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**Fish-finding Fido;
Can Domestic Dogs (*Canis familiaris*) Offer a Solution for
Detecting an Invasive Freshwater Catfish (*Ameiurus nebulosus*)**

A thesis
submitted in partial fulfilment
of the requirements for the degree
of
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by
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Abstract

Invasive species now dominate many aquatic landscapes in most parts of the world, displacing native plants and animals by disrupting and altering ecosystems. The brown bullhead catfish (*Ameiurus nebulosus*) has been significantly correlated with regime shifts from macrophyte-dominated clear water states to de-vegetated turbid states, population declines of endemic species, and the disruption of food webs in New Zealand lakes. Conventional detection methods (e.g., visual surveys, fyke netting, electrofishing, and eDNA) for catfish are limited by their cost, invasiveness, time- consumption, and potential to be prone to error. Given that scent detection dogs are a well-established tool across a variety of fields, it was hypothesised that domestic dogs (*Canis familiaris*) may be able to detect the presence of catfish from water samples that have contained these fish. This could provide a new potential biosecurity monitoring tool.

In this study, five pet dogs were trained to operate an automated apparatus that presented water samples for evaluation. Water samples were presented to dogs from aquaria that had previously contained, catfish, goldfish (*Carassius auratus*), or no fish. Experiment 1 evaluated if dogs could discriminate between samples that had contained catfish or no fish. In Experiment 2, it was evaluated if dogs can discriminate between fish species (i.e., catfish and goldfish), and at what fish biomass concentrations they can do so. Experiment 3 evaluated if dogs could indicate the presence of catfish when samples were presented at two different biomass concentrations in the same session. It was found that dogs were able to correctly identify water that had contained catfish and largely reject water samples that had contained either no fish or goldfish at above 80% accuracy at biomasses equivalent to environmental biomasses of 4.6 x 1,000 kg/ha. Preliminary investigations of lower detection limit thresholds were investigated in the study. These results suggest further investigation is warranted to confirm the dogs' ability to detect catfish at biologically relevant concentrations comparable to real-world sample scenarios. However, these findings support the suggestion that dogs may have an important role to play in waterway conservation and management.

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Chapter One: Introduction

1.1 Impacts of introduced fish

Potable, inhabitable water is a cornerstone value of healthy aquatic ecosystems, and water quality is of inestimable inherent and economic value to the surrounding human communities (Richards, 2018). The demand for fresh water is increasing as the human population grows and land usage changes, but in some places, freshwater quality is decreasing due to these changes (Ministry for the Environment, 2020). The water clarity influences productivity and affects how water can be used, which is why many countries, including New Zealand, have set standards for improving water quality (Vant & Davis-Colley, 1984).

The introduction of exotic fish species can have numerous potential impacts on freshwater ecosystems. These impacts can include reduced water quality, increased competition and predation disrupting food-webs, hybridisation and introgression, and the introduction of associated parasites and diseases (Ellender & Weyl, 2014). In particular, the sediment grubbing feeding style of benthivorous fish has been shown to reduce water clarity and increase turbidity in lakes (Adámek & Maršálek, 2013). In shallow lakes (< 4 m deep), the disturbance of bottom sediments increases water column turbidity, reducing light availability to submerged aquatic plants that stabilise the lakebed by preventing wind-driven sediment resuspension (Badiou & Goldsborough, 2011). At high densities, large-bodied benthic feeding fish such as common carp (*Cyprinus carpio*) and brown bullhead catfish (*Ameiurus nebulosus*) can uproot submerged plants, further increasing turbidity due to wind-driven wave resuspension (De Winton, Taumoepeau & Clayton, 2002; Badiou & Goldsborough, 2011). Furthermore, increased sediment resuspension from benthic feeding fish and wave action can increase nutrient availability, resulting in algal blooms and further reductions in light availability (Dieter, 1990; Roozen et al., 2007). For example, brown bullhead catfish have been significantly correlated with regime shifts from macrophyte-dominated clear water states to de-vegetated, turbid states in New Zealand lakes (Rowe 2007; Schallenberg & Sorrell 2009).

The introduction of an exotic competitor or predatory species can also result in population declines of endemic species and disruption of food webs. For example, Lake Victoria in Africa suffered a loss of almost 200 endemic cichlid species after

piscivorous Nile perch (*Lates nilotica*) was introduced as a potential commercial species (Rahel, 2002). In New Zealand, brown trout (*Salmo trutta*) introduced by European recreational fishers are a recognised threat to many endemic galaxiid species, competing for macroinvertebrate prey and preying on smaller native fish species such as banded kokopu (*Galaxias fasciatus*) and inanga (*Galaxias maculatus*) (McIntosh et al., 2010). Benthivorous species such as brown bullhead catfish are also known to prey on native kōura (*Paranephrops* spp.) (Barnes, 1996; Francis, 2019). Tempero et al., (2019) reported that the removal of common carp and brown bullhead catfish from Lake Ohinewai in the Waikato region of New Zealand was associated with increased abundance and size of native eels (*Anguilla* spp.), presumably through reduced competition. This hypothesis was somewhat supported by comparative stable isotope and gut content analysis of brown bullhead catfish and New Zealand native shortfin eels (Collier et al., 2018). Despite native eels' diet consisting primarily of fish, and the majority of brown bullhead catfish prey being macroinvertebrates, there was considerable dietary overlap between the two species (Collier et al., 2018). These findings indicate that the impacts of fish introductions can extend through the food-web system.

Hybridisation and introgression between native and invasive fish species are of growing concern overseas as the resulting interbreeding can result in outbreeding depression. Outbreeding depression can manifest in three ways; sterility of the resultant offspring, adaptive differentiation, and genetic bottleneck (Largiadier, 2008).

New Zealand has a lengthy history of introducing exotic species, both terrestrial and aquatic. While some of the introductions of exotic fish species to New Zealand waterways were accidental, some were deliberate in order to create new recreational fisheries in lakes where salmonid and coarse fish species were not present (Rowe, 2007). One introduced exotic species of interest in recent years is the brown bullhead catfish.

1.2 The brown bullhead catfish

The brown bullhead catfish is a relatively small catfish species, dark brown to olive green in colour, growing up to 50 cm and weighing upwards of 3 kg (McDowall, 1990). They are characterised by their scaleless stout body, sharp spines at the front

of their pectoral and pelvic fins, and a ventrally flattened head with four pairs of barbels around the mouth. Their native range is the fresh and brackish waters of North America, from the east of the Rocky Mountains of Canada through to the Central United States of America (McDowall, 1990).

The brown bullhead, referred to herein as catfish, is the only species of catfish currently found in New Zealand (Collier & Grainger, 2015). Catfish were first introduced in 1877 when 140 individuals were placed into Saint John's Lake in Auckland as a game fish (Collier & Grainger, 2015). While some of the subsequent introductions of catfish to the smaller and warmer North Island waters were accidental, others were deliberate to create new recreational fisheries (Rowe, 2007).

Catfish are predominantly distributed from Lake Taupō to Northland, as well as having small isolated populations throughout the remainder of the North and South Islands (Figure 1), inhabiting sluggish, slow-flowing weedy streams, sandy shallow lakes, and lagoons (Collier & Grainger 2015). In the wild catfish are known to live up to eight years, maturing between 2-3 years of age. Catfish are one of few freshwater fishes that show parental care for their young, the male will fan the eggs during development and guards the larvae for up to 29 days after hatching (Collier & Grainger, 2015). They are repeat spawners laying up to 6,000 eggs at a time in depressions on the substrate in the shallows. Catfish spawn from September through to December, but they are also known to spawn later in the Waikato Region from December through to February (Patchell, 1977; McDowall, 1990; Barnes, 1996; Bannon, 2001).



Figure 1. Distribution of the brown bullhead catfish in New Zealand. Source: Data from the New Zealand Freshwater Fish Database (NZFFD).

The hardy nature of catfish makes them capable of living in a wide range of temperatures and poor water quality environments that other species cannot, making them resilient and difficult to eradicate (Collier & Grainger, 2015). Catfish can tolerate temperatures from 0°C up to 38°C, depending on acclimation temperatures (Scott & Crossman, 1973). They are well-known for their ability to tolerate poor water quality, including dissolved oxygen levels as low as 0.2 ppm in winter (Scott & Crossman, 1973). They also experience higher fecundity, and larger eggs in polluted areas with sediments contaminated with heavy metals, polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs) (Lesko et al., 1996).

As indiscriminate benthivorous feeders, catfish's diet includes plants, detritus, insects, crustaceans, molluscs, and small fish, competing with carnivorous native fish, such as eels, while also recently being linked to the decline of kōura populations (Collier et al., 2017; Francis, 2019). They are bottom-dwelling feeders, using their barbels as sensors that stir up sediments, resulting in reduced water quality by suspending sediments in the water column and releasing nutrients, especially nitrogen and phosphorus (Collier & Grainger, 2015). Hicks et al. (2001), suggested that increased rates of nutrient cycling caused by catfish may contribute to higher productivity in Lake Ngaroto. Under current legislation, the brown bullhead has no legal status, but they are considered a pest fish in many regions, meaning that they must be killed upon capture and not returned to the water alive (Collier & Grainger, 2015).

An example of how invasive catfish are and how quickly they can populate waterways is the recent incursion in the Te Arawa lakes of the Rotorua district of North Island New Zealand (Figure 2). In 2009, a dead catfish was found on the shore of Lake Rotoiti, and despite extensive dive and net searches, no live catfish were ever caught (Blair & Hicks, 2009).

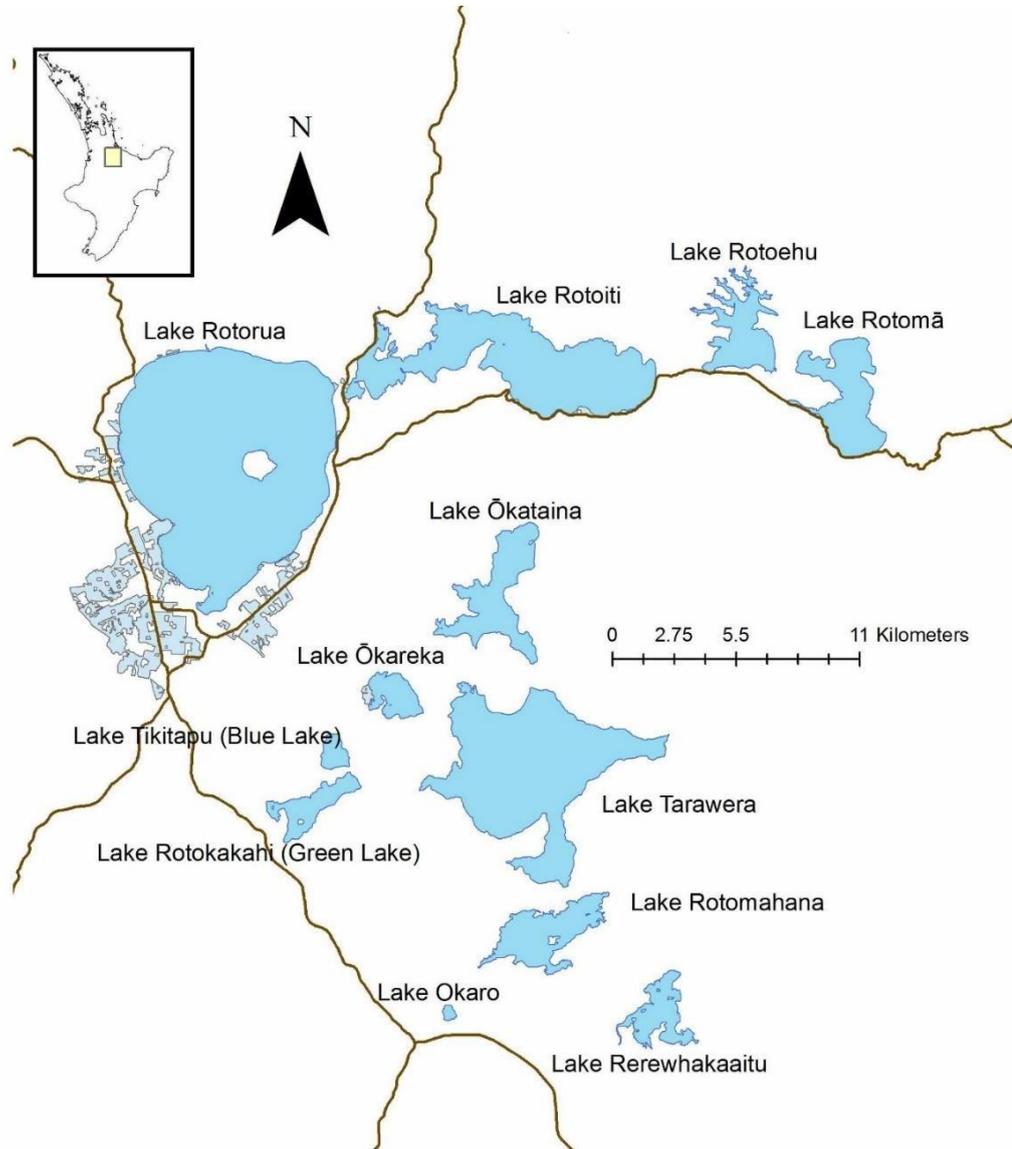


Figure 2. Map of the Te Arawa lakes of the Rotorua District, North Island New Zealand.

In March 2016 one catfish was caught and a second was seen during weed harvester work in Te Weta Bay, Lake Rotoiti. These findings led to more extensive netting and the installation of a cordon to control the catfish population. Despite these efforts, by 2018 catfish were detected in most of western Lake Rotoiti and the Ohau Channel, which links Lake Rotorua and Lake Rotoiti. Since that discovery in 2018, more than 70,000 catfish have been caught and destroyed by the Te Arawa Lakes Trust eradication program (Bay of Plenty Regional Council, 2019).

Currently, catfish are only found in lakes Rotoiti and Rotorua within the Bay of Plenty Region, and organisations such as the Te Arawa Lakes Trust and Bay of Plenty Regional council wish to monitor their population, prevent their spread to the other lakes in the region, and eradicate them if possible. The control

and monitoring of invasive fish species are usually expensive, and it is rare for complete eradication to be possible. It is also important that fish sampling methods do not facilitate the spread of invasive species (Joy et al., 2013). Catfish are known to survive out of water for extended periods so long as they are kept moist, making them easy to spread inadvertently if attached to nets or caught in boat trailers (Collier & Grainger, 2015).

To successfully make accurate decisions regarding freshwater management, the use of a consistent and appropriate sampling method is required. It allows regional councils and other governing bodies to robustly report on freshwater fish diversity, as well as invasive fish incursions and recruitment patterns within river systems, especially in poorly surveyed lowland habitats. Such data are critically needed given that two-thirds of aquatic native fauna is currently ranked with a threat status of declining or worse (Joy et al., 2013).

1.3 Review of current fish detection methods

Freshwater fish are generally visually cryptic, making obtaining measures of their distribution and population sizes difficult (Grainger, Goodman, & West, 2013). Most conventional monitoring and detection methodologies are reliant on visual observation and counting, while others employ the use of offsite technologies (e.g., environmental DNA). Even the most robust method can be compromised by inappropriate application or poor design. The best way to choose a method is to make a cumulative decision based on many factors such as site constraints (e.g., water velocity, depth, vegetation) and catch selectivity (Grainger et al., 2013). Some commonly used detection methods for catfish are discussed below.

1.3.1 Visual survey & fyke netting

Visual surveys of shallow lakes and rivers usually take place in the summer months, as fish are generally more active and susceptible to being seen and captured as compared to colder months (Joy et al., 2013). Spotlighting is a particularly suitable visual survey method, as many freshwater fish species are nocturnal. An example of how this method may be carried out is that a suitable site of 150 m is selected, then marked in sub reaches, and is monitored 45 minutes after sunset. A spotlight beam is scanned over the water surface by one team member, who visually spots fish within the beam and calls out species identifications to another team member

who assigns the fish into a size category. A few fish are captured using dip nets to record their length and aid in size estimations (Joy et al., 2013).

Several considerations must be made when conducting visual surveys such as poor visibility, and spawning or climatic events that can cause fish to be displaced from the survey area or burrow further into cover, as these present the risk that fish may be present but not seen by the surveyor (Joy et al., 2013). In New Zealand, visual surveys are rarely conducted for catfish as they often inhabit waters with low clarity.

Fyke netting (Figure 3) is the most common and effective netting method used to survey for catfish (Joy et al., 2013). Fyke nets are deployed along the edges of rivers and lakes, and they are essentially a type of cylindrical fish trap which contains a series of funnel-shaped openings which make it easy for fish to enter the trap but very difficult for them to make their way out. One or more vertical sections of netting, called leaders, extend from the mouth of the fyke net and guide swimming fish into the net. There are several advantages to these nets; they can usually be set and lifted by one person, they can be set in shallow or deeper water so long as the tunnel is submerged, and fish caught using this method are not normally injured or killed. However, these nets are difficult to set where the substrate is uneven, or if there is an abundance of dense vegetation or obstructions such as logs or stumps. Waterfowl can be caught and drown in fyke nets if the front hoop is not submerged, and diving birds can be drowned even if the entire net is submerged. Predation of smaller fish by larger fish within nets can occur, and equipment can be prone to vandalism or theft if left unattended (Joy et al., 2013). The effectiveness of fyke netting for capturing catfish was reviewed by Hicks et al. (2015), who found that fyke netting was found to be 21-52% efficient for capturing catfish when comparing actual catches to population estimates during mark-recapture and fish removal in Lake Kaituna, Waikato region.



Figure 3. Fyke net being set. Source: provided by Dr Grant Tempero.

1.3.2 Electrofishing

There are two electrofishing options available; backpack electrofishing is generally conducted in hard-bottomed wadable streams or shallow water bodies, while boat electrofishing is commonly used to survey lake and river margins (Hicks, Osborne, & Ling, 2006; Joy et al., 2013; Hicks et al., 2015). Catfish generally inhabit soft-bottomed still or slow-moving waters with depths greater than 1 m, making backpack electrofishing unsuitable for capturing these fish.

The University of Waikato operates New Zealand's only electrofishing boat (Figure 4). A three-person crew (one driver and two netters) operates a pontoon-hulled aluminium vessel equipped with a DC generator that emits electrical pulses between a submerged anode and cathode in front of the boat (Hicks et al, 2006; Hicks, Daniel, Ling, Morgan, & Gauthier, 2015). The electrical field produces taxis in nearby fish, triggering muscle contractions and causing them to swim involuntarily towards the submerged anodes, and they experience narcosis upon arriving at an anode and often float belly-up to the surface where they can be scooped up by dip netters (Hicks, Jones, de Villiers, & Ling, 2015). This method is recommended for shallow water where boating is possible, as the effective fishing field only extends 4 m in width in front of the boat and only reaches a depth of 1-2 m below the surface. The use of this method in deeper water is likely to be inefficient and can underestimate species abundance, as bottom-dwelling catfish would be unaffected by electrofishing in water deeper than 4 m (Magnuson, 1994; Banks & Hogg, 2015).



Figure 4. University of Waikato electrofishing boat in operation. Source: provided by Dr Grant Tempero.

The use of this method is also dependent on habitat conditions, especially conductivity (the range of conductivity in which electrofishing is generally effective is about 50–500 $\mu\text{S}/\text{cm}$), and netting efficiency can be affected by current velocity, underwater obstructions, vegetation, and poor visibility due to the suspension of sediments in soft-bottomed waterways (Magnuson et al., 1994; Joy et al., 2013; Banks & Hogg, 2015). Boat electrofishing is also expensive compared to other capture methods because of the equipment capital cost and the number of crew required (e.g., netting). A conservative estimate of cost (not including expenses associated with travel, equipment, consumables, depreciation, and maintenance) is approximately \$480 (NZD)/per person per day (Hicks et al., 2015). Capture efficiency of catfish using electrofishing is also low compared to fyke netting, only 2–6% compared to 21–52% when comparing actual catches to population estimates during mark-recapture and fish removal in Lake Kaituna, Waikato region (Hicks et al., 2015).

The impact of electrofishing on non-target species (especially vulnerable native species) should also be considered when using this method. There have been studies

that document fish injury and mortality (which may go unnoticed unless externally obvious or severe) as a result of electrofishing expeditions, especially in eels where incidents of spinal damage and haemorrhaging can be high (Dalbey et al., 1996; Snyder, 2003; Reynolds & Holliman, 2004:). However, fish injuries can also result from careless capture, handling, and transport (Snyder, 2003). A comparison of capture methods found that netting and electrofishing produced similar rates of injury and mortality in three New Zealand native fish species (shortfin eel, *Anguilla australis*; grey mullet, *Mugil cephalus*; and common smelt, *Retropinna retropinna*) in the Waikato region (De Villiers, 2013).

1.3.3 Environmental DNA

Environmental DNA (eDNA) offers an alternative option to conventional fish detection methods; it is less invasive and eliminates the risk of damage to non-target species (Taberlet et al., 2018). eDNA is a complex mixture of genomic DNA from many different organisms found in an environmental sample such as sediment or water (Taberlet et al., 2018). Single-species detection is often applied to track rare species, or invasive species in the early stages of invasions, mainly in aquatic ecosystems (Dejean et al., 2011; Jerde et al., 2011; Mächler et al., 2014; Tréguier et al., 2014).

Fish release a relatively large amount of eDNA into their environment (Jerde et al., 2011; Takahara et al., 2012; Thomsen et al., 2012; Civade et al., 2016). DNA fragments from sloughed skin cells are isolated from environmental samples and small sections of genes that contain species-specific primers and synthetic nucleotides are amplified using polymerase chain reaction (PCR) or quantitative PCR (Ficetola et al. 2008; Jerde et al., 2011; Takahara et al., 2012; Thomsen et al., 2012; Biggs et al., 2015). If these complementary portions of DNA match the target species sequence, they bind to a gene template and produce amplicons that can be visualised using electrophoresis on an agarose gel, or detected by fluorescent luminance after DNA synthesis (Dejean et al., 2011; Wood et al., 2013; Banks & Hogg, 2015). Both PCR and quantitative PCR reveal the presence of a target DNA sequence, however, the latter is more informative as it can monitor in real-time the number of target copies present in the mix, whereas simple PCR can only measure their final concentration (Valasek & Repa, 2005; Kim et al., 2013). It has been

suggested that the strength of the eDNA signal detected by PCR assays likely corresponds to the relative abundance of species in the sample area and so an estimate of biomass can be inferred (Jerde et al., 2011). While this may be true for stagnant water systems, flow rates in other areas may affect dilution levels so conclusions based on signal strength alone are not advised (Jerde et al., 2011).

The high sensitivity of the technique and the possibility of remote detection at similar or reduced cost (once initial development of the technique and species-specific primers are complete) are the main reasons for the attractiveness of the eDNA approach (Thomsen & Willerslev, 2015). Historically, eDNA-based single-species detection has been considerably successful in biomonitoring invasive, elusive, or threatened animal species, mainly in aquatic environments (e.g., Bohmann et al., 2014; Lawson Handley, 2015). For example, Sigsgaard, Carl, Moller, and Thomsen (2015) compared eDNA with conventional methods (electrofishing, traps, and landing nets) for the detection of the European weather loach fish (*Misgurnus fossilis*). These researchers found eDNA to be less labour-intensive (60 hours work for eDNA vs. 300 hours for fishing), and less expensive (\$4,250 USD for eDNA vs. \$8,100 USD for fishing) to survey the same area. The traditional methods resulted in the detection of the species in only one of the two localities in 2008, while the use of the eDNA method detected loach in both localities. After this finding, an intensive survey using nets by local managers resulted in the catching of eight live specimens where none were caught previously (Sigsgaard et al., 2015).

With this level of sensitivity, it is possible to have false positive and false negative results, which is particularly concerning as this may lead to misguided assumptions of species abundance (Taberlet et al., 2018). Consistent sampling techniques are still under development and several factors could affect the amount of DNA in environmental samples, such as the volume of water, size, and density of the organism, and volume of secretions (Ficetola et al., 2008). A wide array of molecules can interfere with DNA isolation and/or amplification, either by hindering cell lysis during extraction and thus access to DNA, by degrading or capturing DNA molecules, or by inhibiting polymerase activity (Taberlet et al., 2018). Therefore, specialist laboratories are needed to prevent contamination (Taberlet et al., 2018).

In addition to these technical aspects, eDNA production, and transport/diffusion,

and degradation strongly influence detectability. Allochthonous eDNA can be integrated into the system, for example, DNA may travel far from its point of origin through predation (e.g., leaving remnants of fish in scat), or contamination of other waterways meaning that any DNA confirmed present may originate from sources other than live organisms (Symondson, 2002; Kelly et al., 2014; Stoeckle et al., 2017). Environmental and biological parameters influence eDNA persistence and degradation. eDNA can persist from a few days to a few weeks (Dejean et al., 2011; Thomsen et al. 2012; Pilliod et al. 2014; Dunker et al. 2016; Williams et al., 2018). Water temperature is an important factor, with better persistence occurring at lower temperatures (Pilliod et al. 2014; Lacoursière-Roussel et al. 2016). Sunlight (UV radiation) and a high level of microbial or fungal activity also increase the degradation rate (Taberlet et al., 2018).

1.3.4 Summary

The currently available methods for the detection of invasive freshwater fish have limitations due to their cost, invasiveness, time-consumption, and potential to be prone to error (Table 1). One of the problems of detecting incursions or monitoring fish at low abundance is that cost of effective fishing increases exponentially as the fish abundance declines i.e. it is very easy to detect fish when they are abundant and extremely difficult to do so when they are rare (N. Ling pers. comm.). As a result, a procedure should be developed to support or replace some conventional methods that limit these issues while still being cost-efficient, minimally invasive, and easy to implement. Domestic dogs (*Canis familiaris*) are already used as a detection tool in many fields due to their incredibly sensitive sense of smell, so could they be the answer?

Table 1 Summary of current fish detection methods and their parameters Source: Joy et al., 2013.

Method				Parameter
eDNA	Visual Survey	Electrofishing	Fyke Netting	
Fast	Fast	Moderate	Moderate	Time taken to sample
No	No	No	Yes	Return trip required
High	Low	High	High	Amount of equipment
Moderate	Low	High	Moderate	Expense of equipment
No	Yes	No	No	Impaired by broken water
Yes	No	Yes	Yes	Sampling done during normal working hours
High	Low	High	High	Ease of identification of fish
Low	Low	Moderate	High	Potential harm to fish
High	Low	Low	Low	Reliability of relative abundance estimates
High	Low	High	Low	Effectiveness for collecting size class data

1.4 Domestic dogs as a detection tool

Scent detection dogs are a well-established tool across a variety of fields (Johnen, Heuwieser, & Fischer-Tenhagen, 2013). These include the detection of various cancers and other diseases (McCulloch et al., 2006; Sonoda et al., 2011; Alasaad et al., 2012; Guerrero-Flores et al., 2017; Koivusalo et al., 2017), human remains (Oesterhelweg et al., 2008; Riezzo et al., 2014; Alexander, Hodges, Bytheway, & Aitkenhead-Peterson, 2015) insects (Brookes et al., 2003; Lin et al., 2011; Hoyer-Tomiczek et al., 2016), plants (Goodwin et al., 2010; Sargisson et al., 2010), explosives (Lazarowski & Dorman, 2014) and narcotics (Jeziarski et al., 2014). A recent proof of concept study has even aimed to evaluate the use of dogs to detect the presence of the COVID-19 virus from human sweat (Grandjean et al., 2020).

The olfactory bulb, stria, and tract make up 1.95% in total of a dog's brain volume compared to just 0.03% in a human, indicating the relative importance of olfaction to how dogs perceive their environment (Kavoi & Jameela, 2011). Dogs have bilateral nasal cavities separated by the nasal septum where inhaled air is warmed and moistened, facilitating the volatilisation of odorants. The canine nasal cavity contains hundreds of millions of sensory neurons (Craven et al., 2010). During active sniffing unique air flows are generated, transporting environmental odorants to selectively bind with olfactory receptors in the nose, and exhaled air exits in a ventral-lateral vortex to minimise re-breathing expired air (Craven et al., 2010). Dogs can detect certain odorant concentrations at 1-2 parts per trillion, roughly 10,000-100,000 times that of human perception (Craven et al., 2010). Not only do dogs have much greater sensitivity than humans, but they can detect a much greater variety of odorants as they have ~670 functional olfactory receptor genes vs ~330 in humans (Quignon et al., 2003). This olfactory sensitivity means that dogs reliably outperform even the most advanced biotechnologies available to date (Angle, Waggoner, Ferrando, Haney, & Passler, 2016).

Scent detection dogs have been used for conservation purposes in New Zealand since the 1890s when they were used to locate kiwi (*Apteryx spp.*) and kakapo (*Strigops habroptilus*) (Browne, Stafford, and Fordham, 2005). Dogs have been recognised for their ability to outperform human surveyors in locating and distinguishing between cryptic terrestrial species, especially in circumstances

where visual searches are limited, for example in dense vegetation (Cablk & Heaton, 2006; Long et al., 2007; Savidge et al., 2011; Chambers et al., 2015). Some research has examined dogs' abilities to detect New Zealand's protected and invasive species. For example, Browne et al., (2015) trained pet dogs to detect Marlborough green gecko (*Naultinus manukanus*), forest gecko (*Hoplodactylus granulatus*), and tuatara (*Sphenodon punctatus*) scents with up to 98% correct responses across trials. Trained dogs have also been shown to be able to locate 87% of Norway rats (*Rattus norvegicus*) and 80% of mice (*Mus musculus*) released into a 63-ha fenced forest sanctuary, with only two false-positive detections (Gsell et al., 2010).

While dogs' efficacy of scent detection for terrestrial searches (e.g., flora, fauna, scat [faeces], and latrines) has been widely researched (Browne et al., 2005), there are few studies published regarding their ability to locate aquatic targets. Although dogs were first used to locate submerged corpses in the late 1960s and 1970s, scent detection of water-based targets is still considered a relatively new area of study (Eisenhauer, 1971; Richardson, 1971; Hart, Bryson, Zasloff & Christensen, 1996; Osterkamp, 2011; Richards, 2018). The process of scent transfer in water is poorly understood, but research on the behaviour of volatile organic compounds (VOCs) can provide some clues. Osterkamp (2011) hypothesised that knowledge of how VOCs spread in the terrestrial environment could suggest that certain organic compounds excreted from submerged organisms rise through the water and evaporate into the air above the surface. It is at the surface where VOCs would then be available to be detected by a trained dog. It is proposed that scents within water become available to dogs as molecules diffuse through the body of water from their source, at the surface these molecules volatilise and evaporate, making them available for dogs to sniff (Hart et al., 1996; Osterkamp, 2011). Scent availability depends on the type of water body; it is theorised that in small quiet lakes or ponds scent rises almost vertically, whereas, in large rivers characterised by strong laminar flow, the scent may be carried a considerable distance downstream before becoming immediately available (Richards, 2018).

Studies of dogs' ability to locate aquatic targets have been limited to the search for submerged cadavers (Koenig, 2000), contaminated wastewater (Van De Werfhorst et al., 2014), off-flavour compounds in catfish farm ponds (Shelby et al., 2004), ocean floating whale scat (Rolland et al., 2007; Ayres et al., 2012; Wasser et al.,

2017) and invasive mussel species (Sawchuk, 2018). These studies have demonstrated dog's promising potential to locate other aquatic species. For example, Zebra and quagga mussels (*Dreissena spp.*) pose a threat to ecosystem health in Canada, costing between \$75 and \$91 million CAD per year (Ontario Ministry of Natural Resources, 2012). The ability of dogs to assess whether lake water is harbouring invisible larval mussels (i.e., veligers) and settled veligers has been investigated (Sawchuk, 2018). Seven dogs were presented with an array of six 5-gallon plastic buckets, each containing water that held 0-68+ veligers per litre of water, in addition to plankton and whatever other biomass were present in each waterbody. Dogs located the mussel-infested bucket at least once during each two-repetition exposure (mean 80%, range 69–92%). Even as time went on and the veligers were dying and disintegrating the dogs were able to correctly select the infested bucket at least once each around 57% of the time (Sawchuk, 2018).

1.5 Factors for consideration in scent detection tasks

The ability of dogs to detect a novel scent must be examined robustly so that results cannot be contested. There are limitations to previous scent detection research that should be addressed, including environmental factors, experimental methodologies, handler bias, and cueing.

In both field and laboratory settings, certain environmental factors can impact dogs' scent detection accuracy. Many compounds can undergo biological oxidation (or reduction), and the reactions involved are quite complex and their success depends heavily on the environmental conditions (Naddeo, Belgiorno & Zarra, 2013). Different VOCs become volatile at different temperatures and humidity, so some of these conditions are target-specific. For example, dogs are less likely to detect target scents at low temperatures and humidity, and under strong wind and precipitation conditions (Rebmann, David & Sorg, 2000; Sargisson et al., 2010; Savidge et al., 2011; Chambers et al., 2015). In general, warmer temperatures can release volatile organic compounds, concentrating them at the source and thus making it stronger and easier for dogs to detect; moister and windier conditions can broadly disperse scent at a source too high or far away for dogs to detect or detect accurately (Shivik, 2002; Cablk & Heaton, 2006; Reed et al., 2011).

In an open system (i.e., a system that has external interactions) with a continuous supply of external heat, all VOCs will eventually dissipate, whereas, in a closed

system (i.e., a system without external interaction), a state of dynamic equilibrium becomes established between the quantity of liquid molecules passing to the vapour state and the quantity of vapour molecules condensing to the liquid state (Naddeo et al., 2013). In laboratory settings, we can regulate temperature and humidity and create closed systems to reduce environmental factor impacts.

A line-up arrangement (Figure 5) is often used in experimental studies and is where the dog is faced with a match-to-sample task in which they must indicate the sample that contains the target scent (Rebmann et al., 2000). The dog sometimes accompanied by a handler, assess each sample, and the dog's behavioural response to each sample is recorded (e.g., sitting or lying down to indicate a target). A dog incorrectly indicating the presence of a target (i.e., a false alarm), or a dog incorrectly indicating the absence of a target scent (i.e., a miss) can occur even under ideal conditions if a handler misreads the dog's behaviour or unintentionally provides cues for the dog. Unintentional cueing is when a handler, through their body language, may accidentally give the dog a prompt to give a certain response that the handler expects.



Figure 5. Dog searching for a matching odour in a Dutch scent identification line-up Source: Netherlands National Police Agency.

One of the most famous examples of human cueing involves a horse named Hans, who apparently gave answers to mathematical equations and could answer general knowledge questions, by tapping his hooves to indicate numbers or letters (Samhita & Gross, 2013). Hans was trained by his owner for four years before being presented to the public. It appeared that the horse could count the number of people in the audience, perform equations, read the clock, recognize and identify playing cards, and knew the calendar of the whole year. The phenomenon was finally explained when it was discovered that Hans was unable to answer any question if the questioning person did not know the answer, or a screen obstructed the face of the questioner. Hans was responding to subtle micro-expressions on the faces of the questioner and crowd in order to give the correct answers. Without being able to see faces, Hans could not give the right answers (Samhita & Gross, 2013).

Handler cueing effects have been shown in scent detection tasks with dogs even under double-blind conditions (Lit, Schweitzer, & Oberbauer, 2011; Zubedat et al. 2014). Lit et al., 2011, influenced 18 handlers' beliefs about the presence of a target scent and evaluated the subsequent performance of the handler-dog team. Handler beliefs were influenced either by verbally communicating to the handlers that a specific marker was an indicator of scent location (i.e., human influence), by encouraging dogs to display unusual interest in a specific location with a decoy scent (i.e., dog influence), or by a specific marker that actually indicated the location of a decoy scent (combined human and dog influence). There was no actual target scent present so that any alert identified by handlers was considered a false alert. There was a total of 225 alerts and 85% of experiment runs had one or more alerts. It has also been found by Szetei et al. (2003) that the presence of human cueing is even enough to override both visual and olfactory cues associated with hidden food.

Dogs' accuracy during scent detection tasks has also been shown to decrease when handlers are changed (Nolan & Gravitte, 1977), and idiosyncrasies among owners (e.g., gender and personality) influence dogs' behaviour and subsequent success in operational tasks (Kotrschal et al., 2009). Therefore, to test the true ability of dogs during scent detection tasks, human influence must be removed; i.e., dogs should be working independently, in the absence of a handler.

1.6 Project aim and thesis structure

This research aimed to investigate the ability of dogs to detect the presence of brown bullhead catfish from water samples. Current detection methods for this invasive species have limitations due to their cost, invasiveness and impact on non-target species, time-consumption, and potential to be prone to error. This study was designed to mitigate and reduce some of these key limitations and create an improved scent detection dog procedure by being laboratory-based. In this setting environmental factors can be controlled, and handler cueing can also be mitigated as dogs assess samples independently of a handler.

The researcher aimed to evaluate:

1. If dogs can detect the presence of catfish in water samples taken from aquaria that contained those fish.
2. If dogs can discriminate between species of fish, i.e., catfish and goldfish.
3. If dogs can detect catfish odour in water samples, at what equivalent biomass concentrations can dogs detect their presence?

Chapter 2: Domestic Dogs' Ability to Detect Catfish from Water Samples

2.1 Introduction

The introduction of invasive freshwater fish species is a key issue for protecting and managing indigenous biodiversity (Hicks, 2001). They pose a considerable management challenge in freshwater ecosystems as new incursions are often difficult to detect and are therefore likely to become widespread before discovery, making these fish difficult to control or eradicate (Hicks, 2001).

The brown bullhead catfish (hereafter referred to as catfish) is one of several freshwater fish species introduced to New Zealand (Collier & Grainger, 2015). They have since been spread both deliberately and accidentally throughout the central North Island and have a few scattered populations in the South Island. These fish have the potential to modify native invertebrate and macrophyte communities, as well as the nutrient status of waterways due to their feeding behaviour as a benthic species stirring up sediments (Collier & Grainger, 2015). They compete with native species such as tuna (eels) and kōura (freshwater crayfish; *Paranephrops planifrons*) and have been implicated in their decline (Barnes & Hicks, 2003; Collier et al., 2018; Francis, 2019;). In Māori culture eels and kōura are considered taonga – an important cultural treasure, as a traditional food source (Parliamentary Commissioner for the Environment, 2013; NIWA, 2016).

Catfish are classified as the third-worst pest fish by Fish Risk Assessment (FRAM) score (NIWA, 2020). Despite this, they currently have no status under the Biosecurity Act. However, possessing catfish is prohibited without permission from the Ministry of Primary Industries and they must be killed and disposed of on capture according to regional pest management plans in the Northland, Auckland, Bay of Plenty, Gisborne, and Waikato regions (NIWA, 2020).

In the previous chapter, it was highlighted that current detection methods for catfish (i.e., visual survey, netting, and electrofishing) can be insufficient to locate and determine the successful eradication of catfish. This is due to low catfish biomass, the reduced activity levels of fish in winter months, and limitations of where fyke nets can be placed (Joy et al., 2013). Therefore, another potential detection method, the use of scent detection dogs is worth evaluating. This

experiment aimed to evaluate dogs' ability to independently distinguish the presence of catfish (at a biomass of 15.5 g/L) or no catfish held in aquaria of dechlorinated water.

2.2 Methods

2.2.1 Animal ethics statement

All experiments using domestic dogs and invasive fish (catfish and goldfish) were carried out under the supervision and approval of the University of Waikato Animal Ethics Committee (Protocol 1055). Fish were held at the University of Waikato Aquatic Research Facility under an existing standard operating procedure (SOP) that met AEC standards. Dogs were housed during the day at the Scent Detection Research Group (SDRG) facilities in accordance with an SOP provided in Appendix A. Dogs were only confined to crates when not completing scent detection tasks. Generally, dogs were only present for half days and were provided 15-minute walks for every two hours while at the facility. In the case of a dog needing to remain at the facility for a full day, an additional 30-minute walk was provided. Dogs' participation was entirely voluntary; owners were informed any time changes to protocols were made and were free to withdraw their dog from the study at any time.

2.2.2 Fish collection and care

Thirty-eight catfish and 16 goldfish (used in later experiments) were sourced from wild populations in the Waikato region, either by boat electrofishing or fyke netting. Fish were initially kept in flow-through fiberglass holding tanks supplied with dechlorinated municipal water and continuous aeration. Holding tanks were supplemented with artificial seawater twice per week up to a salinity of 10 ppt to reduce osmotic stress and reduce susceptibility to skin infections. Following collection, fish were acclimated for at least 1 week, before being weighed and examined for obvious health issues. They were then transferred to single species 240 L (dimensions; 55cm wide X 86cm long X 51cm height) high-density polyethylene experimental tanks supplied with continuous flow-through water and aeration (Figure 6). A plastic divider was placed into each tank as in large numbers catfish were known to injure each other with their spines.

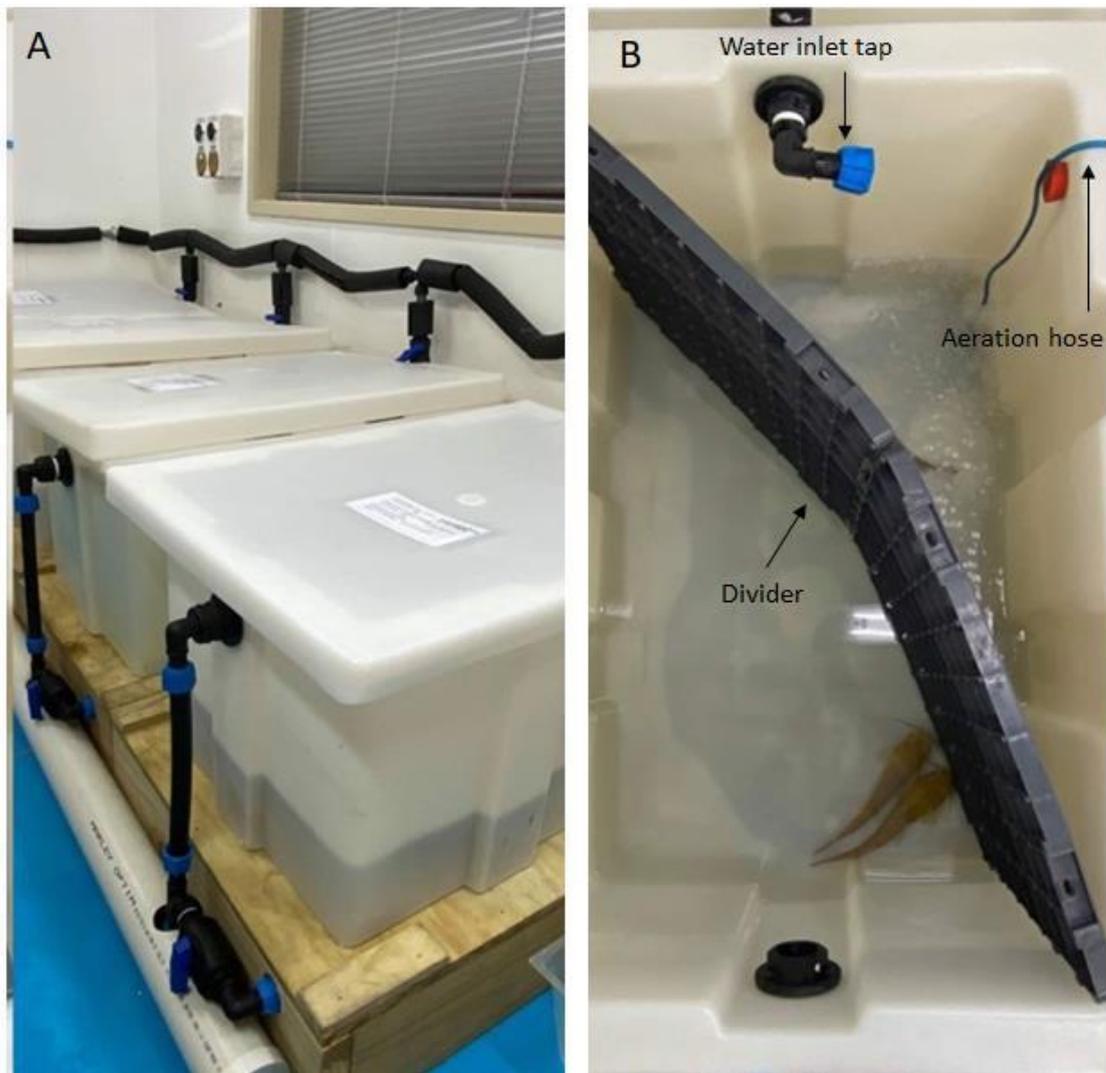


Figure 6. Experimental fish tanks (A) at the University of Waikato Aquatic Research facility, with water inlet tap, supplemental aeration, and a plastic divider to prevent fish injury (B).

The tanks were cleaned several times per week as part of the sample collection procedure. A more extensive tank clean (Appendix B), including the application of a 10% hydrogen peroxide solution was performed once per month. During this extensive tank clean, all fish were transferred back to the holding tanks, and new fish transferred into the experimental tanks once the tanks were cleaned and allowed to flush for 24 h. This change for new fish was done to reduce fish stress and to ensure fish stayed healthy, but also to rule out dogs indicating on individual fish. Fish were fed commercial trout pellet after the last water sample collection of the week.

2.2.3 Water sample collection

The SOP for water sample collection has been provided in Appendix C and was followed for all experiments presented in this thesis. Twenty-four hours prior to water sample collection, both the control (i.e., containing no fish) and catfish experimental tank were cleaned. The water flow-through in the catfish experimental tanks was halted and the water level was adjusted to achieve a standard fish biomass concentration of 15.5 g/L based on tank volume and the total weight of the fish. On the day of testing, as much water as was necessary for the experiments being undertaken that day was collected from the control and catfish experimental tanks using an acid-cleaned (68% HNO₃) glass beaker individually designated for each tank. Sample water was transferred into acid-cleaned (10% HCl) 1 L Schott bottles. If samples were required the next day, the tanks were flushed and refilled to achieve the required biomass concentration of 15.5 g/L. If samples were not required the fish were fed, and the tanks returned to normal water flow through.

Water samples were taken from the fish tanks in order of control to catfish to limit the potential of cross-contamination. Disposable gloves were changed between sample tanks. The water samples were then transported to the testing facility, and stored on separate shelves of a designated water sample refrigerator, at 4°C to prevent cross-contamination and potential sample degradation

2.2.4 Sample preparation

All water samples were prepared on the day of testing following an SOP (Appendix D). Before sample preparation, a stainless-steel bench was covered in boiling water twice and wiped down with 60% isopropanol. Each side of the bench was designated to a single sample type; left, negative (i.e., in this experiment water taken from the control tank, containing no fish, and in later experiments water taken from the control and goldfish experimental tanks) and right; positive (i.e., water taken from the catfish experimental tank). Disposable gloves were changed between handling different sample types.

Non-diluted water samples (100 mL), 8 control and 9 positives, were decanted using individual acid-cleaned (68% HNO₃) glass measuring cylinders and funnels into acid-cleaned (68% HNO₃) 200 mL glass jars. Samples types were marked by the placement of adhesive stickers on the bottom of the glass, so as not to be confused when placed onto the testing apparatus. Negative samples were always

prepared first to avoid cross-contamination. Once all negative samples were prepared, they were transferred to the testing apparatus (see Section 2.2.6). Positive samples were then prepared and transferred to the testing apparatus. The samples were then covered by the apparatus lid and left for a minimum of 10 minutes to allow sample volatiles to diffuse throughout the segment before being presented to the dog.

2.2.5 Selection of scent-detection dogs

Domestic dogs were recruited from within the Waikato region, using flyers, social media, and word of mouth. An SOP for dog recruitment was followed to screen for suitable dogs (Appendix E). Screening criteria included owner commitment/availability and dog behaviours such as aggression towards other dogs or around food, neophobia, separation anxiety, motivation to work for food, and friendliness towards strangers.

If dogs met initial selection criteria, they were brought in for a second assessment during which time they could explore the laboratory off-leash. This provided the researcher with the opportunity to observe the dog's behaviour and discuss details of the study with the owner in depth. After approximately 30 minutes a consensus was reached between the owner and researcher as to the dog's suitability for the research. Written consent was obtained from the owner (Appendix F), and the dog proceeded to a trial period of basic training (see section 2.2.7).

2.2.6 Description of testing apparatus

In order to limit the disadvantages of other methods of scent discrimination tasks as mentioned in the previous chapter, two sets of custom-built testing apparatuses were used during these experiments (Figure 7). Dogs could be observed interacting with the apparatus from an adjacent room via live camera feed (provided by several Logitech® 2 MP HD Webcam C600 with built-in microphones). Each apparatus consisted of an aluminium frame, an omnidirectional switch, and an acrylic glass front panel with a single 90mm diameter porthole cut in the centre. On the interior surface of the front panel, a grid of 3 infrared beam sensors was installed so that the entry of any object larger than 15 mm in diameter (i.e., a dog's nose) into the porthole would result in the breakage of at least one beam. Sample port beam interruption was used to detect observation and indication responses made by dogs.



Figure 7. The testing apparatuses used in this study, A; showing the initial apparatus with opaque plexiglass front panel showing porthole and omnidirectional switch, B; showing the second apparatus with transparent front panel, and C; showing sample segments.

Behind the front panel was a circular stainless-steel carousel plate measuring 760 mm in diameter, which water samples could be placed on. The topside of the carousel plate was engraved with numbers (1-17) so that samples would be correctly placed, while underneath the plate sample positions were marked with matte black tape. This tape created a 5-bit binary code associated with each sample position, which could be read and relayed to the controller by a row of optical sensors positioned under the carousel. The carousel was able to be turned between samples by a rubber tread wheel (85 mm in diameter) that contacted the plate, controlled by a motor mounted inside the frame behind the front panel. The carousel would only move to present the next sample after an appropriate response to the

current sample had been given (see section 2.2.7 for further details), either an indication or rejection (triggering the omnidirectional switch). An additional two sensors on the outer edge of the plate were used to slow the carousel prior to the target position and stop on position, without abrupt movement. While the carousel is rotating, if the infrared beam is broken, the carousel ceases to rotate until the infrared beam is no longer obstructed. This is a safety feature to prevent injury and to prevent the animal from interfering with the rotation of the apparatus, as premature port entry will delay access to the next sample.

Once placed into the correct position on the apparatus, water samples were covered by 17 wedge-shaped (3.57 L volume) removable segments (Figure 9C) constructed out of 1.2 mm thick stainless steel. The segments were laser cut with high precision to minimize air escaping from the segment when the heavy stainless-steel apparatus lid was placed. Each segment was 230 mm high and held in position on the carousel by stainless-steel pins that protruded vertically inside each corner of the segment. The front of each segment had a 100 mm by 100 mm square opening that would align with the porthole when in position. The opening was covered by a stainless-steel flap fastened on by a hinge on the inside. This flap was held closed by a weighted L-bracket unless pushed inward (i.e., when a dog pushed its' nose in to assess a sample). This L-bracket also prevented the flap from opening beyond 28° to stop dogs from being able to open the segment far enough to be able to contact samples. When the segment flap was closed it made a sharp “tap” which could sometimes startle the dogs during initial training.

A circuit board fixed to the frame near the motor sent carousel position readings from the optical sensors and sample port beam breaks to the controlling computer (Dell™ Optiplex 780 running Windows Vista™) in the adjacent room. This computer ran the custom software, referred to as the ‘scent program’. Within the scent program, each dog was assigned a configuration file that could be modified as needed for training and research requirements. Configuration files specified: 1) the maximum session duration; 2) the minimum sniff time (sample port beam-break duration) required for an observation response, usually 500ms; 3) minimum sniff time required for an indication response, usually 5500ms once fully trained; 4) the delay between a response given and the rotation of the carousel, typically 1000ms; 5) the number of times all segments will be presented (if set at “1,” each segment will be presented in the programmed order and if set at “2” the carousel

will complete a full rotation twice); 6) the number of reinforcers (food rewards) to be delivered each time the reinforcement cycle is activated, and 7) the status of each sample/segment. Positive catfish samples were indicated by a “1”, while negative samples were indicated by a “2” for control or “3” for goldfish. At the end of each experimental session, the scent program would update an event file for each dog, recording the infrared beam breaks and omnidirectional switch activations in relation to each sample.

The circuit board was also responsible for operating the feeders that produced food rewards. Two feeders were used during this study. The first feeder could only operate with dry dog kibble, the Treat & Train Remote Reward Dog Trainer manufactured by Premier. The handheld remote-control device for this product was wired directly to the circuit board so that “virtual” button presses activated the feeder on the opposite side of the room approximately 2 m from the apparatus. When the feeder was activated, a relay switch on the circuit board produced a “click” and one or more pieces of dry food were dispensed according to input from the scent program. When food was being dispensed, the feeder also produced distinctive auditory stimuli. The second feeder operated in the same manner as the first but was custom built by the laboratory technician so that it could dispense pieces of a semi-moist dog food roll made from New Zealand common brushtail possum (*Trichosurus vulpecula*) meat called Possyum. Which was sometimes more palatable and preferred by the dogs.

While turned on and operated the apparatus provided several auditory and visual cues (which were added and updated during this study, see further explanation in Section 5.2.3). A strip of LED lights across the top of the front panel illuminated when sessions began and extinguished when the session ended. These lights provided stimuli for the researcher about the status of the apparatus when observing via the camera feed in the adjacent room (e.g., blue light when the carousel was moving, green if a dog had given a correct indication response, red if a dog was incorrectly giving an indication response). The dogs received auditory feedback from the apparatus, i.e., a long “beep” when the infrared beam was broken by the nose held in the port while a sample was ready for assessment, or a “buzz” if the apparatus was interrupted while still moving to the correct placement. Once a new sample was ready for assessment the apparatus provided a brief “double beep”. The circuit board also produced a “click” when triggering the feeders.

2.2.7 Dog training and experimental procedure

Once dogs had been habituated to their new environment and the researcher, training sessions were started. Early training sessions never exceeded 10 minutes and dogs were given short breaks between training sessions. During the initial training stage, the apparatus was turned off so that the additional auditory stimuli other than the tap of the segment flap (i.e., beeping), didn't startle the dog. During the initial stage of training, the researcher was present in the experimental room with each dog during their training sessions. The researcher stood with their hands crossed either in front of their body or behind their back, holding the feeder remote/hand-switch out of view of the dog and avoided eye contact with the dog. The researcher would sometimes provide prompts to interact with the apparatus with an open palmed hand, but this was phased out as soon as possible.

Dogs were first trained to associate the sound produced by the feeders and circuit board with a food reward (i.e., a piece of kibble or Possyum), establishing the sounds as a conditioned reinforcer. The researcher operated the feeder using the remote control, while the dog freely explored, producing a food reward with each feeder operation until the dog began to reliably return to the feeder within three seconds of the food delivery.

Once a dog had returned to the feeder within 3 s five times in a row, they were trained to interact with the apparatus in accordance with an SOP (Appendix G) using the conditioning paradigm known as shaping (the differential reinforcement of successive approximations towards the end target behaviour). For example, dogs were first trained to interact with the porthole. Dogs were initially rewarded for approaching the apparatus, then only when touching the apparatus, then only when they put their nose through the porthole. Once dogs were reliably putting their nose into the port hole far enough to open and close a segment (without showing fear or startle responses to the sharp tap sound) the apparatus was loaded with 17 positive samples and turned on so it would now provide the other auditory cues. The minimum positive indication time was set at 1000 ms in the dogs' configuration file, so that when the sample port beam was broken longer than this specified duration; the feeder was triggered, and the carousel turned to present the next sample.

Once a run (17 samples) at the 1,000 ms indication threshold was completed without prompt, the threshold was increased in 100-500 ms intervals to 1,500 ms. Once a run was completed at 1,500 ms, the apparatus was unloaded of samples, cleaned, and turned off to train the pressing response of the omnidirectional switch (herein referred to as lever). Once again using the shaping paradigm the researcher rewarded successive approximations towards pressing the lever. When the lever press response had been given correctly the lever provided a distinct auditory “click”. This lever activation would later be used to advance the carousel to the next sample following an observation response. The topography of training the lever press response was decided on a case by case basis, based on how the dog initially approached the lever press (i.e., with nose, chest, or head) and how comfortable it was for the dog to manipulate (Figure 8). Once the lever/omnidirectional switch has been activated 10 times in a row without prompt, the dog moved onto the more advanced discrimination training.

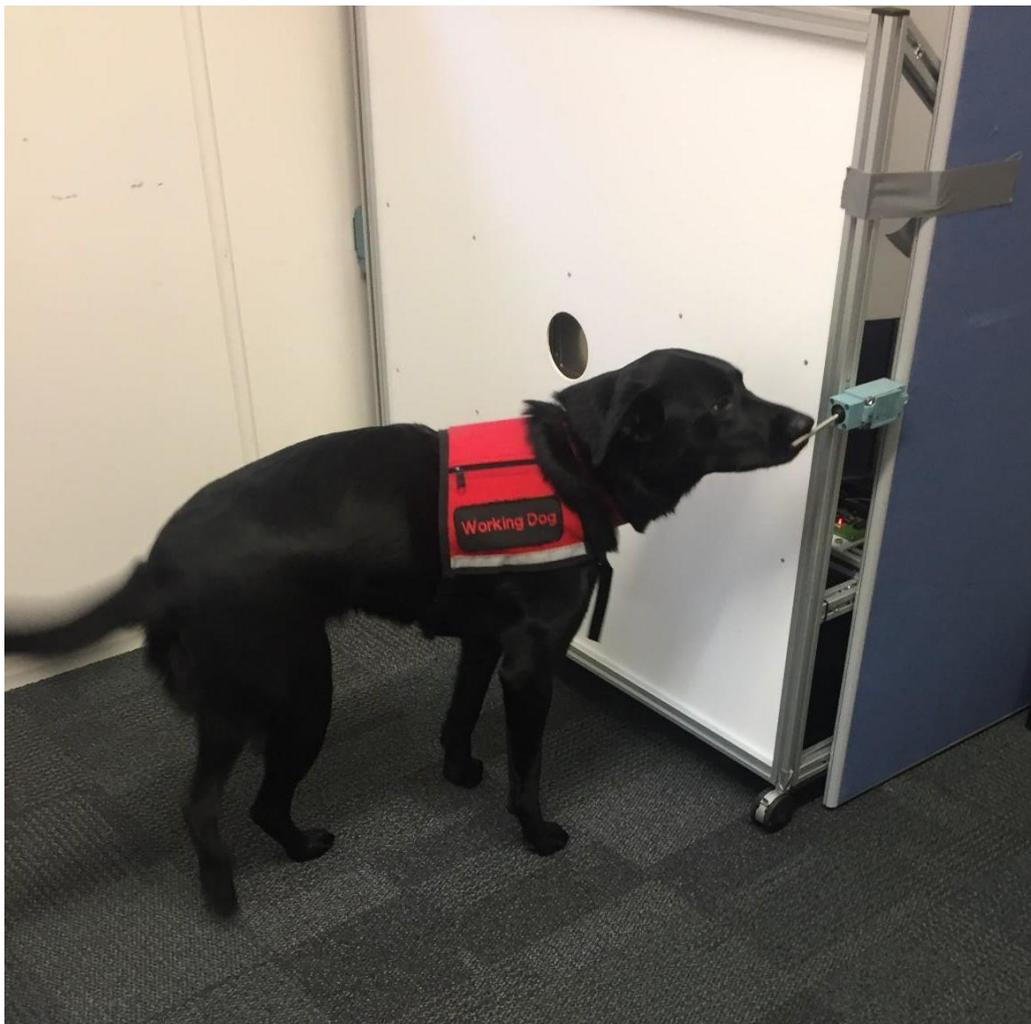


Figure 8. Catfish scent detection research subject, Molly, demonstrating a lever press response during training.

During discrimination training, the lever-pressing response was no longer reinforced (i.e., rewarded with food). The apparatus was cleaned, turned back on, and loaded with samples (i.e., 8 control, 9 catfish), in an alternating positive and negative sample pattern, starting with a positive sample in the first position. This configuration pattern was loaded into the dogs' file, so the apparatus was controlling reinforcement. To show discrimination between samples dogs were expected to assess a sample (i.e., put their nose through the port for the minimum sniff time; 500 ms), then give one of two responses: an indication (i.e., holding their nose in for the programmed minimum indication time; 1500 ms) or a rejection (i.e., pressing the lever).

If an indication was given for a positive sample (Figure 9), this was considered correct (i.e., a hit) and reinforced. However, if an indication was given on a negative sample this was considered incorrect (i.e., a false alarm), this was not rewarded, and the apparatus would not present the next sample until the lever press response was given. If a rejection response was given for a negative sample this was considered correct, but not rewarded, and the apparatus would move to present the next sample. If a rejection response was given for a positive sample, this was considered incorrect (i.e., a miss), not rewarded, and the apparatus moved to present the next sample.



Figure 9. Catfish scent detection research subject, Tommy, demonstrating an indication response to a positive catfish sample.

When the dog encountered the first negative (i.e., control) sample, a period of 20 seconds was given to see if the dog would give the lever press response without

prompt. If necessary, prompting was used and then faded out as quickly as possible. Once one run had been completed without prompting, the sample presentation order pattern was randomised and updated in the configuration file. Randomisation was done using a random number generator to assign samples to segment numbers (1-17). The same randomisation pattern was used for a maximum of 3 sessions in a row.

Once dogs achieved a hit rate (correct positive indication) and rejection rate (correct lever pressing) accuracy above 80% without prompt, the indication threshold was systematically increased in 100-500ms increments until they reached the target threshold (5500ms). The researcher then started to remove themselves from the experimental room. Once the dog was successfully working independently with the researcher observing from the adjacent room, and an additional run was added. This meant the dogs were encountering the same samples twice per session (a total of 34 segments). A dog was considered fully trained and moved onto the experimental phase when they were working independently for two runs.

The criteria set to have completed this experimental phase was initially set at an accuracy above 80% for both correct indications and correct rejections, for three consecutive sessions on the same day. This criterion was later adjusted to an accuracy above 80% for both correct indications and correct rejections, for four out of five sessions. This change was made as dogs would not necessarily be able to perform enough sessions in a day to meet the initial criteria due to availability and avoidance of overfeeding. When dogs reached the completion criterion, they were moved on to the next experiment; sample dilutions and the introduction of another species.

2.2.8 Cleaning

At the end of each day, cleaning procedures for laboratory equipment were followed. Dog bowls, blankets, and crates were cleaned. The whole facility was vacuumed, and steam mopped. The apparatus lid, front panel, omnidirectional switch, and carousel plate were wiped down with a paper towel and 60% isopropanol solution while wearing gloves. Glassware was bagged for transportation to another laboratory space where it was acid cleaned in either 10% HCl or 68% HNO₃ following an SOP (Appendix H). The stainless-steel bench has boiling water poured over it twice and was wiped with 60% isopropanol solution.

Then segments were washed in the sink with hot water and a dishwasher tablet (Sunlight Power Max), rinsed, then submerged in 60% isopropanol solution, and left to dry overnight on the bench.

2.3 Results

Of the 13 dogs that started the training program, only four completed training (Figure 10) and moved onto complete Experiment 1. The remaining nine dogs were removed from the program for various reasons, the most common being voluntary withdrawal by the owner due to changes in circumstances or being withdrawn by the researcher due to the dog's lack of motivation to perform the task for food rewards (Table 2).

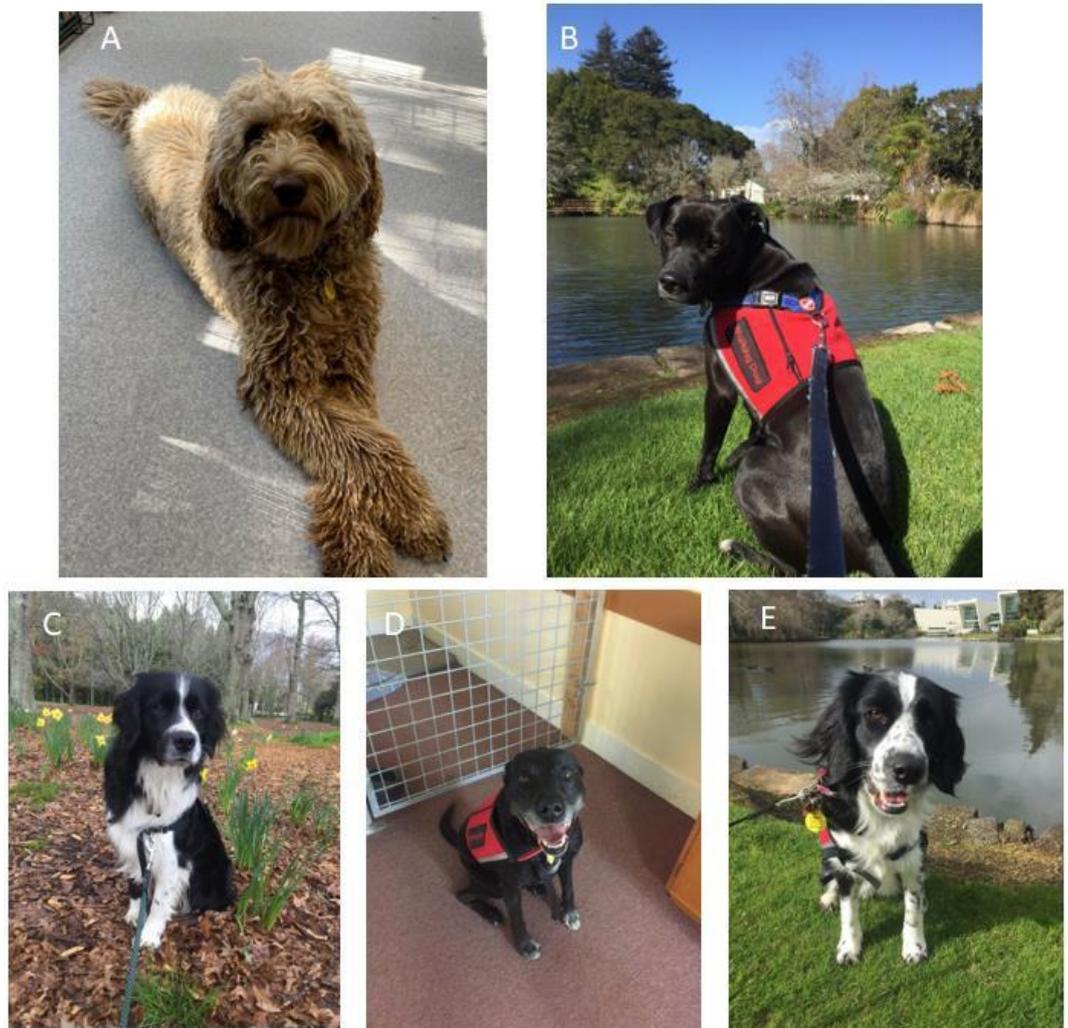


Figure 10. Dogs who participated in the catfish scent detection experiments: Cassie (A); Tink (B); Tommy (C); Cobie (D); Mika* (E). *Mika did not participate in Experiment 1 as she was trained in another experiment using the same apparatus.

Table 2 Details of dogs recruited to participate in the catfish detection research project, NM= neutered male, NF = neutered female. *Mika did not participate in Experiment 1 as she was trained on another project using the same apparatus.

Name	Sex	Age at start	Breed	Passed training	Experiments participated in				Reason for withdrawal
					1	2	3	4	
Tommy	M	1 year	Border collie X	Y	Y	Y	Y	Y	NA
Mika	NF	1 year	Border collie X	Y	N*	Y	Y	Y	NA
Cassie	NF	10 months	Labradoodle	Y	Y	Y	Y	N	NA
Cobie	NF	8 years	Labrador X	Y	Y	Y	Y	N	NA
Tink	NF	2 years	Labrador X	Y	Y	Y	Y	N	Lack of motivation
Molly	NF	1.5 years	Labrador X	N	N	N	N	N	Owner withdrew
Roxy	F	2 years	American Staffordshire bull terrier	N	N	N	N	N	Lack of motivation
Sulley	NM	10 months	Miniature schnauzer	N	N	N	N	N	Lack of motivation
Bonkers	NM	2.5 years	Labrador retriever	N	N	N	N	N	Owner withdrew
Kona	NF	4 years	Rhodesian ridgeback	N	N	N	N	N	Owner withdrew
Raphael	NM	3 years	Cocker spaniel	N	N	N	N	N	Owner withdrew
Barney	NM	10 months	Labradoodle	N	N	N	N	N	Stress response
Pepe	F	1.5 years	Collie X	N	N	N	N	N	Aggression
Poppy	NF	10 months	Border collie	N	N	N	N	N	Lack of motivation

Dogs were scheduled to attend training/experimental sessions at least two days a week. However, dogs did not always attend consistently and there were often large gaps in time between training or experimental sessions. The number of sessions for the dogs to meet completion criteria for Experiment 1 ranged from 4-24 sessions (mean = 13.75 sessions, SD= 10.72 sessions).

Partial runs during sessions were sometimes completed if the apparatus encountered a fault or the dog was not motivated to complete the task. As such, dogs' performance data was only considered and graphed if dogs completed the full two runs. This ensured that dogs encountered the same sample at least twice.

Cassie was the first dog to join the study and complete training. During Cassie's training period some issues were encountered with catfish health. Catfish water samples could not be collected while catfish were being treated for stress and fin rot. Amyl-acetate, a chemical that smells like artificial banana that Cassie would not otherwise contact outside the laboratory environment, was used as a replacement target during training. Cassie was also one of the dogs who was least consistent about attending training/experimental sessions due to the nature of her owner's work and availability.

Cassie took a long time to perform independently (i.e., without the researcher in the room) consistently (Figure 11). This was due to her going through phases of distractibility or not interacting with the apparatus. This distractible behaviour subsequently required the researcher to repeat the training procedure by going back into the experimental room and then phasing herself out of the room again multiple times. Other solutions were also needed to have Cassie work independently; measures tried include increasing the number of food rewards she received during sessions (from one piece of kibble to three) and eventually changing the type of food reward (from kibble to Possum which was a more preferred reward). Despite these issues, Cassie's accuracy for correctly indicating and rejecting samples remained high throughout Experiment 1. At the time Cassie completed Experiment 1 the completion criterion was still three consecutive sessions with above 80% accuracy for correct indications and rejections. Therefore, her data shows she took 25 sessions to meet the criteria, despite her high accuracy. Cassie continued to work at the Experiment 1 fish biomass concentration of 15.5 g/L until she worked alone consistently, and another dog was also ready to progress to the next experiment.

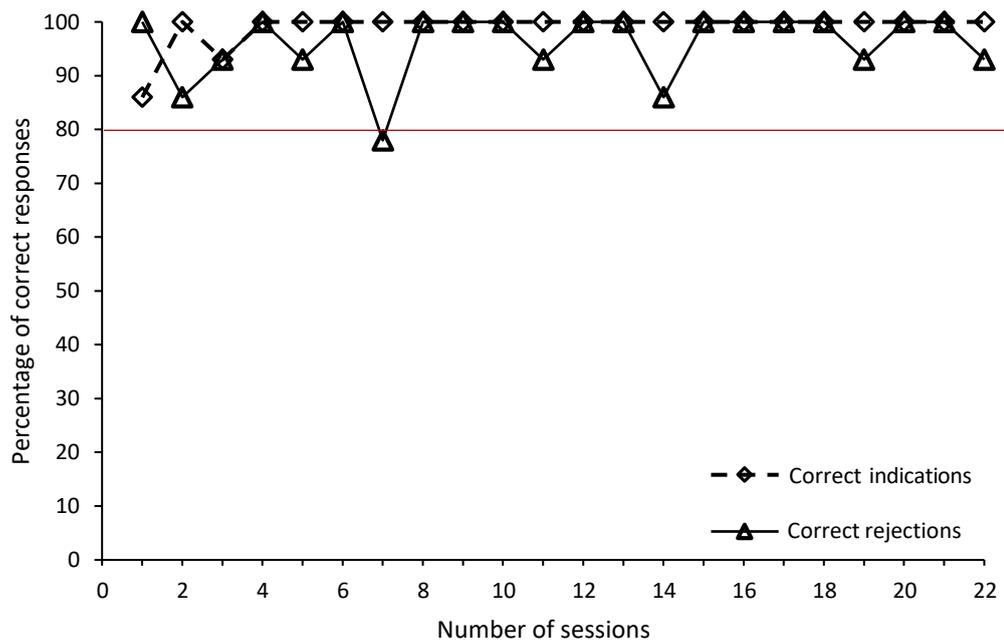


Figure 11. Cassie’s performance accuracy during Experiment 1: correctly indicating the presence of catfish water samples, and rejecting control water samples, with 80% accuracy criterion line shown in red.

Following the difficulty of Cassie being able to meet the completion criterion of 80%+ accuracy for three sessions on the same day, due to not always completing three sessions on the same day, the criterion was changed. The new criterion of 80%+ accuracy for 4/5 consecutive sessions, allowed for the criterion to be met over a couple of days. All other dogs completed Experiment 1 under this new completion criteria.

Tink progressed through Experiment 1 the fastest, and only required the minimum number of sessions to meet the new completion criterion to move onto the next set of experiments (Figure 12).

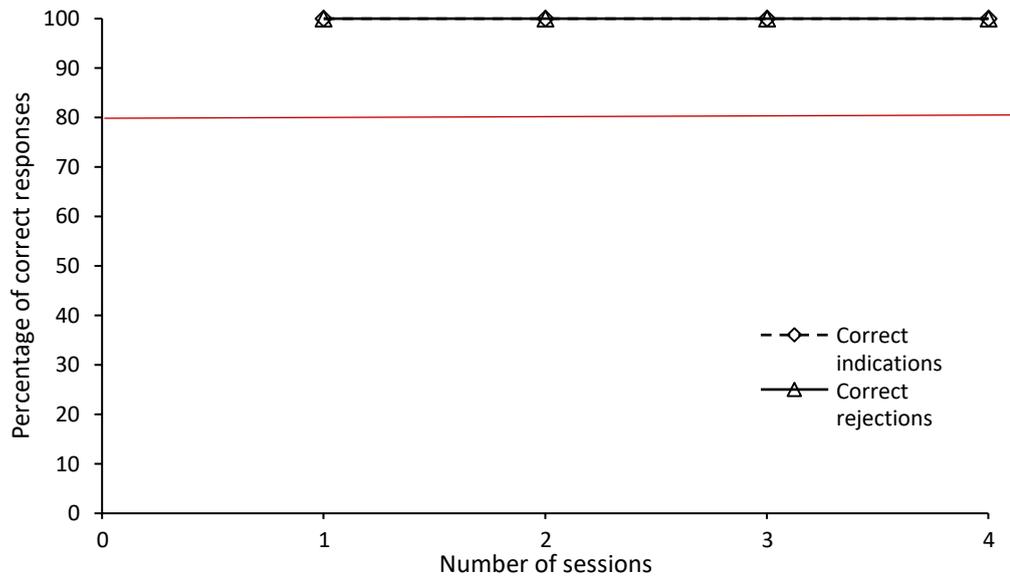


Figure 12. Tink's performance accuracy during Experiment 1: correctly indicating the presence of catfish water samples, and rejecting control water samples, with 80% accuracy criterion line shown in red.

Cobie showed the most variability in her accuracy with negative samples during this experiment (Figure 13). Like Cassie, Cobie's attendance of training/experimental sessions was sporadic, as she experienced health issues during the study.

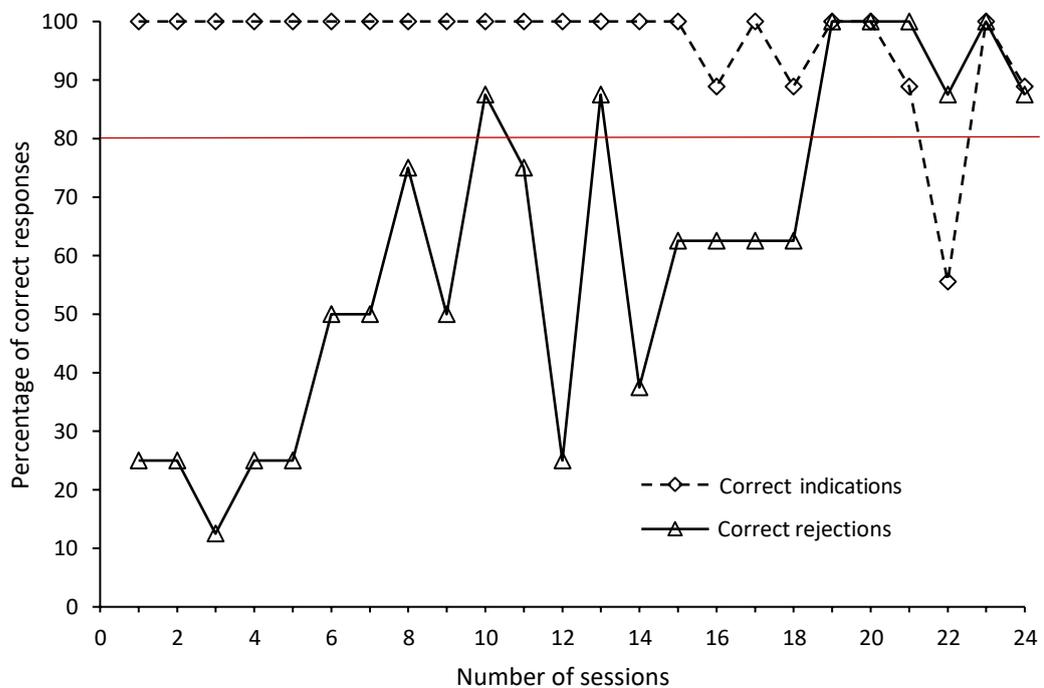


Figure 13. Cobie's performance accuracy during Experiment 1: correctly indicating the presence of catfish water samples, and rejecting control water samples, with 80% accuracy criterion line shown in red.

There was a slight difference in the way that Tommy (Figure 14) was trained compared to the other dogs, as he was the last to join the dogs that completed Experiment 1. While the other dogs met the 5,500 ms target indication criteria before the researcher left the room, Tommy did not have his indication threshold changed until the researcher had already left the room.

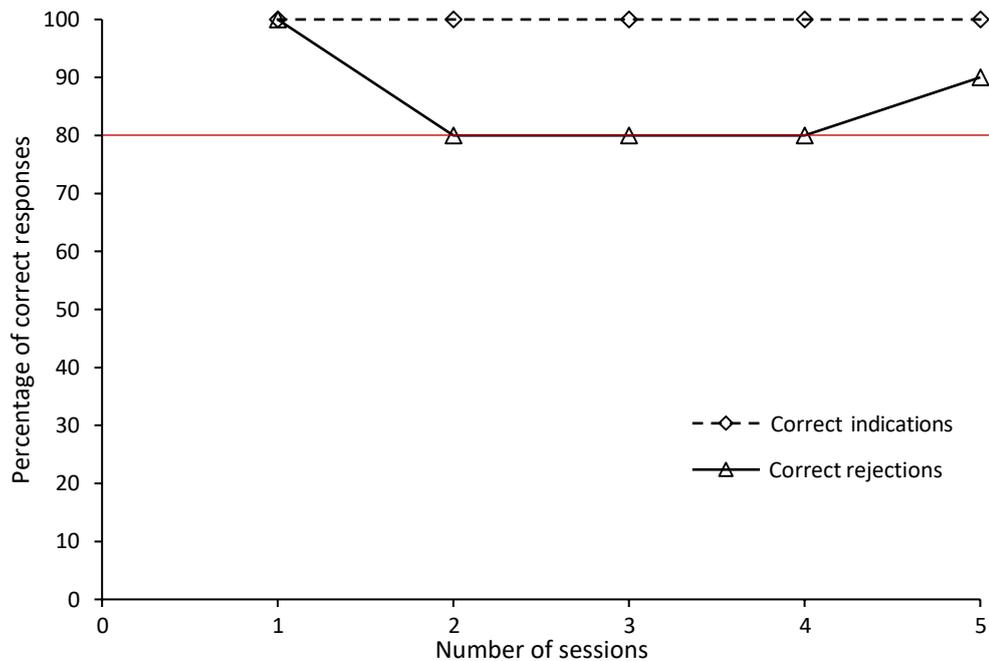


Figure 14. Tommy's performance accuracy during Experiment 1: correctly indicating the presence of catfish water samples, and rejecting control water samples, with 80% accuracy criterion line shown in red.

2.4 Discussion

The four dogs that participated in this experiment were able to discriminate between control and catfish water samples, meeting the accuracy threshold of correct responses at or above 80% for multiple sessions. This study is the first to demonstrate dogs' ability to discriminate catfish from water samples. As samples were not presented in the same order more than three times in a row, it is unlikely that dogs remembered a specific pattern of responses rather than discriminating between sample types. This finding is further supported by the fact that a handler was not present with the dog to provide unintentional cues about the status of samples in the apparatus. These results are also further supported by a previous study using the same apparatus which also found that dogs were able to detect and

distinguish between other freshwater fish species (koi carp and goldfish, from water samples (Quaife, 2018).

During the discrimination stage of training, it was observed that the dogs initially had a bias towards positively indicating on samples, i.e., a false-positive response. This observation is not surprising as only indications on positive samples were reinforced with food. If dogs were presented with a negative sample and unable to discriminate it, they may have indicated on these samples as there was a chance they would receive the food reward, but then through experience with the long indication response (5500 ms) required, learned it was quicker for them to reject that sample for another chance at the possibility of reward for correctly indicating the presence of the target in an up-coming positive sample.

Due to extensive renovations of the laboratory space where this study was undertaken, there were large gaps in training (up to 3 months) because the laboratory space was under construction and unavailable for use. There were also periods of time that involved switching between facilities that were shared by other dogs and researchers due to space and time constraints imposed by these renovations. These changes from the normal routine resulted in delays in dog training progression and experimental performance.

Cassie's distractibility and not interacting with the apparatus could not be attributed to any known cause. It could be speculated that her behaviour was simply a result of the constant changes in her home and the lab environment, a behavioural trait she possessed, or could be a result of her young age (10 months at the start of the experiment). Dogs mature psychologically as they age and are also exposed to more stimuli such as novel environments, conspecifics and humans, and training (Chopik & Weaver, 2019). The examination of dog personality is relatively new, so age differences in dog personality traits are unclear (Chopik & Weaver, 2019). However, it is known that compared to older dogs, younger dogs are more active and excitable (Chopik & Weaver, 2019).

Why Cobie struggled to initially discriminate between samples is also unclear. But she did experience health issues during her training that meant she was unable to come to training consistently, which could have influenced her performance.

The low rate of success in dogs that passed initial training and went on to participate in experiments (4/13 dogs) was expected. The two most common reasons for dog

withdrawal were at the owner's request due to changes in circumstances, and withdrawal by the researcher due to a dog's lack of motivation to perform the task for food reinforcer. As this experiment involved working with household pets and not laboratory-based working dogs, it was expected that some dogs would leave due to changes in their owners' circumstances. This was limited as much as possible by clearly explaining to owners the commitment required before and during their initial interview with the researcher. The selection of an appropriate scent detection dog is not an easy task, as while the olfactory ability is critical there are also many other biological, psychological, and social traits to consider. Strong food and/or play motivation are the most common traits sought after when selecting a conservation detection dog (Beebe et al., 2016). However, other agreeable traits such as problem-solving ability, intelligence, distractibility, and trainability are poorly defined and difficult to measure (Beebe et al., 2016). The failure of a dog to complete training in this study was not necessarily a reflection of the dog's ability to perform scent detection tasks. As this experiment required dogs to be able to work independently without a handler, food rewards were used as positive reinforcement. Some dogs had a strong food drive which allowed them to complete training, while others had a greater preference for human social interaction rather than food.

2.5 Conclusion

All dogs that participated in this experiment met completion criteria, which shows that dogs can detect the presence of catfish from water samples alone, despite the wide range in the number of sessions it took each dog to learn the task. As sample presentation orders were randomised, it can confidently be said that dogs did not learn a pattern of responses, but genuinely discriminated between the presence of fish and no fish. Since the dogs were required to operate the machine alone without human interference it can also be said that there has been an improvement in the scent detection methodology as compared to traditional handler-dog teams, as there was no opportunity for unintentional handler cueing being provided.

Chapter 3: Domestic Dogs' Ability to Discriminate Between Fish Species from Diluted Water Samples

3.1 Introduction

With the ultimate purpose of conservation in mind, any modifications to traditional scent detection procedures must be able to accommodate real-world sample availability to be successful outside of the laboratory. The results of the previous experiment determined that dogs can discriminate the presence of fish or no fish from water samples. However, there was still the need to evaluate if the dogs were able to discriminate between fish species for dogs to be a practical potential detection tool. As brown bullhead is the only catfish species currently present in New Zealand (Collier & Grainger, 2015), a comparison between closely related species was not possible. Instead, another common and widely distributed wild-caught invasive species was selected, goldfish.

To be practical for use as a detection tool, dogs need to be able to detect catfish at biomasses relevant to real-world scenarios. The concentration of catfish in the water samples used in Experiment 1 was a fish biomass of approximately 15.5 g/L, which is equivalent to an environmental biomass of 309 tonnes of catfish in a typically shallow (average 2 m) lake. This biomass is significantly higher than what would be found in a natural environment (Table 3), although the data in Table 3 are based on boat electrofishing biomass estimates and as explained in Chapter 1, electrofishing is relatively inefficient for bullhead catfish; a mark-recapture study of catfish in Lake Mangahia estimated the population biomass at 56 kg/ha compared with the value of 17.6 kg/ha given in Table 3 (N. Ling pers. comm.). It is possible that trained dogs can detect fish at biomass values significantly lower than the 15.5 g/L used for initial training in Experiment 1, as was found for a similar study on koi carp (Quaife, 2018). The aim of this experiment was therefore to determine whether dogs can discriminate between different fish species from water samples and to evaluate at what biomass concentrations they can do so.

Table 3 Catfish biomass estimates from boat electrofishing in water <3 m deep for 15 Waikato lakes before fish removal. Source: Collier & Grainger, 2015.

Lake	Survey date	Area (ha)	Max. depth (m)	Mean catfish biomass (kg/ha)
Hakanoa	19-Oct-09	56	2.5	5.6
Kainui	18-Sep-09	25	6.7	5.9
Kaituna	14-May-09	15	1.3	4.8
Kimihia	21-May-09	58	1	1.4
Kimihia	6-Sep-12	58	1	11.1
Koromatua	4-Jun-09	7	0.8	0
Mangahia	4-Feb-09	10	1.5	17.6
Ngaroto	2-Feb-09	130	3.5	1.7
Ohinewai	28-May-09	16	4.5	0.6
Otamataeroa	28-Apr-09	5	3	1
Puketirini	10-Feb-14	54	64	0
Rotokaeo	12-Dec-08	3.7	1.8	3
Rotokauri	29-Sep-09	77	4	0.8
Rotoroa	9-Jan-12	54	6	3.1
Waahi	8-Mar-07	522	5	7.2
Waahi	23-Mar-11	522	5	2.2
Whangape	24-Aug-10	1450	3.5	4.4

3.2 Methods

All four of the dogs that participated in Experiment 1 (Cassie, Tink, Cobie & Tommy), and an additional dog Mika also participated in this current experiment. Mika had been part of another scent detection project that used the same scent detection apparatus before joining this experiment (Chia, 2020). The previous study Mika was involved in required her to indicate a specific target and reject several other samples that had previously been targeted (Chia, 2020). Due to her previous experience operating the apparatus and discriminating between samples it was believed that Mika would learn to indicate on catfish and reject other samples (i.e., goldfish and control) very quickly. The water sample collection procedure remained the same as that used in Experiment 1.

3.2.1 Sample preparation & experimental procedure

The equipment used remained the same as what was used in Experiment 1, with the exception that auto pipettes were used in this current study to measure dilutions below 10 mL for accuracy. Samples of 100 mL were prepared in order of negative to positive as per the procedure followed in Experiment 1 to limit potential cross-contamination. As goldfish water samples were negative samples, they were prepared after controls, but on the same side of the stainless-steel bench as the control samples.

For the first dilution, goldfish and catfish samples were prepared using 50% water from aquaria containing fish and 50% water from the control tank (i.e., 50 mL fish water diluted in 50 mL no-fish water). This dilution was sequentially halved over the course of the experiment (Table 4) every time dogs met the completion criteria. The completion criteria were correct indications of catfish and correct rejections of both goldfish and control samples above 80% accuracy for 4/5 sessions. It was thought that halving sample dilutions, rather than diluting them logarithmically, would allow the dogs to more readily adjust to the new dilution. The number of positive catfish samples was reduced from nine to seven. This was done to allow for the presentation of five goldfish and five control samples, which were considered negative samples.

Table 4 Dilutions of fish water samples presented to scent detection dogs and their equivalent environmental biomass *based on a water volume of 20,000 m³ per hectare i.e., 100 m x 100 m x 2 m water depth.

Dilution	Fish water (mL)	Control water (mL)	Fish relative biomass in sample (g/L)	Equivalent environmental biomass* (x 1,000 kg/ha)
No Dilution	100	0	15.5	309.6
1	50	50	7.75	154.8
2	25	75	3.88	77.4
3	12.5	87.5	1.94	38.7
4	6.25	93.75	0.97	19.4
5	3	97	0.46	9.3
6	1.5	98.5	0.23	4.6

3.3 Results

This experiment found that dogs can discriminate between two fish species (i.e., catfish and goldfish) in water samples, and can do so at several diluted biomass concentrations. However, it should be noted that there were several impediments to the dogs' ability to progress in this study. Firstly, major renovations were made to the research space from December 2019 through to February 2020. During this time the facility could not be accessed and so the study could not run. Also during this study, the New Zealand Government released a COVID-19 alert and levels system in response to the global virus pandemic, which involved restrictions at four different levels (see: <https://covid19.govt.nz/alert-system/>). These restrictions meant that the study was only operational under COVID-19 Alert Levels One and Two, as New Zealand went into nationwide lockdowns under Alert Levels Three and Four. New Zealand's COVID-19 lockdown occurred from March 23rd to May 13th, 2020, which meant experiments were not able to run during this period.

Two dogs, Cassie and Tink, progressed to the lower dilution of 12.5 mL before goldfish samples were introduced (Figure 15 and 16, respectively). Both dogs' accuracy for indicating catfish remained above criteria across the 50 mL-12.5 mL dilutions, however, there was a drop in their performance for rejecting goldfish samples at the 12.5 mL dilution.

The researcher observed that after the introduction of goldfish, these two dogs struggled to discriminate between samples (i.e., they indicated on all fish samples) and would then not engage with the apparatus (i.e., lying down, barking at the door, reluctance to enter into the experimental room). After this observation, the methodology was changed so that all other subjects were presented with goldfish samples at the 100 mL concentration first before progressing down target sample dilutions.

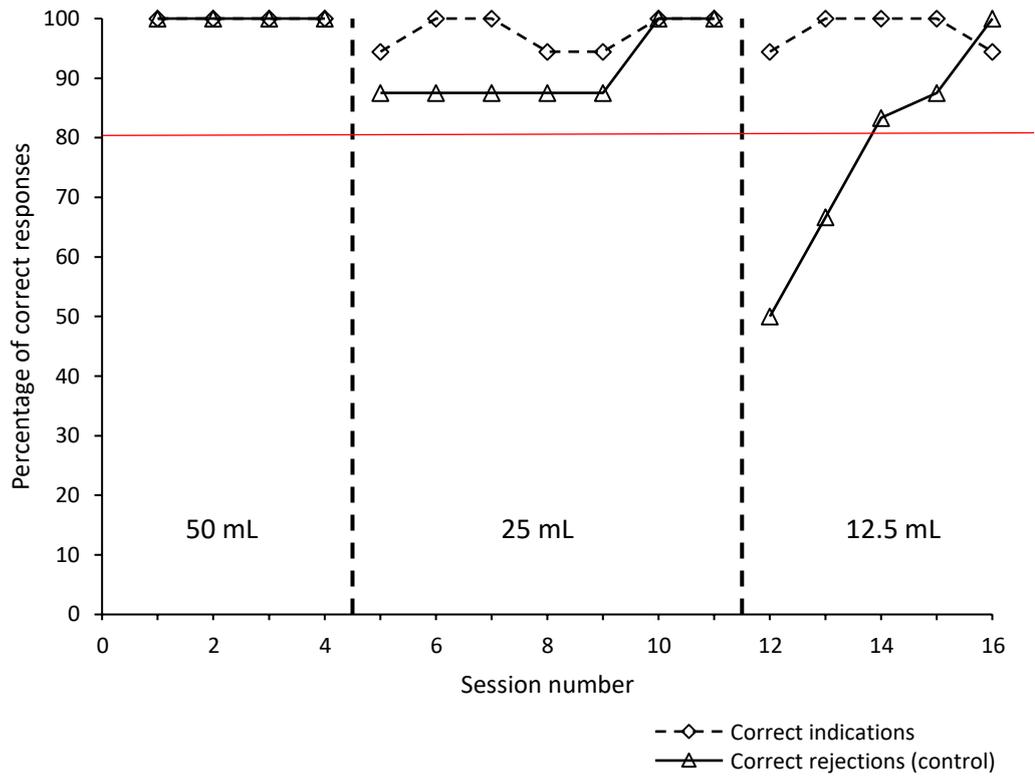


Figure 15. Cassie's performance accuracy across dilutions during Experiment 2: correctly indicating the presence of catfish water samples, and rejecting control water samples, with 80% accuracy criterion line shown in red.

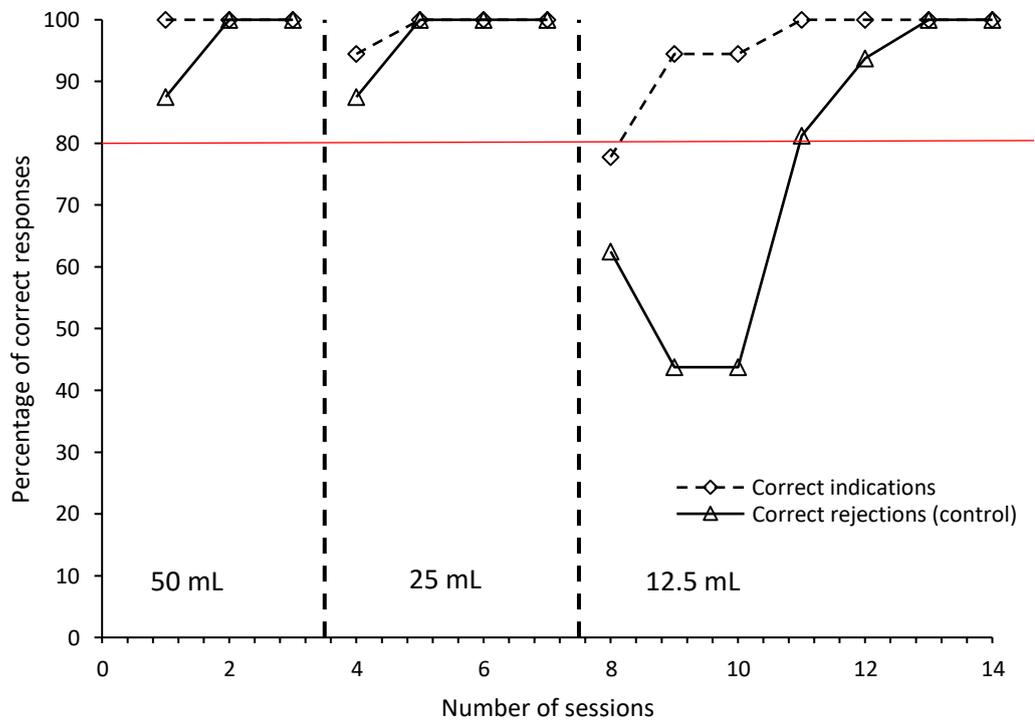


Figure 16. Tink's performance accuracy across dilutions during Experiment 2: correctly indicating the presence of catfish water samples, and rejecting control water samples, with 80% accuracy criterion line shown in red.

Tink never successfully learned to discriminate and rejected goldfish at the completion criteria at the dilution of 12.5 mL (Figure 17). After several attempts were made to improve her motivation to work without success (i.e., the researcher re-entering the room and phasing themselves out again, increasing the number of kibble reinforcers, changing the food reinforcer to Possyum), she was withdrawn from the study.

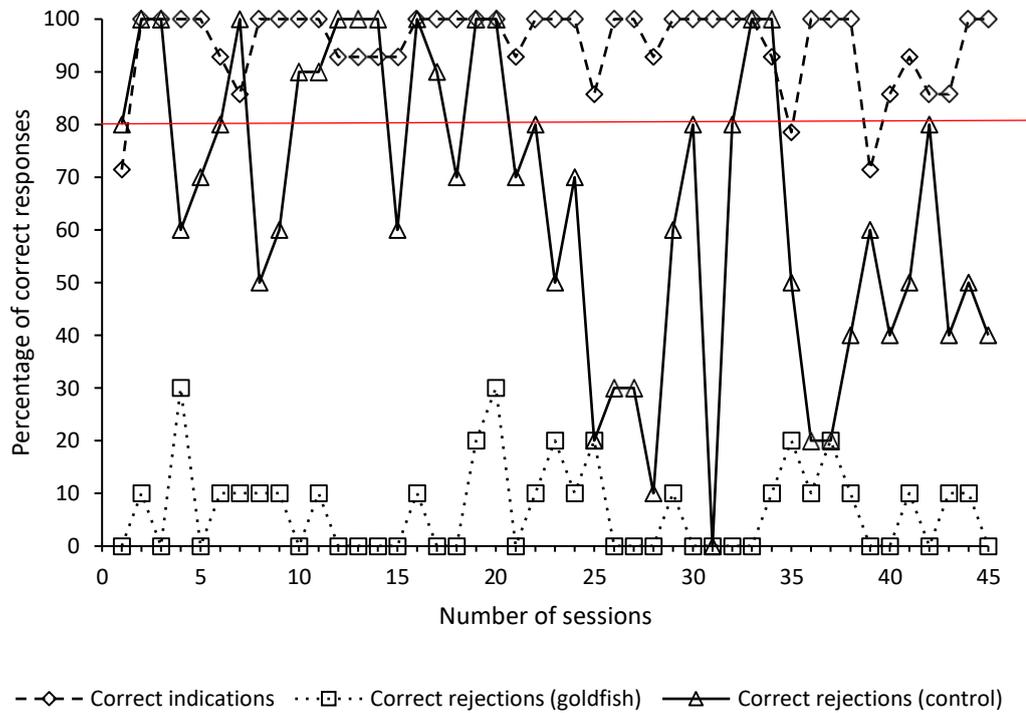


Figure 17. Tink’s performance accuracy at the 12.5 mL dilution during Experiment 2: correctly indicating the presence of catfish water samples, and rejecting control and goldfish water samples, with 80% accuracy criterion line shown in red.

Cassie progressed the furthest through dilutions out of all the dogs, reaching a sample concentration of 1.5% of the original training concentration (Figure 18). Her responding in terms of accuracy and time taken to progress to new dilutions improved greatly after she met the criteria for the 12.5% dilutions. However, her behaviour was variable; some days she would only get through a very limited number of sessions or would not engage with the apparatus.

Cobie consistently indicated on the catfish samples across the dilutions, reaching a dilution of 12.5 mL (Figure 19). Initially, when goldfish samples were introduced, Cobie started to indicate on control samples as well as the goldfish samples. The researcher had to re-enter the room to provide prompts to reject goldfish samples

during the initial concentration (data not graphed), but then faded themselves out

of the room again (as was done during training). After Cobie was working independently of the researcher again, her rejection rate of goldfish samples improved but was still inconsistent across dilutions.

Tommy's accuracy of indicating the presence of catfish remained consistent across dilutions, but his rejection rate of negative samples remained inconsistent, though it improved over time, reaching a dilution of 12.6 mL (Figure 20). Like Cobie, Tommy also initially indicated on control samples after the introduction of goldfish. The researcher had to enter the room to provide prompts at the initial concentration. Tommy's

Mika also reached the 12.5 mL dilution (Figure 21). She progressed significantly faster to the 50 mL dilutions than all other dogs in this experiment. Her accuracy remained consistent for the 50 mL and 25 mL dilutions, but she appeared to initially perform poorly to discriminate between goldfish and catfish at the 12.5 mL concentration.

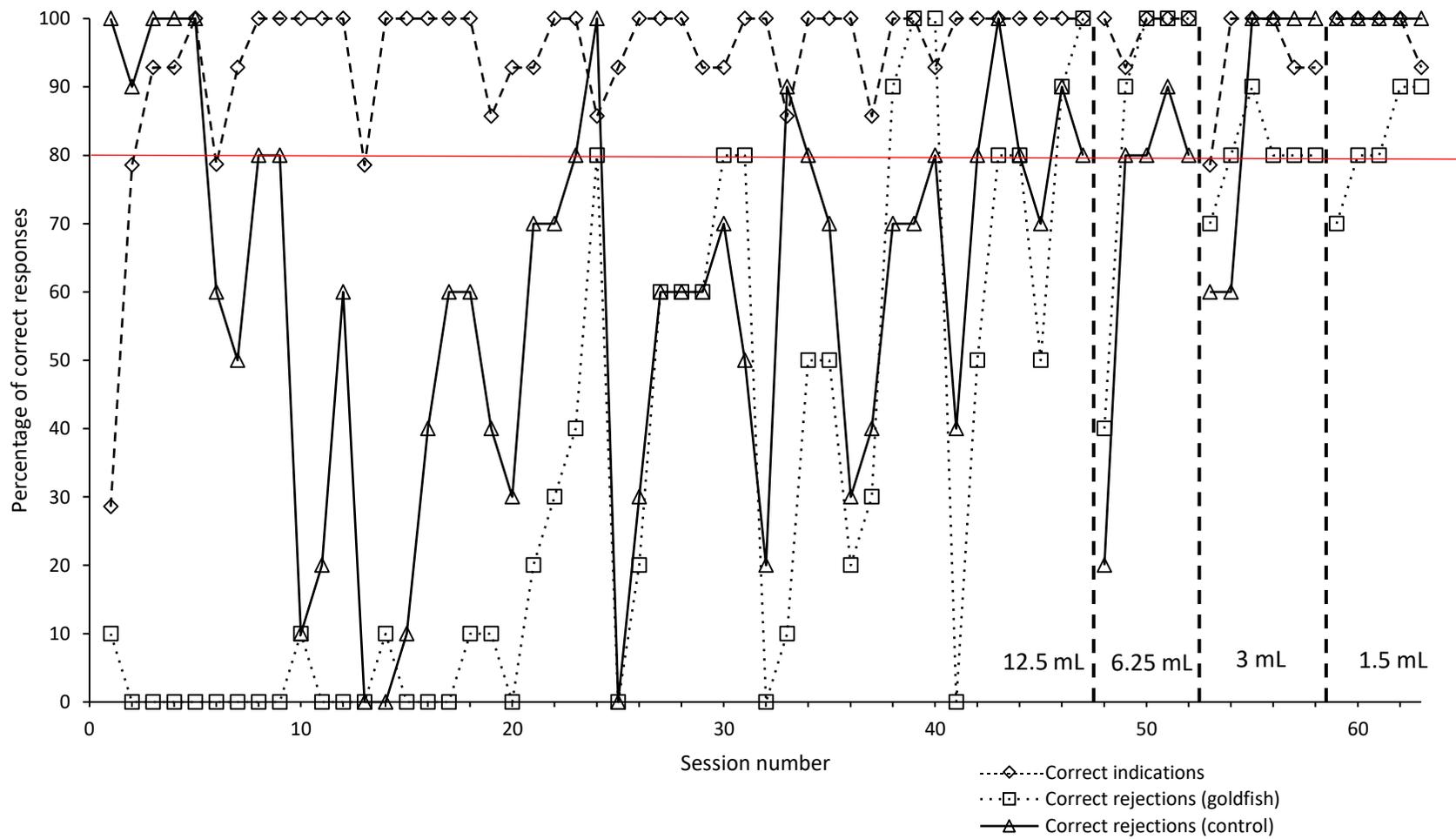


Figure 18. Cassie's performance accuracy across dilutions during Experiment 2: correctly indicating the presence of catfish water samples, and rejecting control and goldfish water samples, with 80% accuracy criterion line shown in red.

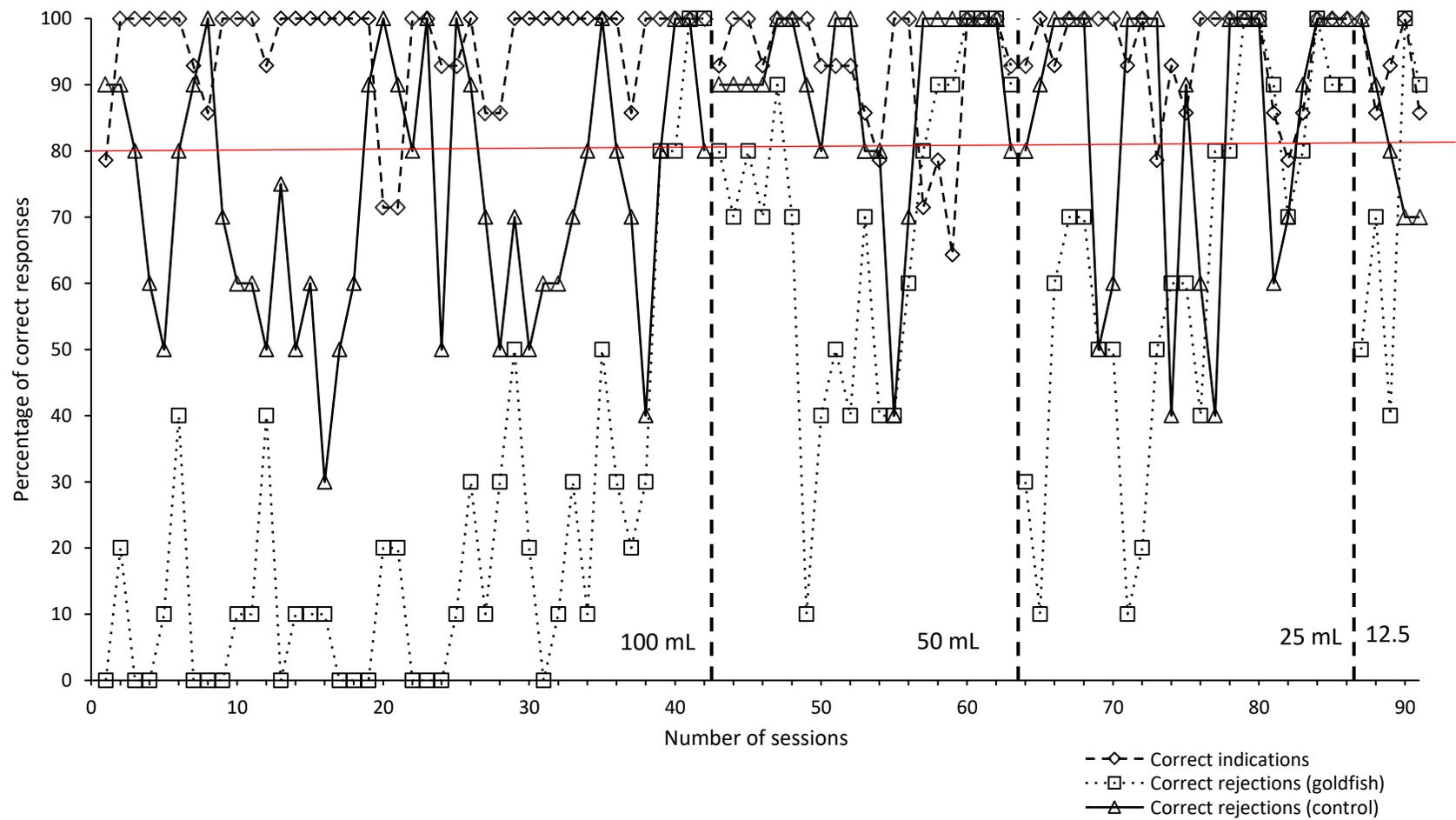


Figure 19. Cobie's performance accuracy across dilutions during Experiment 2: correctly indicating the presence of catfish water samples, and rejecting control and goldfish water samples, with 80% accuracy criterion line shown in red.

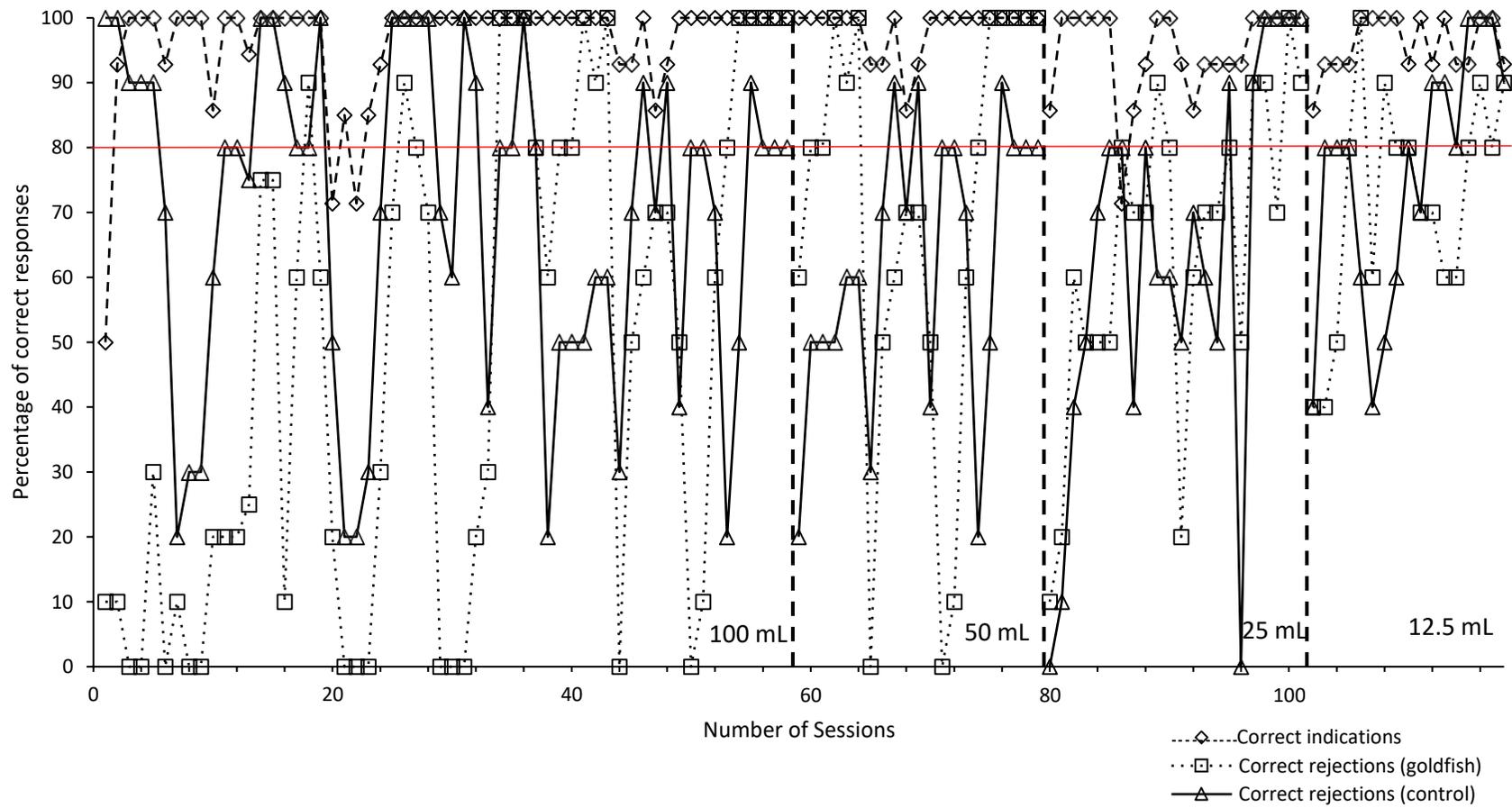


Figure 20. Tommy's performance accuracy across dilutions during Experiment 2: correctly indicating the presence of catfish water samples, and rejecting control and goldfish water samples, with 80% accuracy criterion line shown in red.

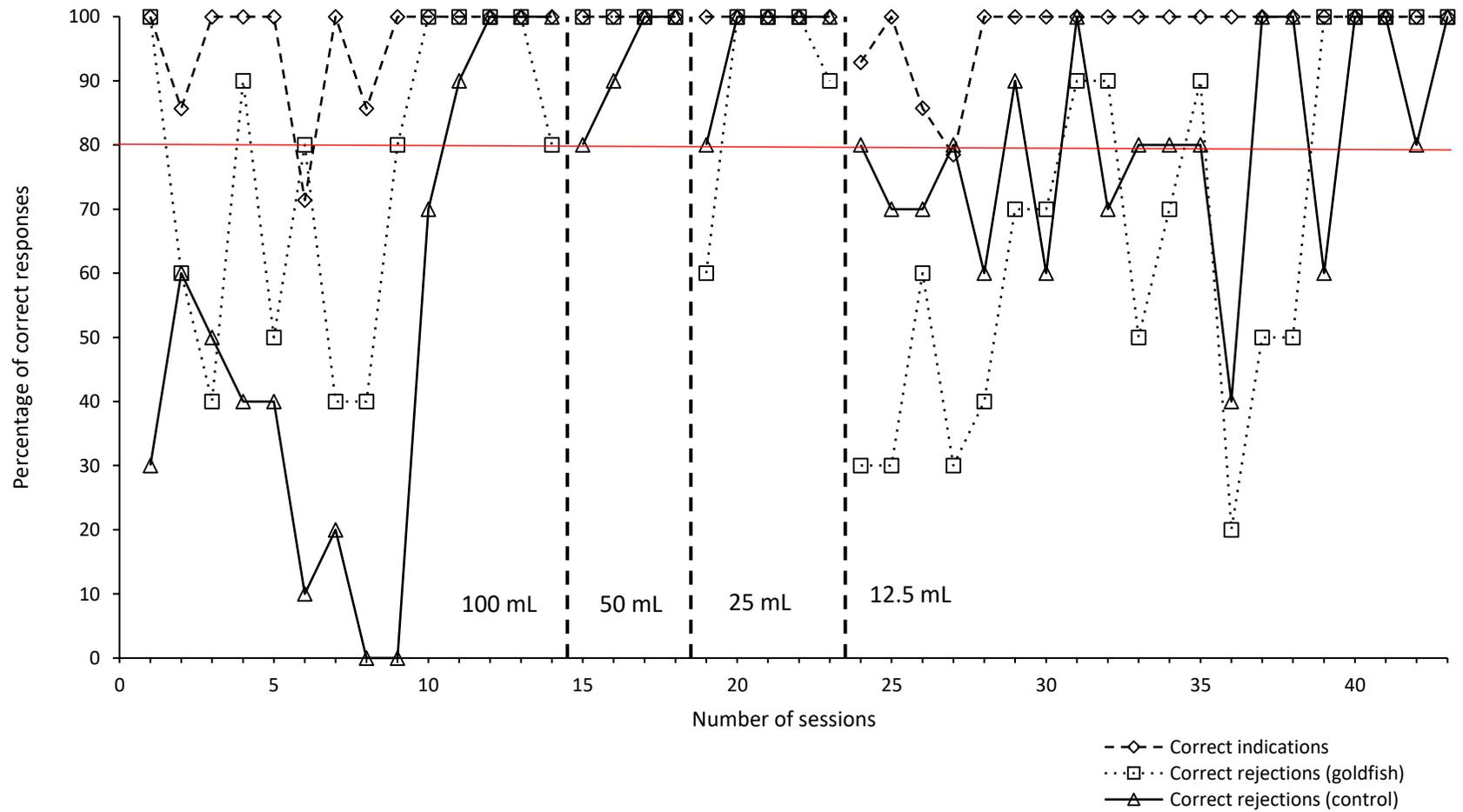


Figure 21. Mika's performance accuracy across dilutions during Experiment 2: correctly indicating the presence of catfish water samples, and rejecting control and goldfish water samples, with 80% accuracy criterion line shown in red.

3.4 Discussion

Conventional detection methods (electrofishing, netting, visual observation) are often unable to detect catfish in water that is deep or sparsely populated (Magnuson et al., 1994; Banks & Hogg, 2015;), due to the benthic behaviour of catfish. Dogs may prove to be a useful tool in this area if they can detect catfish at low biomass concentrations as would be present in the wild (see Table 3). In the present study, all but one dog (Tink) were able to discriminate between catfish and goldfish samples at criteria (80% accuracy for 4/5 sessions) and progressed down several sample dilutions. Cassie progressed to the lowest dilution, reaching concentrations of catfish and goldfish water which were 1.5% the original training concentration, an equivalent of 4,600 kg/ha. Currently, this dilution is not equivalent to environmental catfish biomasses in New Zealand lakes, which are significantly lower, usually under 20 kg/ha (Collier & Grainger, 2015). However, reaching this dilution is a promising start, and should further dilutions at biologically relevant dilutions be investigated, dogs could prove to be useful as a detection tool.

Following mastery of the previous dilution, the performance of each dog typically dropped during the first session in which they were exposed to a reduced sample dilution. Interestingly, it was observed that the dogs demonstrated a greater drop in accuracy for goldfish samples when moving from the 25% dilution to the 12.5% dilution. Without further analysis, it is unclear why this occurred; however, it may be due to the relative drop in signal strength which was perhaps more pronounced between these two dilutions. This phenomenon was also experienced by the dogs in the Quaife (2018) study, who also indicated incorrectly on samples at the 12.5 mL dilution but then improved on mastery of this dilution.

This study has not currently been able to show that dogs can detect the presence of catfish at biologically relevant levels, i.e., biomass concentrations that would be found in the natural environment (Table 3). This was due to the limited time frame in which the study was able to be operational, due to extensive laboratory renovation work undertaken, followed by the nationwide restrictions of the New Zealand Government's COVID-19 lockdown. However, it is possible that had further dilutions been prepared, the dogs that successfully completed this experiment may have been able to detect catfish at even lower concentrations than

in the present study. As such, dogs' detection threshold for catfish in water samples is something that needs to continue to be evaluated.

Cobie's lack of progress may not be reflective of her ability to detect scents. As mentioned in Chapter 2, Cobie experienced health issues during this research. During this experiment, she became increasingly unwell and started to present skin and gastric problems that prevented her from attending sessions for a long period of time. It was suggested that Cobie was having an allergic reaction to the kibble being provided, so upon her return to work, her kibble was provided from her owner's home. However, this new food appeared to not be as great a motivator for Cobie, compared to the previous food. It is possible that had Cobie not become ill she may have shown the higher levels of accuracy and progress shown by the other dogs in the study.

In the study by Quaife (2018), target fish (koi carp) water samples were first diluted to 0.098% of their original state, before other fish species samples to be discriminated between were introduced. Therefore, the researcher in the present study started to dilute Cassie's and Tink's target catfish samples before introducing goldfish. This methodology was changed after discussions with supervisors and it was decided that progressing down dilutions after the introduction of another species was preferable. The dogs in the present study did not progress as far down dilutions as those in Quaife's (2018) study. However, significant improvements to the sample preparation and cleaning procedure were made following Quaife's study, which could explain this result. For example, previously Quaife used plastic syringes to prepare his samples which could have residual odours making it easier for dogs to pick up scents. Whereas acid-cleaned glassware was used in this study to prevent residual odours.

3.5 Conclusions

It was found in this study that dogs can detect and distinguish catfish odour from goldfish at a level equivalent to an environmental fish biomass of 4.5 x 1,000 kg/ha. Whilst this finding is encouraging, this is currently a significantly higher concentration than what would be found in a lake infested with catfish, and so further research into dogs' detection threshold must be done in order to confidently say that dogs can identify catfish at biologically relevant levels in the natural environment.

Chapter 4: Domestic Dogs' Ability to Discriminate When Presented Target Samples at Differing Dilutions

4.1 Introduction

Experiment 2 (Chapter 3) showed that dogs can discriminate between catfish and goldfish present in water samples, the first demonstration of dogs' ability to do so. However, it remained to be seen if dogs can do so at biologically relevant biomasses. Dogs will only be useful as a detection tool if they can detect the presence of catfish relative to real-world scenarios, i.e., at concentrations below the threshold where habitat degradation begins to occur, and native species start to decline (Rowe 2007; Schallenberg & Sorrell 2009). Previous research into dog's detection thresholds has found that dogs can detect lower limit concentrations of n-amylose at approximately one part per trillion; the equivalent of a single drop of liquid in 20 Olympic-size swimming pools (Walker et al., 2006). Thus, it is expected that dogs will be able to detect the presence of catfish at very low thresholds.

Experiment 2 also raised the question of whether dogs would still indicate the presence of catfish when different sample biomasses were presented concurrently during the same apparatus rotation. This was done this is what would likely be done in a real-world sample scenario

This experiment aimed to present samples of catfish at a known concentration (12.5 mL dilution; equivalent to a fish concentration of 1.94 g/L) at which the dogs had previously met success criteria and to evaluate if dogs continued to indicate on lower concentration catfish samples (called 'probes') when they were presented during the same scent detection apparatus rotation.

4.2 Methods

Two dogs from Experiment 2 (Tommy and Mika) were selected to participate in this current experiment. These two dogs were selected as they were the most consistent in terms of their responding to the apparatus and availability to attend sessions.

4.2.1 Sample preparation

For this experiment, in each session, the dogs were presented with 5 control samples, 5 goldfish samples, 3 catfish probe samples (these probe samples progressed to lower concentration sample dilutions as dogs met criteria), and 4 standard positive catfish samples (at the 12.5 mL dilution; a sample concentration at which dogs had previously met criteria). The probe sample dilutions progressed from the 12.5 mL dilution the dogs reached in Experiment 2, as per the dilution procedure explained in Section 3.1.1; i.e., the dilution was sequentially halved over the course of the experiment (Table 4) every time dogs met the completion criteria.

Samples were prepared as described in the previous experiments, in order of negative to positive (i.e., control, goldfish, probe, standard) on the opposite sides of the stainless-steel bench, to prevent cross-contamination. Once prepared, the samples sat in the apparatus for 20 minutes prior to the commencement of sessions, to allow sample volatiles to permeate throughout each segment.

4.2.2 Experimental procedure

This experiment ran at the same time as Experiment 2, so experimental sessions involving the use of probes always ran before dogs that were still participating in Experiment 2. A separate set of segments was used for this current probe experiment, and the apparatus was cleaned with 60% isopropanol solution between different set-ups as per Experiment 2. During this experiment, samples were left on the apparatus for no longer than one hour.

The procedure for this experiment was the same as described in Experiment 2, with the exception that correct indications on probe samples were also reinforced with a food reward. The termination criterion was also changed due to the limited time frame available due to COVID-19 restrictions. The new criterion for dogs to progress to subsequent probe dilutions was the dogs achieving an accuracy of 80% and above for each response (correct indications and rejections) for 2/3 sessions.

4.3 Results

Both dogs met the criteria at the 3 mL probe samples, but no further sample dilutions were prepared due to time constraints (see Chapter 3). Tommy was consistent with correct catfish indications and goldfish rejections, even when two

dilutions of catfish samples were present (Figure 22). Upon introduction of probes, Tommy's performance initially regressed to him incorrectly indicating on control samples (as observed in Experiment 2 after the introduction of goldfish samples), but his responding returned to criterion accuracy (80% and above accuracy) within seven sessions during the 6.25 mL probe sample presentations. Tommy progressed to the 3 mL probes dilutions and met the success criterion at this dilution, but further dilution presentations did not take place due to time constraints.

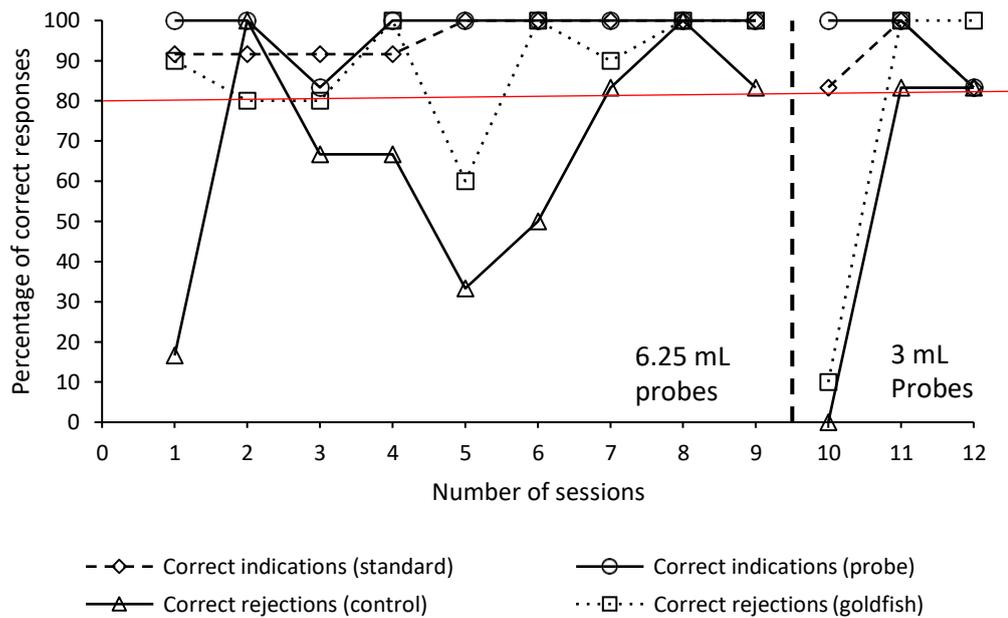


Figure 22. Tommy's performance accuracy across probe sample dilutions during Experiment 3: correctly indicating the presence of catfish water sample (probes and standard 12.5 mL dilution), and rejecting control and goldfish water samples, with 80% accuracy criterion line shown in red.

Mika's performance also remained consistent with correct catfish indications and rejections of goldfish after probes were initially introduced at the 6.25 mL dilution (Figure 23). However, at this dilution, she experienced a regression to indicating on controls as in Experiment 2, but her accuracy improved to criterion within 4 sessions. At the 3 mL dilution, Mika initially indicated on both goldfish samples and control samples, but then met the criteria to continue progressing to the 1.5 mL dilution on the last day of running experimental sessions.

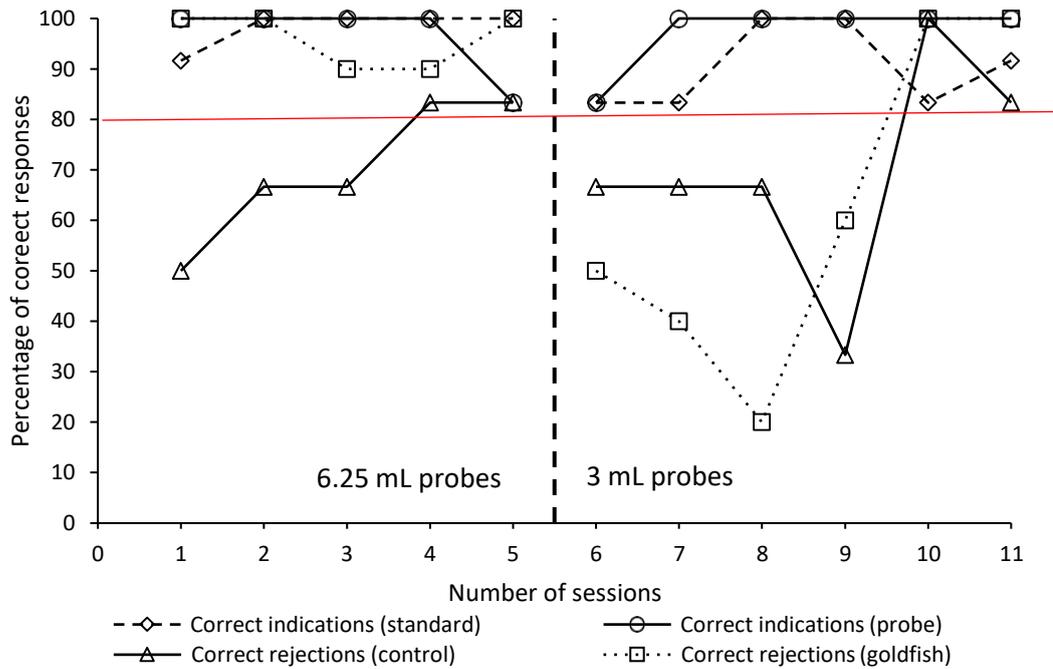


Figure 23. Mika's performance accuracy across probe sample dilutions during Experiment 3: correctly indicating the presence of catfish water samples (probes and standard 12.5 mL dilution), and rejecting control and goldfish water samples, with 80% accuracy criterion line shown in red.

4.4 Discussion

The results of this experiment show that both dogs could have progressed down to the 1.5 mL dilution (equivalent environmental biomass of $4.6 \times 1,000$ kg/ha, that was achieved by Cassie in Experiment 2). While this biomass is still not relative to real-world biomass concentrations of catfish which would be under 20 kg/ha (Collier & Grainger, 2015). This finding further supports those of Experiment 2 that dogs could be useful as a potential detection tool if their detection thresholds are further evaluated. It is recommended that more sample dilution experiments occur in the future, as this study was not able to reach biologically relevant concentrations. A fish biomass concentration relevant to real-world sampling would be a sample concentration equivalent to approximately 50 kg/ha of fish (N. Ling pers. comm.). To achieve this criterion the catfish sample dilution would need to be 0.016% of the initial concentration used in Experiment 1, i.e. 100-fold lower than the criterion achieved during this experiment.

The results of this experiment also show that dogs can indicate the presence of catfish when different dilutions are presented concurrently. This was important to establish as if this method of using scent detection dogs were to be used as an actual

detection tool, the exact biomasses of naturally sourced samples may not always be equal. It would also be interesting to evaluate if dogs would still indicate the presence of catfish in mixed-species samples, as it is a rare occurrence for a single invasive species to be present (Collier & Grainger, 2015).

Data for this experiment was limited by time constraints due to the COVID-19 epidemic, but also due to issues experienced with the operation and testing of the apparatus (discussed further in Chapter 5). As a custom-built machine that was constantly being updated, the apparatus did not always function as intended which halted progress (i.e., not turning to the correct sample, turning itself off, not producing kibble rewards appropriately).

4.5 Conclusions

Dogs can indicate the presence of catfish from water samples at biomasses equivalent to an environmental biomass of 4.6×1000 kg/ha, even when different sample concentrations are presented concurrently. This finding is important as in real-world scenarios samples presented to scent detection dogs may not always be of the same biomass concentration. However, it is necessary to continue to evaluate the catfish detection limit threshold before the use of detection dogs is confirmed as a useful tool in comparison with conventional techniques such as visual survey, netting, electrofishing, and eDNA. As dogs will only be useful as a detection tool if they can detect the presence of catfish at biomass concentrations relative to real-world scenarios, preferably at concentrations below the threshold where habitat degradation begins to occur with invasive species.

Chapter 5: General Discussion

This study was the first to evaluate if dogs could detect the presence of invasive brown bullhead catfish in water samples taken from aquaria that contained these fish. Once it was confirmed that dogs could detect catfish presence, a secondary aim was to investigate if dogs could discriminate between fish species (i.e., between catfish and goldfish) or they were just indicating the presence of “fish” scent. Furthermore, this research also investigated at what biomass concentrations dogs can discriminate the presence of catfish from water samples.

The results of the experiments described in this thesis show that dogs can detect and discriminate the presence of the catfish and goldfish from water samples at a range of concentrations (equivalent to environmental biomasses of $4.6 \times 1,000$ kg/ha that was achieved by Cassie in Experiment 2). To confirm a dog’s usefulness at detecting early incursions of catfish, further study needs to be done with a fish biomass concentration relevant to real-world sampling, i.e., a sample concentration equivalent to approximately 50 kg/ha of fish (N. Ling pers. comm.). To achieve this criterion, the catfish sample dilution would need to be 0.016% of the initial concentration used in Experiment 1, i.e., 100-fold lower than the criterion achieved during this study, as catfish biomasses in real lakes are usually under 20 kg/ha (Collier & Grainger, 2015). Nonetheless, this initial investigation is an encouraging start. This study supports previous findings by Quaife (2018), which demonstrated dogs can discriminate between three invasive New Zealand freshwater fish species (koi carp, goldfish, and catfish), and suggests that there is potential for dogs to detect catfish from water samples at biologically relevant levels. These results add to the growing body of evidence that there is potential for the utility of dogs in detecting invasive freshwater fish species, which has important implications for the management and conservation of waterways in New Zealand and around the world.

5.1 Implications for waterway management and conservation

Invasive species now dominate many aquatic landscapes in most parts of the world, displacing native plants and animals by disrupting and altering ecosystems (Sakai et al., 2001; Molnar et al., 2008; Gallardo et al., 2015). Effective biosecurity requires routine monitoring with sensitive detection to identify new incursions early before invasive species establish. Currently, the detection of catfish using conventional methods (i.e., visual survey, netting, electrofishing) have at times,

been unable to detect this invasive species in areas where they are suspected to exist, due to catfish's benthic behaviour (e.g., as in the case of the Te Arawa lakes, detailed in Chapter 1). As a result, another method of detecting this invasive species would be of value for waterway management and conservation.

Previously, scent detection dogs have proven themselves to be a valuable tool to aid conservation efforts and have been recognised for their ability to outperform human surveyors in locating and discriminating between cryptic species. For example, dogs have been successfully trained to detect imported fire ants and associated nests in the field (Lin et al., 2011); five species of termites and discriminating wood that was termite-damaged vs damaged by other insects (Brooks et al., 2003); invasive brown tree snakes (*Boiga irregularis*) in Guam (Savidge et al., 2011); desert tortoises (*Gopherus agassizii*) and associated burrows (Cablak & Heaton, 2006); Marlborough green gecko (*Naultinus manukanus*), forest gecko (*Hoplodactylus granulatus*), and tuatara (*Sphenodon punctatus*) scents (Browne et al., 2015). Dogs have even been shown to be able to discriminate between 19 different plant species in their natural environment (Sargisson et al., 2010). The present study suggests that dogs could be a useful tool for detecting the presence of catfish, as not only can catfish's presence be detected from water samples alone, but dogs can also distinguish between species. It is possible that dogs' ability to detect catfish from water samples could trigger more intensive investigations (e.g., eDNA and further netting/electrofishing expeditions).

5.2 Advantages of using an automated scent detection method

The results of this study suggest that the ability of dogs to detect freshwater species through water samples alone could provide an advantage over conventional sampling methods. The collection of water samples is minimally invasive, meaning that it is unlikely that aquatic species will be harmed and affected by sample collection, as opposed to accidental bycatch and injury that can occur during netting and electrofishing. Current detection methods (e.g., visual survey, electrofishing, and netting), can need substantial time investment, especially if the terrain is difficult and targets are sparsely located (Rebmann et al., 2000 Hicks et al., 2015). The proposed alternative detection method investigated in this thesis is the use of an automated scent detection apparatus. This apparatus provides a relatively fast and efficient solution for evaluating samples. A typical session involving 17 unique samples lasts approximately 5 minutes and allows each sample to be

evaluated twice by the dog, and multiple dogs can be used to assess the samples over 30 minutes. Some concerns may be raised over the time investment that is needed to train dogs to be able to detect catfish at biologically relevant levels, as the dogs in this study have not yet reached concentrations comparable to real-world scenarios. Training time was not evaluated in this thesis as training was not consistent due to many factors imposing time constrictions as previously discussed in Chapters 3 and 4. This thesis was a proof of concept study, so once it is established that dogs can detect catfish at biologically relevant levels, it may be possible that dogs are trained using lower limit concentrations right from the start of training. Direct comparisons of training investment with other studies are not straightforward because of differences in training protocols, accuracy criteria, and the dogs' previous experience (Edwards, 2019). For example, Williams and Johnston (2002), trained dogs to accurately identify multiple olfactory targets, the dogs required an average of approximately 29 sessions (a unitless measure) to meet criteria with the first target.

Several important factors have been identified in scent transportation processes in water. VOCs can be modified or altered by factors such as water temperature, oxygen content, salinity, pH, buoyancy, and turbulence (Richards, 2018). Increased evaporation enhances scenting conditions, and evaporation can be greater when air is dry, water temperature is high, salinity is high, air pressure is low, the surface area is great and there is increased wind velocity (Rebmann, David & Sorg, 2000; Sargisson et al., 2010; Savidge et al., 2011; Chambers et al., 2015). With the use of an automated apparatus in the current study, some of these factors could be controlled for. Sample water was stored at 4°C when not in use. When presented to dogs, the samples were contained within segments, which allowed for scents to evaporate from sample jars and permeate throughout the segment for dogs to access. The experimental room the dogs worked in was temperature-controlled, so the air was always warm and dry.

Conservation efforts are often underfunded, driving researchers and conservationists to constantly innovate in order to best maximise the funding available (Balmford et al., 2000; Wilson et al., 2007; Waldron et al., 2013). Unlike other survey equipment, dogs are sentient and intelligent animals, which means there are many welfare obligations for the dogs' care. Laboratory dogs need appropriate housing, stimulation, exercise, and a retirement plan (Ng and Fine,

2019; Cristescu et al., 2020;). The use of pet dogs in this study rather than laboratory dogs reduces expenses and ethical issues associated with the housing and care of laboratory animals. In return, pet dogs are provided with stimulation and enrichment by coming into the laboratory to work but go home at the end of the day with their owners.

The use of scent detection dogs provides a useful monitoring tool but also represents an extremely valuable tool in an educational context (Richards, 2018). Public education and engagement are critical for conservation and eradication efforts to remain successful (Sawchuk, 2018). The use of dogs provides an opportunity to engage the general public about conservation issues, as owners and volunteer dog walkers share knowledge about what their pets are working on and why. Dogs also attract media attention which helps disseminate conservation and biosecurity messages. For example, in the initial pilot project of using dogs to detect invasive mussel spawn in Alberta Canada, people outside watercraft inspection stations were educated about invasive mussels and their ability to spread, by canine-related speaking engagements, demonstrations, and media interviews. Seventeen independent media outlets outside of Alberta even carried the story of the dogs nationally. It is estimated that the total captured media exposure generated over \$850,000 of free public education with a projected 111.8 million impressions (Sawchuk, 2018).

5.3 Factors for consideration when working with scent detection dogs

No monitoring tool is entirely infallible, and the effective use of any tool relies on recognising and acknowledging its strengths and limitations. Dogs, like their human counterparts, can never be 100% accurate (Richards, 2018). There are several factors to consider when using scent detection dogs, including their initial selection for work and false negative or positive indications. The more widespread use of scent detection dogs is limited by the uncertainty regarding why some individual dogs succeed while others perform poorly during training tasks, and the costly nature of dog selection and housing (Beebe et al., 2016). There are also negative

aspects associated with using pet dogs rather than working dogs; for example, this study was limited by the availability of owners to drop off their pets and reliability of dogs' performance of the task, which prolonged training time and interfered with sample dilution evaluations during Experiments 2, 3 and 4 respectively.

5.3.1 Dog selection

The scarcity of publicly available knowledge regarding the selection of appropriate scent detection dogs is a significant barrier for organisations who want to use scent detection dogs in conservation settings (Beebe et al., 2016). There is a clear need for the development of standardized tools, which may be deployed reliably throughout the entire industry. However, the development of such tools is an extremely difficult task (Beebe et al., 2016).

While dogs' olfactory capabilities are critical for scent detection work, there is also a range of other characteristics to consider: temperament, personality, and behaviour also play a critical role in determining success or failure (Helton, 2009; Dahlgren et al., 2012). Maejima et al. (2007) identified two factors that are predictive of success during scent detection tasks: (1) desire for work, and (2) distractibility. One of the main difficulties faced by researchers is that many different words may be used to describe very similar behavioural traits, making it difficult to pinpoint exactly which traits are of key importance to scent detection dog success (Beebe et al., 2016). To address this problem, it has been proposed that discrete categories that encompass a broad spectrum of temperament, personality, and behavioural traits should be used (Svartberg, 2002; Jones and Gosling, 2005). For example, it is suggested that boldness may be a particularly important trait in the successful completion of work tasks because dogs that are more fearful and anxious are easily distracted, take longer to train, or may never be successful despite intensive training (Svartberg, 2002). This suggestion is why owners were asked to fill out the initial screening questions (Appendix E), which helped the researcher to evaluate dogs' potential for scent detection work before the commencement of training. The low training-completion rate of dogs in the current study (i.e., of the 14 dogs recruited, only five participated in experiments) reflects that many factors intervene between selection and eventual success.

One of the most commonly selected traits for scent work is a strong motivation for play or food, as this kind of training requires the dog to learn to associate the target

scent with an object of intrinsic pleasure such as play or a food reward (Berridge & Kringelbach, 2013). The more the dog desires play or food, the more successful the training process can be, since this desire increases the dog's focus, decreases distractibility, and increases motivation to work for sustained periods of time (Beebe, 2016). Food motivation was considered important in the present study, as a handler would not be present to provide a play reward as reinforcement. The ordinary dry dog kibble used in this study is not an effective reinforcer for many dogs. Fortunately, during this study, another feeder that was capable of dispensing Possum (a more palatable food) became available for use. This allowed those dogs with low food motivation to work for this reward instead. Not all dogs continued to work when exposed to noise, movement, and other environmental distractions experienced at times during experimental sessions, further making the recruitment of appropriate dogs difficult. However, the data from Experiments 1 and 2 show that, given the right conditions (and the right subjects), dogs can learn to indicate the target scent relatively quickly after the initial training is completed.

5.3.2 False-negative and positive results

Field ecologists have always been aware that observations in nature are prone to detection mistakes, even if sites are surveyed multiple times (Ficetola et al., 2016). A series of procedural and quality control measures must be adopted to limit false-positive impact (Ficetola et al., 2016). This includes following procedures to reduce the risk of cross-contamination, and multiple analyses of the same sample to obtain measures of the reliability of results (Ficetola et al., 2016).

Strict sample collection and preparation procedures were followed in the current study to limit cross-contamination, and multiple dogs were used to assess the same samples. Despite the thorough steps taken to ensure sample fidelity, it was observed that dogs sometimes falsely indicated a sample as positive during both presentation rotations of the apparatus. There is a possibility that negative samples may have become contaminated with the target scent during handling and preparation. If control samples had become contaminated, then in those cases the dogs were still indicating the presence of the target scent, which may support previous reports of dogs' outstanding olfactory sensitivity. Had samples remained uncontaminated, the dogs' correct rejection rates might have been even greater than those reported in this study. Alternatively, it is also possible that the dog's responses may have simply been incorrect during those trials, and that samples did

remain uncontaminated throughout the study, although this cannot be verified. Cristescua et al. (2020) recognised that it is important not only to train dogs to recognise and indicate a target odour, but also to invest time in training to ignore all non-target odours. This additional training step decreases the risk of false positives in field conditions (i.e., where the target odour is not always present), and was a key part of the dog training in this research. The significant time needed to indicate the presence of a sample (5,501 ms) in this study and the fact that rejection of negative samples was not rewarded, helped dogs learn that it was quicker to reject samples for another chance at the possibility of reward for correctly indicating the presence of the target in an up-coming positive sample.

Nevertheless, conventional methods such as eDNA analysis also have the potential to falsely indicate or fail to detect species that are present (Ficetola et al., 2016). False positives can occur due to multiple reasons, such as contamination during sampling or during laboratory work, polymerase chain reaction (PCR), or sequencing errors (Ficetola et al., 2015). If we use eDNA or dogs for the detection of an invasive species and falsely state it is present in a given area of interest, it is possible resources may be wasted in the attempt to locate or eradicate absent species.

5.3.3 Apparatus improvement

Laboratory scent-detection work with dogs has typically been a manual process whereby some or all aspects of the procedures are mediated by researchers or handlers (Edwards, 2019). It is important to note that the novel scent detection apparatus was developed to improve laboratory-based research and operations, not as a general training resource for dogs that would eventually work in the field (Edwards, 2019). This would not present an issue to the future application of catfish detection, as dogs would not go into the field, but would be presented samples taken from field sites.

While automation of the scent detection process does eliminate issues associated with cueing, subjectivity in data collection, and reinforcement delivery, the apparatus employed in this thesis is a complex machine that is an ongoing work in progress. During both training and experiments, several observations were made that highlighted areas of improvement needed for the apparatus and training

methodology. Some of these issues proved difficult to identify and fix as they were triggered by a dog's idiosyncratic behaviours and it was at times difficult or impossible to isolate and replicate the issue by the researcher, which caused delays in training and experiment progress. For example, during training, one dog (Roxxy) learned that breaking the infrared beam but not opening the segment flap would still produce a food reward. Following this, more care was taken to ensure dogs were correctly opening the segment flap, and a camera was placed behind the apparatus so this could be confirmed when the researcher was observing in the adjacent room.

Another issue identified was dogs placing their nose back into the porthole too early, before the apparatus could properly adjust to the next sample. If a dog put its nose in too early then the apparatus would not register a sniff was occurring, the dog needed to take its nose out and put it back in again in order to trigger the appropriate recording. However, if the sample was a positive and a positive indication sniff was not being reinforced sometimes the dog would take their nose out, put it in again and then move to the omnidirectional switch to indicate the sample as negative (recorded in the data as a miss).

After the above-mentioned 'nose too early' observation was made with multiple dogs, several alterations were made to the apparatus and its programming. More salient visual and auditory cues were used to prompt sample port entry or activation of the switch. First, the acrylic glass was made transparent so that dogs could visually see that the apparatus was still moving. Then the programming code was modified so that the apparatus produced a buzzing sound when moving, different from the prolonged beep of a sample being investigated, followed by two brief beeps when the carousel has completed its rotation to the next programmed segment. This change was made with the intention of reducing sample port entries before the next segment was in position. These alterations proved to be successful for some dogs and not others, so further adjustments are still necessary to improve this issue.

Edwards (2019) has also suggested further refinements to the present apparatus could expedite and simplify the training procedure. For example, increased automation of the shaping procedure, whereby the software could be programmed to automatically increase the indication duration threshold following a series of hits or decrease the threshold following a series of misses rather than having a

researcher providing reinforcements. This could potentially reduce the time required for training.

Despite the challenges and improvements to the apparatus faced during this study, it still produced the first data demonstrating dogs' ability to detect and discriminate catfish. As a handler was not required for dogs to perform the task, this finding can confidently be stated as being a result of dogs' scent detection abilities, rather than their ability to read handler cues.

5.4 Future research and considerations

This research was the first to show dogs' ability to detect and discriminate catfish from water samples. To confirm a dog's usefulness at detecting early incursions of catfish, further study needs to be done with a fish biomass concentration relevant to real-world sampling, the catfish sample dilution would need to be 0.016% of the initial concentration used in Experiment 1, i.e., 100-fold lower than the criterion achieved during this study. Experiment 3 ended when the dogs had met the criteria for the 1.5 mL dilution but could have continued working down dilutions, with the target of investigating biologically relevant dilutions. However, due to previously described interruptions (i.e., research space renovations and COVID-19 virus), and thesis time restrictions, it was necessary to end the experiment. These findings suggest the potential for biologically relevant dilutions to be met outside of the scope of the current thesis.

Further investigations of dogs' ability to detect the presence of catfish from water samples should include the use of naturally sourced water, as this contains other compounds that could impact detection (e.g., fine sediments, humic substances from plants, and organic compounds from aquatic fauna). A logical step would be to have dogs evaluate water taken from sites where catfish are known to be present and to be absent. Following this, the preservation methods of samples should also be evaluated, so that if in the future the use of dogs does prove to be a viable option, then how samples can best be collected, sent, and preserved for later analysis will be known. It would also be of interest to conduct an analysis of VOCs in water samples, to determine which compounds might be serving as the discriminative stimuli for dogs, and how these volatiles vary between fish species.

While dogs' ability to detect catfish was selected for investigation in this thesis due to concern over this species' environmental impacts, dogs have the potential to

detect other introduced and native freshwater species as well. A decline of one species in complex ecosystems can have large-scale impacts (Barnes, 1996; Rachel, 2002; Francis, 2019, Tempero et al., 2019). As of 2018, 76% (39 of 51 species) of New Zealand's indigenous fish and 26 % (177 of 670 species) of indigenous invertebrates are classified as either threatened with or at risk of extinction, including many taonga (culturally significant) species (Williams et al., 2017; Grainger et al., 2018). Scent detection dogs could be used to identify areas where these threatened species exist in order to better conserve them.

5.5 Conclusion

The results of this study show that dogs possess the ability to detect the presence of catfish from water samples, the first research of its kind to do so to the author's knowledge. It was found that not only can dogs detect the presence of catfish from water samples, but also that they can discriminate between species (i.e., catfish and goldfish). The catfish biomass concentrations (4.5 x 1,000 kg/ha) dogs were able to detect in this study are 100-fold above those found naturally in lakes. At these concentrations, great ecosystem harm is already occurring. These findings support the suggestion that scent detection dogs have potential as a biosecurity monitoring tool for aquatic ecosystems, as dogs met criteria at the end of Experiment 3 demonstrating the potential for them to progress down further dilutions. However, further study must be undertaken to evaluate the ability of dogs to detect catfish at biomass concentrations below the threshold at which habitat degradation begins to occur as this was not done in the current study. This further investigation must be done using samples of naturally sourced water, as natural water contains many other scents. This further information will necessary to be able to confidently state whether scent-detection dogs provide an advantage over current conventional methods. The use of an automated scent detection apparatus showed that it is possible to improve scent detection procedures and eliminate problem factors such as unintentional handler curing and environmental influences, so long as sufficient reinforcement is given to complete scent detection tasks.

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Appendix A

Handling and Care of Pet Dogs for Research

General:

This document outlines the general procedure for the handling and care of pet dogs being used in research.

This protocol has been developed with reference to the Animal Welfare (Dogs) Code of Welfare (Animal Welfare Advisory Committee, 2010, <http://www.mpi.govt.nz/protection-and-response/animalwelfare/codes-of-welfare/>)

Location/Equipment:

- Dog studies will be carried out in the Scent Detection Research Group (SDRG) facilities on the campus of the University of Waikato. Facility 1 has 5 separate rooms; one room for the apparatus to run the experiment, two rooms with kennels to house the dogs, and other rooms used for sample preparation and working space. Facility 2 has a similar arrangement but can be used as two separate canine research facilities (with separate kennel and experimental spaces). Adjacent to the experimental facilities is a grassy field in which the dogs will be exercised and allowed to toilet while on leash.
- A maximum of 5 dogs will be present in any of the facilities at any one time.
- Dogs will not be held at the facility overnight. Arrangements will be made by the researcher to meet owners when they drop off their dog before each research session and for pickup afterward. Under exceptional circumstances, after consultation with supervisors, a researcher may pick up or drop off the dog from the dog owner's home.
- Each dog will be held in a separate kennel, crate, or tie-up station containing bedding, toys, and a bowl of freshwater.
- A logbook will be kept with the name of the dog, arrival time and collection time, and contact details of the owner. Dog owners will sign in upon arrival and sign out when they have picked up their dogs.
- Dogs must always wear a collar and be on a lead, except when they are in the kennels or in some cases in the experimental room (depending on the exact requirements of the study).
- All dogs must be fully vaccinated up to date for the core diseases: distemper, hepatitis, parvovirus, and leptospirosis. Kennel cough vaccination is also required. Vaccinations are confirmed by sighting and photographing the vaccination certificate; all vaccination records will be stored in a database and reviewed monthly – if a dog's vaccination is due, the owner will be

asked to bring the dog's updated vaccination record into the facility and the photograph/record updated accordingly.

- All food will be stored in labeled sealed containers within the storage room.

Record Keeping:

- Pet dogs will be used in studies. A roster of available pet dogs will be maintained and kept up to date regarding their history of participation and suitability for this type of research.
- The following records will be kept for each dog:
 - Animal's name
 - Owner's name, address, and telephone number
 - Emergency/contact telephone number of the owner or their nominee
 - A photo of the dog
 - A description of the animal including:
 - ✦ sex
 - ✦ breed
 - ✦ colour
 - ✦ age
 - ✦ distinguishing features
 - ✦ any collars, leads, or belongings brought in with the animal
 - ✦ their vaccination status (photograph of the vaccination certificate and storage of expiry dates in a database)
 - ✦ Microchip number (if the dog is microchipped)
 - ✦ the name and contact number of the veterinarian who normally attends to the animal
 - ✦ the dog's normal diet (food type and amount per day)
 - ✦ if the dog has any allergies, or any other relevant health issues (e.g., medication).
- The date and number of trials during each experimental session will be recorded for each dog. In addition, the time the dog was last fed and any adverse/unusual observations will be noted.
- During the recruitment process, owners will be asked if their dog has shown aggressive behaviour in the past and if it is aggressive/protective around food. If a dog has a history of aggression or shows signs of aggression at any time after recruitment, they will not be used for this research. Owners will also be asked if their dog has any other relevant behaviour issues (e.g., fear of certain noises).
- Owners will be asked to sign in their dogs at the lab at the beginning of the day and out at the end.
- Data will be kept for a minimum of 5 years after data collection has ceased.

Cleaning:

- Dog pens and the experimental room will be vacuumed, and soiled surfaces will be cleaned with disinfectant at the end of each day after dogs have left.
- If any faeces or urine is deposited inside the facility, they will be disposed of appropriately and cleaned thoroughly. For example, disposable gloves will be worn, and a plastic scoop will be used to remove any faeces which will then be bagged and disposed of in an external rubbish bin outside of the dog facility. The area will then be cleaned thoroughly using an appropriate cleaner.
- A foot pedal rubbish bin will be used to contain general waste within the dog facility, and this will be emptied as appropriate.
- Bowls will be washed thoroughly each day with a disinfectant that is suitable for food surfaces that kills both viruses and bacteria and then rinsed.
- Pest control for insects and rodents will be applied as necessary. These control methods will be used in such a way that dogs cannot access them. If there is any risk of dogs accessing them, a nontoxic (to dogs) control method must be used.

Animal Handling/General Care:

- One person will be in attendance when dogs are present at the laboratory. Another person will be aware and available on call during the running days.
- Before removing a dog from its kennel, external doors to the facility will be closed, and the dog must be put on a lead.
- Dogs will be taken out to walk and toilet every 2 hours, and dog waste disposed of appropriately. Volunteer dog walkers who have been recruited and trained may be used to walk dogs involved in research at the University of Waikato.
- Dog walkers will take care to prevent dogs from accessing bait stations that are positioned around campus. An up-to-date map of the location of these stations will be posted at the exit of each building. Walkers will also prevent dogs from accessing discarded food/trash and, if there is any indication that they have eaten something inappropriate, they will immediately report this to the lab supervisor.
- Any dog showing signs of aggression towards the researcher will cease participation in the study.
- Any dogs showing persistent signs of distress or fear will cease participation in the research and the owner will be contacted.
- Dogs that are transported in vehicles by researchers will always be transported in a manner that is safe and approved by the dog's owners.

Dogs will only be transported individually, except for dogs that live together.

- If a dog becomes ill/injured, the laboratory manager will call the owner's vet and take the dog to the vet immediately. If the owner's vet is unavailable, the dog will be taken to the local vet (Newstead Vet) or the after-hours clinic if the local vet is closed. The owner will be contacted as soon as practical. A vehicle must be available (i.e., on-campus) for emergency transportation always when dogs are present at the facility. If the dog's illness/injury occurred as a result of involvement in the research project, the School of Psychology will pay for the veterinary services. Otherwise, the owner will pay for the services.
- Multiple dogs will only be present at the facility at the same time if the owners give permission for this to occur, and if they state that their dog is friendly towards other dogs (as part of the consent form). The dogs will be kept on leads around each other and held in separate kennels/crates/tie-up stations (unless they are from the same household and the owner prefers them held together). Only dogs that are confirmed to be reliably friendly toward each other (including those who live together) will be walked together. If any conflict behaviour is seen between dogs, then the dogs will be separated from each other and the senior researcher will be contacted.

Building Security:

All researchers will ensure the building is locked and secure (windows and curtains shut) before leaving. If there are any security concerns, the University of Waikato security will be called on 07 838-4444.

Emergency Evacuation Procedures:

If the personal safety of the staff or researchers are not compromised the dogs will be led one at a time (if possible) to the fence adjacent to the FMD building where tie-stations have been installed for this purpose. Each dog will be secured to one of the tie-stations, which are situated such that no dog can be in physical contact with another. Five chew-proof leads designated for this purpose are hanging by the main exit of both buildings. Dog owners will be contacted as soon as possible, and temporary provision of water will be made to the dogs.

Versions and Reviews: Version 2.1

Date revised: October 2019

Date approved: 18 October 2019

Next revision due: 19 October 2022

Appendix B

Protocol for Monthly Fish Tank Sterilisation

General:

All four housing tanks must be emptied, thoroughly cleaned, and the interiors sprayed with a 10% hydrogen peroxide solution every month. Record the cleaning date and the initials of the person who cleaned them on the room whiteboard. Lab coats, long-cuff nitrile gloves, and safety glasses must be worn when using a 10% hydrogen peroxide solution. *Take care to use only the equipment designated to each tank (e.g., hand nets, scrubbing pads, etc.) to avoid cross-contamination between tanks.*

- 1) Fill white fiberglass holding tanks with dechlorinated water. Ensure there is sufficient water flow through and aeration.
- 2) Turn off water flow-through and aeration to housing tanks.
- 3) Transfer each species from the housing tanks to the labeled holding tanks using the designated hand nets.
- 4) Completely empty all the housing tanks and thoroughly clean all interior surfaces including the lids, plastic grills, aeration tubing, and air stones with designated scrubbing pads. Wipe down exterior tank and lid surfaces with Janola wipes as much as possible. To avoid cross-contamination, *start with the control tank*, clean one tank at a time, and change gloves between each tank.
- 5) Spray the tank interiors (including lids, aeration stones, and tubing) with 10% hydrogen peroxide solution ensuring all surfaces are thoroughly wetted and tank fittings are soaked. Allow tanks to stand for 15 minutes.
- 6) Rinse tanks, lids, and items inside the tanks with dechlorinated water and resume water flow through and aeration.
- 7) Leave tanks for 24 hours before transferring fish back from the holding tanks using the designated hand nets.
- 8) Spray hand nets with 10% hydrogen peroxide solution and store separately.
- 9) Replace old scrubbing pads used to clean the tanks and rinse containers used to store scrubbing pads. As per above, *start with the control tank items* before the fish tank items, clean one container at a time, and change gloves between each container.
- 10) Drain and rinse the white fiberglass holding tanks, ensure aeration is turned off.

Preparation of 10% Hydrogen Peroxide:

Do not store hydrogen peroxide in the flammables cabinet as it is an oxidiser and incompatible with flammable substances.

- 1) Discard any unused solution older than 1 week down the sink with plenty of water.
- 2) Add 1 L of 30% hydrogen peroxide to the sprayer reservoir.
- 3) Add 2 L of dechlorinated water to the sprayer reservoir.
- 4) After use, make sure the pressure is released from the sprayer by pulling the release valve.

Appendix C

Standard Operating Procedure: Fish Water Sample Collection

Purpose

This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during the collection of water samples from the Aquatic Research Centre, located at FC2 on the University of Waikato Hamilton campus. Only those with prior induction training are authorised to collect samples.

General rules

1. Covered shoes must always be worn on the premises.
2. All personal belongings must be placed in the office (room G.01 on facility map).
3. Lab coats should be worn when collecting samples.
4. Gloves should be changed between handling different samples.
5. Samples should be handled in order from negative to positive (e.g., control-negative, goldfish- negative, koi/catfish- positive).
6. Only have the lid of one tank off at a time. Unless there is a gap of at least one tank between them.
7. Use safety signage as appropriate.

24 hours prior to sample collection

Control Tank

1. This tank does not need to be left on standing.
2. Wear a lab coat and a pair of disposable gloves.
3. Turn the blue lever at the top of the tank from a slight angle to the horizontal position. This will turn off the water flow into the tank.
4. Turn the blue lever at the bottom of the tank from a vertical to a horizontal position. This will allow the tank to drain.
5. Remove the lid from the tank.
6. As the water drains using a designated green scrubbing pad (stored in a labeled plastic box), scrub the plastic tank divider, the sides, and bottom of the tank thoroughly.
7. Return scrubber to its appropriate box.
8. Once the tank is drained completely turn the blue lever at the bottom of the tank from horizontal to the vertical position.
9. Turn the blue lever at the top of the tank from the horizontal position to a slight angle so the water supply is flowing (not gushing) again at a steady rate.
10. Ensure sure the oxygen hose is still under the water surface.
11. Replace the lid on the tank.
12. Dispose of gloves before moving onto the next tank.

Fish Tanks

1. Tanks should be cleaned in order from negative to positive. Gloves should be changed between tanks.
2. Wear a lab coat and a pair of disposable gloves.
3. Turn the blue lever at the top of the tank from a slight angle to the horizontal position. This will turn off the water flow into the tank.
4. Turn the blue lever at the bottom of the tank from a vertical to a horizontal position. This will allow the tank to drain.
5. Remove the lid from the tank.
6. As the water drains (take care not to drain tank fully, make sure fish are still covered by water) using a designated green scrubbing pad, scrub the plastic tank divider, the sides, and bottom of the tank thoroughly to remove any food or waste. Take care not to submerge hands further than the gloved area in the water.
7. Once the water level has drained to the standing level, as indicated in marker on the outside of the tank, turn the blue lever at the bottom of the tank from a horizontal to a vertical position.
8. Do not turn the water supply back on; this is to ensure a potent sample for collection the following day.
9. Ensure the oxygen hose is still under the water surface.
10. Replace lid on the tank.
11. Dispose of gloves.
12. Ensure the floor is clear of water, mop, and squeegee as necessary. Be sure to put up appropriate safety signage if the floor is wet.

Day of Sample Collection

1. Samples should be collected starting from negative to positive. Gloves should be changed between sample types. Sample bottles of different types should be kept separately in plastic zip-lock bags.
2. Wear a lab coat and gloves.
3. Remove the tank lid.
4. Use a beaker that has been acid wash cleaned (refer to glassware cleaning SOP), collect water from the tank, take care not to submerge gloves in the sample, or put the beaker down before all sample water required is collected. Also, take care not to agitate the fish when sampling from a tank containing fish.
5. Pour water from the beaker into the corresponding sample specimen bottle (as indicated by the colour of zip-tie around the bottleneck; black- koi, white- goldfish, black and white – catfish, no tie- control). Repeat until the volume needed is collected and secure the bottle lid. Wipe outside of the bottle with a disposable paper towel and replace it in its designated bag.
6. Put the used beaker in the designated “used” beaker bag for acid washing. This will be taken to the acid washing lab at the end of the day.
7. If the tank is to be used for sampling again the following day, follow “24 hours prior to sample collection” instructions.
8. If the tank is not being used for sample collection the following day, turn the blue lever at the top of the tank from the horizontal position to a slight angle so the water supply is flowing (not gushing) again at a steady rate. Feed fish (if applicable).

9. Ensure the oxygen hose is still under the water surface.
10. Put the lid back on the tank.
11. Dispose of and change gloves. Repeat steps 4.1 to 4.9 as necessary for each tank.
12. Ensure the floor is clear of water, mop, and squeegee as necessary. Be sure to put up appropriate safety signage if the floor is wet.
13. Samples are stored in the fridge at the dog laboratory, at approximately 4°C for a maximum of 24 hours. Positive samples are stored on the bottom shelf, negative samples on the top shelf or in the fridge door (e.g., goldfish), in their designated bags.

Appendix D

Standard Operating Procedure: Fish Water Sample Preparation

Purpose

This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during the preparation of samples during fish water sample scent detection on the University of Waikato Hamilton campus. Only those with prior induction training are authorised to do this.

General Rules

1. Samples bottles are stored in the fridge. Control and negative (goldfish) bottles are to be stored on the top shelf, positive (catfish and koi) samples are to be stored on the bottom shelf. Bottles should stay in their bags unless being used.
2. The left-hand side of the bench is to be used for positive samples only. The right-hand side of the bench is to be used for negative and control samples only. Only one sample type should be prepared at a time, starting from negative to positive.
3. Only glassware that has been acid washed is to be used.
4. Gloves must always be worn when handling equipment and must be changed between handling sample types to avoid cross-contamination.
5. Check the wheels of the transport trolley to make sure they are not loose before putting samples on it.

Bench Preparation

1. Fill and boil the kettle, pour boiling water over both sides of the workbench, and up the metal sides. Wipe clean and repeat.
2. While the kettle is boiling and cut and prepare the stickers for the bottom of the sample jars. One sticker per jar. Control – whole sticker, negative – sticker cut in three, positive – sticker cut in two.
3. Wearing gloves wipe the workbench and sides, and the transport trolley with IPA.

4. Collect the number of jars necessary and mark the bottom with stickers as appropriate; control- whole sticker, negative – 3 stripes, positive – plus sign.

Control Samples

1. Control samples should be prepared first.
2. Wearing clean gloves and using the designated glass cylinder and funnel, measure out 100 mL of control water and pour carefully into each sample jar.
3. When all control samples are prepared, place them gently on the top shelf of the transport trolley.

Negative Samples

1. Negative samples should be prepared second.
2. Wearing clean gloves and using the designated glass cylinder and funnel or autopipette and disposable tip, measure out the desired amount of control water and pour carefully into each jar.
3. Wearing clean gloves and using the designated glass cylinder and funnel or autopipette and disposable tip, measure out the desired amount of negative sample water and pour carefully into each sample jar.
4. When all negative samples are prepared, place them gently on the middle shelf of the transport trolley.

Positive Samples

1. Positive samples should be prepared last.
2. Wearing clean gloves and using the designated glass cylinder and funnel or autopipette and disposable tip, measure out the desired amount of control water and pour carefully into each jar.
3. Wearing clean gloves and using the designated glass cylinder and funnel or autopipette and disposable tip, measure out the desired amount of positive sample water and pour carefully into each sample jar.
4. When all positive samples are prepared, place them gently on the middle shelf of the transport trolley.

Apparatus Set Up

1. The apparatus should be loaded with samples while it is turned off.
2. Gloves should be changed between handling each sample type.
3. Control samples should be loaded first, followed by negative, then positive.
4. Change gloves and place the segments over the samples, take care to make sure segments are closed.
5. Place the lid on the apparatus and slide the blue board into place so the dog cannot reach behind the apparatus.
6. The apparatus can now be turned on. Samples should be left in the segments for 10 minutes before starting a session.
7. Change gloves and re-wipe the transport trolley with IPA.

Appendix E



THE UNIVERSITY OF
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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)? Yes / No

If **no**, please explain briefly:

Does your dog enjoy meeting new people?

Yes / No

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

If **no**, please explain briefly:

Is your dog comfortable being handled by other people?

Yes / No

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

If **no**, please explain briefly:

Is your dog comfortable going to new places?

Yes / No

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

If **no**, please explain briefly:

Is your dog comfortable when you leave them, including at home alone and in new places? Yes / No E.g., is your dog relaxed and happy (showing no signs of stress) when you leave them?

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, (e.g., 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?

Yes / No

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

If **no**, please explain briefly:

Is your dog friendly towards other dogs?

Yes / No

E.g., if your dog has shown any aggression or fear towards other dogs, please select '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? Yes / No E.g., if your dog has any joint or other problems that might be affected, please select '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.

Appendix F

CONSENT FORM *Researcher's Copy*



THE UNIVERSITY OF
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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 water that has contained specific species of fish. 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

STUDENT DETAILS

Dr. Tim Edwards, 07 837 9409, tim.edwards@waikato.ac.nz

Dr. Clare Browne, 07 837 9394, clare.browne@waikato.ac.nz

PURPOSE OF THE PROJECT

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.

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 understood 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:

CONSENT FORM

Owner's Copy



THE UNIVERSITY OF
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Thank you for allowing your dog to participate in behavioural research at the University of Waikato. As part of how we care for your dog when they spend time with us, we take them for regular walks during the day. We would like to get help with the dog walking from volunteer dog walkers, as this would free up a lot more of our researchers' time to work with the dogs. As the owner or duly authorised agent for the owner of _____, you are being asked to give consent for your dog to be walked by trained volunteers.

All volunteer walkers will be given training from postgraduate students and/or staff working on dog behaviour research projects before being allowed to walk a dog on their own. This training will include being supervised by a trained researcher for a minimum of three 15-minute walks.

All volunteer walkers will be given information on our standard animal care and health and safety protocols, and all dogs 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. The volunteer dog walking protocol has also been approved by the University of Waikato Animal Ethics Committee.

Before you give consent for your dog to be walked by trained volunteer dog walkers, please read all of the information on this form, 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

Research student:

Supervisors: Dr. Clare Browne, 07 838 4139, clare.browne@waikato.ac.nz

Dr. Tim Edwards, 07 837 9409, tim.edwards@waikato.ac.nz

Authorisation

I certify that I am over the age of 18 and that I have read and understood the foregoing statements. I hereby grant permission for my dog to be walked by trained volunteer dog walkers. Upon signing below, I will receive a copy of this consent

form. I understand that I can change my decision and withdraw this decision by notifying the researcher or supervisors at any time.

Dog's name: _____

Owner's name: _____

Owner's signature: _____

Date:

Researcher's signature: _____

Date:

Appendix G

Training Dogs for Scent Detection Work Using Automated Apparatus

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

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 porthole. 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 3 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 appendix). For initial sessions, the apparatus should be turned off, and not loaded with samples but one segment may be placed on the apparatus for the dog to open. The closing of a segment does make a sharp tap noise which can sometimes initially startle the dog. Prompting (e.g., pointing) may be used, but the prompt must be faded and removed before processing to the next step (lever activation).

As soon as the dog is comfortably placing its nose into the port far enough to open the segment and make the closing noise, the dog should be removed from the room. The apparatus should then be loaded with positive samples only and turned on. The subject's configuration file on the computer should then be edited to set the status of the samples in relation to their placement on the carousel, and the response times as 1000ms for the minimum indication time and 500ms for observations. The apparatus will now make a beep sound when the dog places its nose in the port. Continue shaping as required until the dog begins to trigger the feeder automatically. Once a run (17 samples) at the 1000ms threshold is complete, increase the threshold in 100-500ms intervals to 1500ms. Once a run is complete at 1500ms, continue to the next step.

Shaping – Lever Activation

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

Discrimination Training

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

Bring the dog into the experiment room and stand in place beside the apparatus. If there is no response given to the apparatus within 20 seconds, prompt as required. When the dog encounters the first negative sample, allow 20 seconds before prompting to see if lever pressing occurs without prompt. Continue prompting when necessary, but fade out prompts as soon as possible (e.g., wait for increasing amounts of time before prompting). Be sure to prompt with a consistent cue.

Once one run has been completed without prompting, randomise the sample arrangement in subsequent sessions and update this in the subject's configuration file. The same randomisation pattern may be used for up to a maximum of 3 sessions in a row before it is needed to be randomised again. Continue until hit rate (correct positive indication) and rejection rate (correct lever pressing) are above 80% without prompt.

At this point, the experimenter should gradually remove themselves from the room and, once the dog is successfully working on its own, systematically increase the indication threshold in 100-500ms increments until they reach the target threshold (5500ms is generally optimal based on our preliminary research, but this may vary depending on the dog/application).

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

Advanced Training

Increasing Run Number

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

Introducing New Samples

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

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

Decreasing Sample Concentration

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

e.g.,

Dilution	Volume Control (mL)	Volume Target (mL)
1 st Dilution	50	50
2 nd Dilution	75	25
3 rd Dilution	87.5	12.5
4 th Dilution	93.75	6.25
5 th Dilution	96.875	3.125

6 th Dilution	98.4375	1.5625
7 th Dilution	99.21875	0.78125

Appendix: 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., the 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 the approach and consumption and/or by attempting to shape a simple response. If confirmed, try selecting a different food (using paired choice preference assessment procedure).
- Check factors related to sample quality (make sure that samples have been prepared and arranged as specified in the specific sample preparation SOP)
- Return to earlier stages of training as required (e.g., if the lever press is not occurring reliably in discrimination training conduct another lever press shaping session in isolation).
- If the dog continues to perform poorly consult with the supervisor. The dog may need to cease participation in the study.
- If the dog is putting its nose in the port too early (while the apparatus is still moving):
- Turn on the "noise mode" in the subject configuration file. The apparatus will now produce a "buzz" while the carousel is still moving.
- Use a board to create an obstacle the dog must navigate around in order to reach the lever/omnidirectional switch and return to the port.

Appendix: Guide for Shaping Introduction

This document outlines the basic training hierarchy for shaping by successive approximations. Generally, each step must be completed 3 times in a row before

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

Procedure

The 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 sample port entry

1. For initial sessions, the apparatus should be turned off, and not loaded with samples but one segment may be placed on the apparatus for the dog to open (The closing of a segment does make a sharp tap noise which can sometimes initially startle the dog).
2. Reinforce moving further and further away from the feeder, until the dog is reliably approaching the side of the room near the apparatus.
3. Reinforce attending to the apparatus (putting nose near or on any part of the front panel).
4. Reinforce nose near the port.
5. Reinforce nose in port.
6. Reinforce nose touching and opening the flap (indicated by a tapping noise as it closes).
7. Reinforce pushing flap inwards.
8. Turn the apparatus on – when the sample port beam is broken it will now produce a “beep” sound.
9. Continue to reinforce for the dog breaking the beam and pushing the flap inward until the dog is fully opening the flap (nose is fully inside the port).
10. The shaping of lever press
11. Turn apparatus off. Do not have apparatus loaded with samples.
12. Reinforce any movement towards the lever/omnidirectional switch.
13. Reinforce movement of nose or paw toward the lever/omnidirectional switch (as appropriate).
14. Reinforce any contact with the lever/omnidirectional switch (nose or paw, as appropriate).
15. Reinforce any movement of the lever/omnidirectional switch.
16. Reinforce movement of the lever/omnidirectional switch that produces a “click” (microswitch closure).

Appendix H

Basic Scent Detection (Fish Projects) Acid Washing 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 R?

laboratory on the University of Waikato Hamilton campus. Only those with prior induction training are authorised to do this.

General Rules

1. Laboratory rules should always be followed.
2. Two people must be present to do acid washing.
3. Acid washing may not be completed after hours (after 5 pm), without prior approval.
4. Glassware is to be left in acid overnight.

Putting into Acid

Hydrochloric acid washing – Sample bottles only

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

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

Nitric acid washing – All other glassware

1. All glassware used other than the sample bottles must be washed in nitric acid.
2. A lab coat, safety glasses, disposable gloves, long green gloves, and a protective apron must be worn when interacting with the acid.
3. Place paper towels out on the table beside the fume hood. Empty glassware to be cleaned (cylinders, funnels, beakers, and sample jars) onto the paper towels.
4. Put on the pair of long green gloves in the fume hood. Check the gloves thoroughly for any cracking, if gloves are deteriorated replace them with new ones.
5. Remove the glass lid from the acid bath.
6. Using tongs (located in a container in the fume hood), carefully place the glassware into the acid bath. Ensure there are no trapped air bubbles and the glassware is fully submerged. Cylinders can only be placed in the large acid bath on the left-hand side as it is the only bath deep enough to be able to submerge properly.
7. Replace the acid bath lid.
8. Rinse the tongs and long green gloves thoroughly in the sink and return them to the fume hood. Be sure to make sure you've properly closed the fume hood.
9. Remove protective apron and fold it neatly back on the table.
10. Dispose of gloves.

Taking out of acid

Hydrochloric acid – Sample bottles only

1. A lab coat, safety glasses, and disposable gloves should be worn.

2. There are acid buckets of 10% HCl designated to each sample type, this is labeled on each bucket. There are also labeled long green gloves (need appropriate names for these) designated to each sample type/bucket.
3. Bottles should be handled in order from negative to positive sample type.
4. Wear the designated long green gloves.
5. Remove the lid from the bucket.
6. Remove the sample bottles from the bucket, tip the acid out carefully and slowly to avoid splashing.
7. Place the bottle into the labeled designated rinsing bucket.
8. Remove the lids from the acid solution and place these into the labeled designated rinsing bucket.
9. Replace the acid bucket lid.
10. Rinse the bottles and lids in RO water, and place them on the rinsing bucket lid for transport to the drying incubator.
11. Rinse the long green gloves under the tap and return them to the correct space beside their designated bucket.
12. Take the rinsed bottles 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.
13. Dispose of and change gloves (if applicable).
14. Repeat steps for each sample bottle type.
15. Leave glassware in the incubator to dry overnight.
16. Wearing gloves put clean dry glassware in designated storage containers.

Nitric acid – All other glassware

1. A lab coat, safety glasses, disposable gloves, long green gloves, and a protective apron must be worn when interacting with the acid.
2. Ensure the rinsing buckets are full before interacting with acid.
3. Put on the pair of long green gloves in the fume hood. Check the gloves thoroughly for any cracking, if gloves are deteriorated replace them with new ones.
4. Remove the glass lid from the acid bath.
5. Using tongs (located in a container in the fume hood), carefully remove the glassware from the acid bath. Empty glassware of acid as much as possible, and then submerge in the rinsing bucket.

6. Replace the acid bath lid.
7. Rinse the tongs and long green gloves thoroughly in the sink and return them to the fume hood. Be sure to make sure you've properly closed the fume hood.
8. Remove protective apron and fold it neatly back on the table.
9. Dispose of gloves and replace.
10. Glassware must be submerged in the rinsing bucket for 10 minutes prior to being rinsed with RO water (Can't find this written in old SOP but this was what Emily had passed on to us?).
11. Place rinsed glassware on the rinsing bucket lid for transport to the drying incubator.
12. Place glassware in the incubator.
13. Dispose of gloves.
14. Leave glassware in the incubator overnight to dry.
15. Wearing gloves put clean dry glassware in designated storage containers.