note. Red data point represents first session of the day.

Re-Training

Due to their low correct rejection rates when working with lake water samples, Cobie, Mika and Tommy were moved back to a re-training phase which focused on reinforcing the behaviour of identifying and indicating on the correct target odour. The samples were prepared with control aquaria water to reduce the potential for distractor scents. The criteria were also revised from needing to attain hit rates and accuracy scores ≥80% on two out of three consecutive laboratory days so they instead needed to achieve hit and correct rejection rates ≥80% in two out of three sessions.

Due to poor discrimination rates by Mika, her positive sample concentration was increased from 43.5 kg/ha to 311 kg/ha after 12 sessions (Table 10). While working at 311 kg/ha, Mika's correct rejection rate slightly improved after 10 sessions, ranging between 66.7-68.3% (Figure 14). However, following a large drop in her correct rejection rate, her positive sample concentration was increased to contain undiluted catfish aquaria water (310,968 kg/ha).

Cobie was away from the laboratory for the 2 days Mika was presented with the 43.5 kg/ha sample concentration. Upon Cobie's return, she was moved immediately to the 311 kg/ha concentration with Mika. Cobie met the revised criteria after 21 sessions (Table 10), scoring a hit rate of 100% and a correct rejection rate of 83.3% for two consecutive sessions (Figure 15). Cobie was maintained at this dilution whilst water samples were collected from Lake Rotomā and was subsequently moved back to lake water samples in Part B (Part 2).

Table 10

The Number of Sessions completed by Cobie, Mika, and Tommy until they Achieved Criteria at each Dilution.

Sample dilution number	Equivalent environmental biomass (kg/ha) *	Cobie	Mika	Tommy
1	43.5	-	12 **	-
2	311	21	20 **	23 **
3	310,968	-	18	17
4	155,484	-	3	11

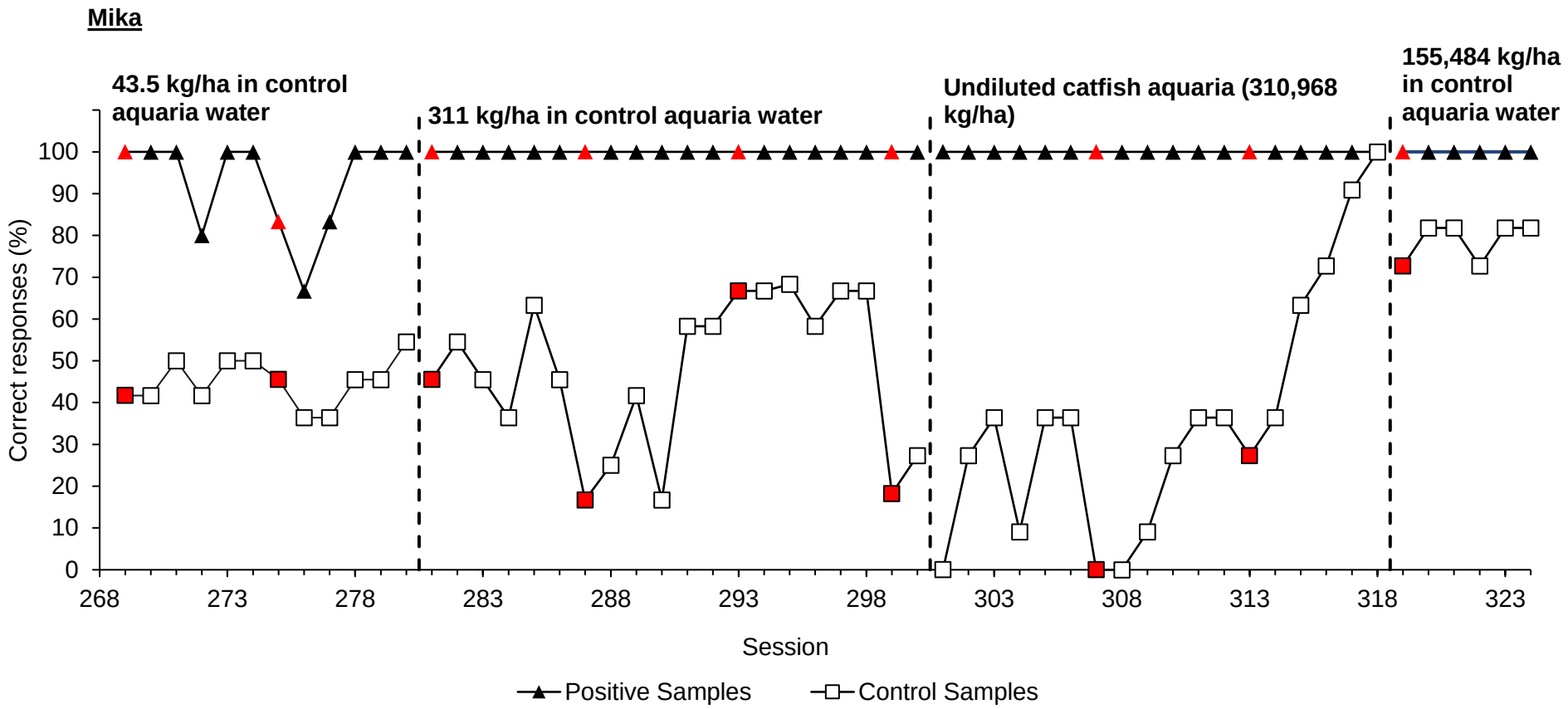
Note. * Based on a water volume of 20,000 m^3 per hectare (i.e., 100 m x 100 m x 2 m water depth).

- The dog was not presented with this sample dilution.

** The dog did not meet criteria at this dilution.

Figure 14

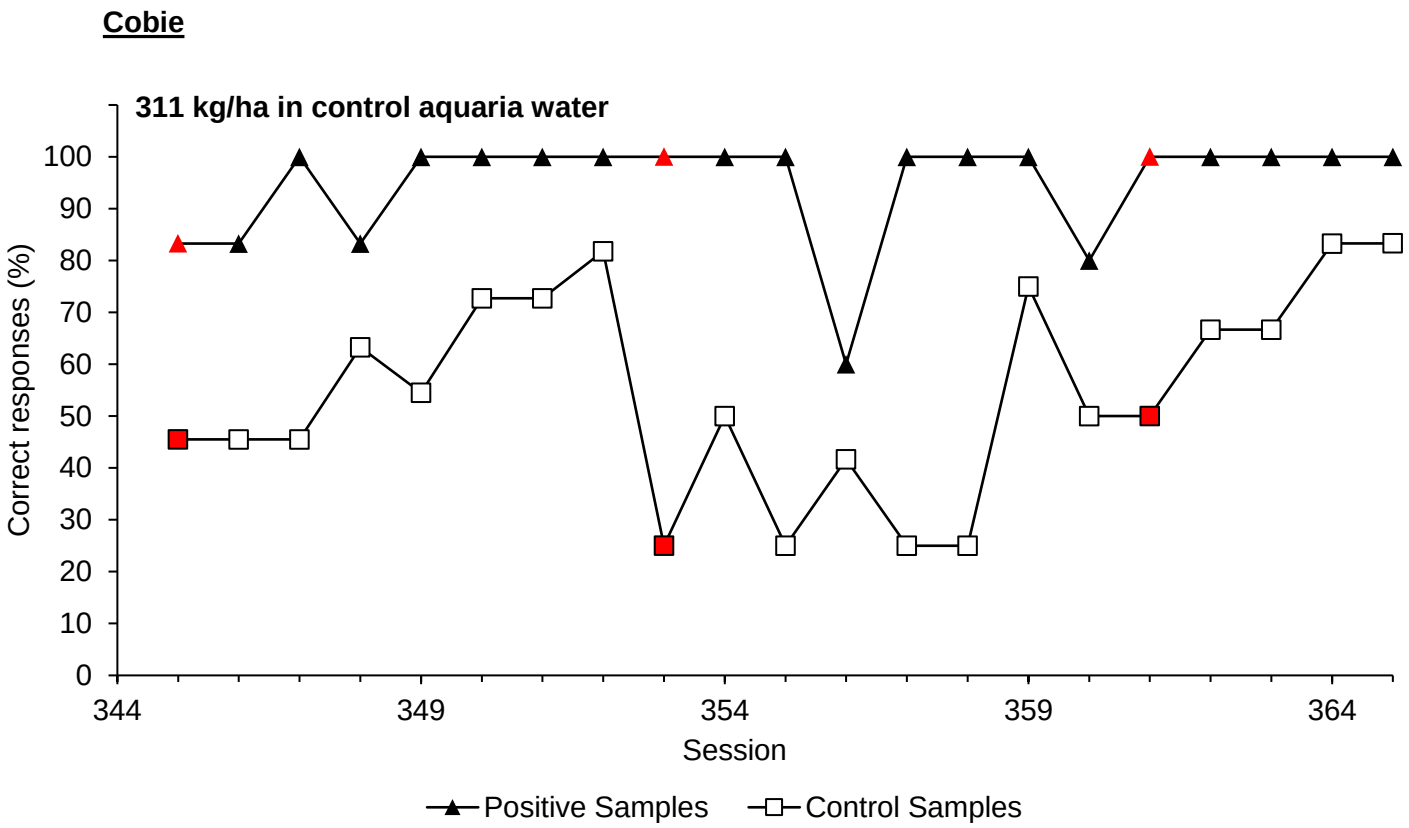
Mika's percentage of Correct Responses to Positive and Control Samples session by session as Positive Sample Concentration Increased then Decreased.



Note. Red data point represents first session of the day.

Figure 15

Cobie's percentage of Correct Responses to Positive and Control Samples session by session while working at a Biomass Equivalent Concentration of 311 kg/ha.



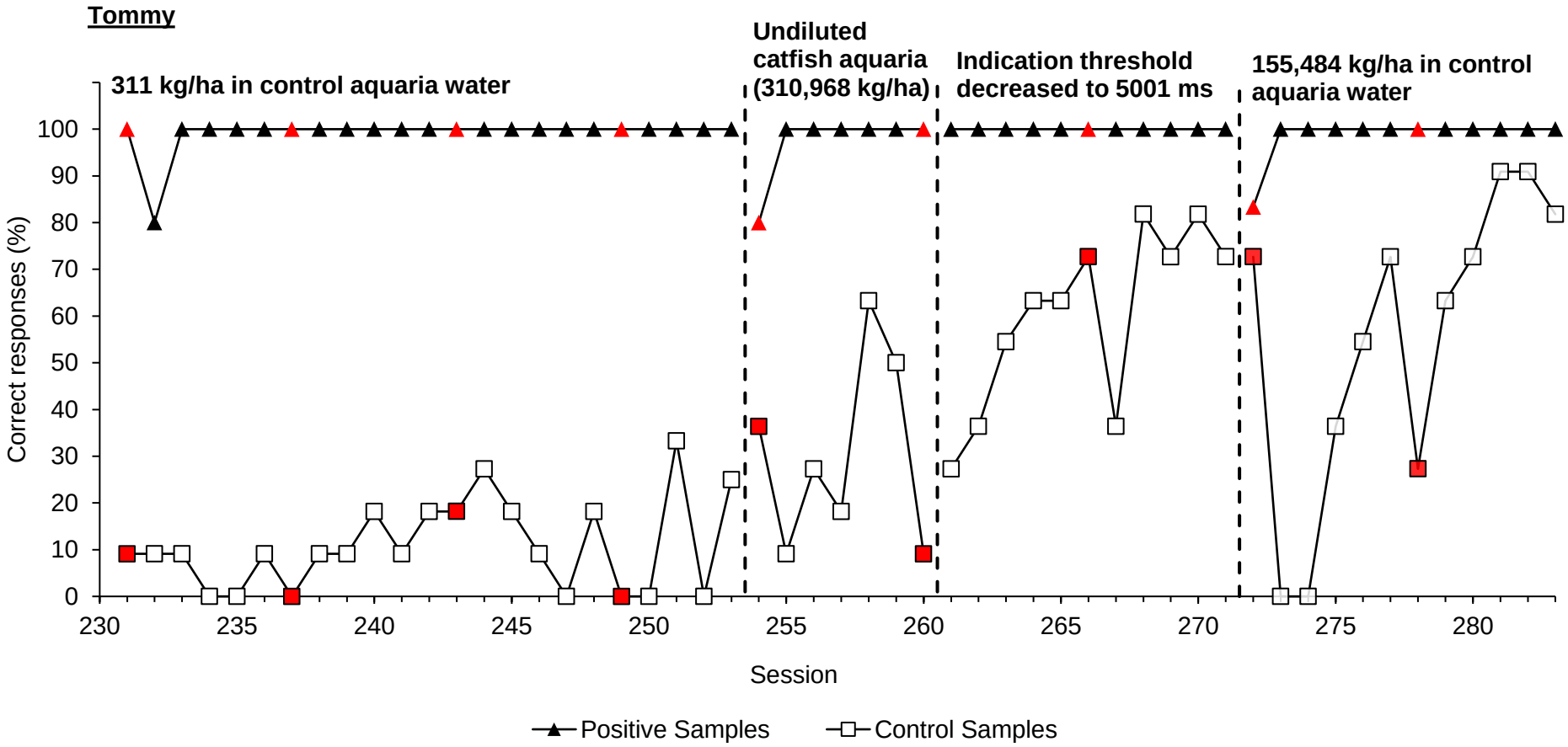
Note. Red data point represents first session of the day.

After 23 sessions at a sample concentration of 311 kg/ha, there were no improvements in Tommy's correct rejection rate (Table 10) and his sample concentration was increased to undiluted catfish aquaria water (310,968 kg/ha) alongside Mika. After Tommy was transitioned to undiluted catfish aquaria water samples, his indication threshold was also reduced from 6001 ms to 5001 ms in an attempt to improve his poor motivation, and he no longer lay down during

his sessions. After 17 sessions of re-training, Tommy met the revised criteria (Table 10, Figure 16), and Mika met the criteria after 18 sessions (Table 10, Figure 14). Tommy and Mika's positive sample dilution was then halved (155,484 kg/ha), with Mika meeting the criteria after three sessions and Tommy after 11 sessions (Table 10). They were then eligible to move back to lake water samples.

Figure 16

Tommy's percentage of Correct Responses to Positive and Control Samples session by session as Positive Sample Concentration Increased then Decreased.



Note. Red data point represents first session of the day.

Part B - Catfish Aquaria Water in Lake Water Samples (Part 2)

The dogs were returned to samples prepared with lake water to determine their ability to detect the presence of catfish in lake water. The dogs were presented with samples containing water from catfish-free Lake Rotomā spiked with either control or catfish aquaria water. The criteria remained the same from the previous phase, meaning the dogs needed to achieve ≥80% hit rate and correct rejection rate in two out of three sessions to move down a dilution step. An overview of the number of sessions completed by the dogs until they achieved the criteria at each sample concentration is provided in Table 11. At this stage of the project, Saydee had finished her training and was moved to this phase of the project alongside the other dogs.

Table 11

The Number of Sessions completed by Cobie, Mika, Tommy, and Saydee until they Achieved Criteria at each Dilution.

Sample dilution number	Equivalent catfish biomass (kg/ha) *	Cobie	Mika	Tommy	Saydee
1	155,484	-	6	4	4
2	31,097	-	3	6	2
3	311	14	39	23	24**
4	155	3	10	14	-
5	43.5	18	4	8	-
6	18.7	7	-	-	-
7	9.33	2	-	-	-
8	4.66	4	-	-	-
9	1.55	2	-	-	-

Note. * Based on a water volume of 20,000 m^3 per hectare (i.e., 100 m x 100 m x 2 m water depth).
- The dog was not presented with sample dilution.
** The dog did not meet criteria at this dilution.

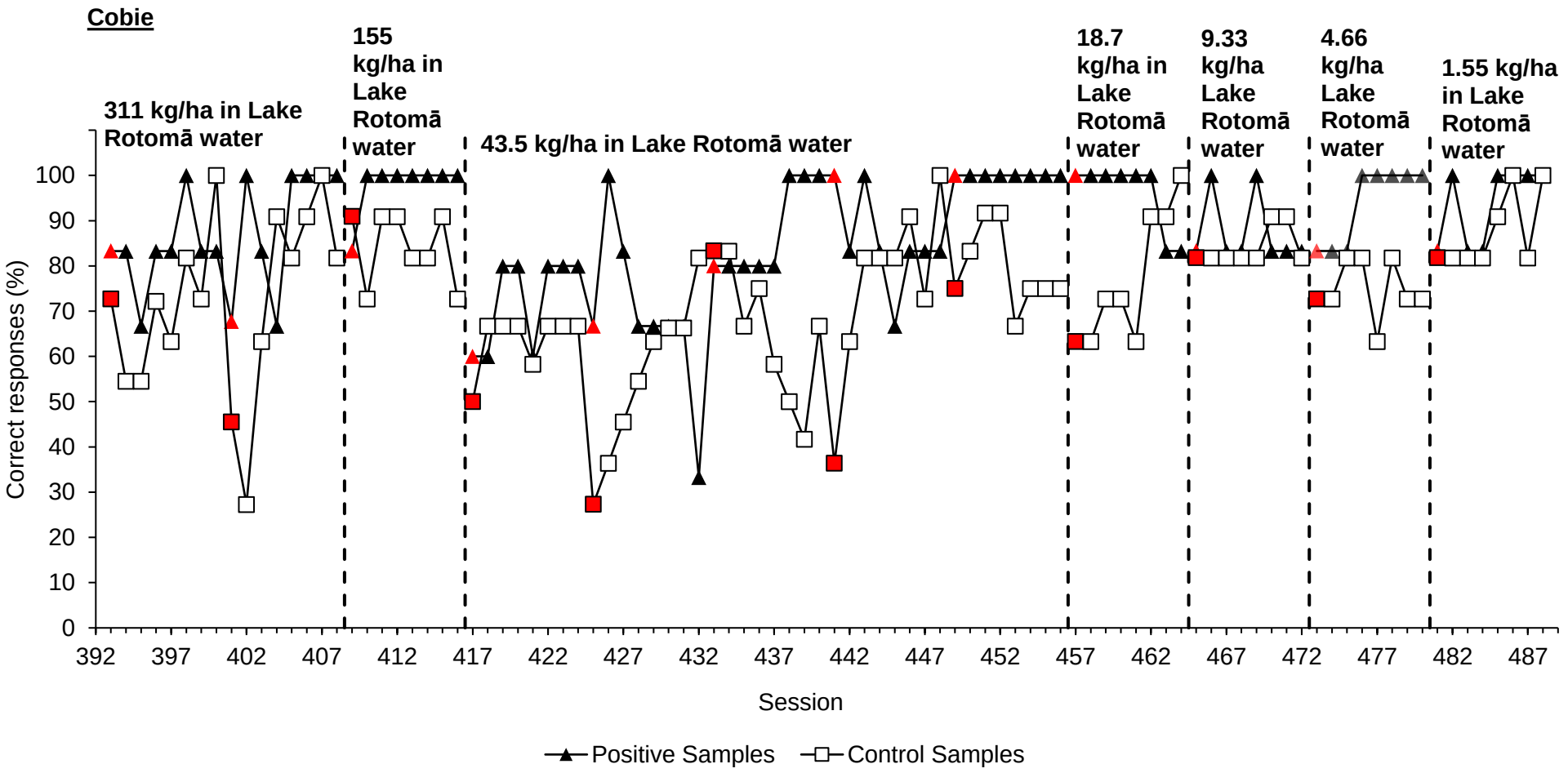
Cobie was presented with a sample concentration of 311 kg/ha and met the criteria after 14 sessions (Table 11). The concentration was reduced to 155 kg/ha and she met the criteria within the following three sessions (Table 11). At the 43.5 kg/ha sample concentration, her correct rejection and hit rate dropped, her hit rate averaging 72.50% across her first day, while her correct rejection rate averaged 63.56% before dropping further (Figure 17). However, her hit and correct rejection rates progressively increased and she met the criteria after 18 sessions

(Table 11), achieving the target positive sample concentration for this project. Her positive sample concentration continued to be reduced to 18.7 kg/ha, 9.33 kg/ha, 4.66 kg/ha, and 1.55 kg/ha, with her meeting the criteria for each of these dilutions within seven, two, four, and two sessions, respectively (Table 11).

Mika's sample concentration was decreased from 155,484 kg/ha to 31,097 kg/ha as she met the criteria for each of these dilutions within six and three sessions, respectively (Table 11). Her sample concentration was decreased to 311 kg/ha which was followed by a large drop in her correct rejection rate (Figure 18). Her correct rejection rate improved after 12 sessions but plateaued for a further 23 sessions at an average score of 55%. Mika met the criteria after a total of 39 sessions at this dilution (Table 11). A reduction in her sample concentration to 155 kg/ha was again met with a decrease in her correct rejection rate and an initial drop in her hit rate (Figure 18). She met the criteria after 10 sessions (Table 11) and her sample concentration was then reduced to 43.5 kg/ha, the target dilution for the project. This was followed by another drop in her correct rejection rate, which then progressively increased (Figure 18) and she met the criteria by her fourth session at this dilution (Table 11).

Figure 17

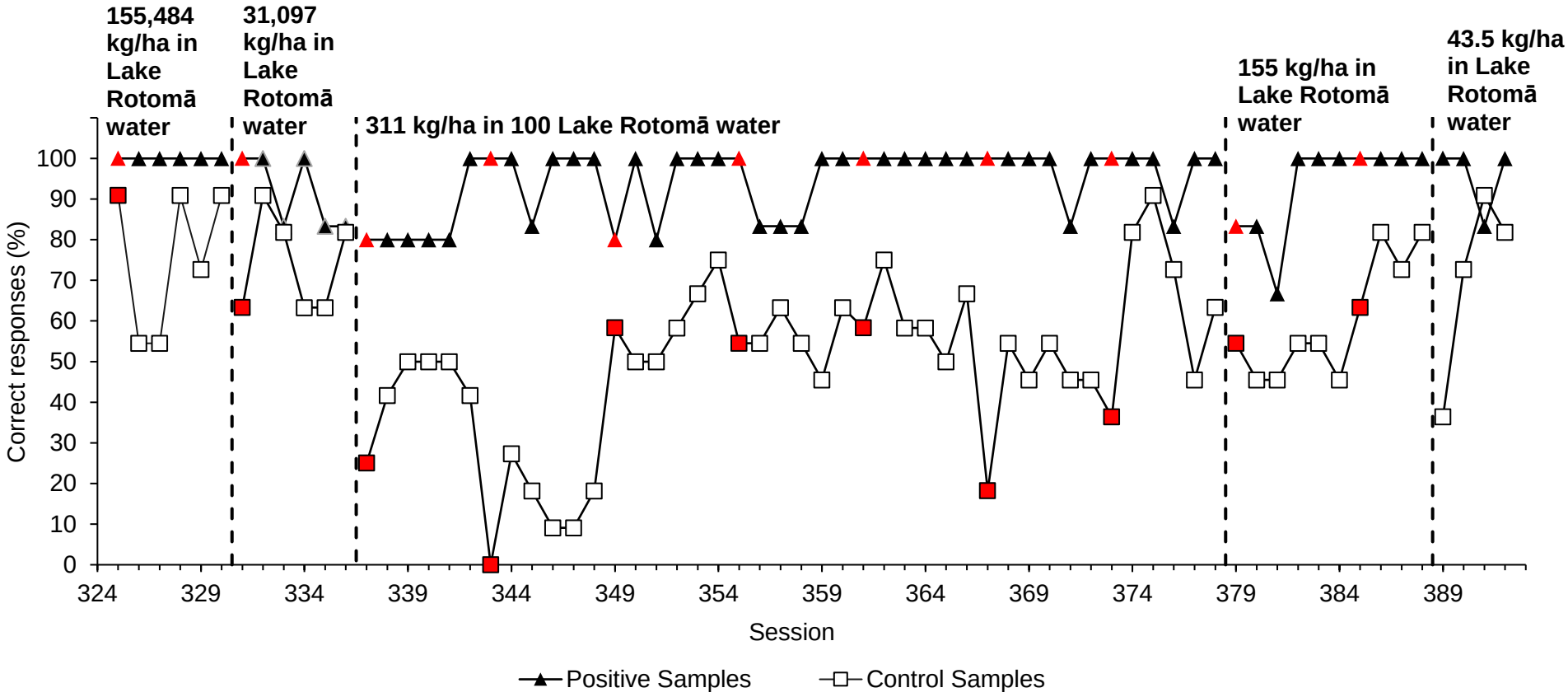
Cobie's percentage of Correct Responses to Positive and Control Samples session by session as Positive Sample Concentration was Decreased.



Note. Red data point represents first session of the day.

Figure 18

Mika's percentage of Correct Responses to Positive and Control Samples session by session as Positive Sample Concentration was Decreased.



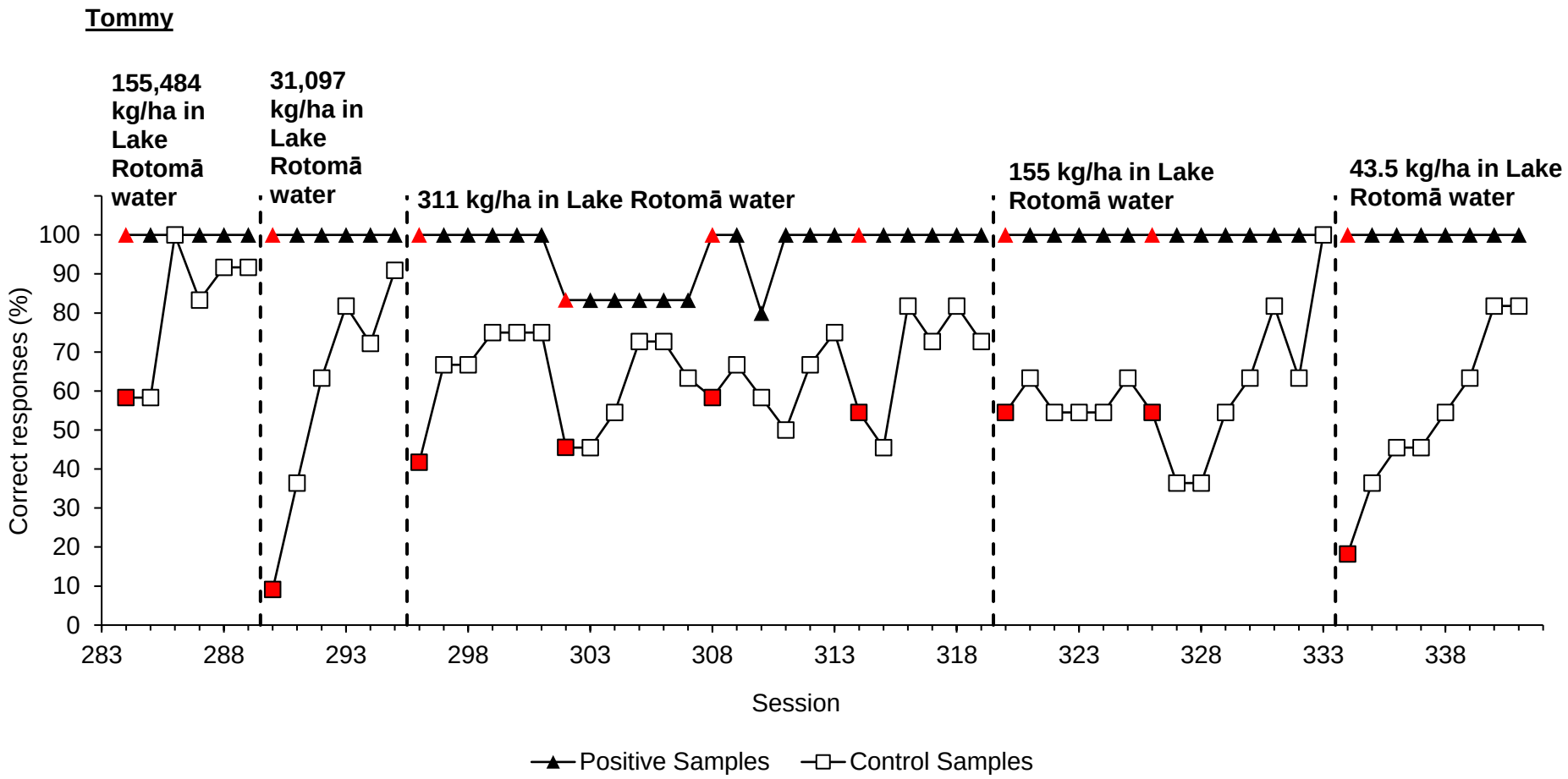
Note. Red data point represents first session of the day.

The concentration of aquaria water in Tommy's samples was decreased from 155,484 kg/ha to 31,097 kg/ha as he met the criteria for each of these dilutions within four and six sessions, respectively (Table 11). At 311 kg/ha, there were small decreases in his correct rejection rate on his first and occasionally second session each day, however this tended to increase again by the end of the day (Figure 19). He met the criteria after 23 sessions and following a decrease in his sample concentration to 155 kg/ha, met the criteria again after 14 sessions (Table 11). At 43.5 kg/ha, there was a large decrease in his correct rate, before it progressively increased (Figure 19) and he met the criteria after eight sessions (Table 11). Although Tommy usually ran six sessions within one laboratory day, on the last day of the project his number of sessions was increased to eight as he had high motivation levels and his correct rejection rate was on an upward trend and close to meeting the criteria.

Saydee was presented with a sample concentration of 155,484 kg/ha and met the criteria within four sessions (Table 11). Her sample concentration was reduced to 31,097 kg/ha and she maintained a perfect hit and correct rejection rate across all sessions that day, except in her final session when her correct rejection rate dropped (Figure 20). Her sample concentration was decreased to 311 kg/ha, which subsequently led to a large drop in her hit rate and a decrease in her correct rejection rate (Figure 20). She remained at this dilution for 24 sessions but was unable to meet the criteria before the end of the project (Table 11).

Figure 19

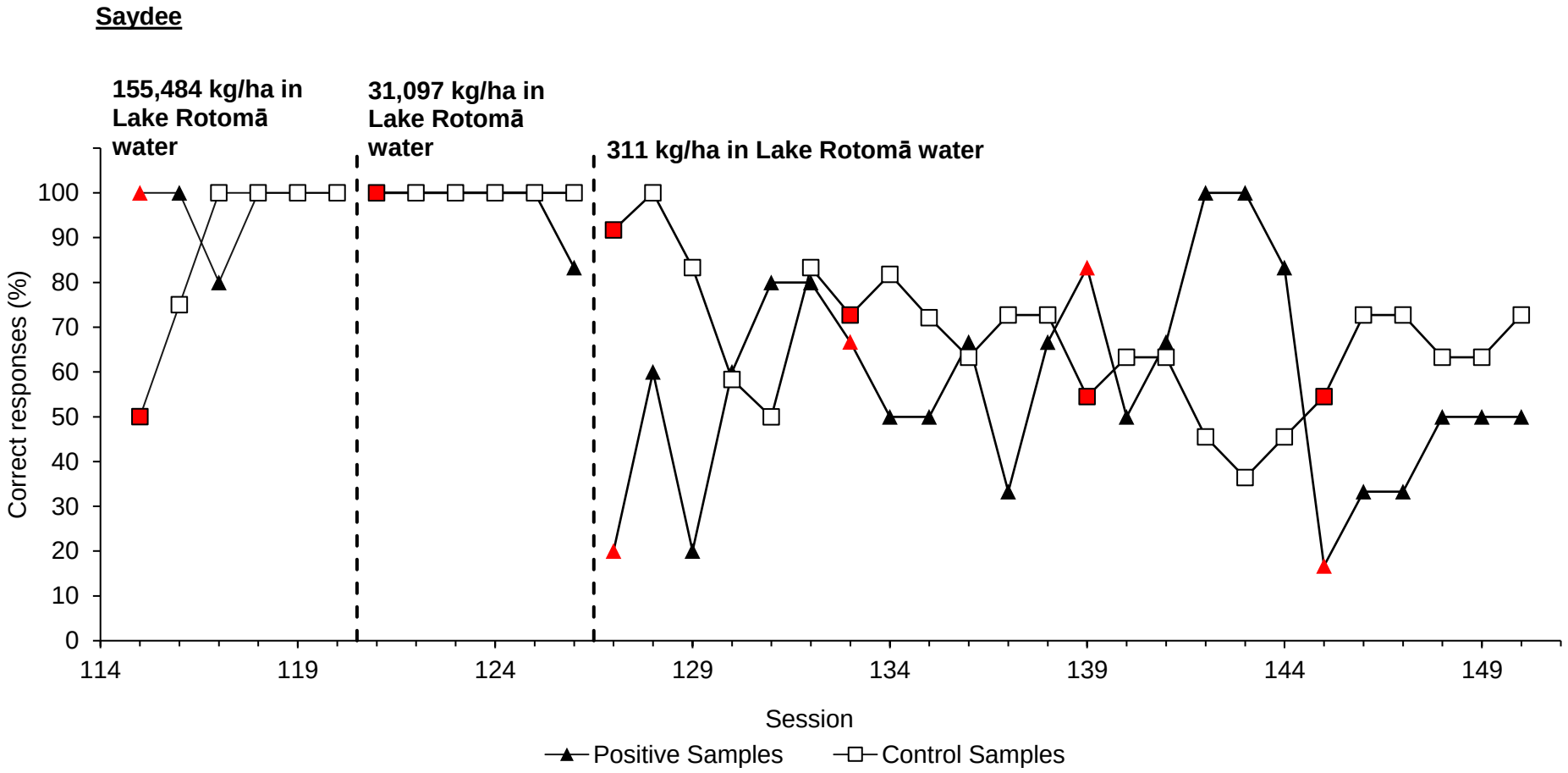
Tommy's percentage of Correct Responses to Positive and Control Samples session by session as Positive Sample Concentration was Decreased.



Note. Red data point represents first session of the day.

Figure 20

Saydee's percentage of Correct Responses to Positive and Control Samples session by session as Positive Sample Concentration was Decreased.



Note. Red data point represents first session of the day.

Discussion

Future Research and Considerations

Future research into determining dogs' ability to detect the presence of catfish in water from more sediment laden or productive lakes is recommended. The lake water used in this study was collected from Lake Tikitapu and Lake Rotomā which have comparatively good water quality with low humic content. This may have made it easier for the dogs to detect the presence of catfish as there is generally lower fish biomass in less productive systems to act as distractor scents, and potentially less VOC inhibitors such as clay particles or humic substances (Downing et al., 1990; McColl, 1977).

The next step in this research is to determine whether dogs can generalise their learning to discriminate between lakes which contain catfish from lakes that do not. This is an important step in the real-world application of dogs in the management of invasive species. Although the results of this study indicate that dogs can detect the presence of catfish in lake water samples, it is unknown how the addition of catfish aquaria water may affect the chemical composition of lake water. Therefore, the chemical composition of the samples used in this study may differ from the composition of lake water that naturally contains catfish. This may result in different findings from those achieved in this study and perhaps the biomass concentrations at which the dogs can successfully detect the presence of catfish may differ.

Aims and Results

Brown bullhead catfish were deliberately introduced to New Zealand from North America in the 1880s, and have since become widespread in the Auckland, Waikato, and Bay of Plenty regions (McDowall, 1990; Collier & Grainger, 2015). A high density of catfish can negatively impact the health and biodiversity of New Zealand's freshwater ecosystems. They reduce water clarity through bioturbation and nutrient release (Rowe, 2007), prey on, and compete with native species for food (Francis, 2019). Hence, preventing the establishment of new catfish populations requires early detection so that containment and eradication measures are more likely to be effective. Scent detection dogs have shown promise in detecting invasive fish species such as catfish and koi carp (*Cyprinus rubrofuscus*) under laboratory conditions (Collins et al., 2022; Denby, 2021). The aim of this study was to determine whether domestic dogs could detect the presence of catfish in lake water samples at a biomass concentration equivalent to 43.5 kg/ha, a concentration consistent with catfish population estimates in several New Zealand lakes (Hicks et al., 2015; Tempero et al., 2019).

This is the first study to demonstrate that dogs can detect the presence of catfish in lake water at operationally useful dilutions, with the majority of the dogs successfully detecting the presence of catfish at a biomass equivalent concentration of 43.5 kg/ha. The dogs initially performed well when presented with Lake Tikitapu water samples spiked with catfish aquaria, with hit rates and accuracy scores around 80%. However, the dogs' average correct rejection rates of control samples fell below 40% after a change in sample preparation to compensate for settling of sediment in the lake water. Despite adjustments in the protocol, such as reducing the

number of positive samples presented, increasing the concentration in these samples, and increasing indication thresholds, there were no improvements in the dogs' correct rejection rates. However, following a re-training phase, the dogs were returned to lake water samples and performed well, achieving hit and correct rejection rates above 80% at decreasing sample concentrations as low as 1.55 kg/ha.

Lake Tikitapu Samples and the Methodology Change

In the first 4 days of testing using water from Lake Tikitapu, the dogs achieved hit rates and accuracy scores of approximately 80% for both control and positive samples. However, after 4 days, it was observed that sediment which had settled to the bottom of the sample container was being disproportionately distributed to the positive samples. Sample preparation was adjusted so that the lake water was shaken every three samples to distribute the sediment more evenly. This change coincided with the dogs' correct rejection rates dropping significantly, while their hit rates remained high, indicating the dogs were no longer differentiating between the control and positive samples and were instead indicating on the majority of samples.

One common issue as to why a dog may fail to discriminate between control and positive samples is that the disincentive for making an indication response on control samples is insufficient. For example, during Little's (2020) study, the dogs had an initial bias towards indicating on samples during the discrimination stage of training, which resulted in a high number of false positives. As only indications on positive samples are reinforced with food, if a dog is presented with a sample that they are unable to discriminate, they may indicate on these samples as there is a chance of receiving food. Therefore, it is important to disincentivise

indicating on these samples, which can be achieved by decreasing the number of positive samples or increasing the dog's indication time threshold (Edwards et al., 2022). Therefore, in the current study, the number of positive samples were reduced to vary between five or six and some dogs' indication thresholds were increased from 4,501 ms to 5,001 ms and later, 6,001 ms. However, these changes were unsuccessful in resolving the dogs' poor discrimination performance.

It was hypothesised that there was instead an issue with the dogs' learning, and they were no longer targeting the catfish odour. Samples were prepared using dechlorinated water to test this hypothesis. If the dogs' correct rejection rates quickly improved, it would indicate that the lake water was preventing differentiation between control and positive samples. If correct rejection rates did not improve, it was likely the dogs were no longer targeting the catfish odour and remedial training would be required. When the dogs were presented with samples prepared with dechlorinated tap water, their correct rejection rates remained low and remedial training was initiated so the dogs would again indicate on the target catfish scent.

When the dogs were once again successfully differentiating between control and positive samples, they were returned to lake water samples. Furthermore, to avoid any learning that may have occurred with the Lake Tikitapu water samples, water from Lake Rotomā was used for sample preparation. In addition, a higher equivalent biomass concentration of 311 kg/ha was initially used for Cobie and 155,485 kg/ha for the other three dogs. These sample concentrations were then progressively decreased as the dogs successfully met the criteria at each concentration.

Higher catfish sample concentrations were presented as no previous literature has presented dogs with lake water samples spiked with catfish aquaria water, so the dilution at which dogs could successfully detect the presence of catfish was unknown. However, detection dogs frequently work in complex environments with many extraneous odours and, accordingly, training often incorporates a shift from less- to more-realistic detection scenarios (Cristescu et al., 2015; Wasser et al., 2004). In the current study, presenting the positive samples at a high catfish biomass concentration allowed for a clear contrast between the positive samples and the control samples which contained no catfish odour. This made it easier for the dogs to discriminate between the two sample types and identify the target odour. As the dogs achieved the criteria, indicating an ability to recognise the target odour at that dilution, the concentration of catfish odour in the positive samples was decreased to create more realistic detection scenarios.

Furthermore, in Little's (2020) study, two dogs, Cassie and Tink were presented with increasingly lower catfish sample concentrations and when they reached a dilution of 38,700 kg/ha, goldfish samples were also presented at the same dilution. This coincided with a drop in Cassie's and Tink's performances as they struggled to discriminate between the goldfish and catfish samples and instead indicated on all fish samples. The methodology was changed so the dogs were presented with undiluted goldfish samples which were then progressively diluted. Following this change in methodology, Cassie was able to discriminate between catfish and goldfish samples at an even lower sample concentration (4,600 kg/ha). Similarly, Cobie, Mika, and Tommy performed better in the current study when they were presented with lake water

samples spiked with a higher catfish concentration that was then progressively diluted. When the sample concentration reached the level presented to them in the first failed phase with lake water samples (43.5 kg/ha), they were successful in detecting the presence of catfish, with Cobie even surpassing that sample concentration and detecting catfish at a 1.55 kg/ha.

Lastly, the chemical composition of the lake water and how it might react with the catfish aquaria water was unknown. Lake water would naturally contain distractor or masking scents, and it is possible that at low catfish biomass concentrations, the catfish odour could be overshadowed or masked. Although Mika had initially achieved the criteria when presented with a positive sample concentration of 43.5 kg/ha in Lake Tikitapu water, this was voided as following the methodology change, she and the other dogs were unable to achieve hit and correct rejection rates ≥80%. As a result, it was unclear whether Mika had been detecting the presence of catfish when she met the criteria or if she was targeting another odour.

Therefore, it appears beginning at a higher odour concentration and progressively diluting it allows for a clearer contrast between the positive and control samples. This makes it easier for the dogs to discriminate between the sample types and may allow for better generalisation to different sample concentrations.

Number of Positive Samples

Through discrimination training, the dogs were taught to make an indication response when they encountered the target odour and to press the lever without indicating when they encountered a non-target. Only indications on the target odour were reinforced and as a result,

the target scent became a discriminative stimulus. That is, the target scent signalled to the dogs the availability of reinforcement on the condition an indication response was made and thus, evoked the indication response. Indications on the non-target odour (termed 'false alarm') were not reinforced, which should lead to the extinction of this behaviour (Edwards et al., 2022). Extinction is a phenomenon where a behavioural response that is not followed by reinforcement will decrease in frequency. Despite indications on the non-target odour being in extinction, the dogs in the current study were still exhibiting high numbers of false alarms when presented with Lake Tikitapu samples. However, the prevalence of a target has been observed to impact responses, with observers increasingly tending to incorrectly indicate that a target is present as the target prevalence increases (Wolfe & Van Wert, 2010). Furthermore, Edwards et al. (2022) demonstrated that when the prevalence of a target was low, domestic dogs demonstrated a bias toward indicating that the target was absent, but when a target prevalence was high, the dogs demonstrated a bias toward indicating that the target was present. However, the dogs' sensitivity to the target remained unaffected, as their hit and correct rejection rates did not change with target prevalence. Therefore, the dogs' high numbers of false alarms observed in the current study may have been due to a high prevalence of the target scent. As a result, the number of positive samples were reduced from seven to vary between five or six in the hope that with a low target prevalence, the dogs would display a bias toward indicating the target was absent on non-target samples while their sensitivity to the target remained unaffected (Edwards et al., 2022). Decreasing the number of positive samples consequently increased the number of control samples presented. Through experience, the dogs would learn that indicating on a non-target

sample produced a longer delay until their next reinforcer but making a rejection response reduced the delay until their next reinforcer and this reduction may also work as a reinforcer (Edwards et al., 2022). However, in this study, decreasing the number of positive samples was unsuccessful in resolving the dogs' poor discrimination performance. Therefore, the high number of false alarms may not have been due to high target prevalence, but rather an issue with the dogs' learning so they were no longer targeting the catfish odour.

The number of positive samples was reduced from seven to vary between five and six to prevent the dogs from ceasing work after the set number of positive samples had been presented and the maximum total of reinforcements attained. Although the dogs had not yet started doing this, the behaviour was observed in previous unpublished research from this laboratory, where the dogs discontinued working after the maximum number of treats they were usually given had been achieved. Therefore, varying the number of positive samples was simply a preventative measure.

Indication Threshold

The indication response required is critical in determining whether a bias exists in favour of indicating or rejecting a potential signal. For example, Edwards et al. (2021) evaluated the influence of response requirements on hens' signal detection accuracy and bias by manipulating the number of pecks required to make an indication response when the target was present. There was a clear relationship between the indication response requirement and bias, with the hens producing higher hit and false alarm rates at lower indication response requirements, and lower hit and false alarm rates at higher indication response requirements. In another study by Edwards

et al. (2022), the indication threshold required by dogs to activate a feeder was systematically increased in increments of 500 ms from 4,000 ms to 13,000 ms. As the indication response requirement increased, the dogs' hit rates declined, and their correct rejection rates improved.

These findings are in line with Fantino's (1969) Delay Reduction Theory, with the time required to make an indication response when the target is absent producing a delay to the next reinforcer. Therefore, by making a rejection response when the target is absent, the delay is reduced, and this reduction may serve as a reinforcer (Edwards et al., 2022). Based on these findings, when Tommy and Saydee's low correct rejection rates persisted, their indication thresholds were increased. However, increasing their indication thresholds was unsuccessful in resolving their poor discrimination performance. This may mean that making a rejection response was already sufficiently reinforcing, but that there was an issue with the dogs' learning so they were no longer targeting the catfish odour.

Factors Influencing VOC Release

Scent detection is based on the binding of VOCs to receptors in the dog's nasal epithelium (Craven et al., 2010). The concentration and transmission of VOCs is influenced by temperature and humidity (Richards, 2018) as greater evaporation of VOCs occurs at high temperature and low humidity (Chambers et al., 2015). The collected sample water was stored at 4°C when not being used to reduce VOC loss through evaporation. Conversely, the temperature of the experimental room was maintained at a warmer temperature (between 18- 20°C) to encourage VOC evaporation within the apparatus and improve scenting conditions for the dogs. The samples

were also contained within individual segments on the apparatus, containing and concentrating the VOCs, and could only be accessed by the dogs through a flap that was otherwise closed.

Turbulence and bubbles bursting are other factors that can enhance VOC release, with higher turbulence resulting in faster evaporation (Richards, 2018). This may have contributed to the large decrease in the dogs' correct rejection rates following the methodology change in Part B (Part 1). Shaking the bottle containing lake water every three samples caused turbulence and bubbles to burst. This may have resulted in VOCs being released and evaporated into the air each time the bottle was opened, and thus decreasing the concentration of VOCs present in the samples. Another possible explanation for the results is that the dogs may have learnt to indicate on certain VOCs present in the sediment. Prior to the methodology change, the dogs were reinforced for indicating on the positive samples that the sediment from the lake water had been disproportionally added to. Incorrectly reinforcing this behaviour could have led the dogs to target any VOCs that may have been present in the sediment rather than targeting the catfish odour. Therefore, when the methodology was changed and the sediment was spread throughout all the samples, it resulted in the dogs indicating on all the samples. However, the experimental design of this study did not allow for either hypothesis to be confirmed. Although it was not the aim of the project, ceasing shaking the lake water bottle for a period and evaluating the dogs' subsequent performances might have been advantageous in explaining the results received in Part B (Part 1).

Experience with Target Odour

Mika and Tommy were able to successfully detect the presence of catfish at the target sample concentration of 43.5 kg/ha and Cobie was successful at 1.55 kg/ha. However, Saydee was unable to meet the target sample concentration and the project ended while she was still working at 311 kg/ha. Cobie, Mika, and Tommy had participated in previous projects related to catfish scent detection, giving them 2-3 years of experience over Saydee, who was only brought onto the team in late 2021. Other research indicates that the more experienced a dog is with a particular scent, the more proficient they are at detecting it (Smith et al., 2003). Hence, a dog's performance on a scent detection task will improve with repeated practice. Experience may have contributed to the difference in dilutions achieved by Cobie, Mika, and Tommy when they were first presented with catfish samples in Little's (2020) study compared to the dilutions they achieved in the current study. Mika and Tommy successfully detected the presence of catfish at a sample concentration equivalent to 4,600 kg/ha in Little's study but achieved a sample concentration of 43.5 kg/ha in the current study. Likewise, Cobie was originally successful at a sample concentration equivalent to 38,700 kg/ha but achieved 1.55 kg/ha in the current study.

Therefore, Saydee's inability to successfully detect the presence of catfish at a biomass concentration of 311 kg/ha and lower, may be attributed to an inexperience with the target odour. Upon the evidence discussed, it is plausible that if Saydee was given further exposure to the odour, she could have met the target concentration. However, her ability in scent detection would need to be further investigated under laboratory conditions.

Relevant Findings in Aquatic Scent Detection

Little (2020) was the first to show that dogs could discriminate between catfish and goldfish in dechlorinated water samples, rejecting the samples containing goldfish and indicating only on the catfish-positive samples. However, the sample biomass concentrations employed in that study were significantly higher than those naturally present in the environment. Denby (2021) followed this up by showing these dogs could discriminate between catfish and goldfish in dechlorinated water samples but at biomass concentrations equivalent to, and lower than, those in New Zealand's aquatic systems. In Denby's study, Mika was able to successfully discriminate between control, goldfish, and catfish-positive samples at a biomass concentration of 4.6 kg/ha. Tommy could successfully discriminate between the three sample types at a biomass concentration of 9.2 kg/ha. At 4.6 kg/ha, his correct rejection rate was below 80% for goldfish samples, but he was able to successfully discriminate between control and catfish-positive samples. Cobie could successfully discriminate between all three sample types at 18.6 kg/ha. Similar to Tommy, her correct rejection rate was below 80% on goldfish samples at a biomass concentration of 9.2 kg/ha, but Cobie could discriminate between control and catfish-positive samples at this concentration. Lastly, the current study demonstrated that these dogs could detect the presence of catfish aquaria in real-world lake water samples and at a biomass concentration consistent with catfish population estimates in several New Zealand lakes (Hicks et al., 2015; Tempero et al., 2019). This also means the dogs could successfully discriminate catfish odour from the other fish odours and potential distractor scents present in the lake water samples. Furthermore, Cobie achieved a dilution lower than the limit reported by Collins et al.

(2022) for koi carp. Collins et al. (2022) found domestic dogs could reliably detect koi carp odour at a dilution equivalent to 9.3 kg/ha in dechlorinated water samples, but were unsuccessful at 4.7 kg/ha.

In Denby's (2021) study, Mika and Tommy could successfully detect the presence of catfish at a biomass concentration as low as 4.6 kg/ha and Cobie at a biomass concentration of 9.2 kg/ha in dechlorinated water samples. Although Mika and Tommy reached a biomass concentration of 43.5 kg/ha in lake water samples in the current study, based on Denby's results and Cobie's ability to detect catfish at a biomass concentration of 1.55 kg/ha in lake water samples, it is likely Mika and Tommy could have achieved even lower dilutions if the project had not had to finish due to time constraints.

Therefore, the findings from the current study suggest that dogs can detect the presence of catfish at a biomass equivalent concentration of 1.55 kg/ha in lake water samples. In New Zealand, it is highly unlikely significant environmental impacts would occur from a catfish biomass of 1.55 kg/ha (Hicks & Allan, 2019; Francis, 2019). However, targeted eradication of a population of this size (equivalent to 5-8 adult fish) would be highly advisable, as they can quickly reach population abundances resulting in negative environmental impacts. Although the effectiveness of an eradication programme would be highly dependent on resourcing, size of the target water body and its connections to other waterways (Collier & Grainger, 2015), these findings indicate the potential utility of domestic dogs as an early detection tool for invasive freshwater species.

Advantages of Scent Detection Dogs for Aquatic Invasive Species Detection

There are many benefits to using dogs over other traditional fish detection methods. For example, the collection of lake water samples for this project was minimally invasive compared to methods such as netting and electrofishing, which can result in by-catch, or the accidental harm or mortality of specimens (de Villiers, 2013; Snyder, 2003). The procedure employed in this research simply required filling a bottle with lake water, albeit with careful controls for cross-contamination. The water was then taken back to the laboratory for presentation to the dogs.

Traditional survey methods such as visual surveying, netting, and electrofishing can be difficult to conduct in areas with poor boat access or in remote locations. Similarly, these methods are restricted to relatively shallow water (<3 m). For example, backpack electrofishing is limited waters of a wadable depth, making it difficult to capture benthic species such as catfish (Ward et al., 2015). The time and labour requirements for such surveys are also extensive, restricting the number of locations that can be surveyed (Hicks et al., 2015).

In comparison, the automatic apparatus used in the current study is able to present up to 17 samples to the dogs per session, with a typical session taking a matter of minutes. With multiple dogs being presented with the same samples six to eight times, a reasonable level of accuracy can also be achieved. As a result, the number of locations that can be surveyed could be significantly greater than traditional fishing methods given restricted resourcing.

The use of pet dogs for scent detection provides advantages in terms of animal enrichment, welfare and ethical considerations, and reduced research costs. The use of

laboratory dogs would require providing appropriate housing, exercise, food, and stimulation for the dogs (Orkin et al., 2016). However, using pet dogs reduced these ethical obligations and their corresponding expenses. Instead, the pet dogs were brought into the laboratory twice per week by their owners and picked up at the end of each day. While the dogs were provided treats as reinforcement for indicating on positive samples, their owners were responsible for feeding them. In return, the laboratory work provided the dogs with stimulation and enrichment.

Using pet dogs also provided a way to reach the community and educate them about invasive fish in New Zealand and the adverse impacts they have on our freshwater ecosystems. For example, the owners and volunteer dog walkers may have shared their knowledge of what the dogs were working on and why. Furthermore, the dogs also attracted media attention, and stories involving dogs are more likely to be spread across media platforms, resulting in a wider reach. There was also the opportunity to reach the community through speaking engagements, interviews, and demonstrations.

Disadvantages of Using Scent Detection Dogs

Over the course of this study and the related previous projects, a large number of candidate dogs were found unsuitable and did not progress to the training stage. While most dogs possess an impressive olfactory acuity, their success in scent detection also depends on their cognitive and learning abilities (Jezierski et al., 2014). In Little's (2020) study, of the 13 dogs found suitable to begin the training program, only four completed training. Three of these dogs continued in Denby's (2021) study and a further two dogs were recruited but later withdrawn. Some dogs were withdrawn due to changes in the owner's circumstances, however one of the

most common reasons was due to the dog exhibiting a lack of motivation to perform the task for food reinforcers. Maejima et al. (2007) identified two key components that may determine a dog's success in drug detection training: desire for work, and distractibility, with desire for work significantly related to their successful completion of training. Therefore, with a requirement for high olfactory, cognitive, and learning capabilities, as well as a strong motivation to work, only a small minority of dogs possess the traits to complete scent detection training (Rooney et al., 2004).

Some dogs were found unsuitable due to them exhibiting a stress response when separated from their owner. However, as handler cueing effects have been observed to occur in scent detection tasks with dogs even under double-blind conditions (Lit et al., 2011), this study required the dogs to work in a room alone. This may be an issue as many domestic dogs have been observed to exhibit separation-related behaviours. For example, 20-40% of the cases at behaviour specialty clinics in North America were for the treatment of separation anxiety (Simpson, 2000, as cited in Ogata, 2016) and in a U.S. telephone marketing survey, 14-17% of pet dogs were reported to experience separation anxiety (Sherman & Mills, 2008, as cited in Ogata, 2016). However, this number is likely to be even higher as 50% of 94 randomly selected dog owners reported separation-related behaviour, yet only 13% of the affected dogs' owners had attempted any kind of professional help (Bradshaw et al., 2002). Therefore, with separation-related behaviours prevalent in dogs, it is likely many domestic dogs would be found unsuitable for this research.

Another disadvantage of using scent detection dogs is that the time taken to train them can be extensive, with research suggesting more than 4 hours per week of training is positively correlated with completion of training in search dogs (SWGDOG, 2005, as cited in Alexander et al., 2011). However, pet dogs are often not available every day of the week and they can miss days or longer periods due to activities by their owners. This can prolong the time taken to train them or they may regress in their training (Helton, 2009). For example, Saydee needed to begin her training again due to a break of around 6 weeks between the end of the previous catfish scent detection project and the start of this project.

Even when trained, there are often setbacks in dogs' progress due to health complications, disruptions in their home routine, or changes in handlers (Jamieson et al., 2018). For instance, when Saydee was presented with sample concentrations of 155,484 kg/ha and 31,097 kg/ha, her hit and correct rejection rates remained mostly above 80% and she met the criteria within two to four sessions. However, the week she was presented with a sample concentration of 311 kg/ha, her owners went away on a trip and this coincided with a drop in her performance. While it cannot be concluded this change in routine influenced her decrease in performance, instances such as this occurred with every dog. For example, Tommy's family reported he was sometimes disturbed during the night due to a new baby in the family. This coincided with a decline in Tommy's motivation and he began to lie down repeatedly throughout his sessions. As a result, the number of sessions he completed per day was reduced in an effort to improve his motivation.

Collins et al. (2022) also found that the dogs were less successful in differentiating between the closely related species of koi carp and goldfish. In Collins et al.'s study, domestic dogs had to discriminate between samples containing goldfish odour or control aquaria water from those containing the target koi carp odour. While false alarms occurred on both non-target sample types, they occurred more commonly on the goldfish samples. Goldfish and koi carp are closely related, meaning they are likely to have similar scent profiles (Chistiakov & Voronova, 2009). This may have made it difficult for the dogs to discriminate between their odours, resulting in the occurrence of false positives. In a real-world scenario, false positives can result in the misplacement of already scarce resources by governmental agencies, local authorities, and iwi/ hapū.

Conclusion

This study was the first to demonstrate that dogs have the ability to detect the presence of catfish in lake water samples at operationally useful dilutions. The scientific rigour employed in this study demonstrated that dogs can successfully differentiate between catfish odour and the other distractor scents present in the lake water and were able to differentiate between control and positive samples at levels much higher than chance. These results add to the growing body of evidence for the potential of domestic dogs in aquatic biosecurity and in the detection and management of invasive freshwater species such as catfish. This has significant ecological implications for the conservation of New Zealand's freshwater ecosystems, with the potential utilisation of domestic dogs as a detection tool for invasive fish. This study also generates more

scientific enquiry into other uses of training domestic dogs for wider commercial and biosecurity purposes.

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Appendix 1: Initial Assessment Forms

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Thank you for your interest in our dog behaviour research. We are looking for dogs who enjoy going to new places and meeting new people – and who really like working for food. We have some other criteria for potential research participants, so if you are interested in your dog possibly taking part, please provide the following information.

Is your dog fully vaccinated (standard vaccines: distemper, hepatitis, parvovirus, leptospirosis)?

Yes No

If **no**, please explain briefly:

_____

_____

_____

Does your dog enjoy meeting new people?

E.g., are they friendly and comfortable around strangers?

Yes No

If **no**, please explain briefly:

_____

_____

_____

Is your dog comfortable being handled by other people?

E.g., is your dog happy to be touched on their body, neck, head, tail, paws, etc.?

Yes No

If **no**, please explain briefly:

_____

_____

_____

Is your dog comfortable going to new places?

E.g., is your dog relaxed and happy (showing no signs of stress) when you go somewhere new?

Yes No

If **no**, please explain briefly:

_____

_____

_____

Is your dog comfortable when you leave them, including at home alone and new places?

E.g., is your dog relaxed and happy (showing no signs of stress) when you leave them?

(Dogs will not be left alone at our training facility, but we would like to know if they might have any separation-type anxieties).

Yes No

If **no**, please explain briefly:

_____

_____

_____

Does your dog like working for food?

Yes No

If **no**, please explain briefly:

_____

_____

_____

Can your dog eat any food, including kibble (biscuits) and different kinds of meat products?

Yes No

If **no**, please explain briefly:

_____

_____

_____

Is your dog comfortable with people getting near their food?

E.g., if your dog has shown any aggression (freezing, growling, snarling, biting) around food, please select 'no'.

Yes No

If **no**, please explain briefly:

_____

_____

_____

Is your dog friendly towards other dogs?

E.g., if your dog has shown any aggression or fear towards other dogs, please select 'no'.

(We will not necessarily have more than one dog at the training facility at once. If we do, it will be with permission of all owners and the dogs will be contained separately.)

Yes No

If **no**, please explain briefly:

_____

_____

_____

Is your dog comfortable with unexpected/loud noises, such as beeping sounds?

Yes No

If **no**, please explain briefly:

_____

_____

_____

Is your dog free of medical conditions that could be aggravated by repetitive walking?

E.g., if your dog has any joint or other problems that might be affected, please select 'no'.

Yes No

If **no**, please explain briefly:

_____

_____

_____

Would you be able to drop off and pick up your dog in the morning/afternoon so that your dog spent just half a day with us (our facility is at the University of Waikato main campus)?

Yes No

Please indicate which times are more convenient:

_____

_____

We want to make sure that all dogs enjoy participating in our research. If you answered "no" to any of these questions, this may indicate that your dog is not suitable for some of this research; however, it does not necessarily exclude them from taking part. A researcher will be in touch with you to discuss the information you have provided here. Thank you for taking the time to complete this form.

Please email this form to: *insert researcher's email*

Thank you for your interest in our dog behaviour research.

Please provide us with the following contact information:

Owner's name	
Mobile phone	
Home phone	
Email address	
Home address	
Most convenient days & times to drop off/collect your dog?	
How did you hear about this research?	

Please provide us with the following information about a nominated emergency contact person (in case we cannot contact you):

Nominated contact person	
Mobile phone	
Home phone	
Email address	

Home address	

Please provide us with the following information about your dog's normal veterinarian:

Normal vet clinic	
Normal veterinarian	
Clinic phone	
Clinic address	

Please provide us with the following information about your dog:

Dog's name	
Breed	
Date of birth	
Age	
Sex	
Are they de-sexed?	
Weight	
Colour & distinguishing features	
Fully vaccinated?	

When are their next vaccinations due? *	
Normal food type	
Normal mealtimes/amounts	
Favourite food type	
Any aggression around food?	
Allergies/illnesses	
Behaviour issues	
Other likes/ dislikes (e.g., other dogs, being touched, being alone, noises, etc.)	

Note. *Please bring your dog's vaccination records with you for your first visit to the training facility.

Thank you for taking the time to complete this form.

Please email this form to: *insert researcher's email*

Appendix 2: Owner Consent Form

CONSENT FORM
Researcher's Copy

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

These protocols have been approved by the Animal Ethics Committee of the University of Waikato.

As the owner or duly authorized agent for the owner of _____ you are being asked to have your pet participate in the project evaluating dogs' ability to identify certain chemicals commonly used in scent detection research. Before giving your consent to your pet's participation, please read the following, ask as many questions as needed to understand what your participation involves, and sign and date the statement at the end of this document.

Principal Investigators

Lauren Hopkins, *insert mobile number, email address*

Dr Tim Edwards, *insert mobile number, email address*

Dr Clare Browne, *insert mobile number, email address*

Purpose of the Project

1. I certify that I am over the age of 18 and hereby grant permission for my pet to participate in a research project designed to evaluate dogs' ability to identify water that has contained specific species of fish.
2. I have been informed about the purpose of the project and what my dog is going to do.

Description of Procedure

Samples will be presented to dogs via an automated carousel apparatus that turns, presenting multiple samples, one by one. The dogs will be trained to sniff each sample, and to indicate if the samples do/not contain certain chemicals commonly used in scent detection research. Training will be achieved using food treats as positive reinforcement.

I understand that my dog will only participate in the project if willing to do so and will be humanely treated at all times as described in the Standard Operating Procedures for Handling and Care of Pet Dogs for Research, which has been approved by the University of Waikato Animal Ethics Committee.

Costs to Owner

I shall be responsible for all costs related to illness or treatment of problems unrelated to the experiment.

Withdrawing my Pet from the Project

I understand that participation in this project is entirely voluntary and that I may withdraw my pet at any time without any negative consequences. I understand that my dog might be withdrawn from the project if a vet finds it is necessary and in my dog's best interest.

If I have additional questions regarding this project, I may phone or email the principal investigators.

Additionally

I understand that participation in this project involves a commitment to bring my pet to the dog facility according to a schedule realised in cooperation with the researchers. Upon

completion of the research, I will have access to my dog's data and the general findings from the research project.

Authorisation

I have read and understand the foregoing statements and agree to allow my pet to participate in this project. Upon signing below, I will receive a copy of this consent form.

I give consent for my dog to be at the research facility in the presence of other dogs:

Yes No

My dog is friendly towards other dogs:

Yes No

I give consent for videos of my dog to be shown for other purposes (presentations, lectures, etc.):

Yes No

Pet's name: _____

Owner's name: _____

Owner's signature: _____ Date: _____

Researcher's signature: _____ Date: _____

Appendix 3: Standard Operating Procedure for Training Dogs for Scent Detection Work Using Automated Apparatus

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

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.

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 three times in a row to continue to the next stage of training.

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 (see Guide for Shaping below). 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 1,000 ms for the minimum indication time and 500 ms for observation time. 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 1,000 ms threshold is complete, increase the threshold in 100-500 ms intervals to 4,501 ms. Once a run is complete at 4,501 ms, continue to the next step.

Shaping – Lever Activation

With the apparatus unloaded and turned off, use the method of differential reinforcement of successive approximations to shape lever pressing (see Guide for Shaping below). 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 proceed to the next step.

Discrimination Training

Load the apparatus with approximately half positive and half negative samples (e.g., eight negatives and nine 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 substance.

Bring the dog into the experiment room and stand 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 of four sessions in a row before it needs 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.

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 two out of three sessions.

Troubleshooting 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 have been no significant changes in the dog's 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
- 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 the dog continues to perform poorly, consult with your 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.

Guide for Shaping

Introduction

This document outlines the basic training hierarchy for shaping by successive approximations. As a general rule, each step must be completed three times in a row before

progressing to the next stage of training. Some dogs may require additional learning trials before progressing. Keep sessions short (under 5 minutes) and finish on a positive note (e.g., reinforcement) when possible to ensure that the process is enjoyable for the dog.

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.

Shaping of nose to port

1. For initial sessions, the apparatus should be turned off and not loaded with samples. 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 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 the dog breaking the beam and pushing the flap inward, until the dog is fully opening the flap (nose is fully inside the port).

Shaping of lever press

1. Turn the 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 4: Cleaning SOP

Basic Scent Detection (Fish Projects) Acid Washing Standard Operating Procedure

Purpose

This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during the acid washing of glassware 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.

Placing into acid bath

1. Separate acid buckets containing 10% HCl and 90% reverse osmosis (RO) water should be designated to each of the different sample types. Separate long rubber latex gloves should also be designated to the different sample types/buckets.
2. Sample bottles should be emptied prior to acid washing and should be handled in order from negative to positive sample type.
3. A lab coat, safety glasses and disposable gloves should be worn. The designated long rubber latex gloves are then worn over the disposable gloves.
4. Remove the lid from the designated bucket.
5. Remove the lid from the sample bottles to be placed in that bucket.
6. 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.
7. Replace the bucket lid.

8. Rinse the rubber latex gloves under the tap and return them to the correct space beside their designated bucket.
9. Dispose of and change disposable gloves.
10. Repeat steps 3-9 for each sample bottle type.

Taking out of acid

1. Once the glassware has been soaking in the acid for at least 12 hours they can be removed.
2. Bottles should be handled in order from negative to positive sample type.
3. A lab coat, safety glasses and disposable gloves should be worn.
4. Wear the designated rubber latex gloves.
5. Remove the lid from the bucket.
6. Remove the sample bottles and lids from the bucket, tipping the acid out of the bottles carefully and slowly to avoid splashing.
7. Place the bottles and lids into the sink
8. Replace the acid bucket lid.
9. Rinse the bottles and lids in the sink with RO water, then place them on the drying rack for transport to the drying incubator.
10. Rinse the long rubber latex gloves under the tap and return them to the correct space beside their designated bucket.
11. 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.

12. Dispose of and change gloves.
13. Repeat steps 3-11 for each sample bottle type.
14. Leave the sample bottles and lids in the incubator to dry.
15. Once dry, wearing gloves, put each sample bottle and their lid into separate zip-lock bags. Change gloves in between each sample bottle type.